GYNECOLOGY IN PRACTICE

Series editor **Aydin Arici**

Infertility

Edited by

Emre Seli

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Infertility

GYNECOLOGY IN PRACTICE: SERIES EDITOR AYDIN ARICI

Infertility

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Series Foreword

In recent decades, massive advances in medical science and technology have caused an explosion of information available to the practitioner. In the modern information age, it is not unusual for physicians to have a computer in their offices with the capability of accessing medical databases and literature searches. On the other hand, however, there is always a need for concise, readable, and highly practicable written resources. The purpose of this series is to fulfill this need in the field of gynecology.

The *Gynecology in Practice* series aims to present practical clinical guidance on effective patient care for the busy gynecologist. The goal of each volume is to provide an evidence-based approach for specific gynecologic problems. "Evidence at a glance" features in the text provide summaries of key trials or landmark papers that guide practice, and a selected bibliography at the end of each chapter provides a springboard for deeper reading. Even with a practical approach, it is important to review the crucial basic science necessary for effective diagnosis and management. This is reinforced by "Science revisited" boxes that remind readers of crucial anatomic, physiologic or pharmacologic principles for practice.

Each volume is edited by outstanding international experts who have brought together truly gifted clinicians to address many relevant clinical questions in their chapters. The first volumes in the series are on *Chronic Pelvic Pain*, one of the most challenging problems in gynecology, *Disorders of Menstruation*, *Infertility*, and *Contraception*. These will be followed by volumes on *Sexually Transmitted Diseases*, *Menopause*, *Urinary Incontinence*, *Endoscopic Surgeries*, and *Fibroids*, to name a few. I would like to express my gratitude to all the editors and authors, who, despite their other responsibilities, have contributed their time, effort, and expertise to this series.

Finally, I greatly appreciate the support of the staff at Wiley-Blackwell for their outstanding editorial competence. My special thanks go to Martin Sugden, PhD; without his vision and perseverance, this series would not have come to life. My sincere hope is that this novel and exciting series will serve women and their physicians well, and will be part of the diagnostic and therapeutic armamentarium of practicing gynecologists.

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Preface

The rapid pace of scientific and technological discovery has greatly influenced the care of the infertile couple. Diagnostic and therapeutic options for infertile couples have undergone dramatic evolution in the past three decades, touching the lives of couples for whom few options existed previously. More than 30 years after the birth of the first child conceived by assisted reproductive technologies, we are now able to stimulate ovaries to obtain multiple oocytes, extract sperm from testes of men with severe infertility, inject sperm into oocytes to achieve fertilization, and biopsy embryos for diagnostic purposes. With increasingly complex modalities available for diagnosing and treating infertility comes the need for a clear, concise, readable, and practical text to serve as a resource for providers.

This text has been written with the aim of providing a clinical "in the office" or "at the bedside" guide for effective patient care for obstetrician gynecologists and trainees. Written by experts in the field of reproductive endocrinology and infertility, this book provides cutting-edge and evidence-based suggestions regarding common and important clinical problems in reproductive medicine. A standard format is adopted throughout the text, intended to streamline access to critical information. Practical guidance is provided through the use of sensible algorithms and treatment guidelines. Key evidence is summarized in "Evidence at a Glance" boxes; "Tips & Tricks" boxes provide hints on improving outcomes, and "Caution" boxes offer suggestions for avoiding potential problems and pitfalls. In addition, "Science Revisited" boxes provide quick reminders of the basic scientific principles necessary to more fully understand the clinical issues at hand.

The overall format of the volume is similarly aimed at facilitating access to information. Following a discussion of factors affecting fertility and an overview of the evaluation of the infertile couple, the diagnosis and management of specific causes of infertility are presented. Treatment options available for infertile couples (including ovulation induction, intrauterine insemination, in-vitro reproductive technologies, and oocyte donation/gestational surrogacy) and complications of infertility treatments are then discussed. Finally, up-to-date reviews of preimplantation genetic screening/diagnosis and fertility preservation are provided; two areas of extensive research in the field of infertility. The volume concludes with a chapter comparing the different approaches to diagnosis and treatment of infertility in different parts of the world, emphasizing that there are differences among practices and resources across continents (and that there is often "more than one way to skin a cat").

I would like to thank all the authors for their outstanding contributions. I would also like to thank Martin Sugden, Lewis O'Sullivan, and Michael Bevan from Wiley, who made this book possible. I hope the readers will find the book a useful and concise review of a rapidly evolving field of medicine.

Emre Seli
New Haven
1 September 2010

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Factors Affecting Fertility

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In order to take optimal care of patients who seek preconceptional counseling and of patients who present with infertility, the treating physician needs to have an extensive knowledge of the multiple factors that influence human reproduction.

Fecundability and time to pregnancy (TTP) are used in the literature as markers of fertility. Fecundability is defined as the probability of conceiving in a single menstrual cycle while TTP is the length of time in months that it takes a couple to conceive. Infertility is defined as the failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse in a woman under the age of 35 years and 6 months without success in a woman 35 or older.

Although the current literature describes a number of factors that are likely to play a role in the ability that a patient or couple has to conceive, it is important to highlight that evidence from randomized controlled trials is lacking for the majority of these regarding quantification and certainly causality of each factor.

In this chapter we summarize the most relevant factors that affect fertility, making evidence-based recommendations where appropriate to better counsel our patients to improve their ability to conceive. Our recommendations will include the practice committee opinions of the American Society for Reproductive Medicine (ASRM) and the American College of Obstetricians and Gynecologists (ACOG) as leading institutions in reproductive medicine and women's health in the United States.

Weight

Body mass index (BMI) is used in the literature as an objective marker to classify underweight, overweight and obesity in adults. BMI is a calculated measurement that compares a person's weight and height and it is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). Even though there is a growing debate on the possible need to develop different BMI cut-off points for each ethnic group, BMI is applicable to all ethnic groups, is the same for both sexes and is age-independent.

The World Health Organization (WHO) and the National Institutes of Health (NIH) describe underweight as a BMI < 18.5, normal weight as BMI 18.5–24.9, overweight as BMI 25–29.9 and obesity as BMI > 30.

☆ TIPS & TRICKS

Always calculate and document your patient's BMI in the first office visit (weight in kilograms divided by the square of the height in meters) and determine if she falls under the category of underweight (BMI < 18.5), normal weight (BMI 25–29.9), overweight (BMI 25–29.9) or obese (BMI > 30). Appropriate counseling should follow.

BMI should then be calculated in the follow-up visits to determine any changes as well as to track response to treatment or interventions.

Infertility, 1st edition. Edited by Emre Seli.

Fecundability has been found in multiple studies to be lower at the extremes of BMI in patients trying to conceive spontaneously. This finding was confirmed in a recent prospective cohort study by Wise et al., in which a longer TTP was seen in women who were overweight, obese and very obese (BMI ≥ 35), compared with normal weight women. Additionally being underweight (BMI < 20) was associated with reduced fecundability among nulliparous women.

Although some authors have linked male obesity with subfecundity, the evidence is not compelling and there are no randomized controlled studies to address this association. Male obesity was not linked to subfecundity in the recent prospective cohort study by Wise et al.

The direct effect of being overweight and obese on assisted reproduction technologies (ART) is less clear than for spontaneous pregnancies. In a meta-analysis by Maheshwair et al., women with a BMI ≥ 25 had a lower chance of pregnancy following *in vitro* fertilization (IVF) [odds ratio (OR) 0.71], required a higher dose of gonadotropins and had an increased miscarriage rate (OR 1.33) in comparison to women of normal weight. In a recent study by Bellver et al., implantation, pregnancy and live birth rates were lower in obese women; in fact, pregnancy and live birth rates were reduced progressively with each unit of BMI, independent of embryo quality, suggesting an alteration in the uterine environment as a likely factor in these patients.

Evidence is accumulating that suggests that effective treatment of women with elevated BMI may improve reproductive outcome. Nonsurgical treatment for patients with overweight and polycystic ovary syndrome (PCOS) was shown to improve fertility in patients that lost at least 5% of their weight in a small prospective study by Crosignani. Surgical treatment may show benefit as well, as evidenced by a recent meta-analysis by Merhi which concluded that although "the majority of the present data tend toward reporting an improvement in fertility status after surgical weight loss, it remains unclear whether this is a direct result of the significant weight reduction per se".

Recommendations

All patients should be advised to follow a healthy diet according to the United States Department of Agriculture (USDA) guidelines. Patients should consume a variety of nutrient-dense foods and beverages within and among the basic food groups while choosing foods that limit the intake of saturated and trans fats, cholesterol, added sugars, salt and alcohol.

ACOG recommends folic acid supplementation of 400 µg/day on all women capable of becoming pregnant, since it has shown to reduce the occurrence and recurrence of neural tube defects.

Weight loss and exercise should be advised for all women who are overweight or obese for all the associated health benefits.

There is not enough evidence to recommend surgical treatment for obesity on the ground of fertility improvement. Patients who wish to pursue bariatric surgery should have appropriate medical management with an expert in the field to monitor possible nutritional deficiencies and other complications.

☆ TIPS & TRICKS

Refer your patients to the USDA website to obtain detailed information on following a healthy diet with help of the USDA Food Guide and the DASH Eating Plan: http://www.health.gov/DietaryGuidelines/dga2005/document/

Age

The incidence of infertility increases with advancing maternal age. In a prospective study by Dunson et al., the percentage of infertility was 8% for women aged 19–26 years, 13–14% for women aged 27–34 years and 18% for women aged 35–39 years.

There is also evidence that the age of the male partner may affect fertility after the age of 35 years. In the study by Dunson et al., the proportion of couples failing to spontaneously conceive within 12 cycles increased from 18% when the male partner was 35 years old to 28% when the male partner was 40.

There is a similar decline in the success of IVF as the age of patient increases. In 2007, the percentage of transfers of fresh embryos from nondonor oocytes resulting in live births in the United States was 46.1 for patients <35 years of age and showed a progressive decline to 16.4 in patients 41–42 years of age.

Recommendations

Although according to the ASRM guidelines a patient is diagnosed with infertility after 12 months or more of being unable to achieve a successful pregnancy, earlier evaluation and treatment may be justified based on medical history and physical findings and is warranted after 6 months for women over age 35 years.

The ASRM additionally states that "times to conception increases with age. For women over age 35 years, consultation with a reproductive specialist should be considered after 6 months of unsuccessful efforts to conceive."

Important reproductive potential and ovarian reserve tests that are commonly performed by specialists include a sonogram to assess general pelvic anatomy and basal antral follicular count (BAFC) as well as the day 3 serum biomarkers follicle stimulating hormone (FSH) and estradiol.

As part of the counseling process for treatment of infertility, the reproductive specialist should be familiar with the new and innovative options that are available for each individual patient, including options that halt the reproductive aging process such as oocyte cryopreservation.

Smoking

Approximately 30% of reproductive age women and 35% of reproductive age men in the United States smoke, and up to 13% of infertility may be attributable to cigarette smoking. Most studies that address the effect of active smoking of the female partner on fertility to date report a decreased fecundability independent of other confounding influences. In the largest available population study by Hull et al., the increasing delay to conception correlated with increasing daily numbers of cigarettes smoked.

In a meta-analysis by Waylen et al., which included 22 studies, patients who smoked had significantly lower odds of live birth per cycle (OR 0.54), significantly lower odds of clinical pregnancy per cycle (OR 0.56), significantly higher odds of spontaneous abortion (OR 2.65) and significantly higher odds of ectopic pregnancy (OR 15.69).

The ASRM in the Practice Committee Opinion on Smoking and infertility concluded that smoking (1) appears to accelerate the loss of reproductive function, (2) may advance the time of menopause by 1–4 years and (3) is associated with an increased risks of spontaneous abortion, ectopic pregnancy and gamete mutagenesis. Additionally, smokers require nearly twice the number of IVF attempts to conceive as nonsmokers.

A definite causality between male partner smoking and infertility has not been proven but there are data that suggest that there may be adverse effects in male smokers, as well. Smokers have an average 23% decrease in sperm concentration and 13% decrease in sperm motility in comparison to nonsmokers.

The largest meta-analysis to date on the effect of female and male smoking on IVF included 22 studies and despite the variations in results between studies, there was compelling evidence that smoking had a negative influence on IVF outcome.

Recommendations

The United States Public Health Service (USPHS) guidelines recommend that advice to quit and brief counseling be done at all or nearly all office visits by a smoker, regardless of the reason for the visit.

☆ TIPS & TRICKS

The USPHS and ACOG recommend using the "5 A's" algorithm for brief counseling in the office:
Ask about smoking status
Advise smokers to quit
Assess their readiness to quit
Assist them with their smoking cessation effort
Arrange follow-up visits or contact

☆ TIPS & TRICKS

Be familiar with the different tools available to help your patient quit, including:

- Telephone counseling (1–800-QUITNOW)
- Pharmaceutical aids:*
  Nonprescription aids:
  Nicotine replacement therapy [pregnancy class D]:
  Gum, lozenge and transdermal patch
  Prescription aids: Nicotine replacement therapy (nasal spray and oral inhaler [pregnancy class D]), bupropion (pregnancy class C) and varenicline (pregnancy class C)
- Computer programs
- Websites:
  http://www.smokefree.gov/
  http://www.cdc.gov/tobacco/

*ACOG states that "The use of nicotine replacement products or other pharmaceuticals for smoking cessation aids during pregnancy and lactation have not been sufficiently evaluated to determine their efficacy or safety. Nicotine gum, lozenges, patches, inhalers, and special-dose antidepressants that reduce withdrawal symptoms, such as bupropion, should be considered for use during pregnancy and lactation **only** when nonpharmacologic treatments (e.g., counseling) have failed."

Caffeine

Caffeine consumption is common in women of reproductive age, as shown in a recent study of women between the ages of 16 and 45 where the mean consumption of caffeine was 173.95 mg/day (1 cup of coffee is approximately equivalent to 100 mg of caffeine). Eighteen percent of women exceeded caffeine guidelines and consumed 300 mg or more of caffeine.

Subfertility has been linked to heavy caffeine consumption (>500 mg). A European multicenter study that controlled for potential confounding factors (e.g. smoking) found a significantly increased OR (OR 1.45) for subfecundity among women that consumed more than 500 mg per day of caffeine (>5 cups of coffee/day), which represented an increase in the TTP of 11%. The OR was even higher in patients who also smoked (OR 1.56).

A meta-analysis by Klonoff-Cohen on the effect of female and male caffeine consumption on IVF found only one study that directly examined the effect of female and male caffeine consumption on IVF. In this study, female caffeine consumption had an effect on spontaneous abortions (OR range from 6.2 to 19.8, depending on the dose and timing of consumption), failure on achieving a live birth (OR 2.9–3.9) and infant gestational age (OR decreases of 3.5–3.8 weeks). Male consumption of caffeine did not have an effect on sperm, IVF or neonatal endpoints.

Given the limitations of available data, more studies are needed to further evaluate this possible association.

Recommendations

Patients attempting to conceive naturally or through ART who consume more than 500 mg of caffeine/day (>5 cups of coffee/day) should be advised to limit consumption to 100–200 mg of caffeine/day (1–2 cups of coffee or equivalent).

The ASRM states in their Committee Opinion that "moderate caffeine consumption (1–2 cups of coffee/day or equivalent) before or during pregnancy has no apparent adverse effects on fertility or pregnancy outcomes."

Alcohol

The data reflecting the effect of alcohol on fertility have shown conflicting results. The most recent prospective study on 18555 women addressing this possible effect, by Chavarro et al., did not find an association between alcohol consumption and infertility after adjusting for other possible confounding factors (e.g. smoking, parity), confirming the findings of a prior prospective trial by Florack et al., where the level of alcohol consumption in the female partner was not related to fecundability. In the study by Chavarro et al., an even split on the results of the available prospective trials is reported (three positive and three null studies).

In the meta-analysis on the effect of female and male alcohol consumption on ART by Klonoff-Cohen, the author found one study that examined female and male alcohol consumption as a primary risk factor for ART. In this study, female alcohol consumption was associated with a 13% decrease in the number of oocytes

retrieved (OR 0.87), a decrease in pregnancy rate (OR 2.86), and an increased risk of spontaneous abortion (OR 2.2). Additionally, male alcohol consumption (1 drink) during the IVF cycle was associated with increased risk of spontaneous abortions, compared with men who did not drink 1 month before the IVF attempt (OR 2.7), or up to 1 week before sperm collection (OR 38.04).

Additional studies are needed to further assess the relationship between alcohol consumption and ART.

☆ TIPS & TRICKS

Assess if your patient has an alcohol-drinking problem by following the simple CAGE questionnaire in the first office visit:

- Have you ever felt the need to cut down on drinking?
- Have you ever felt annoyed by criticism of your drinking?
- Have you ever had guilty feeling about your drinking?
- Do you ever take a morning eye opener (a drink first thing in the morning to steady your nerves or get rid of a hangover)?

One positive response to any of these questions suggests suggest the need for closer assessment; two or more positive responses are very suggestive of alcoholism.

Recommendation

Patients who report heavy consumption of alcohol or who test positive to alcohol consumption questionnaires should be referred to a substance abuse specialist.

Patients attempting to conceive naturally or through ART who consume alcohol should be advised to avoid consumption of more than 2 drinks/day.

The ASRM states in their Committee Opinion "higher levels of alcohol consumption (≥2 drinks/day) probably are best avoided when attempting pregnancy, but there is no evidence to indicate that more moderate alcohol consumption adversely affects fertility."

Alcohol consumption should cease completely once pregnancy is established, since the level of alcohol consumption that is safe during pregnancy is not known. In fact, the U.S. Surgeon General's advisory on alcohol use in pregnancy advises women who are pregnant or considering becoming pregnant to abstain from using alcohol.

Summary

Factors that play an important role in fertility include the age and weight of the patient, as well as maternal consumption of tobacco, caffeine or alcohol.

Weight

Fecundability has been found in multiple studies to be lower at the extremes of BMI in patients trying to conceive spontaneously. The direct effect of being overweight and obese on ART is less clear than for spontaneous pregnancies. In a recent prospective cohort study, male obesity was not linked to subfecundity.

Age

The incidence of infertility increases with advancing maternal age, as does the likelihood of success with ART. There is some evidence that the age of the male partner may affect fertility after the age of 35 years.

Smoking

Smoking is associated with an increased risk of spontaneous abortion, ectopic pregnancy and gamete mutagenesis. Additionally, smokers require nearly twice the number of ART attempts to conceive as nonsmokers.

A definite causality between male partner smoking and infertility has not been proven.

Caffeine

Subfertility has been linked to heavy caffeine consumption (>500 mg/day). Female caffeine consumption has been linked to an increased risk of spontaneous abortion, failure to achieve a live birth and a decrease in gestational age of the infant. Male consumption of caffeine has not been proven to have a deleterious effect on fertility.

Alcohol

The data reflecting the effect of alcohol on fertility have shown conflicting results. A recent prospective study did not find an association between alcohol consumption and infertility.

Recommendations

The following advice should be given to patients attempting to conceive spontaneously or through ART:

- follow a healthy diet according to USDA guidelines (including folic acid supplementation of 400 μg/day)
- quit smoking
- limit caffeine consumption to 1–2 cups of coffee/day or equivalent
- avoid alcohol consumption greater than 2 drinks/day (quit completely once pregnancy confirmed).

Additionally, weight loss and exercise should be advised for all women who are overweight or obese, for all the associated health benefits. Evidence is accumulating to suggest that effective treatment of women with elevated BMI may improve reproductive outcome.

Although the diagnosis of infertility is established until 12 months of not being able to conceive, earlier evaluation and treatment may be justified in some patients based on medical history and physical findings and is warranted after 6 months for women over the age of 35 years.

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http://www.smokefree.gov/

http://www.cdc.gov/tobacco/

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Evaluation of the Infertile Couple

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Introduction

It is estimated that approximately 15% of couples will seek assistance for fertility issues during their reproductive years. This number is increasing as more women elect to delay childbearing and patients are increasingly aware of testing and treatment options. Infertility is generally defined as 1 year of unprotected intercourse without conception. It may take longer to conceive as women age; therefore, it is appropriate to initiate an evaluation after 6 months of infertility for women over the age of 35 years or for women with a history of oligomenorrhea or amenorrhea, a partner who is known to be subfertile, or known uterine/tubal disease or endometriosis.

The basic evaluation of infertility, regardless of patient age, includes a thorough history and examination, assessment of ovulation, a semen analysis, and assessment of the uterine cavity and tubal patency. Additional testing may be warranted based on the patient's history and may include laparoscopy to evaluate the pelvic and ovarian reserve testing (Table 2.1). The recommendations for appropriate testing have been revised over time as the causes of infertility have been investigated and tests to detect these underlying etiologies have been evaluated. Evaluation should be tailored to the patient's history, age and duration of infertility, and should be cost-effective and evidence-based.

Evaluation of the female

History and physical examination

Approximately 40% of infertility can be attributed to female fertility factors which may be elicited from patient history. A thorough history should include both childhood and pubertal development, as these may influence future menstrual function and fertility. A detailed menstrual history is an integral part of the evaluation. Age at menarche as well as length, frequency and amount of menstrual flow should be noted. A history of irregular cycles, menorrhagia or intermenstrual bleeding can indicate an ovulatory disorder or an anatomic condition of the uterus warranting further evaluation. Any complaints of neck tenderness, masses or galactorrhea may indicate a thyroid or prolactin abnormality. Any chronic pelvic pain, pain with menses (dysmenorrhea) or with intercourse (dyspareunia) may indicate pelvic pathology such as infection or endometriosis. Prior Pap smear abnormalities and subsequent treatments (i.e. ablative or excision procedures) should be noted. Other pertinent reproductive history includes prior pregnancies and their outcomes.

A detailed medical history includes ongoing medical conditions, prior illnesses and infections, surgeries (particularly pelvic surgeries), medications and social habits such as tobacco and alcohol consumption. Any family history of reproductive difficulties, birth defects or genetic diseases should also be elicited. Exposure to any toxic environmental agents should be noted, but are not known to be a common cause of infertility in most patients. Physical examination should include assessment of the thyroid for enlargement, masses or tenderness. A breast exam for masses or secretions may reveal galactorrhea. Ay signs of hirsutism, male pattern baldness or acne may indicate the possibility of polycystic ovary

Infertility, 1st edition. Edited by Emre Seli.

Table 2.1 Evaluation of the infertile couple

Hysterosalpingogram	Performed in the follicular phase of the cycle (day 7–12) to evaluate uterine cavity and fallopian tube patency Additional assessment of the uterus and/or uterine cavity may be performed with transvaginal ultrasound or SIS
Semen analysis	Assessment of sperm quality and quantity The man should abstain from ejaculation for 2–3 days prior to semen analysis Semen analysis should be repeated and patient should be referred to a urologist if preliminary testing indicates abnormalities
Assessment of ovulation	BBT OVT Serum progesterone assay (timed 7 days after LH surge or 7 days before expected menses) Luteal phase progesterone assay is the most accurate of the tests;however, any are acceptable to determine ovulation
Additional hormonal testing	*TSH and prolactin:* Only necessary if patient is oligoovulatory *Ovarian reserve testing:* Day 3 FSH and E2 AMH AFC Testing of ovarian reserve is indicated for women >35 years of age, or with a single ovary, a history of prior ovarian surgery or prior poor response to exogenous gonadotropin stimulation No single test of ovarian reserve has been found to be most predictive of fertility potential but increasing evidence suggests that AMH and antral follicle counts may be more predictive than FSH and E2 alone
Laparoscopy	Peritoneal factors such as endometriosis and pelvic/adnexal adhesions should be evaluated with a laparoscopy only if there is a strong clinical suspicion of these conditions or before considering aggressive empirical treatments with significant cost and/or risks

AFC, antral follicle count; AMH, antimüllerian hormone; BBT, basal body temperature charting; E2, estradiol; OVT, ovulation predictor testing; SIS, saline instilled sonography.

syndrome (PCOS). An abdominal/pelvic exam may reveal uterine or ovarian masses, vaginal or cervical abnormalities, or pelvic tenderness suggestive of endometriosis.

Assessment of ovulation

Ovulatory infertility will be detected in approximately 30% of infertile women and is the most common etiology for female factor infertility. There are several methods for evaluating ovulation including menstrual history, ovulation predictor kits, basal body temperature charting, ultrasound, endometrial biopsy and midluteal serum progesterone. Menstrual history is often all that is required to reveal an ovulatory dysfunction. Infrequent or absent menstrual cycles often indicates an ovulatory disorder.

Methods to more specifically evaluate for an ovulatory disorder include home assessment with ovulation detection test (OVT) or basal body temperature (BBT) charting. OVTs include kits for assessing urinary luteinizing hormone (LH) at midcycle and are approximately 90–95% accurate. Other more elaborate electronic monitors that incorporate other factors such as menstrual cycle length tracking are not clearly more effective for most patients than urinary OVT alone.

Ovulation may also be assessed by BBT charting performed by the patient. BBT tracking is performed by measuring temperature each

morning before rising and is based on the principle that temperature rises in response to the presence of serum progesterone after ovulation. Baseline temperature during the follicular phase of the cycle prior to ovulation is 97.0–98.0 °F (36.1–36.7 °C) and increases approximately 0.5 °F (0.25 °C) or more after ovulation in the luteal phase. Since progesterone rises after ovulation, BBT is not helpful in an individual cycle to determine the most appropriate time for intercourse but rather is used retrospectively to determine a range of days in which ovulation generally occurs.

Perhaps the easiest and more accurate way to assess ovulation is a midluteal serum progesterone assay. Serum progesterone is generally low (1 ng/mL or less) in the early follicular phase, rises slightly on the day of the LH surge to approximately 1–2 ng/mL, and then peaks in the midluteal phase approximately 7 days after ovulation. There is no specific quantitative value of progesterone that indicates the quality of ovulation, however, a value >3–4 ng/mL indicates that ovulation occurred. Serum progesterone testing is best scheduled approximately 7 days after an LH surge detected with an OVT. Serum progesterone is otherwise evaluated on cycle day 21–23 in a patient with a 28-day cycle, or approximately 7 days prior to expected menses in a patient with longer cycles (i.e. cycle day 28 in a patient with 35-day cycles). It is important to remember that menstrual cycle lengths may vary and the serum progesterone assessment will not be accurate if not timed properly.

Other methods for assessing ovulation have been suggested such as serial ultrasound and endometrial biopsy. Serial transvaginal ultrasound is useful prior to ovulation to detect the size and number of preovulatory follicles and the growth of the endometrium in response to estradiol production from the ovary. Findings of a collapsed follicle or development of a corpus luteum are presumptive of ovulation; however, these signs are not particularly helpful in establishing the timing of ovulation. In addition, serial ultrasounds are not as cost-effective as other options and are generally not warranted.

Endometrial biopsy is often described as the "gold standard" to assess ovulation. Histologic evidence of secretory endometrial changes indicates a progesterone effect on the endometrium and confirms ovulation. This test has the disadvantages of patient discomfort and increased cost compared to other methods; its use is therefore not indicated in the general infertility evaluation.

In summary, there are several methods for assessing ovulation and the choice of method should be individualized to the patient. If a diagnosis of ovulatory infertility is established, further investigation into the underlying etiology is warranted. Common etiologies for anovulation include thyroid disease, PCOS, hyperprolactinemia, eating disorders, perimenopause and pituitary or hypothalamic conditions. Further evaluation will depend on patient history but will often include a TSH, prolactin, follicle stimulating hormone (FSH) and estradiol to determine the underlying etiology. An ovarian ultrasound may also be used in the diagnosis if PCOS is suspected based on menstrual history and/or signs of hirsutism. Polycystic ovaries are found on ultrasound if multiple small (<10 mm) follicles are crowded along the periphery of the ovary.

Fallopian tube assessment

Tubal disease is responsible for approximately 15% of female infertility. The two primary methods for determining fallopian tube patency are hysterosalpingography (HSG) and laparoscopy with fallopian tube chromopertubation. Laparoscopy has a higher risk of complications, so HSG is preferred unless the patient is otherwise undergoing surgery for another consideration. HSG is able to detect uterine cavity abnormalities such as uterine polyps, müllerian anomalies and fibroids. HSG is also valuable to assess fallopian tube architecture and abnormalities such as salpingitis isthmica nodosum (SIN), polyps, hydrosalpinges and peritubal adhesions. There are two contraindications for HSG: current pelvic infection and pregnancy. Allergy to iodine is also a consideration if agents containing iodine are used for fallopian tube instillation.

There are a variety of instruments to instil dye into the uterine cavity including acorn-top catheters, uterine balloon and cervical cap. The choice of instrument is generally based on operator preference and there is no clear advantage of one instrument over another. There are two types of dye used for instillation into the uterus and

fallopian tubes: water soluble and oil based. A water soluble dye is generally preferred as it provides improved visualization of subtle abnormalities. Fluoroscopic images are obtained intermittently to evaluate the uterus and tubes during slow instillation of dye.

The HSG should be scheduled during cycle days 7–12 to avoid the possibility of pregnancy. No medications are required prior to performing the procedure; however, anti-inflammatories may be recommended an hour prior to HSG or within 30 minutes after to decrease the risk of cramping. Various other agents such as paracervical block and topical analgesia have been evaluated with no conclusive evidence of benefit for pain reduction. Antibiotics are not universally recommended but may be considered in patients with a prior history of pelvic infection or findings of hydrosalpinges on HSG.

HSG is valuable for evaluating both the uterine cavity and fallopian tube architecture. There should be free spill of contrast material into the peritoneal cavity during the HSG if there is no occlusion of the fallopian tubes. The fallopian tubes have three segments visualized on HSG: the interstitial or cornual region attaching the tube to the uterus, the isthmic portion and the ampullary portion nearest the ovary. Tubal abnormalities can be due either to occlusion from prior infection or surgery, or to spasm of the tube in reaction to the contrast material. Proximal spasm of the tube at its junction with the uterus can occur as the cornual portion of the tube is encased by smooth muscle of the uterus. This is difficult to distinguish from a pathologic condition resulting in occlusion. The HSG should be repeated or a laparoscopy performed to differentiate spasm from actual occlusion.

Obstruction of the fallopian tubes in the isthmic (Fig. 2.1) or distal ampullary tube is more likely to be pathologic. SIN may be visualized in the isthmic segment as several small diverticulum of contrast and is secondary to prior infection. Distal tubal obstruction can be secondary to peritubal adhesions or hydrosalpinx (Fig. 2.2) which is identified by significant dilation of the fallopian tube and absence of spill of contrast into the peritoneal cavity.

Fibroids, polyps and uterine synechiae manifest as filling defects on HSG and can have a variety of appearances depending on their size and location. Small abnormalities are best visualized during early contrast filling of the uterus as they may be obscured when the uterus is completely opacified. This provides the most accurate assessment of the architecture of the abnormality to determine the most likely diagnosis. Uterine fibroids and polyps are generally well demarcated with smooth borders, in contrast to adenomyosis and uterine synechiae. Adenomyosis is a condition in which endometrium extends into the myometrium of the uterus. This may be seen on HSG as small diverticula with contrast material extending into the myometrium. If adenomyosis is suspected, MRI is more commonly used to confirm the diagnosis. Uterine synechiae may be present from prior endometritis or uterine surgery such as myomectomy, cesarean

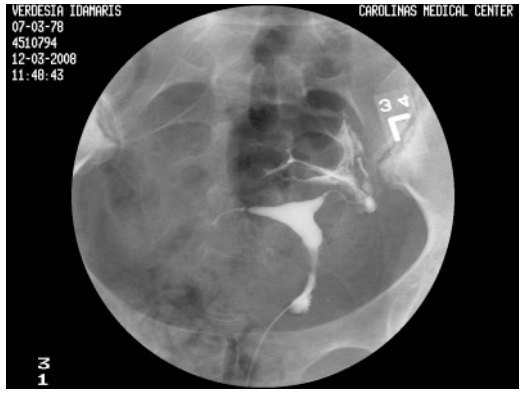

Figure 2.1 HSG with normal uterine cavity, midtubal occlusion of the right fallopian tube, and patency of the left tube.

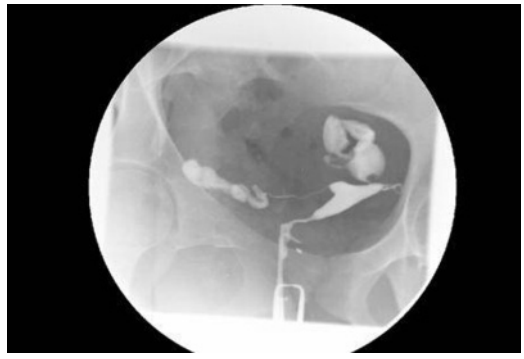

Figure 2.2 HSG with bilateral hydrosalpinges.

section, endometrial ablation or dilation and curettage. This will manifest as an irregular contour within the cavity of the uterus. Further assessment of any abnormality with transvaginal ultrasound, saline instilled sonography (SIS) and/or hysteroscopy is often needed for further assessment.

Alternatives to HSG have been recommended for evaluation of tubal patency including chlamydia antibody titers, sonohysterography (SHG) or hysterosalpingo-contrast sonography (HyCoSy), and salpingoscopy. Chlamydia antibody titers have been suggested as a cost-effective, noninvasive test of fallopian tube status since chlamydia is the primary cause of pelvic inflammatory disease (PID) and subsequent tubal damage. However, other etiologies of possible tubal damage (i.e. gonorrhea, prior tubal surgery, endometriosis) will not be detected with this assay. More direct methods of assessing fallopian tube status include injecting saline (SHG) or air-contrast (HyCoSy) through the uterus and fallopian tubes and observing for presence in the cul-de-sac by ultrasound observation. Benefits of these techniques may include improved patient comfort, decreased cost, and avoidance of radiation exposure, however, the location of tubal blockage cannot be determined if present. Salpingoscopy is assessment of the interior architecture of the fallopian tube by endoscopy. It has the advantage of direct assessment of the tubal mucosa, but is not able to evaluate external tubal anatomy including peritubal adhesions. These alternatives are discussed in more detail in Chapter 3.

Evaluation of the uterus

There are several anatomic abnormalities of the uterus that may impact fertility potential. Abnormalities of the uterine cavity include both developmental abnormalities (unicornuate, biocornuate, didelphic and septate) as well as acquired ones (polyps, fibroids, synechiae), as mentioned previously. Generally, congenital abnormalities may increase the risk of complications during pregnancy (i.e. preterm labor, preterm delivery, breech presentation) but do not significantly impact the risk of infertility. However, these findings should be noted for appropriate preconceptual counseling.

Evaluation of acquired uterine abnormalities such as uterine polyps and fibroids is warranted, although their overall contribution to infertility is debatable. Evidence suggests that submucous and large intramural (≥4 cm) fibroids may impact fertility and miscarriage risk. Polyps are more likely to impact fertility potential if they are larger (≥2 cm), or causing abnormal uterine bleeding. Uterine synechiae (i.e., areas of scarring) are another acquired abnormality that may impact embryo implantation. Uterine synechiae should be suspected in patients were there is a suspicion of intracavitary abnormality and a history of prior endometritis, endometrial curettage or surgery for fibroids/polyps. Thorough assessment and treatment of these conditions may improve fertility potential based on individual findings.

The uterine cavity may be assessed by HSG, transvaginal ultrasound or SIS. Either transvaginal ultrasound or SIS has the advantage of assessing the uterine myometrium for other pathologic conditions such as fibroids or adenomyomas in addition to assessing the uterine cavity for adhesions, fibroids or polyps. HSG is most effective for assessing both the uterine cavity and fallopian tube patency. Assessment should be tailored to the patient's history and examination. Most patients are able to be assessed by HSG and transvaginal ultrasound. Generally, SIS is reserved for more thorough evaluation of abnormalities visualized on standard transvaginal ultrasound or HSG.

SIS enhances endovaginal ultrasound examination of the uterine cavity with minimal patient discomfort. SIS consists of using transvaginal ultrasound to image the uterine cavity while sterile saline is instilled into the cavity. This allows detection of abnormalities within the uterine cavity.

☆ TIPS & TRICKS

Saline instilled sonography (SIS; sonohysterography) enhances endovaginal ultrasound examination of the uterine cavity with minimal patient discomfort. SIS consists of using transvaginal ultrasound to image the uterine cavity while sterile saline is instilled

into the cavity. This allows detection of abnormalities within the uterine cavity. SIS is performed by first cleansing the cervix with an antiseptic solution. A small intrauterine insemination catheter purged of any air bubbles is inserted through the cervix into the uterine cavity. Sterile saline is flushed through the catheter as the uterus is scanned in the long axis and coronal planes. Water appears dark and tissue is light so that filling defects such as polyps, fibroids and adhesions are easily visualized.

If traditional methods for assessing uterine pathology are indeterminant, MRI can used for more detailed evaluation of the uterus. MRI is particularly helpful in differentiating leiomyomas from andenomyosis. It is also considered the standard evaluation for uterine anomalies such as bicornuate and septate uteri. It is particularly useful before considering invasive surgery to more clearly define uterine architecture in patients with uterine anomalies or suspected andenomyosis or adenomyomas.

Evaluation of the pelvic peritoneum

Laparoscopy has traditionally been considered the gold standard for evaluation of the pelvis for intra-abdominal causes of infertility and tubal pathology. Laparoscopy was traditionally considered part of a standard fertility evaluation until those recommendations were revised. Current recommendations from the American Society for Reproductive Medicine (ASRM) indicate that peritoneal factors such as endometriosis and pelvic/adnexal adhesions should be evaluated with a laparoscopy only if there is a strong clinical suspicion of these conditions (i.e. history, physical exam, pelvic ultrasound or HSG suggests endometriosis or pelvic pathology) or before considering aggressive empirical treatments with significant cost and/or risks.

It should be noted that laparoscopy may detect pelvic pathologies that HSG may not identify, specifically pelvic adhesions and endometriosis. However, the risk versus benefit of laparoscopy should be considered on an individual patient basis considering her risk factors and the likelihood of benefit from the surgery. While laparoscopy may identify endometriosis which otherwise would not be detected without surgery, the likelihood of ablative surgery for minimal endometriosis significantly increasing absolute pregnancy rates is relatively small. It is important to consider whether the findings on laparoscopy would change the management plan for an individual patient given her presentation and other fertility factors.

CAUTION

There are two contraindications for HSG: current pelvic infection and pregnancy. Allergy to iodine is also a consideration if agents containing iodine are used for fallopian tube instillation. The exam should be scheduled during days 7–12 of the cycle to avoid the possibility of pregnancy and thickened endometrium which will interfere with most effective imaging of the uterine cavity. Antibiotics are not universally recommended but may be considered in patients with a prior history of pelvic infection or findings of hydrosalpinges on HSG.

Cervical factors

Cervical factors are a relatively infrequent cause of infertility. Abnormalities of cervical mucus are a presumptive cause of infertility for some patients but are difficult to identify. The postcoital test was developed to assess the ability of the sperm to penetrate cervical mucus and assess the quality of the mucus for conception. However, the test results were found to be highly variable and did not clearly correlate with outcomes. This test is not recommended for routine screening in couples and perhaps the only utility is to assess for sperm presence after intercourse in men who are otherwise unable to produce a sperm sample for semen analysis.

Ovarian reserve testing

The most recent U.S. data indicate that the number of women having children after 30 years

of age is increasing. Between 1980 and 2002, the percentage of women in the U.S. giving birth at age 30–34 years has increased from 8.4% to 14.2%. An increase in birth rate from 2.2% to 5.9% has also been documented for women age 35–39 years, with a small increase as well from 0.3 to 1.3% for women age 40–44 years. Ovarian reserve testing is recommended to assess a woman's reproductive potential if over 35 years of age, or for women with a single ovary, a history of prior ovarian surgery or prior poor response to exogenous gonadotropin stimulation. The purpose of ovarian reserve testing is to evaluate oocyte quality and quantity to provide information of reproductive potential beyond the likelihood of successful pregnancy based on chronologic age alone.

Several endocrinologic markers have been evaluated as a marker of ovarian reserve including FSH, inhibin B, estradiol and antimüllerian hormone (AMH), in addition to ultrasonographic techniques such as antral follicle count (AFC), ovarian volume and vascular resistance. Provocative tests have also been utilized such as the clomiphene citrate challenge test (CCCT) and the exogenous FSH ovarian reserve test (EFORT). These tests are often helpful in assessing the ability of a patient to respond to ovulation induction agents, but their utility in assessing likelihood of pregnancy is limited.

Serum endocrinologic assays

Basal FSH values drawn during menstrual day 2–3 have been routinely utilized as a marker of ovarian reserve. As FSH values increase, ovarian responsiveness decreases. The cut-off value used to define a "normal" FSH may vary between laboratories. An FSH value of 10–15 IU/L is generally considered borderline, and values over 15 IU/L are considered significantly elevated and have been correlated with decreased likelihood of conception with fertility treatments. It is important when assessing FSH values to also evaluate basal estradiol as an elevation may suppress FSH and give a falsely reassuring value. A normal basal estradiol may vary between laboratories, but typically is less than 60 pg/mL.

FSH values may fluctuate widely between cycles as age increases. It is important for patients and healthcare providers not to be falsely reassured by a single normal FSH result. A normal FSH level in a 40–45 year old woman with regular menstrual cycles will be elevated in a preceding or subsequent cycle about 30–50% of the time. In addition, a normal FSH level does not negate the impact of age on fertility. Although basal FSH testing is often used as a marker of ovarian responsiveness to gonadotropins, controversy exists regarding its utility as a predictor of spontaneous pregnancy.

Inhibin B is produced by the granulosa cells and levels diminish with age due to a reduction of the recruited cohort of the antral follicle pool. However, both baseline estradiol and inhibin B levels have not been consistently correlated with ovarian response. Estradiol levels rise in the early follicular phase in later reproductive years, and then eventually decrease, but no consistent cut-off value have been correlated with ovarian responsiveness. A fall in inhibin B results in the increase in FSH, but the inhibin B assay is not routinely commercially available and has not been shown to have added benefit to the FSH value alone.

AMH is a member of the transforming growth factor B family and is produced by the granulosa cells of the secondary, prenatal and antral follicles. AMH levels decrease progressively until it becomes undetectable at menopause. Theoretically, this may be a better marker of ovarian reserve as it represents the number of early and developing follicles and appears to have less intercycle variability. One significant advantage of this test is that it does not require assessment on cycle day 2 or 3 since there is limited variability during the menstrual cycle. However, this test may not be routinely available at all laboratories and no international standard has yet been developed for this assay. Due to variations in different assays, there are no specific "normal" values. Further research is needed to determine if cut-off values can be obtained that correlate with ovarian responsiveness. In addition, AMH is useful primarily as a test of ovarian responsiveness, not as a predictor of pregnancy.

SCIENCE REVISITED

Although menstrual cycles do not start to become irregular until a mean age of 45–56

years, endocrinologic changes associated with ovarian aging have been demonstrated for women age 35–40 years. FSH elevations have been demonstrated in the late luteal and early follicular phase for women with increasing age. The FSH elevations are secondary to a decrease in negative feedback on the pituitary from inhibin B and AMH production from the granulosa cells of the ovary. These hormones have been evaluated as a measure of ovarian reserve.

Antral follicle counts

Ultrasound has been evaluated as a tool for predicting ovarian reserve. Neither ovarian volume measurements nor vascular resistance has been correlated with ovarian responsiveness to stimulation. However, the number of antral follicles (2–10 mm) present on a menstrual cycle day 3 on transvaginal ultrasound is correlated with underlying oocyte supply and appears to be a better predictor of ovarian responsiveness to ovarian stimulation than day 2–3 FSH. As with other tests of ovarian reserve, the AFC is a predictor of ovarian responsiveness rather than chance for pregnancy.

Provocative tests

Several provocative tests of ovarian reserve have been reported. These tests require the use of fertility medications to challenge the ovarian response. The clomiphene citrate challenge test (CCCT) requires assessment of day 3 and day 10 FSH levels with administration of clomiphene citrate (100 mg) on cycle day 5–9. A normal result may vary between laboratories, but FSH levels of 10–15 IU/L are borderline and values over 15 IU/L are elevated. Overall, pregnancy rates have been reported to be no higher than 5% with an elevated result; however, recent reviews have reported that the CCCT probably provides no more additional predictive value than a cycle day 3 FSH alone.

Other provocative tests require the use of exogenous gonadotropins and are generally not performed outside of an ART center. A complete cycle of gonadotropins will assess the ability of the ovaries to respond to stimulation. An abbreviated test, the EFORT, was first reported in 1994 as a test to demonstrate the ability of the ovaries to respond to a fixed dose of 300 IU of gonadotropins. A rise of estradiol of 30 pg/mL or higher has been reported as an adequate response. This test may be recommended to patients prior to consideration of assisted reproduction technologies (ART) to determine the likelihood of an appropriate response, but is not utilized as a routine screening assessment of ovarian reserve.

In summary, several methods are available to evaluate ovarian reserve. A recent meta-analysis evaluated multivariate models for predicting ovarian response and found that combining multiple endocrinologic markers is similar to the accuracy of measuring only AFC alone. Although markers may help predict ovarian responsiveness, there is no data to assess these models to predict pregnancy or live birth. Although decreased ovarian reserve is defined by a decline in the quantity and quality of ovarian follicles, ovarian reserve is a better predictor of the ability of the ovary to produce oocytes than of the oocyte quality. Intercycle variability is present with all forms of ovarian reserve testing, and no single test has been consistently recommended. Basal FSH has been the mainstay of screening, but AFC and AMH may prove to be superior. It is important to consider that most research has evaluated these markers in relation to success of ovarian stimulation for in vitro fertilization; therefore, caution must be used in counseling patients about the impact of these test results on the likelihood of pregnancy with other modalities of treatment. Female age is the best predictor of pregnancy despite the results of ovarian reserve testing.

Evaluation of the male

Male factor issues contribute to infertility in about one-third of couples, and may be solely responsible in approximately 20% of couples that seek infertility testing. Testing should be initiated according to the recommendations previously discussed as part of the evaluation of the infertile couple. However, an evaluation may be warranted sooner for men with risk factors for infertility.

Recommendations on evaluation of male fertility have been established by a joint committee of the American Urologic Association and the ASRM. Initial evaluation should include a reproductive history and two properly performed semen analyses. A full evaluation by a urologist or male specialist should be done if there are any concerns present in the initial history or an abnormal semen analysis.

The male history should include developmental history, and childhood illnesses (i.e. cryptorchidism, mumps, serious illnesses), adult medical illness and surgeries (i.e. testicular, hernia or pelvic surgery), sexual history (timing and frequency of intercourse), toxin exposures (pesticides, heat, chemicals), family history of infertility and genetic diseases, and current medications and supplements. Physical examination should note body habitus and secondary sexual characteristics, testis and epididymis size and consistency, presence of vas deferens, and any varicoceles or masses on testicular exam or rectal exam.

Semen analysis

Semen analyses should be performed after a period of abstinence of 2–3 days. Most semen samples for analysis are collected by masturbation, but they may be collected by intercourse provided that the sample is collected in a special condom free of any spermicidal agents. The sample should be kept at room or body temperature, and for most accurate results, should be collected at the laboratory. Specific guidelines for the laboratory evaluation of semen samples have been established by the World Health Organization. Semen analyses provide information on several parameters including semen volume, sperm count, motility and morphology. Reference ranges have been reported (Table 2.2), but semen parameters may vary frequently in any individual. Therefore, two semen analyses are suggested to most accurately assess semen parameters. It is important to be aware when counseling patients that men with semen parameters outside of the normal ranges may still be fertile. However, further evaluation by a male reproductive specialist is warranted.

Additional testing of the male may include hormonal testing to assess for an endocrinopathy (FSH, LH, testosterone, prolactin), as well as scrotal ultrasound to detect varicoceles, spermatoceles, masses and absence of the vas deferens. A postejaculatory urinalysis is indicated for low-volume (<1 mL) or absent semen ejaculate to determine if retrograde ejaculation into the bladder is occurring. This is most common in men with neuropathic conditions that affect normal sperm ejaculation (i.e., diabetes, spinal cord injuries, vascular disease). Genetic screening may also be recommended in men with azoospermia or severe oligospermia. Conditions that may be detected include cystic fibrosis gene mutations which may result in congenital absence of the vas deferens, karyotype abnormalities which impair testicular function, and Y-chromosome microdeletions impacting spermatogenesis.

Table 2.2 Semen analysis: WHO 2009 reference values

Volume	>1.5 ml
Total sperm number	>39 million per ejaculate
Concentration	>15 million/mL
Viability	>58%
Total motility	>40%
Progressive motility	>32%
Morphology	>4.0%

Additional testing of the infertile couple

Many additional diagnostic tests have been recommended for evaluation of the infertile couple (i.e., evaluation of TSH and prolactin in ovulatory women, luteal-phase endometrial biopsy, endometrial integrins, antisperm antibodies and additional sperm penetration tests). Clinicians should consider on an individual basis if the findings of these tests may indicate an effective treatment and if treatment of these conditions has been proven to achieve a higher pregnancy rate.

Summary

Infertility is a common condition that warrants accurate evaluation and treatment. Evaluation should be initiated after one year of unprotected intercourse without conception. It is appropriate

to initiate an evaluation after 6 months of infertility for women over the age of 35 years or for women with a history of oligomenorrhea or amenorrhea, a partner that is known to be subfertile, or known uterine/tubal disease or endometriosis. Clinicians should recommend those tests that are evidence-based and consider history of the couple to determine the most cost-effective evaluation.

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3

Diagnosis and Management of Tubal Factor Infertility

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Introduction

Of couples with infertility, up to 30% have complete or partial blockage of a fallopian tube. Blockage can occur anywhere in the tube, and may be transient (obstruction) or permanent (occlusion). Tubal disease is classified as either proximal or distal and unilateral or bilateral. Tubal obstruction, endosalpingeal destruction, and periadnexal adhesions are the main causes of tubal factor infertility, with pelvic inflammatory disease (PID) being the most common etiology representing >50% of cases. Even one episode of PID increases the rate of subsequent infertility to 8%; two and three episodes result in rates of 20% and 40%, respectively. Evaluation of tubal patency should be part of the standard infertility work-up, and knowledge of the various diagnostic and management strategies is essential in order to maximize a patient's chances of conception.

While a history of PID is a strong risk factor for tubal disease, other pelvic pathology, such as endometriosis, ectopic pregnancy, and complicated appendicitis, can also lead to pelvic inflammation and tubal obstruction. A history of pelvic surgery has also been associated with tubal disease in cohort studies. Based on a meta-analysis of 32 studies, there is only a weak association between chronic pelvic pain, IUD use, or surgical abortion and tubal disease, and history of cervicitis due to chlamydia infection does not appear to play a role in subsequent tubal obstruction.

☆ TIPS & TRICKS

Important aspects of the patient history

Laparoscopy with chromopertubation is considered the gold standard for evaluation of tubal pathology. However, it is invasive, expensive, and requires general anesthesia. Luttjeboer et al. conducted a systematic review of factors that could be identified in the medical history, which put the patient at increased risk for tuboperitoneal disease. In various case-control and cohort studies, tubal pathology was most strongly associated with a history of PID and complicated appendicitis. Strong associations were also found with a history of pelvic surgery, endometriosis, ectopic pregnancy and STD. Associations were less strong but still present for appendectomy, chronic pelvic pain, and history of induced abortion. Patients with these risk factors, in whom suspicion of tubal disease is high, should be offered early diagnostic laparoscopy. It is important to remember that patent tubes on HSG do not necessarily rule out tubal factor infertility as periadnexal adhesions may affect the anatomical relationship between tube and ovary.

Distal tubal disease

Distal tubal obstruction is more common than proximal obstruction. Evaluation with contrast dye will reveal alterations in the mucosal folds

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and rugal pattern of the endosalpinx, and laparoscopy may demonstrate the presence of peritubal pelvic adhesions. Other etiologies include prior tubal sterilization, salpingitis, or endometriosis. Distal fimbrial occlusion can be classified as mild, moderate, or severe based on size of hydrosalpinx, presence of fimbria, and degree of adhesions. Although 80% of women with mild disease may conceive, only 31% and 16% of those with moderate and severe disease, respectively, will become pregnant.

Proximal tubal disease

Only 15% of women have proximal tubal occlusion. Proximal blockage is usually the result of infection, but can also be due to endometriosis, which is present in up to 14% of patients with tubal infertility; tubal polyps, which are found in 11% of hysterectomy specimens; or salpingitis isthmica nodosa (also known as tubal diverticula). The possibility of congenital tubal occlusion also exists. If no contrast dye reaches the fallopian tube during evaluation, the diagnosis of proximal tubal blockage is likely. However, spasm of the tube may occur in up to 30% of cases, preventing evaluation of the distal tube and leading to a false-positive diagnosis of proximal obstruction. Thus, the diagnosis is best confirmed with laparoscopy, which allows for both visualization of spasm and evaluation of distal obstruction. This will be further discussed below. Of note, if both proximal and distal obstruction exist, the fallopian tube is unlikely to function normally, even after surgical repair.

Diagnosis of tubal infertility

Laparoscopic chromopertubation

Laparoscopic chromopertubation is the gold standard for evaluation of the fallopian tubes. This procedure involves injection of diluted indigo carmine into the uterine cavity with simultaneous laparoscopic visualization to evaluate for tubal fill and spill into the abdominal cavity. However, this procedure is invasive, expensive, and requires general anesthesia. It also carries with it a small, but real, mortality risk. This has led some authors to suggest safer, more economical alternatives to laparoscopic chromopertubation, such as hysterosalpingogram (HSG) or hysterosalpingo-contrast sonography (HyCoSy).

Hysterosalpingography

HSG involves injection of a radio-opaque contrast material (either oil- or water-based) into the uterine cavity under fluoroscopic visualization. HSG is excellent at visualizing obstruction, with a specificity of 83%. Its advantages include speed and lack of need for anesthesia. The use of oil-based contrast media may also, in itself, play a therapeutic role, both by flushing tubal debris and preventing mast cell phagocytosis of spermatozoa (a phenomenon that has been observed in vitro). However, water-based dyes, such as Renografin (Squibb, Princeton, NJ), are most frequently used at present because of the slower absorption of oil-based dyes and the small potential risk of pulmonary embolus if oil bubbles extravasate into the vasculature. More recently, use of water-based dyes for HSG has also been demonstrated to have a comparable therapeutic effect**.**

A potential limitation of HSG is tubal spasm, which can lead to a false-positive diagnosis of proximal tubal blockage. It has been estimated that HSG may give a false-positive diagnosis of tubal spasm 50% of the time. It is also limited by its inability to detect peritubal adhesions. The use of iodinated contrast dye is also associated with a small risk for allergic-like reaction (approximately 3% risk of mild reaction, 0.05% risk of severe reaction), particularly in those women who have had prior reactions to contrast dye, those who have allergies to multiple substances, and those with poorly controlled asthma. The widespread belief that women with allergies to shellfish are at particularly increased risk is not supported by the literature, as iodine, which is contained in shellfish and the thyroid requires to make thyroxine, is too small in itself to be an allergen. If other options for evaluating tubal patency are not available, patients can be premedicated with either methylprednisolone (32 mg 12 h and 2 h prior to HSG) or a combination of prednisone (50 mg 13 h, 7 h, and 1 h prior to procedure) and diphenydramine (50 mg 1 h prior to procedure) to minimize the risk of a reaction.

☆ TIPS & TRICKS

Rotation during HSG

While the finding of bilateral proximal tubal obstruction on HSG is strongly associated with tubal pathology, unilateral proximal obstruction (which is found in up to 24% of HSGs) is often a temporary finding that has limited prognostic significance for fertility. It does, however, lead to additional testing and cause unnecessary stress for the patient. Several theories for this temporary obstruction—including tubal spasm, plugging, or blockage by air bubbles—have been postulated. However, more recently it has been suggested that the blockage might be positional, due to kinking of the tube at the uterotubal junction when the patient is supine. Rotating the patient, such that the obstructed tube is inferior to the uterus, resolves 63% of proximal unilateral obstruction.

Sonohysterography and hysterosalpingo-contrast sonography

Sonohysterography (SHG), also known as saline infusion sonography (SIS) is a low-cost and well-tolerated alternative method to HSG for evaluating tubal patency. To perform SHG, a saline solution is injected transcervically, and transvaginal ultrasound is used mainly to assess for intrauterine anomalies. The presence of post-procedure free fluid in the pouch of Douglas can also suggest tubal patency, but is not confirmatory, and is limited by the fact that one cannot determine if the saline spilled from one or both tubes. In addition, proximal versus distal obstruction cannot be delineated with simple sonohysterography For the evaluation of tubal patency, this procedure may also be performed with air contrast to assess the passage of bubbles through the tubes. This method, known as hysterosalpingo-contrast sonography (HyCoSy), increases the accuracy of SHG, putting it in agreement with laparoscopic chromopertubation nearly 80% of the time. A meta-analysis of data from over 1000 women concluded that this method is superior to HSG and comparable to laparoscopic chromopertubation.

Benefits of HyCoSy as compared to HSG include patient tolerance (a majority of patients rated procedural pain as mild to moderate and preferred it to HSG) and avoidance of iodinated contrast medium and ionizing radiation. The air and saline solutions used to provide contrast are less expensive than iodinated contrast. Interestingly, HyCoSy has also been reported to increase fertility rates, but a recent randomized controlled trial did not confirm these results. Although HyCoSy is an excellent primary tool for evaluation of tubal patency, confirmatory laparoscopy may be considered in order to complete the evaluation prior to resorting to assisted reproduction techniques.

For both SHG and HSG there is a small, but real, risk of infection. This risk is increased (from approximately 1% to 11%) in women with a history of PID or hydrosalpinx. Cases of fever and pelvic peritonitis have been reported after tubal imaging studies. The American College of Obstetricians and Gynecologists (ACOG) states that in patients with no history of PID, HSG can be performed without the use of prophylactic antibiotics; however, if dilated tubes are visualized, doxycycline (100 mg orally twice daily for 5 days) should be given to reduce the incidence of post-exposure PID. In patients with a history of pelvic infection, prophylaxis may be started the day before the procedure, and treatment extended 5–7 days if hydrosalpinx is discovered. There are no data upon which to base guidelines for prophylaxis prior to SHG; thus, ACOG recommends individualization of treatment based on patient risk factors. In practice, since pelvic infection can be a devastating consequence for an already infertile patient, many providers err on the side of providing prophylaxis prior to HSG or SHG.

CAUTION

Preoperative imaging

The value of preoperative imaging (such as HSG or SHG) prior to diagnostic laparoscopy, even in patients with risk factors for tubal disease (or known tubal disease) should not be underestimated. Such evaluation may help to delineate potential problems—location of tubal blockage, unilateral or bilateral disease,

potential intratubal disease—prior to surgery, and allow for more efficient and effective surgical repair. Imaging may also identify additional factors, such as fibroids, polyps, or intrauterine adhesions, which contribute to infertility and can be addressed at the time of surgery.

Salpingoscopy

Salpingoscopy, also known as falloposcopy, allows for the endoscopic evaluation of tubal mucosa, including visualization of mucosal flattening and intraluminal adhesions. The severity of endosalpingeal damage directly correlates with poor pregnancy outcome. However, it is not directly correlated with periadnexal adhesions; thus, external tubal anatomy may not reflect the extent of intraluminal damage. As such, lysis of adhesions may not restore tubal function if there is endoluminal damage. Salpingoscopy is rarely used as part of the basic infertility work-up, as it is invasive and often complicated by tubal perforation, but it remains an option for certain patients in those settings where it is an available procedure.

CAUTION

Tubal evaluation in older patients

An age-related decline in female fertility begins many years prior to the onset of menopause, even in women with continued, regular ovulatory cycles. This diminished ovarian reserve is due to depletion of eggs and a decline in oocyte quality. Successful treatment of infertility in these patients depends on an organized plan for diagnosis and aggressive treatment from the time of initial presentation. While younger women may benefit from the use of simpler, cheaper tests initially, diagnosis and treatment should not be delayed, especially in women over 35. Such patients are less likely to benefit from surgical repair of the fallopian tube; thus, such attempts should be limited, and consideration should be given to early assisted reproduction technology (ART).

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Tubal anatomy

The longitudinal fallopian tubes extend from the uterus to the ovary and are supported by the mesosalpinx. The tubes are divided into several portions:

- Interstitial: the narrowest portion of the tube, which lies within the uterine wall and forms the tubal ostia
- Isthmus: forms the segment closest to the uterine wall
- Ampulla: the longest segment of the tube, located lateral to the isthmus; it is the site of fertilization and early cleavage
- Infundibulum: the trumpet-shaped, fimbriated portion of the tube, which opens into the peritoneal cavity and provides a wide surface for ovum pick-up

The tubal epithelium is extensively folded. The majority of cells lining the tubal epithelium are ciliated; the direction of ciliary beat is toward the uterus to facilitate ovum transport. The embryo is capable of developing to the blastocyst stage prior to entry into the uterus.

Chlamydia serology

Chlamydia trachomatis is the main cause of PID. There is evidence that the antibody response to chlamydia heat shock protein 60 (chsp60) predicts subsequent risk of tubal infertility. Thus, evaluation for chlamydia antibody titers has been proposed as a low-cost, noninvasive method of assessing tubal status, with sensitivity and specificity of 75% when microimmunofluorescence is performed. The utility of this testing probably lies in its ability to exclude tubal disease; i.e., if antibody testing were negative, further invasive testing might be avoided. However, the value of invasive testing lies partly in its ability to provide anatomical and prognostic information. As discussed above, invasive testing can also be used therapeutically (as in the case of tubal flushing). Chlamydia serology cannot provide this information, nor can it serve an interventional role, which limits its overall use; however, it remains a useful option for those patients allergic to

contrast dye or to those with limited finances, who do not have infertility coverage for more invasive diagnostic testing.

Management of tubal infertility

Proximal tubal disease

Tubocornual anastamosis

Tubocornual anastamosis, which involves excision of diseased proximal tube, followed by axial incision of patent residual tube along its antimesenteric border and reimplantation (either into the interstitial segment of the oviduct, or into a new uterotomy posterior to the cornua), has a reported term pregnancy rate of up to 50% when microsurgical techniques (10× to 20× magnification) are used. Success rates for macrosurgery are lower, at less than 25%, and associated with higher rates of stenosis. As expected though, the actual rate is related to the extent of tubal removal and interstitial occlusion.

Selective salpingography and transcervical tubal cannulation

This technique involves fluoroscopic or hysteroscopic placement of a cannula at the tubal ostium, followed by injection of contrast dye under fluoroscopic or laparoscopic visualization. Tubal cannulation has replaced microsurgical treatment of proximal blockage at many institutions. Increased hydrostatic pressure from the dye may clear tubal debris; if this fails, an atraumatic guide wire can be threaded through the oviduct to clear any remaining debris. The combination of these methods can overcome up to 85% of occlusions, but the reocclusion rate is high (up to 30%). In addition, perforation of the tube by the guide wire, which is usually minor and heals spontaneously, occurs in up to 10% of cases and occasionally leads to further tubal dysfunction. However, in selected patients, this may represent a minimally invasive, relatively inexpensive, and effective method of resolving proximal tubal blockage.

Distal tubal disease

Salpingostomy

Salpingostomy is performed by creating a new stoma at the occluded part of the distal tube. It results in an overall pregnancy rate of 30%. Not surprisingly, the pregnancy rate is greater for those with mild disease (80%) than for severe disease (16%). A meta-analysis by Watson et al. demonstrated increased term pregnancy rates with microscopic as compared with macroscopic salpingostomy or laparoscopic salpingostomy. However, laparoscopic salpingostomy might provide an economic advantage due to its shorter recovery time in settings where ART is subsequently available.

Salpingostomy is generally recommended only for young women with mild distal tubal disease, though it can be considered for those women who do not have ART as an alternative option. No benefit has been demonstrated when prostheses have been used to maintain tubal patency or provide postoperative irrigation.

One approach to salpingostomy involves the use of the CO_2 or yttrium aluminum garnet (YAG) laser to make a cruciate incision on the occluded distal tube. This is followed by use of thermal energy to coagulate the leaves of the cruciate incision so that they are everted, thereby maximizing the chances of the ostomy remaining patent.

Hydrosalpinges

Salpingectomy for tubal occlusion

The presence of hydrosalpinx fluid decreases live birth rates after ART by about one-half. A number of mechanisms have been proposed to explain this interaction, including an embryotoxic effect of hydrosalpinx fluid, decreased implantation due to leakage of fluid into the endometrial cavity causing alterations in endometrial receptivity, or flushing of the embryo by fluid. A Cochrane review investigating the use of laparoscopic salpingectomy prior to ART looked at three randomized controlled trials, including a landmark multicenter Scandinavian study, and found that the odds of pregnancy increased (OR 1.75, 95% CI 1.07–2.95), as did the odds of ongoing pregnancy and live birth (OR 2.13, CI 1.24–3.65). The authors concluded that unilateral salpingectomy for unilateral hydrosalpinx, and bilateral salpingectomy for bilateral hydrosalpinx, should be recommended.

Of note, the original Scandinavian study found no improvement in outcome in patients with hydrosalpinges that were not visible on ultra-

sound. In keeping with this finding, the Cochrane review found that women with ultrasound-visible hydrosalpinges had the greatest benefit from salpingectomy prior to ART (with a 2.4-fold increase in the delivery rate). Among these patients, however, tubes with healthy-looking mucosa may still be suitable for reconstructive surgery and should not be removed if the patient is a candidate for such a procedure.

With respect to timing, Strandell et al. concluded that salpingectomy prior to ART in women with ultrasound-visible hydrosalpinges is more cost-effective than delaying salpingectomy until after failed ART cycles, with a savings of about $9500. However, although it may be obvious that a particular patient may benefit from salpingectomy, the psychological impact of the procedure can be significant, and it may take several failed ART cycles for a patient to give consent. Ultimately, in those women with limited finances and/or no coverage for ART, it may be best to preserve the tube if possible.

Drainage of hydrosalpinx fluid

Data are insufficient to recommend aspiration of hydrosalpinx fluid, and similarly, there are limited data on surgical proximal tubal blockage, though the latter can be considered if hydrosalpinges cannot be surgically removed. One randomized controlled trial demonstrated an improvement in pregnancy rates, from 17% to 31%, after fluid aspiration; a second and third trial showed a possible benefit and no change in outcomes, respectively. Although the data are limited, this procedure can be considered, particularly in the setting of ART at the time of oocyte retrieval. Prophylactic antibiotics should be administered given the possibility of infection with leakage of the hydrosalpingeal fluid.

Adhesions

Lysis of adhesions

Laparoscopy results in fewer, and less dense, adhesions postoperatively than laparotomy. In addition, there is a trend towards increased intrauterine pregnancies, and reduced ectopic rates, with laparoscopic lysis of adhesions. Routine laparotomy for adhesiolysis should thus be avoided. Intraoperatively, adhesions may be ablated using the cold knife, electrocautery, or laser. In theory the laser minimizes surrounding tissue damage and bleeding, but in practice two randomized controlled trials have not shown a benefit over conventional techniques in prevention of adhesions.

If partial occlusion of the distal tubes is present, with preservation of fimbriae, removal of these adhesions (also known as fimbrioplasty) facilitates oocyte retrieval and results in about a 60% conception rate, which is double that of salpingostomy. However, patients with severe adhesive disease, including thick, vascular, or extensive adnexal adhesions, should probably proceed to ART, as these patients are less likely to benefit from surgery. In addition, spontaneous pregnancy after adhesiolysis will occur in the first 6–12 months after surgery; thus, patients who do not conceive within this window of time should also move towards ART. Although data are limited, antiadhesion barriers, such as 4% icodextrin (Adept, Baxter), can be considered in those patients undergoing laparoscopic adhesiolysis in the setting of infertility.

Sterilization reversal

Tubal reanastamosis

The prognosis for fertility after tubal sterilization depends on multiple factors. The method of sterilization is important; reanastamosis after ring or clip placement has a higher success rate than after electrocauterization (which is associated with increased ectopic pregnancy rates). The location of the planned anastamosis is also important, as isthmus–isthmus anastamoses have the highest subsequent pregnancy rates (up to 81%). Other factors include length of adequate residual tube, the age of the patient, and the presence of other tubal pathology. No randomized controlled trials have compared tubal reanastamosis to ART, and choice of surgery versus ART should be individualized to the patient, with surgery reserved for younger patients with good ovarian reserve and adequate, well-preserved remaining tubes.

Tubal surgery versus in-vitro fertilization and embryo transfer

The aim of most tubal reconstructive surgery is to correct damage at the distal end of the

tube, restoring the normal anatomic relationship between fimbriae and ovary and allowing proper ovum pick-up. Various techniques have been advocated, including open microsurgical laparotomy; laparoscopic surgical techniques, which have been promoted due to decreased morbidity and postoperative hospital stay; and placement of a prosthesis (to be used as a port for postoperative tubal flushing). A Cochrane review of these techniques found very few good-quality studies; most were not randomized or had small sample sizes. From the limited data available, there was no evidence of benefit or disadvantage of tubal surgery versus no treatment or alternative treatments, nor was there evidence that one approach is more effective than another.

With the advent of ART, tubal surgery as a method of improving fertility has become less common. Some authors have advocated complete abandonment of surgical techniques, in favor of liberal referral to an ART program. However, tubal surgery may be performed if the prognosis is good or if assisted reproductive techniques are not available to the patient.

ART has the advantage of being able to completely bypass tubal blockage, offering, on average, an almost 30% delivery rate per cycle, and a rate of more than 70% over four cycles for patients with tubal factor (with or without other coexisting infertility factors). However, ART is expensive and not available to all infertility patients. Cost may become an issue when more than one pregnancy is desired or when insurance companies are involved, as they are more likely to pay for surgical procedures than for ART. More recently, a Cochrane review, designed to compare tubal surgery to expectant management and ART, failed to find any suitable randomized controlled trials.

Based on the limited available evidence, diagnostic laparoscopy with an initial surgical approach is an appropriate first step for young patients with adequate ovarian reserve. If pregnancy does not occur within 6 months to 1 year, patients should be offered ART. However, given the poor prognosis for fertility in patients with severe disease, these patients should proceed directly to ART, but may be offered surgery (such as prophylactic salpingectomy for moderate or severe hydrosalpinx) prior to ART to to maximize the chances of intrauterine pregnancy with ART, such as prophylactic salpingectomy in the setting of moderate to severe hydrosalpinx.

Summary

Fallopian tube damage, whether due to fimbrial injury leading to impaired ovum pick-up, periadnexal adhesions, or intratubal disease,is responsible for a significant number of cases of infertility. The work-up for tubal infertility should be tailored to the individual patient. Diagnostic modalities include laparoscopic chromopertubation (the gold standard), HSG and HyCoSy, and less commonly used techniques such as salpingoscopy and chlamydia serology. The appropriate intervention depends on the patient as well; surgical approaches can be effective, but tubal factor remains one of the major indications for ART. In some situations, ART may be indicated as first-line treatment (with or without prophylactic surgery).

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4

Diagnosis and Management of Uterine Infertility

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Introduction

During the evaluation of female infertility, the uterus deserves thorough assessment, with attention to the myometrium and endometrial cavity. At the most basic level, the uterus is essential for regeneration of the endometrium; sperm migration; embryo migration and implantation; and nurture and protection of the fetus. Although a great deal is known about the function of the uterus during reproduction, many questions remain unanswered. Abnormalities of the uterus, both congenital and acquired, may impact a woman's ability to conceive or her ability to sustain a pregnancy. Congenital and acquired uterine abnormalities may be present in up to 10% of infertile women.

SCIENCE REVISITED

Implantation is a complex process that begins 6–7 days after fertilization. The peri-implantation endometrium has been primed by the ovarian steroid hormones estradiol and progesterone. Biochemical communication between the endometrium and the preimplantation blastocyst occurs prior to and throughout the implantation process. Furthermore, multiple signals are necessary to achieve endometrial receptivity to the blastocyst including hormones, growth factors, cytokines, and immunologic and angiogenic factors. During implantation, the blastocyst apposes and then adheres to the endometrium, and subsequently penetrates the endometrium, after which the blastocyst becomes completely embedded in the stromal epithelium. Successful implantation is followed by placental development.

The processes of implantation, placentation, and embryo development are dependent upon the uterus. Failure of implantation may be due to abnormalities of endometrial receptivity or to abnormalities of the embryo; approximately two-thirds of implantation failure is related to problems with endometrial receptivity. Clinically, implantation failure may be attributed to congenital anomalies or acquired lesions of the uterus, and these abnormalities can present as infertility or recurrent pregnancy loss. Nonuterine abnormalities such as endocrine disorders, thrombophilias, infectious etiologies, or immunologic factors may also contribute to implantation failure, but are beyond the scope of the present discussion.

Evaluation of the uterus

The initial assessment of female infertility should include investigation of the female reproductive tract, evaluating for patency of the fallopian tubes and a normal contour of the endometrial

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cavity. When clinically indicated, and particularly if a uterine abnormality is suspected, more detailed evaluation of the uterus and uterine cavity may be necessary. Each imaging technique has inherent strengths and limitations; therefore, a combination of several techniques may best evaluate a particular abnormality.

Imaging techniques commonly employed to evaluate the uterus and uterine cavity include transvaginal ultrasonography (TVS), hysterosalpingography (HSG), saline-infusion sonography (SIS) which is also known as sonohysterography, and hysteroscopy. TVS is a routine diagnostic tool for assessment of the pelvis, including the uterus and adnexa. Timing the study to the secretory phase of the menstrual cycle provides better visualization of the endometrium, and thus the contour of the uterine cavity. While TVS is considered to have high specificity and high sensitivity for detecting uterine abnormalities, some studies have not identified such a high level of accuracy. When available, three-dimensional ultrasonography offers highly accurate imaging of pelvic anatomy including detailed assessment of the uterus.

HSG is commonly utilized to assess patency of the fallopian tubes, and may provide further information about the contour of the endometrial cavity or the presence of any complex communications in the setting of a müllerian anomaly. However, the sensitivity of HSG to detect intrauterine abnormalities can be as low as 50%, and the lack of information about the external uterine contour limits its utility for evaluating a uterine anomaly. Therefore, exclusive use of TVS or HSG to evaluate the uterine cavity in women with suspected abnormalities may lead to suboptimal assessment of the uterus.

When imaging techniques for the uterine cavity are compared, SIS (Figure 4.1) is considered superior to HSG or TVS and is comparable to hysteroscopy. SIS effectively delineates the intracavitary space, and internal and external uterine contours. When SIS, HSG, and TVS imaging techniques are compared for the evaluation of submucosal myomas, SIS is most accurate for evaluating the size, location, and intracavitary component of the myoma.

Furthermore, when indicated, MRI is an excellent technique for detailed evaluation of the uterus. Considered the "gold standard" for the diagnosis of müllerian anomalies, MRI provides excellent delineation of internal and external uterine contours, can determine the extent of a uterine or vaginal septum, and can identify rudimentary uterine structures and the presence of functional endometrium. MRI can also differentiate between abnormalities such as leiomyomas, adenomyosis and adenomyomas.

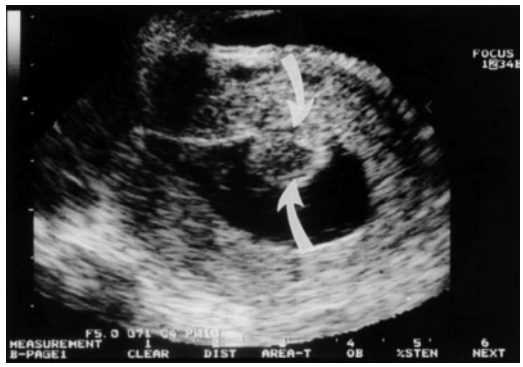

Figure 4.1 Submucosal uterine myoma visualized with saline-infusion sonography. Reproduced from Cohen and Valle 2000, with permission from Elsevier.

☆ TIPS & TRICKS

When ordering an MRI to evaluate a complex müllerian anomaly, consider discussing the case with the radiologist prior to the study. This will enable the radiologist to perform the best study possible to answer your clinical questions, obtaining the appropriate views of the uterus. They may also recommend that the patient place gel in the vagina to better delineate vaginal or cervical anomalies, if indicated.

Congenital abnormalities

Congenital abnormalities of the female reproductive tract, otherwise known as müllerian anomalies, may involve the uterus, cervix, fallopian tubes, or vagina. Uterine abnormalities are the most common müllerian anomaly; however, the true incidence of uterine anomalies is not known since many women are asymptomatic,

and sensitive imaging techniques have not always been available. Normal and adverse reproductive outcomes are seen with uterine anomalies: they are identified in approximately 3–4% of fertile and infertile women, 5–10% of women with recurrent early pregnancy loss, and up to 25% of women with late first or second trimester pregnancy loss or preterm delivery. These anomalies are typically associated with difficulty maintaining a pregnancy, and seldom with subfertility or infertility.

Development of the female reproductive tract involves a complex series of events. The process of müllerian duct development occurs concomitantly with the development of the urinary system, but gonadal development is a separate process. Any defect in müllerian development can lead to one of three categories of anomalies: hypoplasia and agenesis, lateral fusion defects, and vertical fusion defects. The American Society for Reproductive Medicine (ASRM) has classified müllerian anomalies into seven groups: hypoplasia/agenesis, unicornuate, didelphys, bicornuate, septate, arcuate, and diethylstilbestrol drug related. This classification system concentrates on uterine anomalies, thus documentation of associated anomalies of the cervix, vagina, fallopian tubes, or urinary system should be included.

SCIENCE REVISITED

By week 6 of embryonic development the paired müllerian (paramesonephric) ducts are present, arising from the coelomic epithelium along the lateral walls of the urogenital ridge. These solid ducts elongate medially and caudally, cross the wolffian (mesonephric) ducts, fuse in the midline, and reach the urogenital sinus by 10 weeks of gestation. The ducts undergo internal canalization, followed by resorption of the intervening septum; septal resorption generally occurs in a caudal to cephalad direction. Uterovaginal development is completed by 20 weeks of gestation. The fused portion of the ducts becomes the upper vagina, cervix, and uterus, and the unfused portions become the fallopian tubes.

Uterine anomalies are associated with an increased risk of poor obstetric outcomes (Table 4.1). These complications are attributed to abnormalities of space and vascularity, and local defects: diminished uterine cavity size, insufficient musculature, impaired ability to distend, abnormal myometrial and cervical function, inadequate vascularity, and abnormal endometrial development. Hence, pregnancies with a uterine anomaly may be complicated by recurrent pregnancy loss, preterm labor and delivery, cervical incompetence, intrauterine growth restriction, and malpresentation. Surgical intervention to correct the uterine anomaly, when possible, may be offered to women with a history of poor obstetric outcomes such as recurrent pregnancy loss and preterm delivery. However, inherent developmental abnormalities of the myometrium and vascular supply may permanently impair uterine function. When women

Table 4.1 Reproductive outcomes in women with congenital uterine anomalies. Rates are averaged and presented as a percentage.

Uterine anomaly	Number of studies	Number of patients	Number of pregnancies	Abortion rate (%)	Preterm birth rate (%)	Term delivery rate (%)	Live birth rate(%)
Unicornuate	11	151	260	36.5	16.2	44.6	54.2
Didelphys	8	114	152	32.2	28.3	36.2	55.9
Bicornuate	4	261	627	36	23	40.6	55.2
Septate	4	198	499	44.3	22.4	33.1	50.1
Arcuate	3	102	241	25.7	7.5	62.7	66

Data presented from Grimbizis et al. 2001.

with uterine anomalies undergo advanced reproductive technologies (ART), clinical pregnancy rates are similar to women with normal uteri, but they experience higher rates of pregnancy loss and preterm delivery.

Septate uterus

The septate uterus is considered the most common uterine anomaly, occurring in approximately 1% of the fertile population. The degree of septation can vary from a complete septum, which extends from the uterine fundus through the cervix, to a partial septum, in which a portion of the caudal aspect of the septum has resorbed (Figure 4.2). The fibromuscular uterine septum is associated with the poorest reproductive outcomes among the uterine anomalies: 44% pregnancy loss rate, 22% preterm delivery rate, 33% term delivery rate, and 50% live birth rate. Furthermore, when pregnancy loss is evaluated by trimester, a higher rate of loss is seen in the first trimester (25.5%) compared to the second trimester (6%). However, surgical correction of the uterine septum is associated with marked improvement in reproductive outcomes: postoperative live birth rates of 80%, and pregnancy loss rates of 15%.

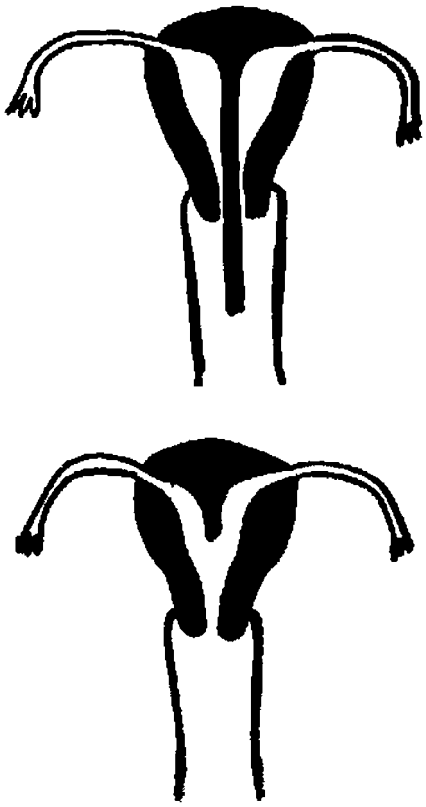

Figure 4.2 A complete septate uterus with a longitudinal vaginal septum (top), and a partial septate uterus (bottom). A longitudinal vaginal septum commonly occurs with a complete septate uterus.

For a uterine septum, the preferred surgical approach is hysteroscopic metroplasty due to its safety, simplicity, and excellent postoperative results. Concomitant laparoscopy can be employed to guide the hysteroscopic procedure and to evaluate pelvic anatomy. Surgical intervention is recommended for women with a uterine septum and a history of recurrent pregnancy loss, second trimester pregnancy loss, preterm delivery, or other obstetric complications.

Although there is no definitive association between a septate uterus and infertility, several recent studies have questioned if a relationship exists. The uterine septum is the most common anomaly in the infertile population, which suggests a possible association. Furthermore, among women with unexplained infertility, hysteroscopic resection of uterine septae has been shown to improve pregnancy outcomes. Similar positive results are seen in women with a uterine septum and recurrent pregnancy loss who undergo hysteroscopic metroplasty. Although women with a septate uterus can have reasonable pregnancy outcomes, prophylactic hysteroscopic metroplasty may prevent pregnancy loss or other obstetric complications, and is commonly recommended to optimize outcomes for women with prolonged infertility, for women older than 35 years of age, or for women planning to pursue ART.

Unicornuate, didelphys, and bicornuate uteri

These congenital anomalies demonstrate different defects of müllerian development: the unicornuate uterus occurs due to unilateral müllerian duct agenesis, the didelphys uterus results from lack of müllerian duct fusion in

the midline, and the bicornuate uterus demonstrates incomplete fusion of the müllerian ducts with a variable degree of septation between the two endometrial cavities. These anomalies are associated with acceptable live birth rates of approximately 55%. Therefore, women with these anomalies do not warrant any intervention prior to conceiving, but should be closely observed during pregnancy. Unlike the septate uterus, these anomalies have not been associated with infertility. Although uterine unification procedures are available to repair the didelphys or bicornuate uterus, these procedures are controversial since they may not improve outcomes, and should only be considered for women with poor obstetric outcomes.

The unicornuate uterus cannot be repaired or augmented. However, women with a unicornuate uterus should undergo MRI evaluation to determine if a rudimentary uterine horn is present; a rudimentary horn with functional endometrium is associated with retrograde menstruation, endometriosis, and pelvic pain, as well as the risk of a horn gestation. Hence, functional rudimentary uterine horns should be surgically removed. However, there is no evidence that removal of nonfunctional rudimentary horns will improve reproductive outcomes.

Acquired anomalies

Acquired uterine anomalies that can affect fertility include leiomyomas, endometrial polyps, intrauterine adhesions, and adenomyosis. The causality between these acquired abnormalities and infertility is not well characterized, and further investigation of these relationships is necessary. Among these abnormalities, the effect of myomas on fertility has been best studied.

Uterine leiomyomas

Uterine leiomyomas are the most common benign tumor affecting women of reproductive age. Myomas are identified in more than 50% of women between 35 and 50 years of age, and the incidence increases with age. Symptoms associated with uterine myomas depend on the number, size, and location of myomas, and include pelvic pressure and discomfort, abnormal uterine bleeding, and distortion of adjacent organs such as the bladder and bowel. However, many women with myomas are asymptomatic. Myomas are present in approximately 5–10% of women with infertility, and are the only identifiable abnormality in 1–2.4% of infertile women. Myomas have been implicated in recurrent pregnancy loss, and the association with infertility continues to be investigated.

Although the size and number of myomas is clinically important, myoma location is most clearly associated with impaired fertility. Any distortion or obstruction of the female reproductive tract may impede normal migration of sperm, ovum, or embryo, or may impair implantation. It has been theorized that myomas impair fertility as a result of alteration of the endometrial contour, enlargement and deformity of the uterine cavity, anatomic distortion of the cervix, altered uterine contractility, persistence of intrauterine blood or clots, or distortion or obstruction of tubal ostia. Furthermore, submucosal myomas may impair implantation by damaging the overlying endometrium and disturbing the endometrial vasculature, causing endometrial inflammation, ulceration, thinning, and atrophy, or altering the biochemical environment.

A recent systematic literature review and meta-analysis demonstrated that women with uterine myomas in any location had worse reproductive outcomes than infertile women without myomas. Therefore, reproductive outcomes were evaluated based on myoma location. When women with submucosal myomas were compared to infertile women without myomas, the submucosal myoma group demonstrated significantly worse reproductive outcomes. However, after women with submucosal myomas underwent a myomectomy procedure, reproductive outcomes became similar to those of infertile women without myomas. Multiple studies investigating the effect of myomas on in-vitro fertilization (IVF) pregnancy rates identified significantly lower pregnancy rates in the setting of a distorted uterine cavity; when myomectomy was performed for these distorting myomas, ART pregnancy rates improved and

were comparable to the pregnancy rates of women with normal uteri.

EVIDENCE AT A GLANCE

Women with myomas in any location had significantly lower relative risks of clinical pregnancy (RR 0.85; 95% CI 0.73–0.98), implantation (RR 0.82; 95% CI 0.72–0.93) and ongoing pregnancy/live birth (RR 0.70; 95% CI 0.59–0.83), and had higher rates of spontaneous abortion (RR 1.68; 95% CI 1.37–2.05). Compared to infertile women without myomas, women with submucosal myomas demonstrated significantly lower rates of clinical pregnancy (RR 0.36; 95% CI 0.18–0.74), implantation (RR 0.28; 95% CI 0.12–0.65), and ongoing pregnancy/live birth (RR 0.32; 95% CI 0.12–0.85), and a higher spontaneous abortion rate (RR 1.68; 95% CI 1.37–2.05). Furthermore, women with intramural myomas and a normal uterine cavity experienced significantly lower implantation (RR 0.79; 95% CI 0.70–0.90) and ongoing pregnancy/live birth rates (RR 0.78; 95% CI 0.69–0.88), and higher spontaneous abortion rates (RR 1.89; 95% CI 1.47–2.43), but no difference in clinical pregnancy or preterm delivery rates. (Pritts 2009)

The meta-analysis also demonstrated that intramural myomas may adversely affect reproductive outcomes. However, when intramural myoma size was analyzed as a variable (sizes 2–6 cm), fertility outcomes were not significantly different compared to infertile women without myomas, and no statistical trends were appreciated. Subserosal myomas did not affect reproductive outcomes. The mechanism by which intramural myomas, in the setting of a normal uterine cavity, affect fertility and pregnancy outcomes warrants further investigation. It is also unclear whether the distance between the myoma and the endometrium impacts fertility. Furthermore, even if intramural myomas impair fertility, it has not been proven that a myomectomy procedure will normalize fertility or be of benefit to this population of women.

CAUTION

For *asymptomatic* women with intramural myomas who do not have a poor reproductive history, or who have not yet conceived, the risk of a surgical procedure to remove intramural myomas must be carefully weighed against the possibility that reproductive outcomes might not improve.

Several medical therapies such as oral contraceptive pills and the levonorgestrel-releasing intrauterine device are commonly used to treat women with myoma-related symptoms such as abnormal bleeding and menorrhagia, and to provide contraception when indicated. Although medications may improve myoma-related symptoms, any decrease in myoma size promptly reverses after discontinuing the medication, and no beneficial effects on subsequent fertility have been identified. Furthermore, most medical therapies have substantial disadvantages: they prevent conception, long-term use can be associated with adverse effects, and upon discontinuation, rapid symptom rebound and a delay in the return of ovulatory menstrual cycles may occur.

Gonadotropin-releasing hormone (GnRH) analogues have been the most effective medical treatment for uterine myomas, and have specific indications: treatment of anemia and/or large myomas prior to surgery, treatment of symptomatic myomas in the perimenopause, and long-term management for poor surgical candidates. The marked hypoestrogenic state due to GnRH agonist treatment results in amenorrhea, and a rapid and significant reduction in uterine and myoma size by 30–60%; this effect is most pronounced after 3 months of therapy. When GnRH agonists are utilized prior to a myomectomy procedure, they achieve a higher preoperative hematocrit and decreased uterine and myoma volumes, and may enable conversion from a vertical to a transverse incision, and may result in decreased surgical blood loss. Although similarly effective, GnRH antagonists are not as well studied as GnRH agonists, and long-term preparations are not currently available. Overall, GnRH analogues are most useful and cost-effective as

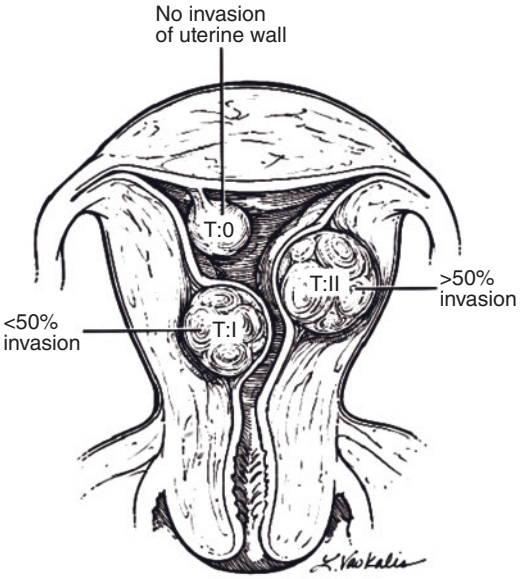

Figure 4.3 The European Society of Hysteroscopy classification of submucosal uterine myomas based on the extent of the intracavitary portion of the myoma: a type 0 myoma is intracavitary, with no intramural component; a type I myoma has <50% intramural extension; a type II myoma has >50% intramural extension. Reproduced from Cohen and Valle 2000, with permission from Elsevier.

preoperative treatment for women with anemia and/or large myomas.

Conservative procedures, both surgical and nonsurgical, are indicated for significant myoma-related symptoms such as abnormal bleeding, anemia, pelvic pain or pressure, ureteral obstruction or urinary symptoms, or for myomas that may affect reproductive outcomes. Detailed uterine imaging is essential to confirm the location of myomas and to plan the most appropriate procedure. Each procedure has inherent risks and benefits depending on the complexity, invasiveness, and duration of the procedure. Surgical treatment of myomas with myomectomy, using a hysteroscopic, abdominal, or laparoscopic approach, is considered the best and safest approach for women with symptomatic myomas and desired fertility. One of the benefits of myomectomy procedures is the ability to achieve complete removal of one or more myomas; other procedures may only shrink the myomas. Furthermore, procedures such as myolysis, uterine artery embolization (UAE) and MRI-guided focused ultrasound (MRgFUS) are not currently recommended for women who desire future fertility since the safety of pregnancy after these procedures has not been established.

Depending on location, submucosal myomas may be appropriate for hysteroscopic myomectomy. The European Society of Hysteroscopy established a classification system to describe the intracavitary component of submucosal myomas: a type 0 myoma resides entirely within the uterine cavity, a type I myoma protrudes more than 50% into the cavity, and a type II myoma has at least 50% of its volume within the myometrium (Figure 4.3). Hysteroscopic myomectomy is an appropriate procedure for submucosal myomas types 0 and I, although type I myomas require significant hysteroscopic skill to successfully resect the myoma. The advantages of hysteroscopic myomectomy include the lack of an abdominal incision, short recovery time, and less blood loss and postoperative discomfort. The procedural risks include bleeding and fluid overload, both directly related to the complexity and duration of the procedure, the potential need for a second procedure to complete the removal of the myoma, and intrauterine adhesions. After hysteroscopic myomectomy, myoma symptoms often improve and fertility rates often normalize. There have been no reports of uterine rupture after hysteroscopic myomectomy.

Myomectomy procedures are also performed using abdominal, laparoscopic, or robotic-assisted laparoscopic approaches. The most common myomectomy approach is abdominal; this is appropriate for women with multiple and large myomas, including those with intramural and transmural myomas. The risks of this major surgical procedure include blood loss and infection, and postoperative adhesions that may impair fallopian tube function and hence impair fertility, or may cause pelvic pain. To allow sufficient time for myometrial healing, it is recommended that women delay conception for 4–6 months after an abdominal myomectomy. Based on the presurgical myoma location, the extent of surgery or the woman's age, postoperative assessment of

fallopian tube patency with an HSG may be reasonable prior to attempting to conceive. Depending on the situation, additional evaluation of the uterine cavity may also be warranted. Although the incidence of uterine rupture after myomectomy is considered to be low, the true incidence is unknown since current practice limits the number of women who labor; a cesarean delivery is commonly recommended when a myomectomy involved a transmural incision that entered the endometrial cavity, or multiple myometrial incisions to remove multiple myomas.

Use of the laparoscopic approach for myomectomy procedures is limited by the size, number, and location of the myomas to be removed, and the technical difficulty of the procedure and of laparoscopic suturing. Compared to abdominal myomectomy, the laparoscopic approach is associated with less postoperative pain, shorter hospital stay, and shorter recovery. Risk factors for conversion to an open myomectomy include myoma size greater than 5 cm, intramural or anterior location, and preoperative use of a GnRH agonist. However, as experience with laparoscopic myomectomy continues to develop, skilled laparoscopic surgeons demonstrate excellent outcomes with technically difficult procedures: large (>5 cm) myomas, transmural and deep intramural myomas. As experience with robotic-assisted laparoscopic myomectomy increases, a greater number of minimally invasive myomectomies may be performed. For women who desire future childbearing, it is controversial whether laparoscopic suturing to reapproximate the myometrium is comparable to the multilayer suturing performed during an abdominal myomectomy. However, with skilled and experienced surgeons, the rate of uterine rupture after laparoscopic myomectomy appears comparable to abdominal myomectomy. Further outcomes data for laparoscopic myomectomy procedures are warranted.

The effectiveness of myomectomy is limited by the recurrence rate for uterine myomas. Furthermore, small myomas may remain after a myomectomy procedure, thus myoma persistence, not recurrence, can also become clinically significant. Women with multiple myomas at myomectomy have a higher myoma recurrence rate compared to those with a single myoma, and a higher rate of additional surgical procedures for myomas. A recent review of life table analyses determined that 5 years after abdominal myomectomy, there was a 10% cumulative risk of clinically significant new myomas.

Endometrial polyps

Endometrial polyps are localized hyperplastic overgrowths of the endometrium, containing both endometrial glands and stroma, and the large majority of lesions are benign. Polyps can occur as individual or multiple lesions, can range in size from millimeters to centimeters, and can be sessile or pedunculated. Although polyps may be identified during the evaluation of abnormal uterine bleeding, many polyps are asymptomatic and only revealed during the infertility work-up; up to 25% of women with unexplained infertility have endometrial polyps on hysteroscopy. Polyps are commonly visualized during TVS or SIS, and may present as a uterine cavity-filling defect on an HSG. The preferred method of polypectomy employs hysteroscopic visualization, not blind transcervical curettage, since small polyps and other structural abnormalities of the uterus could be missed. Hysteroscopy-directed polypectomy using microscissors and grasping forceps, or a loop electrode, enables excision of the entire polyp, including the stalk if the lesion is pedunculated.

The association between endometrial polyps and infertility is unclear. However, it is plausible that polyps can cause infertility as a result of mechanical interference with sperm and embryo transport, impairment of embryo implantation, or altered endometrial receptivity. Furthermore, the size, number, or location of endometrial polyps may influence any effect on reproductive outcomes.

Several studies have investigated the effect of endometrial polyps on pregnancy rates after intrauterine insemination (IUI) or IVF. Although one randomized trial identified a significant improvement in IUI pregnancy rates after hysteroscopic polypectomy, several IVF studies have not demonstrated impairment of pregnancy rates in the presence of endometrial polyps less than 2 cm in size. However, these IVF studies may have been insufficiently powered to detect a difference in pregnancy rates. In contrast, for

Table 4.2 American Fertility Society classification of intrauterine adhesions. Stages of disease severity are based on the cumulative score: stage I (mild) 1–4; stage II (moderate) 5–8; stage III (severe) 9–12

Extent of cavity involved	<1/3	1/3–2/3	>2/3
Score	*1*	*2*	*4*
Type of adhesions	Filmy	Filmy and dense	Dense
Score	*1*	*2*	*4*
Menstrual pattern	Normal	Hypomenorrhea	Amenorrhea
Score	*0*	*3*	*4*

women with unexplained infertility who underwent polypectomy, a higher rate of spontaneous conception has been demonstrated. Hence, the effect of endometrial polyps on fertility and pregnancy rates is uncertain, and further studies are warranted. However, in the absence of sufficient data on the effect of polyps on pregnancy rates, and in light of the negative impact of submucosal myomas or myoma-associated cavity distortion on pregnancy rates, polypectomy prior to infertility treatment is reasonable and often recommended. Lastly, polypectomy is also indicated in women who are at high risk for endometrial hyperplasia, including those with chronic anovulation, obesity, or a personal history of endometrial hyperplasia.

Intrauterine adhesions

Intrauterine adhesions are caused by trauma to the basalis layer (regenerative layer) of the endometrium, with subsequent scarring between opposing areas of myometrium. Intrauterine adhesions (IUA) can achieve partial or complete obliteration of the uterine cavity, resulting in menstrual abnormalities or amenorrhea, pelvic pain, infertility or recurrent pregnancy loss; symptomatic intrauterine adhesions are also known as Asherman syndrome. However, some women with IUA are asymptomatic. Trauma to the endometrium and uterine cavity may occur during uterine curettage of the pregnant or recently postpartum uterus, operative hysteroscopy, endometrial ablation, pelvic irradiation, or hysterotomy at the time of myomectomy or cesarean delivery, and the risk of IUA may increase in the presence of infection. In the developing world, genital tuberculosis is a significant cause of IUA. After surgical treatment for retained products of conception, up to 40% of women demonstrate IUA.

Although IUA are often identified during SIS or HSG, hysteroscopy is considered the gold standard procedure for diagnosing IUA. Several classification systems for IUA exist; the American Fertility Society classifies IUA based on the extent and type of adhesions, and the effect of adhesions on menstrual pattern: eumenorrhea, hypomenorrhea, or amenorrhea (Table 4.2). However, the degree of menstrual disturbance does not necessarily correlate with the extent of IUA.

Women with IUA demonstrate poor reproductive outcomes, with high rates of subfertility and infertility, spontaneous abortion, and preterm delivery. Treatment of IUA has been shown to improve reproductive outcomes by improving fertility and reducing the rate of pregnancy loss. Management of IUA is best achieved with hysteroscopic adhesiolysis. Lysis of adhesions can be performed with hysteroscopic scissors, monopolar or bipolar electrosurgery, or Nd:YAG laser ablation; these techniques are preferred to blind lysis of adhesions with a sharp curette, as further damage to the basalis layer may occur. Concomitant laparoscopy or transabdominal ultrasonography can be used to guide the hysteroscopic procedure. The goal of the procedure is to normalize the anatomy of the uterine cavity, although more than one procedure may be necessary to achieve satisfactory results: restoration of anatomy and menstruation, and reproductive success.

Postoperative adhesion reformation occurs in 20–50% of cases. Hence, techniques to prevent reformation of IUA are necessary. These methods include the placement of intrauterine balloon

catheters or intrauterine devices, and administration of estrogen (with or without progestins) or antibiotics. Hormone regimens commonly involve a 30-day course of estrogen (such as estradiol 4 mg twice daily) with the addition of a progestin (such as medroxyprogesterone acetate 10 mg daily) for the last 10 days. If an intrauterine balloon or pediatric Foley catheter is left in place for 1–2 weeks, prophylactic antibiotic treatment is recommended; doxycycline 100 mg twice daily is commonly used. Although evidence supporting these interventions is lacking and there is no consensus on the best postoperative regimen, aggressive adhesion prevention is warranted for moderate and severe IUA cases. Furthermore, due to the high rate of adhesion reformation, postoperative evaluation of the uterine cavity is recommended, usually 1–2 months after the procedure.

Although surgical treatment of IUA is associated with postprocedure pregnancy rates of approximately 60% and live birth rates of approximately 40%, and with an improvement in the rate of pregnancy loss, success is directly related to the extent and severity of the adhesions. However, despite successful normalization of the uterine cavity, poor endometrial development can persist due to deficiency in the residual functional endometrium or due to impaired myometrial perfusion. Lastly, Asherman syndrome and its treatment have been associated with pregnancy complications such as placenta accreta, premature delivery, second trimester pregnancy loss and uterine rupture, so a discussion about possible obstetric complications is imperative.

Adenomyosis

Adenomyosis is a condition in which endometrial glands and stroma have invaded the uterine myometrium. This abnormal tissue can be present in focal areas, in nodules called adenomyomas, or throughout the myometrium which causes diffuse uterine enlargement. Symptoms associated with adenomyosis include dysmenorrhea, chronic pelvic pain, menorrhagia and abnormal uterine bleeding; approximately one-third of women with adenomyosis are asymptomatic. The frequency and severity of symptoms seems to correlate with the extent and depth of adenomyosis. Adenomyosis can be suggested by heterogeneous myometrial echotexture at transvaginal ultrasonography, or by the identification of increased signal intensity within the myometrium and/or a thickened junctional zone on MRI, and a definitive diagnosis can be made upon histologic evaluation of a hysterectomy specimen.

Although adenomyosis is usually diagnosed in the fourth and fifth decades of life, it can be identified in younger women and may present in the setting of infertility. Furthermore, as an increasing number of women of advanced reproductive age attempt conception, the incidence of symptomatic and asymptomatic adenomyosis in women with desired fertility may rise. Adenomyosis has been diagnosed in both multiparous women and infertile women, and in women with concomitant endometriosis, which complicates our understanding of this condition. Hence, the exact association between adenomyosis and infertility is unclear, and further studies investigating the impact of adenomyosis on fertility are necessary, including research on treatment options and pregnancy outcomes.

Although medical treatments such as continuous oral contraceptive pills, the levonorgestrel-releasing intrauterine device, GnRH agonists and aromatase inhibitors have demonstrated improvement in adenomyosis-related symptoms, all treatments are contraceptive and preclude pregnancy. Surgical treatments for adenomyosis such as endoscopic endometrial ablation and hysterectomy are not appropriate for women with desired fertility. Therefore, conservative treatment options for adenomyosis such as hormone therapy, vessel embolization, and combined surgical and hormonal treatments should be further studied in women who wish to conceive. Successful pregnancies have been reported for women treated with GnRH agonists (depot-leuprolide acetate 3.75 mg every 4 weeks for 24 weeks), and for women who underwent microsurgical resection of adenomyosis followed by GnRH agonist therapy. Because of the inability of conservative surgery to completely clear adenomyosis from the uterus, the surgical risks including pelvic adhesions, intrauterine adhesions and reduced uterine capacity, and the risk of uterine rupture during pregnancy, surgical intervention is commonly reserved for women

with adenomyosis in whom GnRH agonist treatment was not effective. In the future, MRgFUS or similar technologies may be available for conservative management of adenomyosis.

Summary

During the evaluation of an infertile couple, as possible etiologies of infertility are investigated, the uterus deserves careful assessment. While symptoms such as abnormal uterine bleeding, dysmenorrhea, or pelvic discomfort may raise suspicion for a uterine disorder, many of these disorders can be asymptomatic. Initial imaging of the uterus can be accomplished with TVS; HSG may be part of the infertility work-up, and SIS should be performed if indicated.

For women with congenital uterine anomalies, available management options depend on the specific anomaly. If a partial or complete uterine septum is identified in an infertile woman, hysteroscopic metroplasty is recommended to improve reproductive outcomes. Management of a unicornuate, didelphys, or bicornuate uterus depends on the woman's reproductive history. Surgical procedures to achieve uterine unification are not routinely recommended for didelphys or bicornuate uteri. However, if a functional rudimentary uterine horn is identified with a unicornuate uterus, prompt removal of the uterine horn is recommended.

If an infertile woman is known to have myomas, then detailed evaluation of the uterus and endometrial cavity is recommended. Uterine myomas that are submucosal or intramural with cavity distortion clearly have an adverse impact on fertility; the exact impact of nondistorting myomas on fertility is unclear. Myomectomy procedures are indicated for symptomatic myomas or those in problematic locations, and the appropriate surgical procedure is determined by myoma location. Furthermore, since myomas may impair fertility and can adversely impact pregnancy, and often enlarge over time, it is generally recommended that women with myomas do not postpone pregnancy, if possible. However, prophylactic surgical procedures to prevent potential myoma-related infertility or pregnancy complications are not recommended.

Based on available evidence and expert opinion, endometrial polyps and intrauterine adhesions should be surgically managed when identified during the infertility evaluation. Although there are limited data about the effect of endometrial polyps on infertility, the clear association between submucosal myomas and impaired reproductive outcomes encourages active management of endometrial polyps with hysteroscopic polypectomy. Intrauterine adhesions are associated with poor reproductive outcomes, and pregnancy rates improve after a hysteroscopic procedure. In contrast, the affect of adenomyosis on infertility remains uncertain; further research on medical and conservative surgical and nonsurgical management options is warranted, as well as evaluation of subsequent reproductive outcomes.

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5

Diagnosis and Management of Infertility Due to Endometriosis

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Introduction

Endometriosis is a gynecologic disease that affects 5–10% of women of reproductive age. It is defined as the presence of endometrial tissue outside the uterine cavity. This disease, first described approximately 300 years ago, is found in 70% of patients with chronic pelvic pain. Early retrospective studies suggested that 25–50% of infertile women have endometriosis and 30–50% of women with endometriosis are infertile.

Clinical presentation

The mean age at diagnosis of endometriosis is 25–29 years. A family history of endometriosis is relevant: there is a sevenfold greater chance of developing the disease if there is a first-degree relative of the patient who has the disease. Some women with endometriosis are asymptomatic but, when present, it involves a vast spectrum of symptoms. The primary presenting symptoms are dysmenorrhea, dyschezia, dyspareunia, pelvic pain, dysuria, hematuria, and infertility. Dysmenorrhea is the most commonly reported symptom and its severe form, although not entirely predictive, is highly suggestive of endometriosis. The common signs are tenderness in the cul-de-sac or uterosacral ligaments, nodularity along the uterosacral ligaments, adnexal tenderness, and pelvic masses. There is no consensus on the development of this disease, but several theories have been put forth in explaining the underlying pathogenesis of the disease. Here we will discuss the different theories of histogenesis and some of the current thinking on the contribution of the stem cells and immune system to the etiology of endometriosis.

Pathogenesis of endometriosis

Retrograde menstruation theory

This mechanism of histogenesis is often referred to as Sampson's theory; it proposes that viable endometrial tissue refluxed through the fallopian tubes during menstruation and implants at ectopic sites in the peritoneal cavity. Retrograde menstruation is a common phenomenon which occurs in 76–90% of women who have patent fallopian tubes. Some of these cells remain viable and have the capacity to invade the peritoneum and implant at ectopic sites. This implantation theory is supported by cases of patients with müllerian anomalies and obstructed menstrual flow. In this population of patients, there is an increased frequency of endometriosis. However, if retrograde menstruation happens in the majority of women in each cycle, one may wonder why endometriosis only occurs in 5–10% of the female population. Are there any other factors contributing to the disease?

Coelomic metaplasia theory

At the turn of the 20th century, Meyer and Iwanoff proposed that endometriosis may develop from metaplasia of cells lining the pelvic peritoneum. This theory is based on embryologic studies showing that müllerian ducts and ger-

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minal epithelium of ovary are derived from epithelium of the coelomic wall. These differentiated structures contain cells that are able to acquire the capacity for further development into endometrium. This theory is able to account for the occurrence of endometriosis anywhere in the abdominal cavity and in the thoracic cavity. The rare occurrence of endometriosis in men and in prepubertal adolescent girls who are not menstruating is often taken as proof of this theory.

Induction theory

The induction theory is an extension of the coelomic metaplasia theory which proposes that hormonal or inductive stimuli can induce peritoneal tissues to form endometriosis by subsequent metaplasia.

Embryonic rest theory

In the 1890s, Von Recklinghausen and Russell were credited with the theory which proposed that cell remnants of müllerian origin could be activated to differentiate into endometrium in the presence of a specific stimulus. This hypothesis could account for the presence of endometriosis of the rectovaginal septum as well as in any location along the migration pathway of the embryonic müllerian system. This theory could also account for the presence of endometriosis in men because the male embryo initially develops female-specific embryological structures that regress with activation of the male genome.

Lymphatic and vascular metastasis theory

An extensive communication of lymphatics has been shown between the uterus, ovaries, tubes, and vaginal lymph nodes, and considerable evidence has suggested that endometrial cells can metastasize via lymphatic and hematogenous routes. In the 1920s, Halban suggested that endometriosis also could result from lymphatic and hematogenous dissemination of endometrial cells. This theory provides an explanation for rare cases of endometriosis occurring in locations remote to the peritoneal cavity. Previous research has demonstrated that intravenous injection of endometrium can produce pulmonary endometriosis in the rabbit, further supporting the theory of venous metastasis.

Stem cell theory

Stem cells are undifferentiated cells that have the ability to self-renew as well as to produce more differentiated daughter cells. Stem cells can be broadly divided into two groups: embryonic and adult. Embryonic stem cells are derived from blastocyts; adult stem cells are derived from postembryonic cell lineages. Adult stem cells have been described in a number of different organ systems such as the gastrointestinal system and also the hematopoietic system. Adult stem cells reside in an anatomic structure called the niche. The stem cell niche is a microenvironment of surrounding support cells that signal the stem cell population to maintain an undifferentiated state or alternatively to provide signals that would stimulate the stem cells to differentiate and proliferate. Du and Taylor generated an experimental model to test whether extrauterine-derived cells could track to and populate endometriotic implants. Endometriosis was generated experimentally by ectopic wild-type endometrial implantation in the peritoneal cavity of hysterectomized LacZ transgenic mice. LacZ-expressing stem cells of extrauterine origin were incorporated into the endometriotic implants and were capable of differentiating along epithelial and stromal cell lineages at a frequency of 0.04% and 0.1% respectively in just 2 months time. Taylor's group demonstrated genetic male-derived cells incorporating into the endometrium by analyzing female mice that had received bone marrow transplantation from male donors. The model demonstrated the generation of endometrium de novo. Furthermore, Taylor also showed that women receiving mismatched bone marrow transplantation displayed a similar phenotype, suggesting that bone marrow derived stem cells may contribute both to normal tissue homeostasis and to the pathogenesis of human endometriosis. This demonstrated that bone marrow derived cells contributed to endometriosis, providing an alternate explanation for the origin of endometriotic implants.

Immunologic dysfunction in the peritoneal environment

Inflammation is a feature of endometriotic tissue, which is associated with the overproduction of prostaglandins, metalloproteinases, cytokines,

and chemokines. Peritoneal immunologic alterations result in an inflammatory environment in the abdominal cavity promoting endometrial stromal cell proliferation, lymphocyte and macrophage activation and proliferation, and the increasing presence of non-organ-specific autoantibodies. This enhances the adhesion of shed endometrial tissue fragments onto peritoneal surfaces. There is an increase in the level of acute inflammatory cytokines such as intereukin-1β, interleukin-6, interleukin-8, and tumor necrosis factor, that likely enhance the adhesion of shed endometrial tissue fragments onto peritoneal surfaces. Some of the cytokines also attract the granulocytes, natural killer cells, and macrophages that are typical of endometriosis. Autoregulatory positive feedback loops ensure further accumulation of these immune cells, cytokines, and chemokines in established lesions.

SCIENCE REVISITED

Theories for the pathogenesis of endometriosis

- Retrograde menstruation theory
- Coelomic metaplasia theory
- Induction theory
- Embryonic rest theory
- Lymphatic and vascular metastasis theory
- Stem cells theory
- Epigenetics

Diagnosis of endometriosis

Physical examination

The physical examination can sometimes involve a broad spectrum of findings. Bimanual and rectovaginal examination of the pelvic structures should optimally be performed during menstruation when patient is having symptoms, because this is the easiest time to localize endometriotic lesions. The external genitalia and the vaginal surface are usually unremarkable. Speculum inspection in the vagina may reveal implants that are reddish and hypertrophic lesions that bleed on contact in the posterior fornix. Some of the common locations of endometriosis are ovary, posterior fornix of the vagina, and the cul-du-sac (listed in Table 5.1). Lateral cervical displacement due to scarring of the ipsilateral uterosacral ligament and cervical stenosis are some of the physical findings that can be associated with endometriosis. Endometriomas may be detected as tender or nontender adnexal masses, often fixed to the uterus or to the pelvic sidewall. Bimanual or rectovaginal examination may reveal decreased or absent mobility of the uterus and also tenderness. Table 5.2 lists some of the common location of endometriosis that can be palpated during physical examination. The sensitivity of physical examination in the diagnosis of endometriosis is very poor. A normal clinical examination does not rule out the diagnosis of endometriosis. Endometriosis should not be diagnosed solely from physical examination. Just like any other diseases, caution should always be exercised and differential diagnosis should include other conditions such as neoplasms, infections, etc.

Laboratory testing

Many attempts have been made in the past to identify serum markers as a screen test for endometriosis. CA-125 is the only serum marker that has been commonly utilized by clinicians for

Table 5.1 Possible physical findings of endometriosis

Bluish or red implants, or hypertrophic lesions bleeding on contact in the vaginal posterior fornix
Absent mobility and tenderness on palpation of the uterus
Lateral cervical displacement
Cervical stenosis
Tender masses, nodules and fibrosis
Adnexal fullness or mass (i.e., endometrioma)

Table 5.2 Common locations of endometriosis

Posterior fornix of the vagina
Cul-de-sac
Ovary
Uterosacral ligaments
Rectovaginal septum

the screening test for endometriosis even though it has poor sensitivity and specificity. CA-125 is the cell surface antigen expressed by the derivatives of coelomic and müllerian epithelia, including endocervix, endometrium, fallopian tube, peritoneum, pleura, and pericardium. Originally, the increased serum levels of CA-125 were detected in patients with invasive epithelial ovarian cancer. However, elevated CA-125 levels have been observed in serum, menstrual effluent, and the peritoneal fluid of women with endometriosis.

The level of CA-125 is highest during menstruation whereas the lowest levels were encountered during the midfollicular and periovulatory phases. CA-125 level during the midfollicular phase has been shown to be reproducible and has the best diagnostic accuracy. Some reports have suggested that CA-125 may be used as a marker to follow the effectiveness of the medical or surgical treatments of endometriosis, but there have been conflicting studies indicating that the CA-125 level may not be a reliable marker for both the progression and the diagnosis of the disease. As a result, we suggest that following the patients' symptoms and clinical presentation are much more reliable than the CA-125 level.

However, in recent years, other investigators have evaluated the combined use of putative serum markers for the diagnosis of endometriosis, rather than the use of each singly. Serum concentrations of 7 markers (i.e., interleukin-6, tumor necrosis factor α, macrophage migration inhibitory factor, macrophage chemotactic protein-1, interferon-γ, leptin, and CA-125) were compared between the 63 women with surgically confirmed stage II–IV endometriosis and 78 women who were surgically confirmed to be free of endometriosis. Using classification tree analysis, Barnhart et al. used a three-marker panel of CA-125, macrophage chemotactic protein-1, and leptin to diagnose 51% of subjects as to the presence of endometriosis with 89% accuracy. A four-marker panel of CA-125, macrophage chemotactic protein-1, leptin, and macrophage migration inhibitory factor is able to diagnose 48% of subjects with 93% accuracy. Further studies are needed using a prospective cohort to identify markers that are able to diagnosis endometriosis in the majority of the subjects prior to surgery.

Imaging techniques

Transvaginal ultrasonography

There are two main imaging modalities that can be utilized in diagnosing endometriosis: transvaginal ultrasonography (TUS) and MRI. TUS is particularly helpful in diagnosing endometriomas but is limited in detecting adhesions or superficial implants. TUS should be performed preferably using high-frequency probes (6–7.5 MHz) and with the aid of color Doppler imaging. Diagnostic performance of ultrasound in the detection of endometriomas is reported to have up to 92% sensitivity and 99% specificity. Doppler flow studies can increase diagnostic accuracy. Blood flow in endometriomas is usually pericystic pattern, especially noticeable in the hilar region, and usually visualized in regularly spaced vessels. Furthermore, the transrectal ultrasonography is reported to be a useful tool in the diagnosis of deep infiltrating endometriosis. Endoscopic rectal ultrasound using 7.5- and 12-MHz probes has also been shown to be very useful in detecting endometriotic implants in the rectum. TUS is of limited use in diagnosing peritoneal implants.

MRI

MRI is also most helpful in the identification of endometriomas, and has a sensitivity of up to 90% and specificity up to 98%. A classic MRI finding seen in endometriomas is "shading," which is a hypointense signal on T_2-weighted images due to the high concentrations of iron in the cyst, and relatively homogeneous high signal intensity on T_1-weighted images because of degenerated blood products. Another MRI finding seen in endometriomas is a mixed spectrum of presentation depending on the age of the hemorrhage. Endometrial implants are often small and their signal intensity is similar to that of normal endometrium which is usually hypointense on T_1- and hyperintense in T_2-weighted images; however, it can be variable, which makes this an unreliable tool in detecting endometriotic implants.

Diagnostic laparoscopy

Laparoscopic assessment in combination with histological examination of the excised lesions

Table 5.3 Typical presentation of endometriosis

Powder burn lesions
Black lesions
Chocolate ovarian cysts
Peritoneal windows

Table 5.4 Atypical presentation of endometriotic lesions (n = 154)

White opacification	81%
Red flame-like lesions	81%
Glandular lesions	67%
Subovarian adhesions	50%
Yellow-brown peritoneal patches	47%
Circular peritoneal defects	45%

remains the gold standard for diagnosis of endometriosis. The appearance of the peritoneal implants is divided into two groups, typical and atypical appearing, as listed in Tables 5.3 and 5.4 respectively. The typical appearing lesions are hard to miss, such as dark lesions and endometriomas. The typical appearance of peritoneal implant is a bluish-black powder burn lesion with variable degrees of pigmentation and surrounding fibrosis. It is representative of an advanced stage of the disease. The typical dark coloration is the result of hemosiderin deposits from entrapped menstrual debris. Furthermore, the endometrioma is another typical presentation where they are identified as smooth-walled dark brownish cysts containing chocolate-like fluid. The direct inspection of the ovaries has 97% sensitivity and 95% specificity in identifying endometriomas.

However, most of the peritoneal implants appear as atypical lesions, usually red flame-like lesions (81%) or white opacifications (81%). Red lesions are highly vascularized and proliferative, usually representing an early stage of endometriosis. Other endometriotic lesions can also appear vesicular and yellow in color. However, the clinical significance of microscopic endometriosis remains uncertain. Because endometriotic implants vary in appearance, the expertise of the surgeon may greatly influence the selection of the biopsy area and the likelihood of a diagnosis of endometriosis.

Conclusion

In summary, diagnosis of endometriosis remains challenging. At present there are no simple noninvasive diagnostic tests for the diagnosis of endometriosis. Misdiagnoses and underdiagnoses of endometriosis are due not only to the limitations of diagnostic tools but also to a lack of recognition of the symptoms by patients and physicians. One should keep in mind that endometriotic implants vary in appearance. The experience and expertise of the surgeon may greatly influence the selection of the biopsy area and hence the likelihood of a diagnosis of endometriosis.

Treatment

In treating reproductive-aged women with endometriosis, the success of a therapy, surgical or medical, has been judged by means of assessment of pain and infertility. Medical treatment is commonly used as a first-line therapy for treating endometriosis and can be used in conjunction with surgical therapy for pain. Endometriosis is an estrogen-dependent inflammatory disease, and medical treatments have therefore focused on the hormonal alteration of the menstrual cycle resulting in pseudo-pregnancy or pseudo-menopause, creating an acyclic, hypoestrogenic state. As a result, the medical treatments listed in Table 5.5 are not suitable for patients who are seeking fertility management.

Medical treatment of pain in women with endometriosis

Analgesics

Prostaglandin synthesis by endometriotic lesions may be responsible for the characteristic symptoms of endometriosis, such as pelvic pain and dysmenorrhea. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit biosynthesis of prostaglandins and alleviate these symptoms. These drugs are well tolerated, safe, and inexpensive and are recommended as first-line treatment in women with mild symptoms. It is important to ask patients to start the medication 1 day before the initiation of menses in preparation of the release of the prostaglandins. The reason for starting the drug in advance of menses is to prevent the release of pain-inducing prostaglan-

Table 5.5 Medical treatment for pain in women with endometriosis

Medication	Dose
Oral contraceptives	1 pill/day, continuous (PO)
Medroxyprogesterone acetate	20–100 mg/day (PO)
Depo medroxyprogesterone	150 mg every 3 months (IM)
Megestrol acetate	40 mg/day (PO)
Norethindrone acetate	5–15 mg/day (start at 5 mg/day, increase dose as required)
Danazol	200–800 mg/day (PO)
Leuprolide acetate	3.75 mg/month (IM) or 11.25 mg/3 months (IM)
Nafarelin acetate	200 µg twice a day (intranasal)
Aromatase inhibitor	5 mg/day (PO)
IUD	

IM, intramuscular; IUD, intruterine device; PO, by mouth.

din prior to the start of menstruation. Although NSAIDs are very well tolerated, clinicians must be mindful of their side effects on the gastric lining and also, but rarely, renal damage. Patients who have a history of gastric ulcer or renal disease with elevated creatinine, as well as patients who are pregnant, should avoid this type of medication.

Oral contraceptives

The combination of an estrogen and progesterone to induce a hormonal pseudo-pregnancy state has been used for more than 40 years. The modern version of this treatment is the oral contraceptive (OC). Treatment usually involves the use of one low dose (20–35 µg of ethinyl estradiol) OC pill per day continuously (no placebo pills) or cyclically (21 active pills followed by 7 days of placebo). There is no evidence that one approach is superior to the other, although amenorrhea obtained with continuous OC administration can be advantageous for women with dysmenorrhea. There is no objective data or evidence to show that one OC is any more effective in endometriosis than another. Common side effects include weight gain, nausea, spotting, hypertension, depression, and an increased risk of formation of clots; however, these side effects are usually mild and well tolerated.

Progestins

Progestational drugs are frequently used in the treatment of endometriosis. They cause the decidualization of endometrial tissue, with eventual atrophy. Medroxyprogesterone acetate is the most commonly used progestin to treat endometriosis. Oral medroxyprogesterone acetate, 20–100 mg/day for 6 months, or injection of depot medroxyprogesterone acetate, 150 mg every 3 months, results in significant amelioration of pain symptoms. A major drawback of depot medroxyprogesterone is that the resumption of ovulation after the cessation of therapy may take as long as 1 year. Also, the depot form is not easily titrated and may be less effective than oral administration. The most common side effects consist of spotting, breakthrough bleeding, breast tenderness, depression, and fluid retention. Fortunately, all these effects resolve after the discontinuation of the medication. Norethindrone and medgestrol acetate have similar side effect profile.

Danazol

Danazol is a derivative of a synthetic steroid which is known to have progestagenic and androgenic effects. The drug causes anovulation by abolishing the luteinizing hormone surge and inhibiting the effect of ovarian steroidogenic enzymes on the growth of normal and ectopic endometrium. The recommended dosage of danazol for the treatment of endometriosis is 600–800 mg/day for 6 months. This drug is effective in relieving symptoms and suppressing endometriotic lesions. Studies using lower doses have usually been uncontrolled or small. However, these higher doses have substantial androgenic side effects including increased hair growth, mood changes, a deepening of the voice (which may be irreversible), adverse effects on serum lipids, and rarely liver damage. In practice, the dosage of danazol should be individualized and adjusted to the need of the patient and the

severity of side effects. Pregnancy is an absolute contraindication to this medication. Patients receiving danazol therapy should use barrier contraceptives during the course of the treatment to avoid causing side effects to the fetus (i.e. virilization of the external genitalia of a female fetus). Other side effects include muscle cramps, flushing, mood changes, depression, and edema.

Gonadotropin-releasing hormone agonists

The continuous and repeated administration of gonadotropin-releasing hormone (GnRH) agonists decreases the secretion of follicle-stimulating hormone and luteinizing hormone, resulting in hypogonadotropic hypogonadism which subsequently resulting in endometrial atrophy and amenorrhea. This treatment also results in the down-regulation of pituitary GnRH receptors, resulting in attenuating gonadotropin secretion and decreasing steroidogenesis. The inhibitory effects are fully reversible. The GnRH agonist can be given intranasally, subcutaneously, or intramuscularly, with a frequency of administration raging from twice daily to every 3 months. GnRH agonist is a highly effective medication but the side effects of this medication can be significant including hot flashes, vaginal dryness, decreased libido, breast tenderness, insomnia, depression, irritability and fatigue, headache, and most importantly, osteoporosis. Bone loss is significant after 6 months of GnRH agonist use. Because of the significant side effect profile of this medication and its subsequent effect on bone mineralization, it is usually limited to a 6-month treatment period. A recent modification to GnRH agonist treatment is to "add back" an estrogen and/or a progestin, which has been shown to be able to maintain the efficacy of the treatment and to eliminate most of the side effects of the GnRH agonist. In a 52-week randomized double-blind placebo-controlled trial, Surrey et al. found that GnRH agonist and norethindrone acetate alone or combined with low-dose conjugated equine estrogens administered to symptomatic endometriosis patients for 12 months provide extended pain relief and bone mineral density preservation after completion of therapy. With comparable pain relief and loss of most of the troubling side effects, most women receiving GnRH therapy for endometriosis should receive add-back therapy, which also enables treatment for a prolonged period of time.

Aromatase inhibitors

Aromastase is an enzyme located in estrogen-producing cells in the adrenal glands, ovaries, placenta, testicles, adipose tissue, and brain, that acts by catalyzing the conversion of testosterone to estradiol. Surprisingly, while aromatase is not expressed in the endometrium, it is expressed in endometriosis. Aromatase inhibitors are novel candidates for the medical management of endometriosis and their use is now supported by several studies. The original use of third-generation aromastase inhibitors was to treat estrogen-dependent breast cancer. The two most common use aromatase inhibitors are anastrozole and letrozole. They suppress estrogen production locally and systemically. GnRH agonist can only inhibit estrogen production in the ovary, while aromatase inhibitors can inhibit estrogen production not only in the ovary but also locally in the endometriotic lesion. The recommended dose of letrozole is 2.5–5 mg daily for 6 months continuously; the common dose of anastrozole used for this indication is 1 mg daily. Aliawadi et al. have shown that the combination of letrozole and norethindrone acetate achieved marked reduction of laparoscopically visible and histologically confirmed endometriosis in the small group of patients in the study. The side effects of letrozole include hot flashes and bone pain. Coadministration of norethindrone acetate or a GnRH agonist is essential with aromastase inhibitors, to suppress the ovarian stimulation seen with these medications. Letrozole alone is used as medication for ovulation induction. Moreover, add-back therapy with norethindrone acetate can also decrease the theoretical risk of bone loss in the setting of induced prolonged hypoestrogenic state by aromatase inhibitors.

Surgery

Surgical management is very commonly utilized in patients with pelvic pain that is refractory to medical treatment, and the efficacy of surgical therapy depends heavily on the surgeon's skill and experience. The surgical procedures include excision/resection, vaporization, and coagulation of endometrial implants, excision of ovarian

endometriomas, and lysis of adhesions. These can be accomplished at laparoscopy with surgical excision, laser (carbon dioxide, argon, yttrium–aluminum–garnet [Nd: YAG]), and monopolar or bipolar electrocautery. No significant difference has been observed in the treatment outcomes of the individual laparoscopic surgical management. The 2009 Cochrane review of the effectiveness of operative laparoscopy in patients with endometriosis includes three randomized control trials and two conference reports; the review concluded that laparoscopic surgery results in better pain outcomes than diagnostic laparoscopy alone. Because only a few women with severe endometriosis are included in the meta-analysis, the study outcome may not apply to patient with severe stages of endometriosis. This review does not have sufficient numbers to compare the effectiveness of each laparoscopic surgical intervention in patients with endometriosis.

There are circumstances where medical therapy works in conjunction with surgery, where preoperative and postoperative treatment may play a role in improving the efficacy of endometriosis management. One may consider the use of postoperative treatment in patients with endometriosis when residual disease is expected, or when pain is not relieved, or to extend the pain-free interval following surgery. Several studies have supported the use of postoperative GnRH agonists and oral medroxyprogesterone acetate to reduce pain associated with endometriosis. The efficacy of other hormonal therapy in conjunction with surgery for treating women with endometriosis remains unclear. For patient with the diagnosis of endometriomas on ultrasound, GnRH agonist preoperative treatment resulted in a greater than 25% reduction in the diameter of endometriomas for more than 80% of the women observed compared with 30% of those treated with danazol. Three months of GnRH agonist preoperative therapy decreases cyst wall thickness and inflammation. However, no studies to date have shown the efficacy of the medical therapy for pelvic pain in patients with endometriomas. Even though the reliability of ultrasonography for diagnosing endometrioma is high, be mindful that when medical treatment is used in woman with an ovarian mass, the possible mistake of missing or delaying the diagnosis of a more serious condition (i.e. ovarian malignancy) can be made.

One prospective randomized trial has been conducted to evaluate the effect of surgery on the pain associated with endometriosis. A total of 74 women with stage I, II, or III disease were randomly assigned to operative laparoscopy with ablation of implants, lysis of adhesions, and ablation of the uterosacral nerve, or to diagnostic laparoscopy alone. In the operative laparoscopy group, 62.5% of the women had significant pain decreased compared to 22.6% of those in the diagnostic laparoscopy group. However, the efficacy of the uterosacral nerve ablation component of this treatment is low. In a recent randomized controlled trial of 487 women with history of chronic pain (i.e., without or with minimal endometriosis, adhesions, or pelvic inflammatory disease), laparoscopic uterosacral ablation did not result in improvements in pain, dysmenorrheal, dyspareunia, or quality of life compared with laparoscopy without pelvic denervation. This trial is four times larger than any previously published trial evaluating neuroablation for chronic pelvic pain.

Finally, the surgical definitive management such as total abdominal hysterectomy and bilateral salpingoophorectomy are indicated for patients who have completed childbearing or have significant persistent pelvic pain after conservative treatment. One or both ovaries may be spared if they are completely uninvolved. According to a retrospective analysis, for women who conserved their ovaries, there is 60% likelihood of recurrent symptoms and for women who underwent bilateral oophrectomies, there is 10% chance of recurrence of symptoms within 5 years after surgery.

Treatment of infertility in women with endometriosis

Medical therapy

Although medical therapy is effective in pain management associated with endometriosis, there is no evidence that medical treatment of endometriosis improves fecundity. Small randomized trials have been done in the past involving treatments including danazol and GnRH agonists, but none has shown effectiveness in

increasing fecundity in patients with minimal to mild endometriosis. Patients with endometriosis who undergo ovulation induction or assisted reproduction technology (ART) have improved fertility. A significant increase in cycle fecundity is evident in patients with mild or moderate endometriosis who undergo four cycles of clomiphene citrate and intrauterine insemination compared with controls having intercourse (0.095 vs. 0.033). Cycle fecundity for gonadotropins and intrauterine insemination also compare favorably with no treatment for women with stage I or stage II endometriosis and infertility (0.15 and 0.045).

Assisted reproduction technology

There are no large randomized controlled trials that demonstrated that in-vitro fertilization (IVF) is more effective than expectant management on the treatment of stage-specific infertility associated with endometriosis. However, because of the high pregnancy rates for IVF in women with endometriosis, it is assumed to be a highly effective treatment. According to 2006 American Society for Reproductive Medicine (ASRM) guidelines, for women who have stage III/IV endometriosis and who has had one or more infertility operations, IVF with embryo transfer (IVF-ET) is a better therapeutic option compared to another operation. There are limited data available in showing the effect of surgery in addition to IVF-ET on the outcome of pregnancy in patients with minimal or mild endometriosis-associated infertility. Moreover, several studies have suggested that in women with advance endometriosis, long-term treatment with GnRH agonist before the initiation of the cycle can improve fecundity.

Surgical treatment

Women with minimal and mild endometriosis have decreased fecundity, approximately 3% when no treatment is provided. Information available from a prospective randomized study demonstrates the efficacy of operative laparoscopy (i.e., ablation of endometriotic implants or resection of endometriosis) on fertility. Surgery for minimal or mild endometriosis nearly doubles the pregnancy rate compared to diagnostic laparoscopy. Theoretically speaking, more severe endometriosis may have adhesions that distort the pelvic anatomy resulting in interference with the oocyte passage from the ovary to the fallopian tube. Retrospective studies have indicated that the operative laparoscopy increases fertility in women with advanced endometriosis. It should also be pointed out that there is no randomized control trial in defining the efficacy of operative laparoscopy treatment in increasing fertility in patient with stage III/VI endometriosis.

Summary

It is clear that there are some effective treatments for women with endometriosis, but all have limitations. In treating pelvic pain, both medical and surgical treatments are effective; however, each of the various medications has its own side effects. The optimal surgical approach for the pain associated with endometriosis has yet to be defined but it is clear from several studies that operative laparoscopy is more effective than diagnostic laparoscopy. Finally, combination treatment with drugs and surgery may offer an advantage in treating pain, but the extent of the advantage is unclear because of the limited data available.

The clinical approach to infertile patients with endometriosis remains controversial because there are a few randomized controlled trials on the treatment of endometriosis-associated infertility. However, there are several key points, as listed in the 2006 ASRM guidelines for the treatment of endometriosis:

- Medical therapy is generally effective in treating endometriosis-associated pain symptoms but has no significant effect on endometriosis-associated infertility.
- Female age, duration of infertility, family history, presentation of symptoms, and the stage of disease should be taken into account before formulating a management plan.
- When a patient presents with both infertility and pain, surgical intervention as first-line therapy is appropriate; because operative laparoscopy has shown to be effective in decreasing pain associated with endometriosis

- In asymptomatic patients under 35 years of age with mild or minimal endometriosis presented with infertility concerns, ovulation induction or expectant management can be considered. In patients over 35 years of age with the same presentation, IVF-ET or ovulation induction using gonadotropins should be considered. This is because after age 35 there is a significant decrease in fecundity and a significant increase in spontaneous abortion rate.
- In patients with stage III or IV endometriosis presenting with infertility concerns, IVF-ET or operative laparoscopy may be considered. IVF will provide the highest pregnancy rate per cycle.

No matter what the treatment plan is for pain or for fertility in patient with endoemtriosis, the management plan each patient should be highly individualized and take into account the patient's illness presentation and also her priorities at that point in her lifetime.

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6

Diagnosis and Management of Infertility Due to Anovulation

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Introduction

Ovulation is the final process of an orchestrated cascade of signals from the hypothalamic–pituitary axis to the ovaries. Ovulatory disorders may account for 20–30% of the causes of infertility and often women present with oligomenorrhea, defined as menstruation that occurs at intervals of 35 days to 6 months, or amenorrhea (absence of menstrual periods).

Classification of anovulation

Classification of ovulatory disorders was first proposed more than 40 years ago based on the effectiveness of gonadotropin. The World Health Organization (WHO) classification (Table 6.1) provides a valuable guide for a clinical approach to ovulation disorders. This classification relies on the estrogen secretion. Patients are categorized into WHO group I if they present low levels of estrogens and low to normal levels of gonadotropins, without bleeding after progesterone. WHO group II accounts for 85% of ovulatory disorders, and most of these patients have polycystic ovaries/polycystic ovary syndrome (PCO/PCOS). They will bleed after a progesterone challenge test. In WHO group III estrogen levels are low but gonadotropins are high. The ovary is not able to respond to gonadotropin stimulation. This accounts for 4–5% of ovulatory disorders. A fourth group is represented by the hyperprolactinemic ovulatory disorder.

Another classification of the causes of anovulation can be established based on the location of the physiologic defect (Table 6.2). A further classification may differentiate causes that are suitable or unsuitable for induction of ovulation.

Diagnosis of anovulation

Hypothalamic anovulation

Hypogonadotropic hypogonadal anovulation (WHO group I)

There is a decreased hypothalamic secretion of gonadotropin-releasing hormone (GnRH), or an unresponsiveness of the pituitary to GnRH, hence failure of the gland to produce follicle stimulating hormone (FSH) and luteinizing hormone (LH) is the common finding. Low to low-normal gonadotropin secretion with low concentrations of estradiol (E2) can be demonstrated. These women should also exhibit normal levels of prolactin and thryroid stimulating hormone (TSH). They do not have withdrawal bleeding after progestin administration.

WHO group I may be associated with strenuous exercise and/or weight loss. A detailed dietary history including recent weight gain or loss, as well as a psychological history, will exclude one of the most common causes of amenorrhea.

In addition, a record of physical activity is essential. Intense physical activity, as in athletes

Infertility, 1st edition. Edited by Emre Seli.

Table 6.1 WHO classification of ovulatory disorders

Group I	Hypogonadotropic hypogonadal anovulation
Group II	Normogonadotropic normoestrogenic anovulation
Group III	Hypergonadotropic hypoestrogenic anovulation

Table 6.2 Classification of ovulatory disorders according to defect location

Hypothalamic
Pituitary
Ovarian
Endocrine
Chromosomal

or gymnasts—sometimes accompanied by stress and a critical level body fat—causes reduced production of GnRH.

Eating disorders (anorexia and bulimia nervosa) have to be excluded in secondary amenorrhea, as there may be different presentations of GnRH suppression.

☆ TIPS & TRICKS

Detailed medical history (surgery, radiotherapy, massive hemorrhage, absent sense of smell, exercise, and eating habits) and physical examination with body mass index (BMI) calculation are indispensable for the diagnosis of anovulation disorders.

Hypogonadotropic hypogonadism associated with anosmia (Kallman syndrome) is a rare hereditary condition. There is an abnormal development and migration of GnRH neurons from the olfactory placode. Patients present with primary amenorrhea, low gonadotropin levels, normal female karyotype, infantile sexual development, and anosmia.

Patients included in WHO group I also have a long-term risk of bone mass loss and its related complications, due to low estradiol production.

Pituitary anovulation

Hyperprolactinaemia (WHO group IV)

The secondary amenorrhea relating to hyperprolactinaemia is caused by an inhibition of the pulsatile secretion of GnRH by interaction with the hypothalamic dopamine and opioid system, leading to a decrease in the production of FSH and LH. Most clinicians consider serum prolactin levels above 20–30 ng/mL to be high in women of reproductive age, although levels of 20–50 ng/mL may only cause a short luteal phase with insufficient progesterone.

There are three principal etiologies apart from the idiopathic causes (30%): pituitary prolactin-secreting adenomas, psychotropic medication, and primary hypothyroidism. The most frequent cause is a microprolactinoma (50%). Women with pituitary microadenomas may also present galactorrhea, whereas patients with other symptoms (headache, disturbed vision) need further studies (MRI) to rule out a macroadenoma.

Differential diagnosis can be established on the basis of serum prolactin levels. Prolactin levels greater than 100 ng/mL are almost always due to prolactinomas, and prolactin levels greater than 500–1000 ng/mL represent macroprolactinomas. Hypothyroidism should also be excluded on the differential diagnosis by evaluating TSH levels.

Sheehan syndrome (WHO group I)

Sheehan syndrome is defined by the spontaneous infarction and necrosis of the pituitary in acute obstetric hemorrhagic shock.

Other rare pituitary causes of anovulation include craniopharyngiomas or hypophysectomy.

Cerebral radiotherapy is another local pituitary insult resulting in hypogonadotropic hypogonadism.

Ovarian anovulation

Premature ovarian failure (WHO group III)

This irreversible condition affects 1% of all women before the age of 40. Sometimes the term “premature menopause” is used, and in most cases the etiology is unknown. Apart from this idiopathic cause there are some data relating to genetic alterations, especially mosaicisms.

Table 6.3 Causes of premature ovarian failure

Idiopathic
Autoimmune disorders
Environmental insults
Defective gonadotropin secretion or action
Other known genetic alterations in specific genes
Enzymatic defects
Cytogenetic abnormalities in the X chromosome

Table 6.4 Revised 2003 diagnostic criteria of PCOS (two out of three criteria required for diagnosis)

1	Oligo- and/or anovulation
2	Clinical and/or biochemical signs of hyperandrogenism
3	Polycystic ovaries and exclusion of other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumors, Cushing's syndrome)

Premature ovarian failure has also been described in female carriers of fragile X premutations. Autoimmune processes, infections, and medical procedures (surgery, chemotherapy, or radiotherapy) are additional causes for ovarian failure (Table 6.3).

Elevated levels of gonadotropins and hypoestrogenism are the characteristics of these WHO group III patients.

The objective of the evaluation of a young woman with hypergonadotropic hypoestrogenic amenorrhea is to identify a treatable cause in a timely fashion. Excluding pregnancy is the first diagnostic step in a young woman with amenorrhea. A diagnostic algorithm for evaluation of premature ovarian failure is shown in the box below.

☆ TIPS & TRICKS

Evaluation of premature ovarian failure

- Exclude pregnancy
- Basal serum tests—prolactin and TSH
- At least two basal serum tests—FSH, LH, and estradiol
- Karyotype
- Family history and pedigree
- Premutations in familiar mental retardation 1 gene (*FMR1*)
- Adrenal and thyroid antibodies

FSH levels are usually more than 30–40 mIU/mL in these cases. Patients may have diminished ovarian reserve, which is represented by lower levels of gonadotropins and a low antral follicle count (AFC) assessed by transvaginal scan. Irregular uterine bleeding indicates estradiol production by functional follicles. These patients have difficulties in conceiving and a high miscarriage rate has also been described, usually due to oocytes of advanced age.

Polycystic ovary syndrome (WHO group II)

PCOS is one of the most common disorders in women of reproductive age. It is also a major cause of hyperandrogenism and oligoanovulation. It accounts for 75% of anovulatory infertility.

PCOS was first described by Stein and Leventhal in 1935, and has a prevalence of 4–8% in unselected populations. Different definitions of PCOS have raised an intense debate. The 1990 National Institutes of Health (NIH) criteria required the presence of chronic anovulation plus clinical or biochemical signs of hyperandrogenism. In 2003 a consensus workshop group including members of the European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) defined new criteria for PCOS, requiring two or more of the following three features: oligo-and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries (see also Table 6.4). According to the Rotterdam criteria some authors distinguish four phenotypes of PCOS: severe PCOS, hyperandrogenism and chronic anovulation, ovulatory PCOS, and mild PCOS.

In order to establish the diagnosis of PCOS, related disorders must be excluded:

- 21-hydroxylase-deficient nonclassic adrenal hyperplasia (NCAH) is excluded by measuring

basal morning 17-hydroxyprogesterone levels below 3 ng/mL.
- Thyroid disorders are easily excluded, as routine measurement of TSH in all women of reproductive age is advisable.
- Routine prolactin tests should be performed in hyperandrogenic patients.
- Other endocrinopathies presenting as syndromes of severe insulin resistance have also to be excluded—hyperandrogenic insulin-resistant acanthosis nigricans (HAIRAN) syndrome, Cushing syndrome, androgen-secreting tumors, or exogenous androgens.

Clinical features of polycystic ovarian syndrome

Hyperandrogenism Clinical hyperandrogenism is often subjectively diagnosed by the presence of hirsutism. The cutaneous manifestations of hyperandrogenism also include acne (more often in young women), seborrhea, and androgenic alopecia (especially in older women). Hirsutism is present in 60% of PCOS patients, although it is less prevalent is women of East Asian origin or in adolescence. Hirsutism is often treated with new dermatology technologies before the patient presents to a reproductive endocrinology unit. The use of standardized methods for evaluation of hirsutism is not common.

In a proportion of PCOS patients biochemical hyperandrogenism cannot be demonstrated in terms of high circulating androgens. Measurement of serum free testosterone and the free testosterone index (free androgen index, FAI) are the more sensitive methods for assessing biochemical hyperandrogenemia. Radioimmunoassays that measure free testosterone directly have limited value, and some authors claim they should not be used. Recommended methods to calculate free testosterone are based on the levels of serum total testosterone (T) and sex hormone binding globulin (SHBG).

To date there is limited knowledge about measuring androstendione and dehydroepiandrosterone sulfate (DHEAS) routinely in hyperandrogenic patients.

Chronic anovulation The major clinical data are oligomenorrhea or amenorrhea. Amenorrhea is defined by the absence of menstruation for more than 3 months (excluding pregnancy). Oligomenorrhea is defined by cycles longer than 35 days, or fewer than eight menstrual periods each year.

Hyperprolactinemia (prolactin >20–30 ng/mL) has to be excluded by doing a serum prolactin test in cases of chronic anovulation. Testing of the serum LH level is also necessary to exclude gonadotropin deficiency (LH <2 mIU/mL).

The differential diagnosis also includes hypothalamic amenorrhea caused by exercise and/or diet (no bleeding after progesterone challenge test, low estrogens, normal or low to normal gonadotropins).

High levels of LH can be observed in 60% of PCOS patients. LH/FSH ratio may be elevated in 95% of the subjects. The general opinion is that measurement of serum LH is not necessary for the clinical diagnosis of PCOS, although it can be useful as a secondary parameter. More data is needed regarding the effects of LH suppression with GnRH analogues and potential effects of LH administration in different stimulation protocols in assisted reproductive technology (ART) cycles.

Polycystic ovaries The ESHRE/ASRM PCOS Consensus Workshop participants felt that PCO should be considered as one of the possible criteria for PCOS. The criteria for PCO are the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume. There are two omissions here: follicle distribution and increased stromal echogenicity. Increased stroma echogenicity is a feature of PCO. The use of the oral contraceptive pill modifies ovarian morphology, thus this definition is not applied to those women taking the pill. Investigation has to be repeated if there is evidence of a dominant follicle or a corpus luteum. Only one ovary fitting the definition is sufficient to define PCO.

Asymptomatic PCO (PCO in the absence of an ovulatory disorder or hyperandrogenism) cannot be considered PCOS.

PCO morphology on ultrasound (Figure 6.1) is a good predictor of the risk of ovarian hyperstimulation in patients undergoing ART. In some patients this ovarian ultrasound appearance is seen in the absence of PCOS, and the ovaries behave like those of women with PCOS when

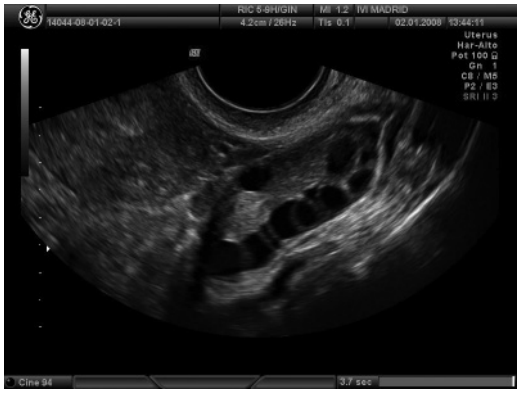

Figure 6.1 PCO ultrasound appearance.

stimulated for intrauterine insemination (IUI) / or in-vitro fertilization (IVF).

☆ TIPS & TRICKS

Recommendations for PCO ultrasound

- State-of-the-art equipment and trained personnel
- Transvaginal approach
- Calculation of ovarian volume (0.5 × length × width × thickness)
- Follicle number estimated in both longitudinal and antero-posterior cross-sections
- The size of the follicles <10 mm should be expressed as the mean of the diameters measured in both cross-sections.

In adolescence, PCO should be assessed by transabdominal ultrasonography based on ovarian volume, as the reliability of the number of follicles decreases, especially in obese young girls.

The measurement of serum antimüllerian hormone (AMH) is promising, as the production of AMH by granulosa cells is related to the AFC.

Insulin resistance Insulin resistance is defined as decreased insulin-mediated glucose utilization, but currently there is not a validated clinical test for this condition in the general population. Some tests, such as the euglycemic clamp or frequently sampled glucose tolerance tests, are restricted to research use because of their complexity.

Table 6.5 Diagnostic criteria for impaired glucose tolerance and type 2 diabetes

	Basal glucose	2-hour glucose
Impaired glucose tolerance	110–125 mg/dL	140–199 mg/dL
Type 2 diabetes	>126 mg/dL	>200 mg/dL

Insulin resistance is a common feature in PCOS, and it is present in 80% of obese PCOS women, and 30–40% of nonobese PCOS women. However, these prevalence values may vary according to the different tests used, as well as their sensitivity and specificity.

Fasting levels of insulin and glucose tests are frequently used in the clinical setting, although they also have some limitations. An oral glucose tolerance test (OGTT) after 75 g oral glucose is also recommended in obese (BMI > 27 kg/m^2) PCOS patients (see Table 6.5).

Although more studies of the metabolic syndrome (see Table 6.6) are necessary in both lean and obese PCOS women, there is a higher risk of type 2 diabetes in PCOS women.

Insulin resistance contributes to hyperandrogenism in a number of ways. Elevated insulin concentrations reduce SHBG levels, thus the bioavailability of testosterone is increased. It may also stimulate adrenal and ovarian androgen synthesis.

☆ TIPS & TRICKS

Summary of 2003 PCOS consensus regarding screening for metabolic disorders

- No test of insulin resistance is necessary to make the diagnosis of PCOS, or to select treatments.
- Obese women with PCOS should be screened for the metabolic syndrome, including glucose intolerance with an oral glucose tolerance test (OGTT).
- Further studies are necessary in nonobese women with PCOS to determine the utility of these tests, although they may be

Table 6.6 Criteria for the metabolic syndrome in women with PCOS (three out of five criteria required for diagnosis)

	Risk factor	Cut-off
1	Abdominal obesity (waist circumference)	>88 cm (>35 in)
2	Triglycerides	≥150 mg/dl
3	HDL cholesterol	<50 mg/dl
4	Blood pressure	≥130/≥85 mmHg
5	Fasting and 2-h glucose from OGTT	110–126 mg/dL and/or 2-h glucose 140–199 mg/dL

OGTT, oral glucose tolerance test.

considered if additional risk factors for insulin resistance, such as a family history of diabetes, are present.

Obesity Obesity has an important role in the different manifestations of PCOS, and exacerbates metabolic and reproductive alterations. The prevalence of obesity varies among countries and regions. In an unselected North American population, 24% of PCOS women were overweight (BMI 25–29.9) and 42% were obese (BMI > 30). European populations have lower mean BMI. The high prevalence of obesity in the general population in North America is probably due to the diet and/or inadequate or insufficient physical activity.

Dyslipidemia, inflammatory markers, and cardiovascular disease The most common features in women with PCOS are hypertrigliceridemia, elevated low density lipoproteins levels and total cholesterol, and low concentrations of high density lipoproteins. Other implications of the metabolic syndrome are the findings of high levels of indices of low-grade chronic inflammation and markers of a prothrombotic state. The evidence for increased risk of cardiovascular disease is limited, despite the fact that cardiovascular risk factors are increased.

Sleep apnea Obesity may not be the only explanation for the high incidence of sleep apnea in PCOS. Insulin resistance is clearly related to the onset of this disorder.

☆ TIPS & TRICKS

Summary of lifelong health complications of PCOS

- Prenatal and childhood: Abnormal fetal growth, premature adrenarche and menarche.
- Adolescence and reproductive life: Cycle irregularity, hirsutism, acne, infertility, miscarriage, pregnancy complications, and endometrial cancer. Obesity and depression, impaired glucose tolerance, insulin resistance, dyslipidemia, type 2 diabetes, sleep apnea.
- Postmenopausal**:** Delayed menopause? Cardiovascular disease?

Chromosomal abnormalities

Turner syndrome

Turner syndrome is the most common genetic abnormality causing ovarian failure. It is characterized by short stature, webbed neck, shield chest, undeveloped breasts, and cubitus valgus. Underdeveloped (streak) ovaries result in primary ovarian failure.

Androgen insensitivity syndrome (testicular feminization)

Androgen insensitivity syndrome is another presentation of primary amenorrhea. A phenotypically female individual with a 46XY karyotype and intra-abdominal testes is typical. There is an absence or nonfunctionality of androgen receptors. Scanty axillary and pubic hair may suggest the diagnosis of androgen resistance. The vagina is blind ended and no uterus is present.

Psychological intervention is usually needed when the diagnosis is confirmed.

Treatment of ovulatory disorders

WHO group I anovulation

This type of anovulation is characterized by decreased hypothalamic or pituitary function and decreased estradiol levels. The use of clomiphene citrate (CC) for ovulation induction has no effect. GnRH administration (subcutaneous or intravenous pump) or subcutaneous/intramuscular gonadotropins are the indicated treatment to restore the hypothalamus–pituitary–ovarian axis.

If the patient has a purely hypothalamic disorder, treatment with pulsatile GnRH will maintain a normal pituitary–ovarian feedback, and increasing concentrations of serum estradiol will restrain FSH secretion, developing a single dominant follicle.

The pregnancy rate per cycle is 20–30%, similar to the that in the general population. Lower multiple pregnancy rates have been shown in patients with pulsatile GnRH compared to a human menopausal gonadotropin (hMG) regimen.

A cumulative pregnancy rate of 89% in 6 months treatment and a 26% pregnancy rate per cycle with gonadotropins were observed.

WHO group II—treatment of polycystic ovary syndrome

PCOS is the principal finding in patients with WHO group II ovulatory disorder (normogonadotropic normoestrogenic anovulation)

Lifestyle modifications and weight loss

Between 35% and 50% of PCOS patients are overweight or obese. Ovulation is profoundly affected by weight, as menstrual cycles are dependent upon BMI. PCOS patients commonly present upper body obesity. Peripheral aromatization to estrogens is increased in obese patients; consequently an estrogenic negative feedback loop is started, resulting in a reduction of FSH secretion. Insulin levels are also high in a proportion of obese patients, leading to hyperandrogenemia, which has also been observed as a result of the reduction of SHBG levels. Poor pregnancy and perinatal outcomes have been described: miscarriage, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, cesarean section; and preterm delivery, perinatal mortality, and admission to neonatal intensive care unit. Obesity has also been related to reduced response and the need for high dose of gonadotropins in ovarian stimulation.

A small weight reduction (2–5%) improves menstrual function by 71% as a result of increased insulin sensitivity, hence there was a lower insulin resistance resulting in spontaneous ovulation. Weight loss in obese patients with PCOS is the first treatment, prior to any pharmaceutical intervention, with an ideal target of a BMI less than 27 kg/m^2.

Weight loss results in improvement in reproductive outcome for all forms of infertility treatment, including ART. Weight loss, if possible combined with exercise, should be promoted in obese PCOS patients, although limited data are available about the pattern of exercise. Long-time weight maintenance is a complicated issue, but rapid weight loss with severe caloric restriction is easily achieved with high-protein supplement diets.

☆ TIPS & TRICKS

Lifestyle changes

- Weight loss should be the first step, before ovulation induction
- Loss of abdominal fat is essential as it is associated with insulin resistance
- A 5% decrease in weight might be clinically meaningful

Clomiphene citrate

Induction of ovulation is the first-line treatment supporting the development of a single follicle in order to achieve a single pregnancy.

CC is a selective estrogen receptor modulator that binds with the estrogen receptor in the hypothalamus, thus antagonizing the negative estrogenic feedback on the hypothalamic–pituitary axis. The levels of LH and FSH rise and stimulate follicular growth and ovulation, by interfering with GnRH secretion.

TIPS & TRICKS

Clomiphene citrate (CC)

- Gold standard treatment for ovulation induction in women with PCOS
- The starting dose should be 50 mg/day for 5 days and the recommended maximum dose is 150 mg/day

SCIENCE REVISITED

Clomiphene citrate (CC)

CC was effective in increasing pregnancy rate compared to placebo (OR 5.8, 95% CI 1.6–21.5) as was CC plus dexamethasone treatment (OR9.46, 95%CI 5.1–17.7) compared to CC alone. No evidence of a difference in effect was found between CC versus tamoxifen or CC in conjunction with human chorionic gonadotrophin (hCG) versus CC alone.

Ultrasound follow-up and serum estradiol tests will avoid multifollicular development and the risk of multiple pregnancies. Resistance to CC (failure to induce ovulation after 3–4 cycles) is present in 20–25% of PCOS patients, with higher incidence in those with obesity, hyperandrogenemia, high ovarian volume, and amenorrhea. It is known that CC is successful at inducing ovulation, but detrimental effects on cervical mucus and the endometrium affect the implantation rates. In addition, there are some side effects such as luteal phase defects, breast tenderness and hot flushes.

Typical ovulation induction doses range from 50–150 mg for 5 days starting on day 3 or day 5 of the cycle, after a spontaneous or progesterone-induced menstrual bleed. Although doses up to 250 mg/day have been used, most clinicians are unwilling to administer doses greater than 150 mg/day. Ovulation induction with CC should be limited to six cycles, and further fertility studies are required.

CC associated with dexamethasone may be considered in PCOS patients with serum DHEA-S greater than 2.0 μg/mL after using CC without success.

Another antiestrogen drug that has been used for ovulation induction in women with PCOS is tamoxifen.

Insulin-sensitizing agents

These drugs can be used before or during the treatment with CC, or instead of CC in those patients who are resistant to it.

Metformin is the most frequently used, but it is not approved by the U.S. Food and Drug Administration (FDA) to induce ovulation. It is an oral hypoglycemic agent which increases glucose uptake in peripheral tissues, and decreases intestinal glucose absorption and liver glucose production. Since there is no increase in insulin levels, metformin does not produce hypoglycemia. Common side effects of metformin are nausea, vomiting, and diarrhea. It is contraindicated in women with renal failure because of the risk of lacticacidosis. There is no evidence of any known fetal toxic effect or teratogenicity, and it appears to reduce first trimester spontaneous miscarriage, although more data is needed. Metformin is administered at a low dose (500 mg), and increased up to 1500–2000 mg/day.

According to a recent meta-analysis metformin has a substantial benefit in the induction of ovulation in women with PCOS. On the other hand, a study comparing CC and metformin alone showed that CC was better for the treatment of infertility. Finally, the combination of CC and metformin compared to CC alone showed no benefit for ovulation, pregnancy rate or live birth rates.

SCIENCE REVISITED

Metformin and ART

There is no evidence that metformin treatment before or during ART cycles improved live birth or clinical pregnancy rates. The pooled odds ratio (OR) for live birth rate (3 RCTs) was 0.77 (95% CI 0.27–2.18) and for clinical pregnancy rate (5 RCTs) it was 0.71 (95% CI 0.39–1.28). The risk of ovarian hyperstimulation syndrome (OHSS) in women with PCOS and undergoing in-vitro fertilization (IVF) or intracytoplasmic sperm

injection (ICSI) cycles was reduced with metformin (pooled OR 0.27, 95% CI 0.16–0.47).

Aromatase inhibitors

Letrozole and anastrazole are third-generation aromatase inhibitors (AI) that have been used for ovulation induction in PCOS patients. The decrease in estrogen levels caused by the inhibition of the enzyme leads to an increase in gonadotropin secretion and stimulates follicular development. In addition to this, the AI may act locally in the ovary by blocking the conversion of androgens to estrogens, the accumulating intraovarian androgens may increase follicular sensitivity mediated by an amplification of FSH receptor gene expression. Unlike CC, the AI does not have a detrimental effect on the endometrium.

Although the use of letrozole for ovulation induction is not approved by the FDA, recent data support the safety of its use. Data from five centers in Canada (911 newborns) demonstrate similar congenital malformations and chromosomal abnormalities among children born to women treated with CC (4.8%) or letrozole (2.4%), and the incidence of major malformations is not significantly higher with CC (3%) than with letrozole (1.2%).

More conflicting evidence about the effectiveness of letrozole is available, according to recent publications. A meta-analysis of randomized trial found significantly higher pregnancy and delivery rates in favor of AI. Nevertheless, more recent data showed no difference between letrozole and CC.

Gonadotropins

Gonadotropins are used for ovulation induction if treatment with CC and/or metformin fails. Gonadotropin treatment is more expensive, time consuming, requires intensive monitoring, and has more complications (OHSS and multiple pregnancy).

The more frequent and advisable protocol for ovulation induction in PCOS starts with 37.5–75 IU/day, and is increased if there is no response (after 14 days) by 37.5 IU/day (low-dose step-up protocol). The maximum is 225 IU because of the high incidence of OHSS. The reported data for this protocol are 13–50% clinical pregnancy rate (cumulative pregnancy rate after six cycles of 60%), multiple pregnancy rate 0–20%, and OHSS 0–7.6%.

Another suggested protocol starts with the administration of 150 IU/day, with the dose decreased by 37.5 IU every 3 days until a dominant follicle appears (step-down approach). This protocol tries to mimic the physiological events in an ovulatory cycle.

The information about different gonadotropin preparations is so conflicting that there is no general consensus on the best option for ovulation induction. Recombinant FSH (rFSH), urinary FSH (uFSH), and hMG are all available; the choice depends on clinician experience, efficacy, safety, cost, ease of administration, previous experience of the patient, etc.

The use of GnRH agonist during ovulation induction with gonadotropins in women with PCOS is not justified, because of the higher OHSS rate, multiple pregnancies, and associated cost, without improving cycle outcome.

Surgery

Surgical ovarian wedge resection was the first established treatment for anovulatory PCOS patients, but this practice was abandoned because of the complications.

Laparoscopic ovarian drilling (LOD) is considered the last line of therapy for PCOS women, and is indicated in women who are both resistant to CC and have no response to gonadotropin administration. A monopolar/bipolar electrode or laser is used to puncture the ovaries; the procedure can be done on an outpatient basis. The exact number of punctures is a matter of debate and there are concerns about long-term effect on ovarian function. Another disadvantage of LOD may be the formation of adhesions which have a negative effect on tubal function.

A recent review showed no evidence of a difference in live birth or clinical pregnancy rate between LOD and gonadotropins. Multiple pregnancy rates were lower with LOD than with gonadotropins. There was no evidence of a difference in miscarriage rates between the two groups.

☆ TIPS & TRICKS

Laparoscopic ovarian drilling (LOD)
Four to ten holes in each ovary, to a depth of approximately 6 mm.

Assisted reproduction in polycystic ovary syndrome

The next step after failure with 4–6 months of ovulation induction is IUI. Four IUI cycles are usually recommended and, if this option fails, IVF and ICSI will be indicated. The clinical pregnancy rate per IUI cycle ranges from 11% to 35%. IVF/ICSI is often needed when infertility is related to a male factor or other nonfemale factors.

The results of ART in PCOS patients appear to be similar to those for women with other types of infertility, suggesting that implantation is not affected in PCOS: the optimal stimulation protocol is not yet established.

A recent meta-analysis compared outcomes of conventional IVF in PCOS and non-PCOS patients. Significantly more oocytes per retrieval were obtained in PCOS patients compared with controls, but the number of oocytes fertilized did not differ significantly. No significant difference was observed in the clinical pregnancy rates per started cycle. The incidence of OHSS after oocyte retrieval was rarely reported. The clinical pregnancy rate per initiated cycle is approximately 35%.

Ovarian hyperstimulation syndrome

Women with PCOS are at increased risk for OHSS because of their particular sensitivity to FSH stimulation. Multiple follicular development will lead to mild, moderate, or severe grades of OHSS. Mild OHSS is characterized by ovarian size less than 5 cm, abdominal discomfort, and nausea. In moderate OHSS (5%) there is vomiting, abdominal distension, and ascites together with larger ovaries. It needs hospitalization. Ovaries larger than 10 cm, tense abdominal ascites, and pleural effusion are signs of severe OHSS (<1% of all cases).

The most advisable intervention is prevention of OHSS by means of recognizing possible patients, lower gonadotropin doses, and strict ultrasound and serum estradiol monitoring.

Other options that may be used to avoid OHSS include early cancellation, coasting (i.e., withholding gonadotropins for a few days), oocyte maturation triggering with GnRH agonists in antagonist protocols, and oocyte or embryo vitrification.

WHO group III anovulation

The only effective option in women with depleted ovaries and high gonadotropins is IVF with oocyte donation.

Large oocyte donation programs with extensive experience show 54.9% pregnancy rate, 50.3% clinical pregnancy rate, and 40.2% ongoing pregnancy rate per embryo transfer. Cumulative pregnancy rates did not differ significantly among different indications for oocyte donation, age groups, or origin of sperm.

Different strategies with gonadotropins have failed to induce ovulation in hypergonadotropic hypogonadism.

Treatment of hyperprolactinemia

Medical therapy with dopamine agonists is the common approach. Bromocriptine is usually started at a dose of 1.25 mg every evening for 7–14 days. It can be increased by 1.25 mg every 2 weeks. Bromocriptine treatment is associated with side effects in up to 60% of patients; nausea and vomiting are the most common adverse reactions. Cabergoline 1–2 mg every week achieved better results compared to 5–10 mg/day of bromocriptine in terms of normalization of serum prolactin and resumption of ovulation.

The expected pregnancy rate is 60–80% if the sole cause of infertility is anovulation due to hyperprolactinemia.

Summary

Ovulatory disorders account for about 30% of infertility problems. Oligomenorrhea or amenorrhea may be present. A valuable therapeutic guide is provided by the World Health Organization classification. Another approach can be established based on the location of the defect. A detailed medical history and physical examination are indispensable for the diagnosis of anovulation disorders.

The most common disorder is polycystic ovary syndrome (PCOS), which is one of the most

prevalent disorders in women of reproductive age. Different definitions of PCOS were defined but a consensus was established in 2003 in Rotterdam. Life style changes and simple treatments can be offered as a first approach for many couples.Long term complications of PCOS patients are a major interest area in reproductive endocrinology.Clomifene citrate is the gold standard treatment for ovulation induction of polycystic ovary syndrome, and along with insulin sensitizers or aromatase inhibitors represents the most frequent approach. ART in PCOS patients show similar results compared to other infertility problems, but the risk of ovarian hyperstimulation syndrome is particularly high in these patients. However other anovulatory disorders are not suitable for ovulation induction. Apart from adoption, oocyte donation appears as a realistic option for these patients, with clinical pregnancy rates of 50%.

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Diagnosis and Management of Infertility Due to Diminished Ovarian Reserve

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Introduction

Along with the introduction of reliable contraception in the 1960s came the birth of fewer children per family in the United States. Simultaneously, there was growing participation of women in the workforce, with a stronger focus on career and education. These societal changes have resulted in postponement of childbearing by many couples, leading to an increased incidence of couples with infertility as a result of female reproductive aging. Recognition of the process of reproductive aging led to the term, first coined by Navot et al. in 1987, 'diminished ovarian reserve'. It was first described as a woman with a follicle stimulating hormone (FSH) level of 26 IU/L or more by radioimmunoassay (>2 standard deviations over control value) during a clomiphene citrate challenge test. As a growing number of couples with infertility due to diminished ovarian reserve sought treatment, numerous tests were developed over the years with threshold values suggesting diminished ovarian reserve, and more importantly, response to fertility treatment. Identifying these patients prior to fertility treatment is essential for all reproductive clinicians to provide patient counseling regarding the most appropriate treatment and/or alternatives to treatment. Furthermore, if the couple proceeds to in-vitro fertilization (IVF), the diagnosis of diminished ovarian reserve is a major factor to consider when determining the type of IVF protocol and/or the use of possible adjuncts to treatment. However, there is still a large degree of controversy and variability regarding the definition of diminished ovarian reserve in the literature, complicating patient diagnosis and treatment. This chapter will attempt to further clarify the definition of diminished ovarian reserve and its potential treatment options.

Ovarian physiology and the ovarian follicle pool

The number of germ cells in human ovaries expands through mitosis to 6–7 million by 20 weeks of gestation. From this point on, the rate of oogonial mitosis decreases while the rate of oogonial atresia increases. In contrast to the continuous process of sperm production throughout a human male's lifetime, oogonial mitosis ends by about 7 months of intrauterine life. As a result, by birth, only 1–2 million germ cells remain in the ovaries (Figure 7.1). This number decreases to approximately 300 000 by the onset of puberty, and only 400–500 of these will ovulate during a woman's reproductive lifespan. Through monthly ovulation and follicular atresia, the pool of oocytes drops to less than 1000 at the time of menopause.

Female reproductive aging

It is well known that female fertility declines after the age of 30, as seen by a drop in monthly fecundity from a baseline of 20–30% per cycle along with an increase in the miscarriage rate

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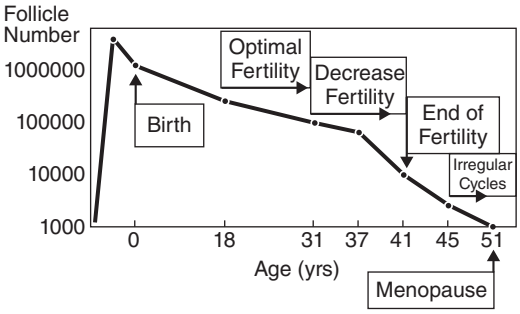

Figure 7.1 The decreasing follicle pool (from Velde, ER. Age at menopause as a marker of reproductive ageing. Maturitas, 1998. 30(2): 119–125)

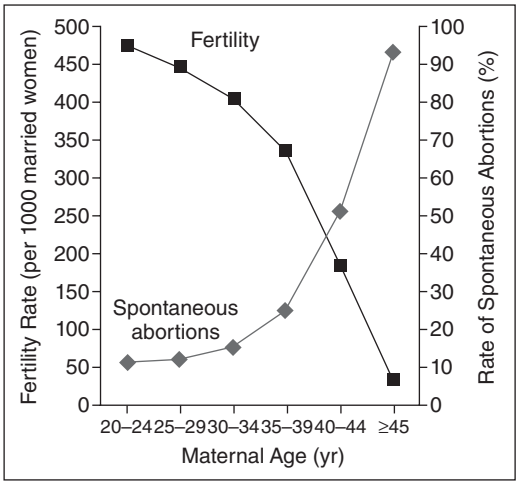

Figure 7.2 Fertility and miscarriage rates as a function of maternal age (from Heffner, L. Advanced maternal age – how old is too old? NEJM, 2004. 351: 1927–1929)

(Figure 7.2). Further understanding of this comes from the study of historical and contemporary populations, and more recently, the outcomes from assisted reproduction technologies (ART). Despite these general tendencies among populations, there is also a substantial degree of individual variation among women, with respect to the point at which their fertility declines.

Clinically, there are subtle findings to suggest that ovarian aging has begun. For example, there is a slight shortening of the menstrual cycle by 2–3 days which occurs as a result of a diminishing follicle reserve driving up serum FSH levels, leading to early follicle recruitment and a shortened follicular phase. Typically, however, this change in the menstrual cycle goes unnoticed. It is not until marked irregularity of the menstrual cycle occurs, as a result of more significant oocyte depletion, that women first notice the signs of ovarian aging. Eventually, the absence of follicles leads to an increasing length between cycles or missed menses, and this is known as the menopausal transition. This period begins at an average age of 46, and eventually leads to the last menstrual period, referred to as menopause, occurring at a mean age of 51.

Diminished ovarian reserve: oocyte quantity versus quality

As mentioned above, the menopausal transition is related to a gradual decline in the follicle pool, and its timing varies significantly among women. Along with a notable decrease in oocyte quantity, the reproductive aging process is also related to a marked loss of oocyte quality. In the human ovary, primary oocytes are formed in the fetus at month 2–3 of development, and remain arrested in prophase of the first meiotic division until just prior to ovulation. Due to this extended period of quiescence, abnormal chromosome segregation occurs during subsequent resumption of meiosis I, leading to oocyte aneuploidy. As women age this period of senescence lengthens, further diminishing oocyte quality through unclear mechanisms. Furthermore, women of advancing reproductive age have been shown to have a greater percentage of aberrant mitotic spindles, suggesting a possible mechanism leading to aneuploidy. Although the ovarian aging process generally leads to a gradual decrease in both oocyte quantity and quality, individual variability among women may lead to more significant decline in one variable over another. Additionally, a woman's chronologic age does not always correspond with her ovarian age. This variability among women makes the evaluation of ovarian reserve a challenging yet essential part of the work-up for all women presenting with infertility.

Diagnosis of diminished ovarian reserve

Determination of diminished ovarian reserve is not only an important part of the infertility work-

up, but also plays a vital role in determining the appropriate fertility treatment. Evaluation of ovarian reserve provides the physician with information used to counsel patients regarding their prognosis, to optimize stimulation protocols, or even to consider alternative treatment options. It is also particularly important to identify the unexpected subset of young women with normal ovulatory cycles who have diminished ovarian reserve, prior to initiating fertility treatment. These patients may have a significantly lower chance of achieving pregnancy than expected, requiring alternative treatment protocols and/or further counseling regarding different treatment options. Despite the value of this information regarding ovarian reserve, there is still a large degree of controversy surrounding which screening methods are most predictive of response to treatment and ultimately, achievement of pregnancy.

Patient age

The value of a patient's age in predicting ovarian reserve and response to fertility treatment is well established. Fecundity in both natural cycles and controlled ovarian hyperstimulation cycles declines with advancing maternal age, with a gradual decline after the age of 30. According to the most recent data from the Society for Assisted Reproductive Technology (SART) registry, delivery rates per IVF cycle in the United States for 2001 in women aged under 35, 35–37, 38–40, and over 40 years of age were 35.5%, 28.5%, 19.7%, and 8.4%, respectively. However, because women of the same age are heterogeneous with regard to their ovarian reserve and response to fertility treatment, relying on patient age alone is of limited predictive value. Therefore, other measures of ovarian reserve have been developed to help predict the potential response to fertility treatment and ultimately, the reproductive potential of an individual woman.

Basal follicle stimulating hormone levels

Evaluation of early follicular (basal) levels of FSH on day 2 or 3 of the menstrual cycle was one of the first widely used measures of ovarian reserve. Levels of FSH have been shown to increase as a result of follicle depletion with decreasing serum inhibin levels. An early study reported that women may have subtle increases in their basal FSH concentrations as early as their mid-30s, at the time when fertility begins to decline. Subsequently, Scott et al. described the utility of basal FSH concentrations in predicting IVF outcome, and several further studies suggested that it might actually be superior to age in predicting response to treatment. Chuang et al. found that women with a FSH level of 10 mIU/mL or less by newer detection methods (chemiluminescence immunoassay) had the best IVF performance, but that women under 35 years old with elevated FSH levels could still have a favorable IVF outcome. This highlights an important delineation between depletion in oocyte quantity reflected by an elevated FSH, and maintenance of oocyte quality and higher reproductive potential due to a relatively young age. Chuang et al. also noted improved predictive value of FSH in women over the age of 40.

Thus, both age and FSH levels are independent predictors of ovarian reserve that should both be taken into account. However, FSH levels are more predictive at the extremes of age, or in the minority of patients with extremely high basal FSH levels, as shown in a recent meta-analysis. Furthermore, basal FSH levels have high intercycle variability from one cycle to the next. These limitations in the utility of serum basal FSH levels have led to the development and utilization of several other tests of ovarian reserve.

Basal estradiol levels

One consequence of elevated FSH levels in women with diminished ovarian reserve is early follicular phase follicle recruitment. As a dominant follicle emerges, it secretes estradiol, which can be detected as early follicular phase elevations in serum estradiol. Furthermore, early follicular phase elevations in serum estradiol above 75–80 pg/mL can be an independent marker of diminished ovarian reserve.

☆ TIPS & TRICKS

When checking cycle day 3 FSH levels, estradiol levels should always be measured concurrently. Elevations in estradiol negatively feedback at the level of the hypothalamus, lowering the level of

gonadotropin releasing hormone, and thus FSH secretion from the pituitary. This could results in an FSH level appearing falsely normal if not measured together with estradiol.

Inhibin B

Inhibin B is a dimeric polypeptide of the transforming growth factor-beta (TGF-β) superfamily, which is secreted by the granulosa cells of the developing antral follicles in the follicular phase. It is thought to play a paracrine role in controlling the early stages of folliculogenesis. Early studies suggested a correlation between low inhibin B levels and poor response to IVF. However, the predictive value of inhibin B has been called into question by several more recent investigators. A recent systematic review of tests predicting ovarian reserve found inhibin B to be of modest accuracy in predicting both poor response to treatment and failure to achieve pregnancy. Furthermore, inhibin B is thought to be a relatively late marker of a diminishing follicle pool, and has been shown to have both inter- and intracycle fluctuations. These numerous limitations make it an overall inferior test when compared to other measures of ovarian reserve.

Antimullerian hormone

Antimüllerian hormone (AMH), a dimeric glycoprotein, is also a member of the TGF-β superfamily. It is produced by the granulosa cells after birth, and the highest levels of AMH in women are achieved after puberty. AMH has been shown to play an important role in regulating early follicular development, and is secreted by preantral (primary and secondary) and early antral follicles as they begin to grow. It can be detected in human serum, and it has recently been shown that serum AMH levels are strongly correlated with ovarian follicular status. As the antral follicle pool declines with age, AMH serum levels also decline, approaching undetectable levels by menopause. Interestingly, it has also been shown to correlate well with the number of oocytes retrieved during IVF. Hazout et al. recently compared AMH to the currently used markers (FSH, estradiol, and inhibin B) as a prognostic marker of response to controlled ovarian hyperstimulation for IVF and of pregnancy outcomes. AMH levels had the strongest correlation not only with the number of oocytes and embryos achieved but, most importantly, with pregnancy outcomes. Higher serum AMH concentrations were strongly associated with higher pregnancy rates. Another major advantage of AMH is that as opposed to FSH, estradiol, and inhibin B, which need to be evaluated on menstrual cycle day 2 or 3, AMH serum levels remain relatively stable throughout the menstrual cycle. This allows for testing at any point during the cycle. The cut-off level used with reasonable sensitivity and specificity to predict poor response to treatment during IVF is generally less than 1 ng/mL, although there is some variation between investigators, ranging from 0.5 to 1.25 ng/mL. The ease of testing and strong predictive power of AMH make it a very promising marker of ovarian reserve.

Dynamic tests

Several investigators have evaluated the use of dynamic tests of ovarian reserve, such as the clomiphene citrate challenge test (CCCT), and the GnRH agonist stimulation test. For the CCCT, after baseline FSH and estradiol levels are measured on cycle day 3, 100 mg of clomiphene citrate is given on cycle days 5–9. FSH is then rechecked on cycle day 10 and the test is considered abnormal if either day 3 or day 10 FSH levels are elevated (>10 mIU/mL by radioimmunoassay). Although the dynamic nature of the test seems promising, comparative studies have shown that the CCCT is no better than other, easier to measure, tests of ovarian reserve such as the antral follicle count (AFC) or FSH levels, while adding the inconvenience of taking over a week to complete. The GnRH agonist stimulation test, which involves measuring changes in estradiol and inhibin B levels from cycle day 2 or 3 after its administration, has similarly shown no additional benefit over other simpler measures of ovarian reserve.

Antral follicle count

Others have taken a different approach, investigating sonographic markers of ovarian reserve. It is known that folliculogenesis starts several

months before the actual cycle, and that follicular recruitment is initiated at the end of the luteal phase of the previous cycle. By the early follicular phase, there is a cohort of early antral follicles measuring 2–5 mm, which can be readily detected by transvaginal ultrasound owing to the presence of a small pool of hypoechoic antral fluid. As opposed to a natural cycle where one dominant follicle is selected and the remainder undergo atresia, in a cycle stimulated with gonadotropins, several follicles have the chance to reach maturity through exogenous gonadotropin recruitment. The higher the dose of gonadotropins used, the more antral follicles will be recruited, up to a certain threshold of medicine. It was postulated that the number of antral follicles (defined as the total number of antral follicles in both ovaries measuring either 2–5 or 2–10 mm) in the early follicular phase could predict a patient's ovarian responsiveness. In fact, this number has been shown to correlate highly with the number of oocytes retrieved and embryos achieved during IVF. Therefore, basal AFC has developed into a high utilized, relatively convenient and cost-effective test to predict ovarian reserve. It is particularly useful in identifying the subgroup of patients with a low AFC who have diminished ovarian reserve and will likely have a poor response to fertility treatment.

Ovarian volume measurement

A decline in ovarian volume has been associated with increasing age, and thus ovarian volume was proposed as a measure of ovarian reserve. However, when used alone it has shown very little accuracy in predicting outcomes of fertility treatment, and therefore is not routinely used as a measure of ovarian reserve.

Treatment of diminished ovarian reserve

Although some patients initially undergo less invasive fertility treatments such as ovulation induction with gonadotropins, once the diagnosis of diminished ovarian reserve is made, most couples will either proceed with IVF or consider alternative treatment options. The traditional long IVF protocol involves the initiation of a GnRH agonist during the midluteal phase of the preceding cycle. The purpose of the GnRH agonist is to achieve pituitary down-regulation, preventing a premature surge of luteinizing hormone (LH) and ovulation, after gonadotropin administration is initiated. Gonadotropins are started in the early follicular phase to induce multifollicular development. Determination of the initial starting dose of gonadotropins is based on the patient's age, weight, results of ovarian reserve testing, and any prior response to gonadotropins, if available. The most commonly used exogenous gonadotropins include recombinant FSH (rFSH), and urinary menotropins (hMG), and often a combination of both is used during stimulation. The response to treatment is monitored with serial estradiol measurements and transvaginal ultrasounds of the growing follicles, adjusting doses if needed. In general, when at least two follicles measure 17–18 mm in mean diameter, human chorionic gonadotropin (hCG) is administered, which completes follicular maturation. There is subtle protocol variation between centers, with some initiating hCG administration when just one follicle reaches maturity, while others measure maturity based on the leading rather than mean follicle diameter. Approximately 36 h later, oocytes are retrieved transvaginally using ultrasound guidance.

More recently, the use of GnRH antagonists to prevent a premature LH surge during IVF cycles was introduced as an alternative to GnRH agonists. Because of their rapid onset of action and lack of a 'flare' response, GnRH antagonists can be initiated during the follicular phase of the cycle after gonadotropins have been started. This allows for greater scheduling flexibility, and less patient discomfort with fewer injections and side effects. Furthermore, antagonist cycles have been shown to require lower levels of gonadotropins and a shorter duration of administration. Although early investigators revealed lower pregnancy outcomes using GnRH antagonists as seen in a recent meta-analysis, as experience grows with the antagonist protocol, more recent investigators have shown equivalent outcomes. Early differences in pregnancy outcomes could relate in part to less follicle synchrony as that seen with the use of an agonist in the preceding luteal phase. This is commonly overcome by administering the oral contraceptive pill (OCP) in the preceding cycle in order to synchronize the follicle cohort. With increasing experience and

improving pregnancy outcomes, the GnRH antagonist protocol has continued to gain popularity in recent years, becoming the protocol of choice in some centers.

Poor responders to IVF

Approximately 9% to 24% of women undergoing fertility treatment with gonadotropins for IVF have a poor response to treatment. Compared to the previously mentioned tests of ovarian reserve, it makes sense that a poor response to gonadotropin stimulation would be the ultimate prediction of a recurrent poor response. A 'poor responder' was first defined by Garcia et al. as a patient with a peak estradiol level less than 300 pg/mL with fewer retrieved and fertilized oocytes, and fewer embryos to transfer. Subsequently, numerous criteria have been used to further quantify a poor response. Some have suggested that the most important criteria are fewer than 3–5 dominant follicles on the day of hCG administration, and/or fewer than 3–5 retrieved oocytes. Others have proposed using a peak estradiol of less than 300–500 pg/mL to define poor response, as it correlates with the number of developing follicles. Other suggested criteria include at least one cancelled IVF cycle, increased (>300 IU/day) gonadotropin use, and prolonged duration of gonadotropin stimulation. Despite the lack of uniformity in definitions, diminished ovarian reserve is consistently shown to be one of the principal etiological factors of poor ovarian response. As one of the greatest challenges in reproductive medicine affecting an increasing number of women, a variety of strategies have been employed to improve response and ultimately pregnancy outcomes in these women.

IVF protocols for diminished ovarian reserve

High gonadotropin dose protocol

Naturally, most patients who fail to respond to standard gonadotropin stimulation protocols receive higher doses in subsequent IVF cycles. Several early reports did in fact show that higher than typical doses of gonadotropins achieved improved follicular growth in the majority of previously poor responders. Hofman et al. showed in a retrospective study that raising urinary FSH doses from 300 to 450 IU/day reduced cycle cancellation rates, while raising pregnancy rates per retrieval, in prior poor responders.

Several other investigators have been unable to repeat these findings, calling into question a dose-related improvement in outcomes. Van Hooff et al. prospectively compared outcomes after doubling the starting hMG dose from 225 IU to 450 IU per day. These authors found that increasing the dose was not effective in enhancing ovarian response for poor responders, although they did not specifically evaluate pregnancy outcomes. Other retrospective studies have evaluated the use of similar doses (450 IU/day) and found no significant improvement in pregnancy rates. This was confirmed in another study where hMG doses were increased to 600 IU/day.

The aforementioned studies are somewhat limited by their heterogeneous nature in terms of cycle protocols and type of gonadotropins used, and/or retrospective design. However, they strongly suggest that although it seems logical to increase gonadotropin doses in poor responders, there is a paucity of data demonstrating a benefit to increasing doses over 450 IU/day.

Low-dose long agonist protocol

The traditional long protocol involves daily administration of 1 mg of the GnRH agonist leuprolide acetate for pituitary suppression followed by a dose reduction to 0.5 mg daily once the gonadotropins are started to prevent oversuppression. Oliviennes et al. prospectively evaluated the effect of attenuating the GnRH agonist dose even further in prior poor responders. They showed that dropping the GnRH agonist dose to 0.5 mg of leuprolide acetate daily starting in the midluteal phase, followed by 0.25 mg daily at the start of gonadotropins, significantly lowered the total gonadotropins used and the cycle cancellation rate, while improving the number of oocytes retrieved and embryos obtained. Felberg et al. also evaluated lowering the midluteal GnRH agonist dose, and similarly showed lower cycle cancellation rates along with increasing numbers of oocytes retrieved and embryos available for transfer. On the basis of these findings, this option has become a viable modification to the traditional long protocol.

GnRH agonist 'stop' protocol

Because lowering the dose of the GnRH agonist appeared to improve IVF success in poor responders, several authors postulated that complete cessation of the GnRH agonist prior to initiating gonadotropins would be even more effective. This would further reduce any ovarian suppression caused by the GnRH agonist during the stimulation, and was referred to as the 'stop' protocol. However, a major concern was the risk of spontaneous LH surge during an IVF cycle as a result of rising estradiol levels with no pituitary suppression, leading to premature ovulation. This protocol was evaluated in two prospective studies, which compared treatment with leuprolide acetate from the midluteal phase of the previous cycle only until menses occurred, to the traditional long protocol continuing leuprolide acetate throughout the entire stimulation. Although a significantly higher number of oocytes were retrieved using the stop protocol in the study by Garcia-Velasco, et al., this did not translate to an improvement in implantation or pregnancy rates. In fact, Dirnfeld et al. found that patients using the stop protocol had a higher cancellation rate, with no improvement in pregnancy rates. Although the risk of premature LH surge was extremely low in both studies, there also seemed to be no benefit in pregnancy outcomes, and thus the stop protocol is not a routinely utilized protocol for poor responders.

GnRH agonist 'flare' protocol

Other investigators tried taking advantage of the flare response that occurs after starting a GnRH agonist, by starting it in the early follicular phase rather than midluteal phase of the preceding cycle. In doing this, the endogenous pituitary stimulation and release of gonadotropins from the GnRH agonist would potentially enhance or augment the ovarian response to exogenous gonadotropin administration. Although several early reports using this approach seemed promising, other investigators have not been able to show any benefit. More recently, Weissman et al. prospectively compared a modified 'flare' protocol to the traditional long protocol and found that pregnancy rates were actually lower in the flare protocol group. Interestingly, other investigators also found that the flare protocol resulted in lower pregnancy rates compared to other traditional protocols. Furthermore, multiple studies have reported increased circulating LH, androgens, and progesterone, as well as a higher rate of rescue of the residual corpus luteum, when starting the GnRH agonist in the follicular phase. The deleterious effects of the above findings could explain a reduction in pregnancy rates using the flare protocol, overall making this protocol a less favorable option.

Microdose flare protocol

A slightly different and much more successful approach to poor responders was to administer a very low dose of GnRH agonist (typically 40 μg twice a day) starting in the follicular phase, referred to as the microdose flare protocol. Initial administration leads to a milder flare response with eventual pituitary suppression. The idea was to give just enough GnRH agonist to prevent the LH surge, while minimizing the suppressive effects of the GnRH agonist, the possibility of corpus luteum rescue, and unwanted elevations in LH, androgens, and progesterone. The first report of a microdose flare protocol by Scott et al. described initiation of leuprolide acetate 20 μg every 12 h starting on cycle day 3, followed by gonadotropins 2 days later. Although this particular study, comparing the microdose flare protocol to results of the same patient's previous cycles as a control, did not show any difference in pregnancy rates, subsequent studies did reveal an improvement in outcomes. Schoolcraft et al. studied a protocol using 40 μg leuprolide acetate every 12 h, with the addition of growth hormone (GH) and gonadotropins, 2 days after the GnRH agonist. They found a significantly lower cycle cancellation rate and higher ongoing pregnancy rate compared to the same patient's prior treatment with a long protocol. Although the addition of GH in this study adds a variable (see below for more information on GH), other studies presented similar findings using 40 μg of leuprolide acetate every 12 h without the use of growth hormone. Additionally, Surrey et al. found no elevations in circulating LH, progesterone, or testosterone levels during a microflare stimulation, as was seen in the earlier agonist flare protocol. Patients in this study, however, received an OCP for 21 days prior to stimulation, which could also

suppress corpus luteum function. Based on the above findings, the microdose flare protocol with or without OCP pretreatment has become one of the most commonly used protocols for poor responders.

Antagonist protocol

In contrast to the long protocol, one of the major advantages of the antagonist protocols is its lack of ovarian suppression in the preceding luteal phase and early follicular phase. Generally, antagonist is started using a fixed or flexible protocol, meaning it is started on a fixed cycle day (typically day 6 of the menstrual cycle), or when the lead follicle reaches a mean diameter of 14 mm or the estradiol reaches 400 pg/mL, respectively. Without the suppression of LH and FSH by the GnRH agonist, follicle recruitment with gonadotropins may proceed more effectively, with initiation of the antagonist only when the follicles begin to grow and the risk of a premature LH surge increases. In fact, several studies have shown that compared to the long protocol, poor responders undergoing the antagonist protocol use less gonadotropin, have shorter stimulations, and have fewer cancelled cycles, while increasing the number of oocytes retrieved.

As the antagonist protocol gained in popularity, several investigators compared it to other regimens used for poor responders. Because the microdose flare protocol was one of the leading contenders, many investigators sought to determine which protocol was superior. A prospective, randomized trial by Schmidt et al. compared the microdose flare protocol to the antagonist protocol in poor responders, and found no difference in any outcome parameters. A similarly designed prospective study by Akman et al. showed an increased number of follicles with the microdose flare protocol, but as in the previously mentioned study, there was no improvement in pregnancy outcomes. Another recent study comparing the two protocols similarly found that although patients in the microdose flare group had more oocytes, higher-quality embryos, and higher fertilization rates, pregnancy rates were ultimately similar between the two groups. Thus, it is extremely difficult to conclusively demonstrate the benefit of one protocol over another, and clinician judgement must be used when determining the optimal protocol.

EVIDENCE AT A GLANCE

Compared to the long protocol, poor responders undergoing the antagonist protocol use less gonadotropins, have shorter stimulations, and have fewer cancelled cycles, while the number of oocytes retrieved is increased. When the antagonist protocol is compared to the microdose flare protocol, another commonly used protocol for poor responders, pregnancy outcomes are similar. Therefore, clinical judgement must be used when determining the optimal protocol for each patient with diminished ovarian reserve.

Natural cycle IVF

Several authors have proposed natural cycle IVF as a viable alternative to standard IVF protocols in women who have responded poorly to conventional IVF. This option is appealing because it would be more cost-effective and less invasive for the patient. The successful retrieval of an oocyte ranges from 48.5% to 82%, and the ongoing pregnancy rate ranges from 2.08% to 18.8%, depending on the study. Although there are a few studies suggesting at least equivalent pregnancy rates with natural compared to conventional IVF, most are flawed due to not clearly defining 'poor responders' at study initiation, and/or using historical controls. Thus, due to limited data at this time, no clear conclusions can be made regarding the utility of natural cycle IVF.

Adjuncts to treatment for diminished ovarian reserve

Corticosteroids

Interestingly, an early prospective trial by Kemeter et al. showed significantly higher follicular response and IVF pregnancy rates with prednisolone cotreatment when compared to controls. There are several proposed mechanisms by which glucocorticoids could influence ovarian responsiveness. They have been shown to stimulate the secretion of both GH and insulin-like growth factor-1 (IGF-1), which act

synergistically with FSH in vitro, stimulating follicular development. Dexamethasone is also a substrate for the enzyme type 1 11-β hydroxysteroid dehydrogenase (11-βHSD), which acts primarily to generate cortisol from its inert counterpart, cortisone. 11-βHSD is differentially regulated across the menstrual cycle, controlling intrafollicular cortisol concentrations, ultimately influencing follicular development. In fact, conception by IVF has been associated with elevated intrafollicular cortisol:cortisone ratios, reflecting modulation of 11-βHSD activity, which could potentially be influenced by administration of dexamethasone. Recently, Keay et al. showed in a randomized, prospective trial that 1 mg of dexamethasone cotreatment during gonadotropin stimulation during IVF resulted in significantly fewer cycle cancellations for previous poor responders, and a trend towards higher pregnancy rates. Although the mechanism is not entirely clear, low-dose dexamethasone is a relatively cost-effective, safe adjunct that can be considered for poor responders undergoing IVF.

Growth hormone

Interest in GH stemmed from animal studies showing increased ovarian production of IGF-1 after GH treatment, and a possible synergistic effect of GH on exogenous gonadotropin treatment in humans. IGF-1 has been shown to stimulate follicular development through several mechanisms. It augments aromatase activity, increasing 17-β-estradiol production in granulosa cells, and also has been shown to protect granulosa cells from apoptosis. Reports on the benefit of cotreatment with GH during IVF have been highly controversial, most likely due to small studies with variation in GH doses and target populations. Importantly, the most recent Cochrane review on the role of GH in IVF showed a small but significant improvement in both pregnancy and live birth rates with the use of GH in poor responders. Results of another recent meta-analysis evaluating various interventions to improve outcomes in poor responders also showed a small but significant improvement in live birth rates with the use of GH during IVF. The results of both meta-analyses are promising, revealing a positive effect on live birth rate, the most relevant outcome parameter. However, it is advisable to interpret these results with caution because of the small number of trials included and the high degree of heterogeneity between studies. Larger studies will help to clarify the optimal dose and target population for GH.

Androgens

Transdermal testosterone

Early in-vitro and primate studies showed that granulosa cell stimulation by FSH is an androgen-mediated process. Treatment of rhesus monkeys with androgens was shown to promote primordial follicle growth and markedly increase the number of growing preantral and small antral follicles, inducing the appearance of polycystic ovaries. This suggests that androgens could potentiate the ovaries response to gonadotropins. With this in mind, Balash et al. conducted a prospective trial showing that 80% of patients with two prior cancelled IVF cycles using pretreatment with transdermal testosterone in a third cycle produced a fair number of oocytes, 2–3 embryos to transfer, while achieving an acceptable pregnancy rate of 30% per retrieval. Although these results seemed promising, other investigators have not been able to reproduce an improvement in ovarian response using transdermal testosterone. Differences in findings may be related to variations in the timing, duration, and dose of androgen supplementation between studies. There has been some suggestion that an optimal threshold of androgens on follicular function exists, beyond which an antagonistic action could manifest. Further randomized studies on the timing, dosage, and potential benefit of androgen therapy are needed before they are a widely used adjunct for poor responders.

DHEA

Interest in pretreatment with dehydroepiandrosterone (DHEA) was sparked after case reports showed an improvement in oocyte and embryo yield in patients using DHEA supplementation. DHEA is a precursor of sex steroids and is the prehormone for up to 48% of follicular fluid testosterone. Through this mechanism, it could also play a role in stimulating follicular development and enhancing ovarian response to gonadotropins. A recent case control study by Barad et al.

showed significantly higher cumulative pregnancy rates in women taking up to 4 months of 75 mg of daily DHEA prior to their IVF cycle. However, prospective randomized trials are nonexistent to date and are needed to further clarify the role of DHEA pretreatment in women with diminished ovarian reserve.

Letrozole

Along those lines, several investigators have proposed the use of letrozole to directly increase intrafollicular androgen concentrations and improve IVF response. Letrozole is a potent aromatase inhibitor, which blocks the conversion of androgens into estrogens, increasing intraovarian androgen levels. Due to safety issues initially raised regarding possible teratogenicity, Garcia-Velasco et al. prospectively studied the use of 2.5 mg of letrozole cotreatment for just the first 5 days of gonadotropin stimulation in poor responders. This timing ensured that the drug would be completely cleared by the time of organogenesis, if the patient conceived. They showed higher levels of intrafollicular androgens in the letrozole-treated group compared to a control group, along with a significantly higher number of oocytes retrieved, higher implantation rates, and a trend towards higher pregnancy rates. Conversely, although a small prospective study by Goswami et al. showed lower gonadotropin requirements in a letrozole-treated group, they failed to show improvement in any outcome parameters. Furthermore, a meta-analysis combining the studies by Goswami and Garcia-Velasco failed to show a significant improvement in pregnancy rates using letrozole. More recently, Schoolcraft et al. compared a microdose GnRH agonist flare protocol to a GnRH antagonist/letrozole protocol for poor responders, and found significantly lower pregnancy rates using the letrozole protocol. However, large differences in treatment protocols make it difficult to conclude that outcome differences are specifically related to the use of letrozole.

Although data presented as an abstract initially suggested teratogenicity with the use of letrozole, several more optimally designed follow-up studies with larger patient numbers did not corroborate these findings. Overall, additional studies are needed to clarify the adjunctive role of aromatase inhibitors for IVF in poor responders.

Aspirin

Because of its antithrombotic and vasodilatory effects, aspirin (acetylsalicylic acid) has been investigated as a possible agent to enhance response to IVF. Aspirin works by inhibiting the platelet enzyme cyclooxygenase, and in low doses, by inhibiting synthesis of the vasoconstrictor thromboxane A2 more than that of prostacyclin, a vasodilator. Some of the proposed mechanisms through which aspirin could improve IVF outcomes include increased ovarian blood flow, enhanced folliculogenesis, or improved endometrial blood flow, thickness, and thus implantation.

Several early studies on the effect of aspirin in IVF showed a potential benefit, but subsequent randomized, prospective trials failed to confirm these findings. In an attempt to clarify conflicting results, Khairy et al. conducted a meta-analysis of prospective randomized trials, and found insufficient evidence to support the routine use of low-dose aspirin to improve clinical pregnancy and live birth rates. A recent Cochrane review and meta-analysis also found no evidence of an improved pregnancy or live birth rate with the use of low-dose aspirin. Of note, there is marked heterogeneity among treatment protocols in the different studies. Most clinicians initiate 80–100 mg of aspirin daily at pituitary down-regulation or with the start of gonadotropins, but the duration of treatment varies widely, with some continuing aspirin until pregnancy is achieved or until 9–10 weeks post embryo transfer, while others continue aspirin for the entire length of the pregnancy. This, along with the small number of trials and a lack of only poor responders included, limits the conclusions of these meta-analyses with respect to the use of aspirin in these women. Additional well-designed trials on this subgroup of women are needed to justify routine use of aspirin in poor responders.

Clomiphene citrate

Interest in enhancing exogenous gonadotropin administration during IVF by concurrently increasing endogenous LH and FSH secretion using clomiphene citrate (CC) was an early

attempt at cotreatment for poor responders. In the earliest protocol, patients received 5 days of 100 mg of CC from menstrual cycle day 3 to day 7, followed by gonadotropins for the remainder of the cycle. However, more recent protocols have combined gonadotropin and CC start at the same time, or overlapped treatment. There are a few trials evaluating protocols incorporating CC with gonadotropins in poor responders that show potential benefit, although these are older studies using outdated gonadotropin preparations and protocols. More recently, Sadaat et al. found that FSH levels were substantially higher using a CC/gonadotropin protocol compared to a microdose flare protocol, however this did not result in any improvement in outcome parameters. CC has been shown to exert an antiestrogenic effect on the endometrium, which could potentially blunt any potential benefit from enhanced endogenous gonadotropin release. Due to a lack of recent, compelling data on the use of cotreatment with CC, it is infrequently used in clinical practice during IVF for poor responders.

EVIDENCE AT A GLANCE

- Corticosteroids: Low-dose dexamethasone has shown promise as an adjunct for poor responders undergoing IVF, and is a relatively cost-effective, safe adjunct to consider.
- Growth hormone: The most recent Cochrane review on the role of GH in IVF showed a small but significant improvement in live birth rates with its use in poor responders, and is a reasonable adjunct to consider.
- Androgens: Several studies have suggested that androgens potentiate the ovaries response to gonadotropins, however further studies on the timing, dosage, and potential benefit are needed before they are widely used.
- DHEA: Early case reports were promising, but randomized, prospective trials are needed to further clarify the role of DHEA pretreatment.
- Aromatase inhibitors: Current evidence regarding the benefit of letrozole in IVF for poor responders is unclear, and additional studies are needed.
- Aspirin: A recent Cochrane review and meta-analysis found no evidence to support the routine use of low-dose aspirin as an adjunct to IVF; thus, further studies are needed.
- Clomiphene citrate: Because of a lack of recent, compelling data on the use of cotreatment with CC, it is infrequently used during IVF for poor responders.

Alternative treatment options

Depending on the individual clinical scenario, alternative treatment modalities should typically be discussed with any patient struggling with diminished ovarian reserve. It is particularly important that couples who have failed multiple IVF cycles, or in whom multiple ovarian reserve tests point to futility of conventional fertility treatment, receive counseling regarding alternative options. For couples willing to use nonautologous oocytes, IVF using donor oocytes provides a unique and highly successful treatment modality to achieve a pregnancy. Other couples not interested in using donor oocytes but still desiring to raise a family may choose to pursue adoption, and thus both options should be presented to all couples. Other less aggressive options that can be beneficial include stress reduction, acupuncture, or depending on the couple's desires, no further treatment. Although patients with diminished ovarian reserve remain one of the greatest challenges facing reproductive endocrinologists, additional methods of detection and treatment will hopefully continue to improve pregnancy outcomes in this growing subset of women.

Summary

The diagnosis of diminished ovarian reserve has become an integral part of the infertility work-up. Other than patient age, many tests have been used to measure ovarian reserve, including basal FSH levels, estradiol, inhibin B, AMH, dynamic tests, and ultrasound parameters including ovarian volume and AFC. Although basal FSH, estradiol, and AFC remain the mainstay of

ovarian reserve testing at many centers, AMH has been increasingly utilized as an accurate and convenient marker.

The diagnosis of diminished ovarian reserve is also extremely important for clinicians when determining the appropriate treatment options. Most women will proceed to treatment with IVF, and a variety of protocols have been used over the years in an attempt to maximize pregnancy outcomes. Currently, antagonist and microdose flare protocols are commonly used for patients with diminished ovarian reserve, but clinician judgement plays a large role in determining the best protocol for each individual. Lastly, adjuncts to treatment that have been used in an attempt to maximize pregnancy outcomes in women with diminished ovarian reserve include corticosteroids, GH, androgens, letrozole, aspirin, and CC.

Women with diminished ovarian reserve remain one of the greatest challenges facing clinicians, but with the evolution of new diagnostic and treatment modalities we hope to continue to improve pregnancy outcomes in these patients.

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8

Diagnosis and Management of Male Infertility

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Introduction

A problem in the male partner may be the sole cause or, more often, a contributing cause of couple's infertility. Although history-taking can reveal factors that may be indicative of a male component in a couple's infertility (see Table 8.1), generally semen analysis is the cornerstone by which a possible male factor is diagnosed. Because semen analysis abnormalities may be indicative of problems in the male reproductive organs or genital tract, the World Health Organization (WHO) lower reference limits are the criteria most widely used to define a "male factor." WHO has recently published updated reference values for normality of semen analysis (see Table 8.2). These one-sided lower reference values are the fifth percentile values obtained from a large population of men impregnating their partner within 12 months. Together with these reference values, the WHO has provided guidelines allowing a standardized assessment of semen parameters, which is of great importance for quality control.

CAUTION

Often the WHO reference values are used to categorize a man as having a fertility problem whenever one or more of his semen or sperm parameters are below the lower reference limit. Yet these values can only be used to predict whether a man can father a child in a reasonable time-frame, not to predict that he will not be able to do so!

A couple's fecundity is always the result of an interaction between both partners. For infertile men who suffer from azoospermia, a "male factor" is a certain and often sole cause for the couple's infertility. However, in case of oligozoospermia, the couple's inability to have children may be influenced by a "female factor" as well. Given the trend to postpone pregnancy to a later age, female age has become one of the most important factors interfering with a couple's fecundity.

Therefore, in order to correctly diagnose a "male factor," a thorough work-up of the female partner including assessment of her ovarian reserve is mandatory, besides appraising at least two semen analyses.

CAUTION

Never underestimate the "female factor." An oligozoospermic man may have problems conceiving a child when in association with a partner who is 39 years old, and thus be categorized as having a "male factor." But when he is associated with a partner who is 30 years old, he may father three children and come to see you for a vasectomy!

Investigating the infertile male

When a man is suspected of having a semen profile contributing to the problem of infertility, a proper investigation should be performed.

WHO has published authority-based guidelines for a standardized investigation and

Infertility, 1st edition. Edited by Emre Seli.

Table 8.1 Factors indicative of a male factor

History	Undescended testicles Bilateral hernia repair Prostatitis or genital infection Testicular trauma or torsion Puberty occurring at a young age or at an older age Mumps after puberty
Lifestyle	Heavy cigarette or marijuana smoking Heavy alcohol consumption
Environmental	Exposure to toxic substances or hazards(heavy metals, radioactivity, etc.) Exposure of the genitals to high temperatures
Drug-related	Prescription of drugs for ulcers, psoriasis Use of anabolic steroids anti-ageing treatments including testosterone

Table 8.2 WHO 2010 reference values for sperm

Volume of ejaculate	≥1.5 mL
pH	7.2–7.8
Sperm concentration	$\geq 15 \times 10^6$/mL
Total count	$\geq 39 \times 10^6$
Progressive motility (type A#+#B)	≥32%
Total motility (type A + B = C)	≥40%
Normal morphology (strict criteria)	≥4%
Vitality	≥58%

Source: Cooper et al., 2010.

treatment of male infertility. These guidelines recommend physically examining the male partner in every couple unable to conceive within 1 year of unprotected intercourse. However, the evidence available from the literature indicates that further investigation, including a comprehensive anamnesis and physical examination, is indicated whenever an abnormal semen analysis is encountered. WHO further advises performing scrotal ultrasonography on every patient presenting with abnormal semen parameters because scrotal ultrasonography is a simple, inexpensive, and noninvasive examination. But evidence available in the literature does not indicate that routine scrotal ultrasonography in every patient with an abnormal semen analysis will change the way the majority of oligozoospermic subfertile men will be treated or managed according to evidence-based guidelines. Ultrasonographic scrotal evaluation in a selected subpopulation of subfertile men, e.g. with a history of cryptorchidism or orchitis, may be a more cost-beneficial alternative to routine ultrasonographic scrotal screening.

Table 8.3 History-taking and examination in the male

Duration of infertility
Children from previous relationship
History of fertility treatments
Risk factors in history or daily life (see Table 8.1)
Erectile function
Ejaculatory function
Coital frequency and timing
Psychological and/or relational problems
Virilization
Position and volume of testes
Penis and foreskin
Presence of vas deferens
Valsava test

CAUTION

Men with a history of cryptorchidism or orchitis, with scrotalgia, or with a palpable testicular mass must have a scrotal ultrasonography because of their increased risk for testicular tumors.

Although evidence is limited, it appears that a systematic evaluation of every man in any couple with unresolved infertility, as proposed by WHO, is not indicated. Thorough history-taking and physical examination must, however, be performed in every man with abnormal semen parameters (see Table 8.3). According to the findings, additional tests can be indicated in selected patients.

An endocrine test should be performed if sexual function is impaired, if any clinical finding

is suggestive of an endocrine disorder (see Table 8.4), or if semen analysis shows less than 10 million spermatozoa or is indicative of a specific disorder of the male reproductive tract (see Table 8.5). Endocrine evaluation in the male includes measurements of plasma follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone in order to assess the pituitary–gonadal axis. A generalized interpretation of these endocrine tests is summarized in Table 8.6.

Genetic testing is indicated in men with less than $5 \times 10^6\,mL^{-1}$ spermatozoa, not only because genetic abnormalities are associated with deficient spermatogenesis but also because of the risk of transmitting these abnormalities to their offspring. Further specific genetic tests may be indicated according to specific clinical findings.

Table 8.4 Risk factors indicative for endocrine disorder in the clinical work-up

History	Puberty occurring at a younger age or at an older age Abnormal sexual differentiation or genital development Loss of libido Use of anabolic steroids Use of anti-ageing treatments
Examination	Abnormal virilization Eunuchoid appearance Small testis volume (<10 mL) Gynecomastia
Semen analysis	Azoospermia Hypovolemia Severe oligozoospermia (<10#×#10^6 spermatozoa)

Table 8.5 Examples of semen analysis results indicative for specific disorders of the male reproductive tract or testis

Volume (mL)	Density ($\times 10^6$/mL)	Motility (%)	Vitality (%)	Specific feature	Indicative for:
1.2	2.0	2	10	WBC 4×10^6/mL	MAGI
0.8	0.0	NA	NA	ph < 7	Agenesis vas deferens blockage ejaculatory ducts
0.5	0.5	2	NA	ph < 7	Retrograde ejaculation
3.0	77.0	0	75	–	Immotile cilia Toxin in collecting recipient
3.0	1.0	0	0	–	Necrozoospermia
3.0	50.0	2	75	Sperm agglutination	Antisperm antibodies
2.5	33.0	54	65	Extremely small heads	Globozoospermia

MAGI, male accessory gland infection; WBC, white blood cell count.

Table 8.6 Interpretation of endocrine tests assessing the pituitary–gonadal axis

Disorder	FSH	LH	Testosterone
Primary testicular failure(e.g. Klinefelter syndrome)	>	>	= or <
Testicular failure with germ cell loss	>	=	=
Testicular failure with maturation arrest	=	=	=
Obstructive azoospermia	=	=	=
Androgen receptor deficiency	=	> or =	> or =

>, elevated; <, decreased; =, normal.

In recent years, DNA damage testing has been proposed as a tool in the evaluation of infertile males. Although sperm DNA damage has been widely studied, so far there is no evidence available that routine testing of DNA damage has any relevant clinical value. DNA damage assessment should therefore currently be viewed as experimental.

SCIENCE REVISITED

Men with azoospermia and extreme oligozoospermia have a higher prevalence of chromosome abnormalities and mutations on the Y chromosome: 47,XXY and 47,XYY and their variants, reciprocal translocations, Robertsonian translocations, and inversions. Occasionally a 46,XX karyotype can be encountered (SRY+ sex reversal). Microdeletions of the Y chromosome can be found in up to 5% of these men too.

Azoospermic men with congenital absence of the vas deferens should have cystic fibrosis transmembrane conductance regulator gene testing.

Treating male infertility: the role of non-ART treatments

Before embarking on any treatment for male infertility, all relevant lifestyle factors should be evaluated and corrected. In women the effect of different lifestyle factors on fecundity and outcome of assisted reproduction (ART) has been well documented, but only few studies address the impact of lifestyle on male fertility. Yet preliminary data indicate a significant negative effect of smoking (at least one cigarette per day) and alcohol (at least one drink per day). Although correcting lifestyle factors may not bring immediate success, correcting such factors may improve the success rate of future treatments, e.g., improved embryo quality in case of tobacco abuse.

The interaction between female and male fertility factors renders any evaluation of treatments trying to alleviate the "male factor" difficult.

Semen parameters show intraindividual variations over time and because of the phenomenon of "regression towards the mean," studies evaluating treatment of male subfertility must include placebo and must have at least pregnancy as their main outcome. To date, the Cochrane library includes only six reviews on non-ART treatments of male subfertility (ART: assisted reproductive techniques) (see box).

EVIDENCE AT A GLANCE

Cochrane reviews on the treatment of male infertility with their conclusions

Non-ART treatments

- Surgery or embolization for varicoceles in subfertile men
  "This review found no increase in pregnancy rates of varicoceles treatment compared to no treatment in subfertile couples, in whom varicoceles in the man is the only abnormal finding"
- Gonadotrophins for idiopathic male factor subfertility
  "This review did not find enough studies to draw strong conclusions about the use of gonadotrophins for the treatment of idiopathic male infertility. However, analysis of the results of the four included studies showed a significant increase in the pregnancy rates, during and within three months, after gonadotrophin treatment of men with idiopathic subfertility"

Withdrawn

- Androgens versus placebo or no treatment for idiopathic oligo/asthenospermia
  "There is not enough evidence to evaluate the use of androgens for male subfertility"
- Bromocriptine for idiopathic oligo/asthenospermia
  "Bromocriptine appears to reduce prolactin levels in subfertile men with normal gonadotrophic function. There is not enough evidence to show that bromocriptine is helpful in improving fertility"
- Clomiphene or tamoxifen for idiopathic oligo/asthenospermia
  "Anti-oestrogens appear to have a beneficial effect on endocrinal

outcomes, but there is not enough evidence to evaluate the use of anti-oestrogens for increasing the fertility of males with idiopathic oligo-asthenospermia"

- Kinin-enhancing drugs for unexplained subfertility in men
  "This review does not provide conclusive proof of effectiveness. The benefit on pregnancy rates suggested by observational studies and low quality controlled trials is not confirmed by more rigorous trials. A modest effect on sperm motility is technically possible but was again not confirmed in the better quality trials. The clinical significance of a modest increase in sperm motility is uncertain. Until effectiveness is proven, kallikrein should only be used in the context of clinical trials"

ART treatments

- Intrauterine insemination for male subfertility
  "The review found no evidence of effectiveness of either treatment. There were few trials that supplied data with our main outcome of interest; live birth rate per couple. Large, high quality properly randomized studies are needed to draw a firm conclusion"
- Techniques for surgical retrieval of sperm prior to intracytoplasmic sperm injection (ICSI) for azoospermia
  "There is insufficient evidence to recommend any specific sperm retrieval technique for azoospermic men undergoing ICSI. In the absence of evidence to support more invasive or more technically difficult methods the reviewers recommend the least invasive and simplest technique available. Further randomized trials are warranted, preferably multi-centered trials. The classification of azoospermia as obstructive and nonobstructive appears to be relevant to a successful clinical outcome so a distinction according to the cause azoospermia is important for future clinical trials

Since most of these Cochrane reviews have not had any substantial update for a long time because of the lack of new evidence, four out of six have recently been withdrawn. Apart from the review on varicocele treatment, the Cochrane reviews cover treatments for unexplained male subfertility, a subgroup accounting for at least 70% of men with abnormal semen parameters. Overall, conventional (i.e., non-ART) treatments for this condition have currently no treatment with a proven benefit (see box for consensus evidence).

EVIDENCE AT A GLANCE

Consensus on non-ART treatment of unexplained male infertility*

Studies with pregnancy as an outcome measure

• Androgens	no benefit
• Dopamine agonists	no benefit
• Glucocorticoids	no benefit
• Kalikrein	no benefit
• Aromatase inhibitors	no benefit (only one RCT)
• Antiestrogens	no benefit (need for more subgroup research)
• HMG (human menopausal gonadotropin)/FSH	potential benefit but needs further evaluation
• Antioxidants	potential benefit but needs further evaluation
• Mast-cell blocker	potential benefit but needs further evaluation

Studies with only sperm parameters as an outcome measure

• GnRH	no benefit
• Growth hormone	no benefit

* Compiled evidence from Cochrane library, National Institute for Clinical excellence (NICE) guidelines and European Association of Urology (EAU) guidelines on male infertility.

In a few men (<1% according to WHO data) infertility is caused by hypogonadothropic hypogonadism, and in these men with gonadotropin deficiency hormonal treatment with FSH or HMG combined with hCG has a proven effi-

ciency for inducing spermatogenesis. The classic, though rare, example is Kalmann syndrome, characterized by both a gonadotropin deficiency and an olfactory dysfunction. Many men presenting with hypogonadothropic hypogonadism will have the idiopathic variant. Men with hypogonadothropic hypogonadism should be referred to an endocrinologist because this condition may be associated with other endocrinopathies.

☆ TIPS & TRICKS

The classic treatment for hypogonadothropic hypogonadism involves intramuscular or subcutaneous doses of 1000–2500 IU hCG given twice a week (e.g. Monday and Friday) and 37.5–150 IU hMG or FSH given three times a week (e.g. Monday, Wednesday, and Friday)

Most patients will start producing sperm with a simplified scheme applying 5000 IU hCG once a week SC (e.g. Monday) together with 150 IU hMG twice a week IM (e.g. Monday and Friday).

Allow up to 6 months before checking the semen for sperm.

Because of their altered acidity and hence shorter half-life, recombinant FSH preparations should be administered three times a week. In the near future long-acting recombinant FSH preparations may reduce the scheme to a single injection session weekly.

There is also a definite role for treatments for anejaculation and retrograde ejaculation in men with ejaculatory disorders. In these men, ART including surgical sperm recovery procedures must only be viewed as a second-line treatment option.

Ejaculatory failure is common in men with spinal cord injury but may be occasionally associated with diabetes or multiple sclerosis. The therapeutic approach involves penile vibrostimulation or electroejaculation, the latter with or without general anesthesia depending on whether the spinal cord is (partially) intact or completely sectioned.

☆ TIPS & TRICKS

Because success rates of ART after using ejaculated sperm are better than those using testicular sperm, it is preferable to refer patients with chronic anejaculation, especially patients with spinal cord injuries, to specialized services where assisted ejaculation can be performed by vibro- or electrostimulation in order to collect and cryopreserve ejaculated sperm for later use in ART.

CAUTION

Since scrotal hematoma may take a long time to heal in anejaculatory men with spinal cord injuries, surgical sperm retrieval techniques are indicated only where noninvasive techniques such as penile vibro- or electrostimulation has failed to produce an ejaculate that can be used for ART.

In ART clinics acute ejaculatory failure is encountered more frequently. Therefore, any patient who reports occasional ejaculatory failure under stress conditions should be advised to cryopreserve semen before embarking on ART.

☆ TIPS & TRICKS

Patients undergoing ART presenting with unexpected acute anejaculation can be given 50 mg sildanefil in order to obtain a semen sample.

Retrograde ejaculation is a condition in which semen is ejaculated into the bladder instead of out through the penis because of dysfunctional urethral valves that control the segregated flow of urine and semen. Men with diabetes or multiple sclerosis, or who have had their prostate removed, are at risk for this condition. Medical therapy is available (a combination of chlorpheniramine and phenylpropanalamine) and will help about one-half of these men to produce an antegrade ejaculation. If this is unsuccessful, spermatozoa can be retrieved from urine. However, because of

the acidity and osmolality of urine, sperm can be damaged instantly. Therefore, certain precautions must be taken before sperm is collected so as not to expose the sperm to the acidic urine for longer than necessary.

TIPS & TRICKS

Always prepare your patient with retrograde ejaculation for optimal sperm collection. Alkalization of the urine can be achieved by taking sodium bicarbonate (available in baking powder), and drinking plenty of water before providing the sample can lower the urinary osmolality. The following instructions may be given to your patient:

- Abstain from any sexual activity involving ejaculation during the 3 days prior to delivery.
- The evening before providing the sample, drink 250 mL of water containing about 8 g of sodium bicarbonate (or an equivalent amount of baking powder) before going to bed.
- 2 h before providing the sample, drink 1 L of water containing about 5 g of sodium bicarbonate.
- Make sure the bladder is not empty when going to the laboratory to provide a sample.

In the laboratory the patient needs to be given three labelled containers:

- Container 1: A small semen container to collect any semen that may be released antegradely through masturbation.
- Container 2: A container to collect the first 10 mL of urine immediately after masturbating, whether or not the patient managed to collect anything in container 1. Container 2 should be marked to help the patient control the volume to be delivered, and an equal volume of albumin-containing medium must be added to the container prior to ejaculation.
- Container 3: A large container to collect the rest of the urine. This third sample will be later processed by centrifugation.

Surgical treatments for reversing obstructive azoospermia after vasectomy are very successful, with 60% of men fathering a child in unselected multicentre follow-up series. However, data available on live birth rates after surgery for reversing postinfectious obstructive azoospermia, i.e. vasoepididymostomy, are scarce and merely based on incomplete follow-up, hence prone to important bias.

For men presenting with antibodies against their spermatozoa, low-dose prednisolone therapy has been proposed as an efficient therapy (20 mg twice daily on days 1–10 of the female partner's menstrual cycle, followed by 5 mg on days 11 and 12).

CAUTION

Autoimmunity against sperm is a tricky diagnosis. This condition cannot be diagnosed solely on the basis of the mixed antiglobulin reaction (MAR) test or immunobead test. First, such test should show high levels of binding (>50%), tail-tip binding being not so relevant, and then these men must also have impaired mucus penetration testing.

CAUTION

Although corticosteroids are a specific treatment for infertility caused by antisperm antibodies, the efficiency of this approach is only supported by one single, small RCT. Furthermore, the potential benefit of this treatment should be balanced against the important side effects experienced by up to 60% of patients: dyspepsia leading to peptic ulcers, weight gain, folliculitis, headache, musculoskeletal pain, mood changes, cardiovascular effects, and even aseptic necrosis of the hip when higher doses are used.

TIPS & TRICKS

The treatment for male subfertility should first focus on finding the cause of the problem, then on assessing whether a specific

treatment with a proven benefit is available. When such a treatment is not available, the chances for spontaneous conception should be estimated integrating the female fertility potential and the benefit of optimizing the female factor whenever feasible. Then, although it is not a specific treatment, ART may be considered.

Treating male infertility by assisted reproductive techniques

The aim of ART is to bring the spermatozoon closer to the oocyte in an attempt to enhance fertilization. While ART should not be viewed as first-line treatment options, they are indicated when no conventional treatment with a proven benefit is available and optimizing the female factor has failed or is not indicated.

Intrauterine insemination (IUI) is an efficient technique to increase fecundity in couples with male subfertility: compared to timed intercourse, three times more couples will achieve pregnancy thanks to IUI when at least 0.8×10^6 motile sperm can be obtained after semen preparation. Although IUI is the simplest technique available, its success remains subject to many interfering factors of which some can be optimized. Unfortunately, to date controlled data on live birth rates after IUI are lacking. Therefore, the Cochrane collaboration has recently changed its favorable conclusion about IUI into a plea for RCTs with live birth as their main outcome.

CAUTION

Note that in male subfertility, superovulation in the female partner has not been proved more efficient but increases the risk for multiple pregnancies.

TIPS & TRICKS

Improving the success rate of IUI for treating male subfertility

- Make sure the female partner is ovulatory and has patent tubes.
- Endometriosis (stage 2 and up) will reduce success rate; this factor may need correction.
- Insemination in female partners aged 40 years or more is not efficient.
- Inseminate $>0.8 \times 10^6$ washed motile spermatozoa.
- Double insemination may be more effective (OR 1.8 with 95%CI 1.4–2.4, but based on inclusion of a large RCT in which double IUI was extremely successful).
- Immobilization of the patient for 15 min after insemination increases the ongoing pregnancy rate (OR 1.6 with 95%CI 1.1–2.4).

How many cycles of IUI are needed is difficult to answer because of the complete lack of prospective studies that take the female age factor into account. The current data available from retrospective life-table studies indicate that at least three cycles and a maximum of six cycles can be proposed, depending on the age of the female partner (more when older).

When no pregnancy ensues, the next proposal would be in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Although theoretical models conclude that a "straight IVF" approach may be more cost-beneficial than a "first IUI, then IVF" approach, only two prospective studies have compared IUI with IVF for male subfertility. Neither of the two studies showed any difference in pregnancy rates between IUI and IVF for moderate male subfertility, but one RCT concluded that IUI was more cost-effective than IVF.

There is currently no good evidence regarding cut-off values before or after sperm preparation for proposing either IVF or ICSI to a couple with male subfertility. Complete fertilization failure is stressful event occurring in up to 50% of conventional IVF cycles performed to alleviate male subfertility. ICSI has been proposed as the most reliable option, because after ICSI complete fertilization failure occurs in less then 3% of started cycles. For nonmale indications the evidence shows that conventional IVF is not inferior to ICSI in establishing a live birth, but such data are currently lacking for male subfertility.

The choice between IVF and ICSI is either made according to experience-based preset cut-off values (ranging from 0.2 to 1×10^6 motile sperm post-wash) or is based on the assumption that ICSI is a more robust insemination technique. Alternatively, a split IVF–ICSI set up can be used as a strategy for a first treatment cycle.

There are, however, strict male indications for ICSI: use of surgically retrieved sperm, use of spermatozoa with flagellar dyskinesia (immotile cilia syndromes) and the use of round-headed spermatozoa (globozoospermia).

☆ TIPS & TRICKS

100% immotile sperm—what now?

- Start with a vitality test: are spermatozoa dead (necrozoospermia), or alive but immotile?

If all spermatozoa are dead:

- Check exposure of semen to toxic substances (e.g., container).
- Rule out chronic prostatitis.
- If correction is impossible: consider testicular sperm recovery.

If spermatozoa are alive:

- Rule out important urinary infections (e.g., *E. coli* can immobilize spermatozoa).
- Rule out chronic prostatitis.
- Check for presence of antisperm antibodies.
- Rule out flagellar dyskinesia (electron microscopy).
- If correction is impossible: consider ICSI with ejaculated sperm and ask the ICSI laboratory to select living sperm by using hypo-osmotic swelling test or laser-assisted immobilized sperm selection (LAISS).

CAUTION

When using immotile sperm from patients with flagellar dyskinesia, results after ICSI are unpredictable due to variable fertilization and embryonic development. Patients should therefore be informed that the success rate of this approach may be limited.

The same applies to patients with round-headed sperm: even with artificial oocyte activation, results remain unpredictable for some patients. A diagnostic heterologous ICSI or mouse oocyte activation test (MOAT) may select patients in whom artificial activation may be successful.

Surgical sperm recovery for treating male infertility

Thanks to ICSI, spermatozoa from different anatomical origins can be used successfully. The recovery of testicular spermatozoa involves a simple surgical procedure, i.e., testicular aspiration or biopsy. As a result, there is a tendency to treat all patients with infertility due to azoospermia by ICSI using testicular sperm, including men who have had a vasectomy. However, for the latter subgroup of men, reconstructive surgery remains the standard because the results in terms of live birth are good and the costs are generally lower than those of ICSI. Epidemiological demographic studies show that in general about 60% of men who have a vasectomy reversal will father a child again after their surgery. In azoospermic men where vasoepididymostomy is to be performed because of vasectomy or postinfectious obstructions, the results are less convincing. Thus surgical sperm recovery may be a good alternative here, certainly when there is any associated female factor.

In men with obstructive azoospermia different recovery techniques are available to retrieve sperm. A first choice to be made is whether testicular sperm or epididymal sperm will be recovered. Although epididymal sperm is known to accumulate DNA damage, the motile fraction of this surgically recovered sperm shows DNA damage rates similar to those of fresh donor sperm.

Epididymal sperm can be easily obtained after percutaneous epididymal sperm aspiration (PESA) under local anesthesia or by microsurgical epididymal sperm aspiration or MESA during a scrotal exploration.

CAUTION

When sperm are to be recovered from the epididymis by MESA or PESA, one should ensure that enough motile epididymal sperm are obtained in order not to jeopardize the success rate after ICSI because of DNA damage. Sperm with good motility ensure good fertilization rates and embryo development in vitro, they can be easily cryopreserved, and in terms of live birth rates there are no differences between cryopreserved or fresh epididymal sperm after ICSI.

When epididymal sperm recovery fails in a patient with obstructive azoospermia, then testicular sperm can be easily obtained either by aspiration or by taking a biopsy. The advantage of fine-needle aspiration (FNA) is that this procedure can be performed even without any anesthesia on an outpatient basis. However, although the number of sperm obtained is sufficient to perform ICSI, it is too low to consider cryopreservation using routine cryopreservation protocols. Hence for a one-stop approach for sperm recovery, a testicular biopsy should be performed under local anesthesia (TESE or testicular sperm extraction). In contrast to FNA, patients undergoing TESE will experience some scrotal discomfort for about 1 week. Fertilization and ongoing pregnancy rates do not differ between ICSI using either fresh or frozen-thawed testicular sperm.

In patients with azoospermia because of primary testicular failure, i.e., men with nonobstructive azoospermia (NOA), surgical sperm recovery combined with ICSI has become an important alternative to donor insemination. In about one-half of men with NOA spermatozoa can be observed in the testis although azoospermia is present in different semen analyses.

CAUTION

Overall, testicular sperm can recovered in about one-half of azoospermic men with primary testicular failure. The recovery rates reported can, however, vary between different studies. This may be the result of differences in inclusion/exclusion criteria, e.g., azoospermic patients with the histological finding of "hypospermatogenesis" will always have testicular sperm and may be included or excluded. Often data on a series of TESE procedures are reported in which patients with successful recovery are reallocated, hence increasing the overall recovery rate.

Unfortunately, there are no good parameters available to predict whether a patient will harbor spermatozoa or not. The classic clinical parameters such as testicular volume, result of semen analysis including centrifugation, serum FSH, and even testicular biopsies of testicular histopathology have insufficient predictive power in order to exclude a given patient from a testicular sperm recovery procedure.

If only studies are considered in which patients with normal spermatogenesis are excluded, even inhibin B assessment has no predictive power. Doppler ultrasound of the testis has been proposed to predict successful recovery, but even this testicular vascularity assessment has a sensitivity not exceeding 50%. The most invasive predictive strategy is testicular mapping, a technique in which the testis is aspirated in different locations according to an organized pattern, followed by a cytological examination of the aspirates. Unfortunately the sensitivity of this approach is less than 50%.

From a surgical viewpoint it is important to know that in many NOA patients testicular sperm can only be recovered after taking multiple biopsies. These multiple biopsies can be taken at random or under magnification, a technique referred to as microsurgical TESE. At present well-controlled studies in well-defined patient populations of nonobstructive azoospermic patients are not available, so it is not possible to accept this approach as the gold standard. More important in improving the recovery rate during a TESE procedure is the laboratory phase where a wet preparation is assessed microscopically.

☆ TRICKS AND TIPS

How to Improve Your Sperm Recovery Rate in Nonobstructive Azoospermic Patients

- Include patients who had a previous biopsy showing spermatozoa.
- Exclude patients with high FSH and small testes.
- Include patients that have sperm after testicular aspiration.

  (yet these actions may exclude patients who actually have sperm present in their testes!)
- Perform TESE in a setting where wet preparation can be examined during surgery.
- Take multiple biopsies if necessary.
- Take biopsies at the contralateral testis if necessary.
- When no sperm are observed in the wet preparation include an erythrocyte-lysing buffer and possibly an enzymatic digestion of the testicular tissue.

Patients with NOA need adequate counseling with regard to both realistic sperm recovery rates and their chance to have a child. Even when testicular sperm are recovered, the chances of establishing an ongoing pregnancy are less than for men with normal spermatogenesis.

When no spermatozoa are recovered after TESE in a patient with NOA, at present the only alternative is adoption or the use of donor sperm. In the past, the use of round spermatids and even secondary spermatocytes has been proposed. However, a critical evaluation of the successes published in the literature shows that when round spermatids are used for ICSI, the viable clinical pregnancy rate is lower than 2% per ICSI cycle when men with normal spermatogenesis are excluded from the analysis.

Summary

Some 10–15% couples suffer from infertility. Whenever repeated abnormal semen analyses are observed in the male partner an appropriate clinical work-up should be performed; specific cause interfering with sperm output or function should be treated, and lifestyle and potentially interfering female factors should be corrected. If the male's problem remains unexplained and a specific treatment cannot be applied, then ART can be proposed.

Although insufficient data for valid conclusions are available, IUI in a unstimulated cycle seems indicated when at least 0.8 million motile sperm can be inseminated after sperm preparation. After six treatment cycles or when less than 0.8 million motile sperm are available, then IVF or ICSI can be proposed with ICSI being the method of choice whenever surgically retrieved spermatozoa are to be used.

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9

Diagnosis and Management of Unexplained Infertility

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Introduction

One of the main emphases in reproductive medicine is the finding of causal diagnoses of subfertility and accompanying fertility treatment. So ovulation induction is used in women with anovulation, tubal surgery and/or assisted reproductive technologies (ART) are used in women with tubal disease or endometriosis, and intrauterine insemination (IUI) is used for cervical factor subfertility.

There is a twilight zone where cause and relationship with the infertility is less clear and consequently rationally tailored therapies are less obvious, such as a mild male subfertility with moderately reduced sperm quality, mild endometriosis, or one-sided tubal pathology. Finally, in 30% of the cases the basic fertility work-up does not find a reason for the failure. In fact it is not a diagnosis but a qualification of a situation in which a couple has attempted to achieve pregnancy for more than a year without success. Many defects are still unknown. Possible factors might be limited oocyte or sperm quality that cannot be revealed by tests and failure of uterine receptivity and implantation. Total fertilization failure does not seem to be a dominant factor. In this chapter we first briefly discuss the standard work-up of a couple with subfertility (a detailed discussion of infertility work-up is provided in Chapter 2) followed by the prognostic aspects in unexplained subfertile couples, and finally the treatment options.

Basic fertility work-up in subfertile couples

Traditionally the work-up starts with the female partner in combination with just one or two sperm analyses; only when these latter are abnormal is male work-up started too. For the purpose of this chapter we do not further address detailed investigation of the male since unexplained subfertility implies presence of normal sperm counts (see Chapter 8 for further details of male infertility).

The work-up includes a medical history, a physical examination, cycle monitoring, semen analysis, postcoital test, imaging including investigation of the tubal patency and ultrasound, and some laboratory tests.

History and physical examination

Aside from age, the general history-taking should gather information about (1) the circulatory, respiratory, gastrointestinal, the urinary, nervous, and endocrine systems and the locomotor tracts; (2) current medications and allergies; (3) occupation and use of tobacco, coffee, alcohol, and other drugs; (4) past surgery, its indications and outcome, previous hospitalizations, serious illnesses or injuries; (5) family history of birth defects, mental retardation, or reproductive failure.

Specific history-taking includes (1) gravidity, parity, pregnancy outcome, and associated complications; (2) methods of contraception;

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(3) duration of subfertility and results of any previous evaluation and treatment; (4) coital frequency, dyspareunia; (5) age at menarche, cycle length and characteristics, onset/severity of dysmenorrhoea and symptoms of pelvic or abdominal pain; (6) pelvic inflammatory disease or exposure to sexually transmitted diseases; (7) previous abnormal Pap smears and any subsequent treatment; (8) galactorrhoea and hirsutism.

Physical examination should note the patient's weight and body mass index (BMI) and identify pubertal development. Furthermore, signs of thyroid disease, scars, signs of androgen excess, breast disease and nipple discharge, pelvic or abdominal tenderness and masses should be noted. Gynecologic examination should note vaginal or cervical abnormality, secretions, or discharge; the size, shape, position and mobility of the uterus; adnexal mass or tenderness; and fornix posterior mass, tenderness, or nodularity.

EVIDENCE AT A GLANCE

- In young couples the monthly chance of becoming pregnant varies between 20% and 40% with 1% chance of a dizygotic twin. When the woman is >35 years of age it will reduce to <10% with a doubled chance (2%) of a twin pregnancy.
- Most pregnancies (80%) occur in the first six cycles of unprotected intercourse.
- After 12 unsuccessful cycles 55% will become pregnant in the next 36 months. These couples should be assigned as subfertile. After this (48 months) 5% are definitively infertile.

Specific diagnostic procedures

Ovulation detection

Ovulation should be demonstrated by (1) a biphasic temperature chart of the basal body temperature (BBT)—after ovulation the basal body temperature will increase by 0.3–0.5 °C; or (2) a midluteal increase of progesterone; or (3) ultrasonographically observed follicle growth and timely transformation of the graafian follicle into a luteal body.

When a biphasic temperature pattern cannot be observed, verification by the progesterone measurement is mandatory because some women do not show the typical increase of their body temperature despite ovulation.

Semen analysis

One and preferably two normal sperm counts (volume ≥1.5 mL, count $\geq 15 \times 10^6$, progressive motility >30%) is likely to rule out male factor infertility.

Postcoital test

The test examines whether sperm is able to penetrate through the cervical mucus. This ability is dependent on the semen quality and the cervical mucus. The cervical mucus changes during the menstrual cycle and should be accessible to semen at the time of ovulation.

The test is planned according to the BBT curve or ultrasonography findings. If the timing is based on the BBT curve, the postcoital test should be scheduled the day before the expected ovulation. This is usually 15–16 days prior to expected onset of the next period. In case of an abnormal test result, i.e., if no progressive motile spermatozoon is seen, the test should be repeated every 48 h until the test is normal or when the temperature on the BBT goes up, i.e., ovulation has taken place. If the postcoital test is performed under ultrasound guidance, it should be done when the dominant follicle is at least 18 mm in diameter and ovulation is expected. The postcoital test result is regarded normal if at least one progressive spermatozoon is seen in one of five high-power fields at 400× magnification.

Evaluation of tubal patency and evaluation of uterine and intra-abdominal factors

There are two different approaches to the assessment of tubal function.

In the first approach, tubal function is circumventially assessed by serum chlamydia immunofluorescence antibody testing. If the result is positive, the patient should be scheduled for hysterosalpingography (HSG) or laparoscopy. In this scenario tubal pathology is considered to be absent if the chlamydia antibody test is negative or when the chlamydia test is positive but a subsequent laparoscopy and/or HSG shows two normal patent tubes.

In the second approach, tubal function is directly assessed by HSG or laparoscopy. HSG defines the size and shape of the uterine cavity and will reveal developmental anomalies (unicornuate, septate, bicornuate uteri) or other acquired abnormalities (endometrial polyps, submucous myomas, synechiae) with potential reproductive consequences. It can document proximal and distal tubal occlusion, demonstrate salpingitis isthmica nodosa, reveal tubal architectural detail of potential prognostic value, and suggest the presence of fimbrial phimosis or peritubular adhesions when escape of contrast is delayed or becomes loculated, respectively.

Laparoscopy and "chromopertubation" with a dilute solution of methylene blue introduced via the cervix can demonstrate tubal patency or document proximal or distal tubal occlusive disease. This procedure can also identify subtle tubal factors such as fimbrial phimosis or peritubular adhesions which may escape detection with less invasive methods such as HSG. Moreover, the diagnostic laparoscopy will identify or exclude the presence of endometriosis and subtle pelvic adhesions.

Ultrasonography

Ultrasound can trace myomas and adenomyosis. Furthermore, periovulatory ultrasound can reveal a temporary hydrosalphinx that may interfere with embryo implantation. Ultrasonography after introduction of saline is useful for the diagnosis of polyps and other intracavitary structures that may interfere with fertility. Ultrasound can rule out ovarian abnormalities as cysts, polycystic ovary syndrome (PCOS), and tumors. During ultrasonography in the early follicular phase antral follicles can be counted. A low of antral follicles count (AFC) is a sign of limited ovarian reserve. The AFC can predict quite accurately the oocyte yield during an ART cycle, but cannot be used for the prediction of a natural (spontaneous) pregnancy.

Laboratory testing

Follicle stimulating hormone

The measurement of follicle stimulating hormone (FSH) in the early follicular phase or basal FSH is performed to assess the ovarian reserve. Elevated FSH suggests a limited ovarian reserve. These women usually have a lower response to hormonal stimulation during treatment using ART. If these women have a normal cycle despite this increase it does not have any value in the prediction of the couple's chance of pregnancy.

Thyroid stimulating hormone and prolactin

Routine determination of thyroid stimulating hormone (TSH) is valuable because subclinical thyroid disease may have an influence on fertility. Furthermore, subclinical hypothyroidism may have a subtle adverse effect on fetal brain development. Preconceptional detection and treatment can therefore be useful. Prolactin should be measured only in case of galactorrhea and/or oligoamenorrhea.

Rubella and syphilis

In view of preconceptional treatment options, screening for rubella and syphilis can be of potential benefit. It allows timely treatment/vaccination in order to prevent serious congenital malformations in the offspring.

CA-125

CA-125 (cancer antigen 125 or carbohydrate antigen 125) is a protein that is used as a biomarker for ovarian cancers. It may also be elevated in a number of relatively benign conditions, such as endometriosis. It tends to be elevated in the presence of any inflammatory condition in the abdominal (peritoneal) area. Increased serum concentrations measured outside the menstrual period may be an indication of endometriosis. When increased levels are found, a diagnostic laparoscopy can follow for further examination.

Prognosis of unexplained subfertility

Treatment for subfertility is expensive and burdensome. It should therefore be offered to a couple only when the expected success rate substantially exceeds the probability of a naturally conceived pregnancy. In particular, in case of unexplained subfertility it is relevant to determine the prognosis. In prognostic models for calculating the chances of pregnancy the most important negative determinants for couples with unexplained subfertility are (1) female age, (2) duration of subfertility, and (3) primary infertility (never been pregnant before). One example

of such a prognostic model, based on the study by Hunault et al., can be found at http://www.freya.nl/probability.php. Several individual findings in the basic fertility work-up of a couple such as the female age, the duration of subfertility, primary or secondary subfertility, and the quantity of semen should be provided and the chance of conceiving a liveborn child as a result can be calculated.

The use of such models makes it possible to distinguish couples with a good (>40%), intermediate (30–40%), or poor prognosis (<30%) for a pregnancy within 1 year. Depending on such a prognosis, the couple together with the physician can then determine whether expectant management or treatment is of additional value.

Management

In absence of any specific known cause of the couple's subfertility, treatment is empirical. Here we discuss the four options: (1) expectant management; (2) timed intercourse (TI) with and without hormonal treatment; (3) IUI with or without ovarian stimulation; and (4) ART, i.e., in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI).

Expectant management

Whether expectant management should be advised depends on the prognosis of a couple. If the prognosis of a pregnancy, established using the publicly available calculators, is more than 30%, then expectant management for 6–12 months is advised. In these patients, it is clearly demonstrated that immediate start of IUI in combination with ovarian stimulation has no benefit over 6 months of expectant management. If the prognosis is lower than 30%, further treatment should be considered without delay.

In women over 38 years of age, further treatment should start immediately. There are practical reasons for this, but it is in fact a paradoxical approach: prolonged subfertility is a normal feature of aging and older premenopausal women are not intrinsically infertile. Because of the reduced quality of the oocytes, these women will require more time to become pregnant. Therefore, a longer rather than a shorter period of expectant management could be advised. However, the success of such treatments, in particular IVF, is negatively influenced by older age, so these treatments should be preferably offered without further delay.

CAUTION

Lifestyle and general precautions

- Smoking, coffee and alcohol abuse, and overweight in women have a negative influence on the natural chance of pregnancy, the success of ART, and the outcome of a pregnancy.
  - Weight loss, smoking cessation, and reduction of alcohol, and coffee intake should therefore be advised
  - So far, however, there is no proof that such adaptations do indeed improve the outcome.
  - Thus, there may be insufficient grounds to refuse patients for fertility treatments if they are not able to adjust their lifestyle.
- Women who are trying to become pregnant should take an oral daily dose of 0.5 mg folic acid.

Timed intercourse

Well-informed couples seeking pregnancy usually focus their sexual activity around midcycle, although TI as such is not a natural spontaneous human sexual activity. Since TI involves interfering with natural coital habits by asking couples to refrain from intercourse until some marker shows that ovulation is imminent, it may theoretically reduce the likelihood of pregnancy. Indeed, several studies suggest that while timing intercourse according to the luteinizing hormone (LH) surge is appropriate for IUI such timing might allow the optimal period for spontaneous conception to pass.

There are no published trials comparing TI with expectant management (ordinary intercourse). There are indications that a beneficial effect of IUI is slightly higher when compared to a control group with TI whereas such a beneficial effect was less when compared with ordinary intercourse (expectant management), which is indeed consistent with the possibility that pregnancy may be less likely in TI controls than expectant management controls. There are now

no scientific grounds to justify TI as part of the management of unexplained subfertility.

Clomiphene citrate therapy

Clomiphene citrate (CC) for the use of ovarian hyperstimulation is a common treatment for unexplained subfertility. However, it has been suggested that the empiric use of CC in ovulatory women can cause alterations in the normal endocrinology of ovulation. CC combined with intercourse has been evaluated. The overall effect of CC treatment is small. Women with unexplained fertility problems should be informed that CC may only slightly increase the chance of pregnancy, with a possible risk of multiple pregnancies.

Intrauterine insemination

In couples with unexplained subfertility, IUI is often the first choice of treatment. The semen is processed in the laboratory. Seminal plasma is removed by centrifuging spermatozoa through density gradients followed by resuspension in culture medium, thus resulting in motile sperms being concentrated in a small volume (0.2–0.5 mL). This allows IUI without strong uterine contractions and prevents pelvic infections. This treatment was introduced in the 1960s as an attractive way of bringing the gametes closer together. The suspension should contain at least 2 million motile sperms in case of mild male subfertility, and preferably >3 million. This yield is usually easily reached with unexplained infertility since normal sperm counts are required for this diagnosis. The insemination should be performed 32–42 h following the injection of human chorionic gonadotropin (hCG) for induction of final oocyte maturation. There is no apparent advantage of two inseminations compared to one. Usually up to six cycles of treatment are advised. In a multicenter retrospective cohort analysis among couples treated with IUI up to nine cycles cumulative ongoing pregnancy rates were 18% after the third cycle, 30% after the seventh cycle, and 41% after the ninth cycle. It may therefore be reasonable to conduct up to nine cycles.

The number of randomized trials assessing the effectiveness of IUI is limited, and most trials are small in size. Evidence for clear effects either for IUI in the natural cycle or IUI with hormonal stimulation is scarce.

Intrauterine insemination in the natural cycle

IUI without hormonal treatment is associated with a slightly higher nonsignificant pregnancy rate compared to expectant management with natural intercourse. This treatment modality may therefore not seem appropriate for an unexplained subfertile couple.

Intrauterine insemination with ovarian stimulation

The rationale of hormonal stimulation of the ovaries is to induce multiple follicle growth thus improving the monthly fecundity and optimizing the timing. Subcutaneously injectable gonadotropins are generally used for this purpose. The start of treatment is typically around day 3 of menstruation with a daily injection of a 50–75 IU dose of a preparation containing FSH. This is continued until the largest follicle has reached a diameter of 18 mm. Then final maturation of the oocyte and ovulation is induced with a subcutaneous injection of hCG. When no multiple follicle growth occurs, and no pregnancy, then in the next cycle the daily dose is increased to 25–37.5 IU of gonadotropin. When three or more follicles of 17 mm are seen by ultrasound monitoring then the treatment should be cancelled and if possible converted into an in-vitro fertilization procedure with the transfer of a limited number of embryos. This will prevent multiple pregnancies. Rates of multiples with IUI and ovarian stimulation should not exceed 10% of the pregnancies.

Currently there is insufficient evidence that IUI should be combined with CC. Remarkably, however, there is quite firm evidence that IUI with hormonal ovarian stimulation is about twofold superior to IUI without hormonal stimulation. Therefore, when IUI is considered as treatment of choice for a couple with unexplained subfertility, a combination with hormonal stimulation should be considered.

ART

Overall, approximately one-third of ART treatments are in couples with unexplained subfertility. For ART ovarian hyperstimulation is

established by a daily injection of FSH-containing preparation until the largest follicles have reached a diameter of 18 mm and then hCG is given for the final oocyte maturation and oocytes are harvested 36 h later. Preferably 10 oocytes or more should be obtained. These are inseminated with sperm and incubated for a few days. The resulting embryos are then transferred to into the uterus usually 2–5 days later. In order to prevent large multiple pregnancies it is preferable to transfer a limited number of optimal embryos, especially in younger women. In Europe nowadays often only one embryo is transferred. During hormonal stimulation most patients undergo treatment with a daily injection of a gonadotropin releasing hormone (GnRH) analogue for prevention of premature luteinization that otherwise would happen in 25% of the cycles.

ART is an expensive and burdensome treatment and has some potential side effects such as ovarian hyperstimulation syndrome (OHSS) and infections.

CAUTION

Drawbacks of ART or IUI with hormonal stimulation

- High costs
- Multiple pregnancy
- Ovarian hyperstimulation syndrome (OHSS)

There are no indications that intracytoplasmatic sperm insemination (ICSI) (with a view to overcoming unknown fertilization problems) is more effective than traditional in-vitro fertilization (IVF).

When ART is considered as the initial treatment for unexplained infertility, it is important to evaluate the effectiveness of ART against other treatment options in these couples. In the context of unexplained subfertility, a recent systematic review determined whether ART improves the probability of live birth compared with expectant management, IUI without ovarian stimulation, or IUI with ovarian stimulation. There was no evidence of a difference in live-birth rates between IVF and IUI either without or with ovarian stimulation. There were significantly higher clinical pregnancy rates with IVF in comparison to expectant management. Any effect of IVF relative to expectant management or IUI with or without ovarian stimulation in terms of live-birth rates for couples with unexplained subfertility remains unknown, however.

Summary

Unexplained subfertility is a diagnosis by exclusion in the diagnostic work-up of known causes of subfertility such as anovulation, tubal occlusion, endometriosis, cervical hostility, and abnormal sperm counts. The basic fertility work-up consists of history-taking, physical examination, ovulation detection, evaluation of tubal patency, a postcoital test, and a sperm analysis.

In case of unexplained subfertility a prognosis of a natural conception in can be calculated by the women's age, history of a previous pregnancy, and duration of the subfertility. The formula for the prognosis can be determined using calculators publicly available on the Internet. Treatment is empirical and depends on the prognosis of a natural pregnancy for the couple. The treatment modalities varies from expectant management, six or more cycles of IUI with mild hormonal ovarian stimulation, to three or more ART cycles.

TIPS & TRICKS

Treatment options for unexplained infertility

- Expectant management: Rates of pregnancy chances are dependent on the couple's prognosis. Advise to use the prognostic models for natural pregnancy.
- IUI with mild hormonal ovarian stimulation: Pregnancy rates 5–10% per cycle; 10% multiple pregnancy.
- IVF: Pregnancies 25–30% per cycle; 5–15% multiples when protocols for single- and double-embryo transfers are used.

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10

Endocrine Disorders and Infertility

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Pituitary disorders that affect reproduction

The pituitary (also known as the hypophysis) is often referred to as the "master gland" in that it is responsible for the secretion of tropic hormones essential to the regulation of the other major endocrine glands. The pituitary gland extends inferiorly from the hypothalamus and rests in the sella turcica at the base of the brain. The gland is divided into two lobes, the posterior pituitary (neurohypophysis) and the anterior pituitary (adenohypophysis). The posterior pituitary plays a role in regulating water balance via the release of vasopressin. Through the release of oxytocin it is also involved in facilitating contractions during labor and in milk let-down during lactation. The anterior pituitary, made up of five major cell types, regulates the secretion of hormones from the thyroid, adrenals, and gonads, through the regulated release of thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and luteinizing hormone (LH) plus follicle stimulating hormone (FSH), from the thyrotroph, corticotroph, and gonadotroph cells, respectively. The anterior pituitary also secretes growth hormone (GH) and prolactin from the somatotroph and lactotroph cells, respectively. Complex pathways regulate the release of the anterior pituitary hormones including stimulatory and inhibitory factors secreted by the hypothalamus and also positive and negative feedback pathways mediated by hormones that are produced by endocrine glands in the periphery (i.e., ovarian and adrenal steroid hormones). Pituitary function can be affected by many things including genetic and acquired disorders, recreational drugs, lifestyle, medications, exogenous hormones, inflammatory processes, and some tumors.

Hyperprolactinemia and prolactinomas

Regulation of prolactin release

Hyperprolactinemia is one of the most commonly diagnosed endocrine disorders among women and is most commonly caused by benign prolactinomas or psychotropic medications. Unlike the other anterior pituitary hormones (i.e., ACTH, LH, FSH, TSH, and GH) that are secreted in response to hypothalamic stimuli—i.e., corticotropin releasing hormone (CRH), gonadotropin releasing hormone (GnRH), thyrotropin releasing hormone (TRH), and growth hormone releasing hormone (GHRH)—prolactin secretion is primarily regulated by the tonic inhibitory response to hypothalamic dopamine. Conditions affecting the hypothalamus or the median eminence may impede dopamine inhibition of prolactin release (leading to enhanced secretion of prolactin) while release of the other pituitary hormones will decline. This is in contrast to direct pituitary insults which can result in a lowering of all of the pituitary hormones. To a lesser extent, prolactin secretion is also inhibited by GABA and is stimulated by factors such as TRH, vasoactive intestinal polypeptide (VIP), estrogen, serotonin, and opioids.

Presenting symptoms of hyperprolactinemia

The clinical presentation of hyperprolactinemia in women is usually related to symptoms of

Infertility, 1st edition. Edited by Emre Seli.

Table 10.1 Causes of hyperprolactinemia

Hypothalamic, Pituitary, and Endocrine Causes	Prolactinoma (microadenoma or macroadenoma) Macroadenoma (non-prolactin-secreting) Invasive tumors Acromegaly Primary hypothyroidism Adrenal insufficiency Cranial irradiation Pituitary stalk trauma/interruption/displacement Infiltrative diseases
Pharmacologic	Antipsychotics Antidepressants Antihypertensives Protease inhibitors Estrogens, hormone replacement therapy Opiates Cocaine Heroin
Reduced clearance	Renal disease/failure Hepatic insufficiency/cirrhosis Macroprolactinemia
Neurogenic stimulation	Nipple stimulation Chest wall injury Spinal cord lesion
Physiologic	Stress Pregnancy Breastfeeding Exercise Sleep Idiopathic

hypogonadism. Galactorrhea may or may not be present. Hyperprolactinemia produces a secondary disruption in gonadotropin-releasing hormone (GnRH) release from the hypothalamus leading to reduced secretion of FSH and LH. These effects, in turn, produce menstrual irregularities which can manifest clinically as oligomenorrhea, amenorrhea, luteal-phase insufficiency, and infertility. The resultant estrogen-deficient state may produce hot flashes, vaginal dryness and, if prolonged, osteopenia or osteoporosis. The presentation of hyperprolactinemia in men also results from hypogonadotropic hypogonadism. Men typically present with manifestations of hypogonadism and may complain of decreased libido, erectile dysfunction, and infertility. Occasionally, men will present with gynecomastia and galactorrhea. Some peri/postmenopausal women may attribute signs of hypoestrogenism to the menopausal transition and are more likely to delay seeking medical attention. These patients may present with mass effects such as headaches, visual changes, and cranial nerve palsies caused by large pituitary tumors.

Screening for hyperprolactinemia

Fasting prolactin levels are typically less than 20 ng/mL in men and in women who are not pregnant or breastfeeding. There are many causes of hyperprolactinemia (see Table 10.1). Mild elevations of up to two times normal should cause the test to be repeated because recent orgasm, nipple stimulation, sleep, exercise, stress and even venepuncture can elevate prolactin levels. Values above 40 ng/mL warrant a thorough evaluation which includes a review of recent medications, a pregnancy test, evaluation for primary hypothyroidism, and an insulin-like growth factor-1 (IGF-I) level to rule out acromegaly. GH is a prolactogen and, in addition, some pituitary tumors secrete both GH and prolactin.

Imaging the brain

A dedicated MRI test with gadolinium contrast should be performed to evaluate for a pituitary tumor. If a tumor is present, the MRI can also distinguish between a microadenoma (<1 cm) and a macroadenoma (>1 cm), both of which can respond to medical therapy but the latter of which may be more likely to produce a mass effect or to infarct when unresponsive to medical therapy or during pregnancy. MRI can also be used to diagnose less common tumors including craniopharyngiomas, germinomas, and meningiomas which may produce secondary hyperprolactinemia and may require surgical intervention. A neuro-ophthalmologic evaluation should be considered for patients with large or symptomatic tumors near the optic chiasm. Patients with pituitary tumors should also undergo an endocrine evaluation for hypopituitarism.

A dual-energy X-ray absorptiometry (DEXA) scan to measure bone density is warranted for patients with prolonged hypogonadism, and bone-sparing medications may be indicated.

CAUTION

A pregnancy test, serum TSH, and serum IGF-I should be assayed prior to initiating treatment for hyperprolactinemia because pregnancy, primary hypothyroidism, and GH-secreting tumors can each elevate prolactin levels.

Neuroimaging with MRI is also important when discussing prognosis and treatment options with the patient. The size of a prolactin-secreting tumor helps predict behavior; microadenomas generally secrete less prolactin and respond better to medical therapy than macroadenomas. Large non-prolactin-secreting tumors which interfere with dopamine inhibition of prolactin release rarely produce serum prolactin levels greater than 250 ng/mL. However, patients with large, invasive, prolactin-secreting tumors may also present with normal to modestly elevated serum prolactin concentrations mimicking a nonfunctioning macroadenoma. This is due to the "hook effect" wherein the serum prolactin concentration determination is artificially low because the detection assay is overwhelmed by high prolactin levels. Where clinically indicated, serum prolactin levels should be repeated and tested using serial dilutions to distinguish between a clinically nonfunctioning macroadenoma and an invasive prolactin-producing tumor.

Two prolactin isoforms (dubbed "big prolactin" and "big big prolactin/macroprolactin") have been identified in about one-quarter of patients with hyperprolactinemia. Both big prolactin and macroprolactin are detectable using standard prolactin immunoassays but demonstrate limited bioactivity. When clinically suspected, screening for big prolactin and macroprolactin is recommended to avoid unnecessary diagnostic, medical, and surgical interventions in patients with hyperprolactinemia solely attributed to clinically irrelevant prolactin forms.

CAUTION

Locally invasive prolactin-secreting tumors with prolactin levels greater than 5000 ng/mL saturate both the capture and signal antibodies commonly used in prolactin sandwich immunoradiometric assays, producing falsely low prolactin levels and giving the appearance of a nonfunctioning macroadenoma. This phenomenon is known as the high-dose "hook effect." A true assessment of prolactin concentration can be ascertained using serial dilutions of patient serum.

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The majority of circulating prolactin consists of a 23-kDa monomeric protein with up to 20% undergoing post-translational modification and glycosylation to form a 25-kDa molecule. In addition, there are two prolactin isoforms that have been identified in patients with hyperprolactinemia. "Big prolactin" is 45–60 kDa in size and consists of covalently or noncovalently bound prolactin dimers or a prolactin monomer bound to another serum component. "Big big prolactin/macroprolactin" consists of a prolactin monomer bound to an antiprolactin antibody. Both of the latter forms of prolactin display limited biological activity and can be detected using special laboratory tests such as gel filtration chromatography or polyethylene glycol (PEG) precipitation.

Initiating therapy

When treating a prolactin-secreting pituitary adenoma, the goals are to normalize prolactin levels, restore gonadal function, reduce tumor size, and reverse pituitary dysfunction (when present). Prolactinomas are generally amenable to medical therapy. The decision to start treatment should be individualized. Often treatment is directed at alleviating symptoms of hyperprolactinemia. A patient desiring fertility with hyperprolactinemia and either oligomenorrhea or amenorrhea will benefit from treatment with a

dopamine agonist. Alternatively, a patient with irregular menses or amenorrhea without galactorrhea who does not desire fertility may be treated with either oral contraceptive pills or a dopamine agonist to regulate her menstrual cycles. In regularly cycling infertility patients with mildly elevated prolactin levels, treatment with dopamine agonists has been shown to improve fertility outcomes.

Microadenomas rarely progress in size and are unlikely to grow without displaying a significant increase in serum prolactin. It is therefore reasonable for regular cycling asymptomatic women with microadenomas to forego treatment when not attempting to conceive. Patients with microadenomas and mildly elevated prolactin levels with regular menses and mild intermittent galactorrhea that is not bothersome may also elect to forego any intervention and can be followed with intermittent observation and yearly prolactin screening. This is in contrast to patients with macrademomas whom often require chronic therapy and are more likely to progress and become symptomatic.

Medical therapy

Dopamine agonists are the treatment of choice for prolactinomas. These agents suppress prolactin secretion and will generally shrink prolactinomas. These effects will generally lead to the resumption of normal gonadotropin pulsatility and restoration of the hyphothalamic–pituitary–ovarian (HPO) axis. Treatment effects of dopamine agonists are rapid. The two most commonly used dopamine agonists in clinical practice are bromocriptine and cabergoline and both appear to be safe in pregnancy. Bromocriptine has a half-life of 4 h and therefore must be given either once or twice daily. Common side effects include nausea, headache, dizziness or postural hypotension, abdominal pain or dyspepsia, fatigue, and nasal congestion. Less commonly, vomiting, constipation, and leg cramps can occur, and, at high doses, valvular heart disease has been reported with dopamine agonists. The half-life of cabergoline is 63–69 h, so the drug can be administered on a twice-weekly basis. Cabergoline tends to be better tolerated by patients, with a lower side effect profile and a lower patient discontinuation rate.

Several studies have demonstrated the superiority of cabergoline to bromocriptine in the treatment of hyperprolactinemia; however, bromocriptine is often preferred in women attempting to conceive because of the longer clinical experience using the drug. Repeat serum prolactin testing should be performed 1, 3, and 6 months after the initiation of treatment, with yearly testing thereafter in patients who remain well controlled and asymptomatic. For patients with macroadenomas, a repeat MRI should be performed after 6 months of treatment to evaluate for tumor shrinkage. These patients should be followed with MRIs on a yearly basis. In the absence of symptoms, repeat MRI testing in not warranted in patients with microadenomas given the low likelihood of silent progression to macroadenoma. If a previously seen tumor is no longer visible on MRI and normal serum prolactin levels persist for more than 2 years, a trial off therapy (i.e., a drug holiday after a drug taper) can be attempted with frequent follow-up in the first year and subsequent yearly screening.

Surgical therapy

Trans-sphenoidal surgery remains an important therapeutic option when patients cannot tolerate medical therapy or when medical therapy does not restore normal gonadal and neurologic functions. Surgical therapy for microadenomas is more successful than for macroadenomas. Studies have demonstrated that the success of trans-phenoidal surgery is dependent on the size of the tumor and the experience of the neurosurgeon. Significant surgical morbidity may occur in up to 3% of patients with complications such as loss of vision, stroke, meningitis, oculomotor palsies, hypopituitarism, cerebrospinal fluid rhinorrhea, and diabetes insipidus.

Radiotherapy

Radiation therapy is rarely necessary in the management of prolactinomas and is generally reserved for invasive tumors that do not respond to medical or surgical therapies. Initial complications reported with radiotherapy included hypopituitarism, secondary malignancies, stroke, optic nerve damage, radiation brain necrosis, neurologic dysfunction, soft tissue reactions, and

radiation-induced encephalopathy. Newer forms of radiotherapy, including the stereotactic gamma knife, may result in lower complication rates.

☆ TIPS & TRICKS

Side effects from dopamine agonists can be minimized by dosing at bedtime (to minimize symptoms of postural hypotension) and slowly titrating to the effective dose, as tolerated. Vaginal administration of dopamine agonists is also effective and is associated with fewer side effects than oral administration.

CAUTION

Caution is warranted in patients that require high doses of dopamine agonists to control their hyperprolactinemia. Cardiac fibrotic valvular disease has been observed in patients on chronic long-term dopamine agonist therapy. This side effect has been observed in patients taking high doses of dopamine agonists to control the symptoms of Parkinson's disease. Patients on dopamine agonists should be followed with periodic cardiac examination and an echocardiogram is warranted if a cardiac murmur is appreciated or long-term high-dose therapy is required.

Hyperprolactinemia in pregnancy

In response to rising estrogen levels, prolactinomas grow and produce more prolactin during pregnancy. This physiologic rise in prolactin during pregnancy generally means that asymptomatic patients with microprolactinomas can discontinue medical therapy once pregnant. Microadenomas rarely become symptomatic in pregnancy and can be followed by screening for symptoms in each trimester. Up to one-quarter of patients with macroadenomas may become symptomatic during pregnancy, particularly those with suprasellar extension of their tumors. Patients with macroadenomas should have a baseline MRI and formal visual field testing each trimester: some may be candidates for continued dopamine agonist therapy throughout gestation. If symptoms present or visual field defects are discovered, an MRI should be performed to evaluate for tumor enlargement. Medical therapy should be reinstituted if the MRI confirms enlargement. If the tumor is unresponsive to medical therapy or symptoms worsen, transsphenoidal surgery may be warranted.

Acromegaly

Acromegaly is an uncommon disorder, affecting women and men in equal numbers, and results from hypersecretion of pituitary GH. Most cases of acromegaly are caused by benign GH-secreting adenomas with a small percentage stemming from GHRH-secreting tumors or, quite rarely, ectopic GH-secreting tumors. GH stimulates hepatic secretion of insulin-like growth factor-1 (IGF-1), previously know as somatomedin C, which causes most of the clinical manifestations of acromegaly. The hypothalamus produces GHRH which provides the positive stimulus for growth hormone secretion from the anterior pituitary, while IGF-1 and somatostatin (SS) feed back to inhibit GH release.

Presenting signs and symptoms of acromegaly

The clinical presentation of acromegaly is often insidious and the disease generally progresses slowly, making early diagnosis rare. Affected patients typically present after years of symptoms that include acral (peripheral bony/limb) enlargement, arthropathy, changes in facial features, hyperhidrosis, soft tissue swelling, and, in some cases, symptoms related to pituitary tumor mass effects such as headaches and visual changes. Often old pictures are compared with the patient's current appearance to help make the diagnosis. Patients typically present with an enlarged jaw, coarse facial features, and swollen hands and feet. Common soft tissue changes include macroglossia, deepening of the voice, and carpal tunnel syndrome. Most organ systems can be affected and often there is enhanced growth of the thyroid gland, heart, liver, kidneys, and prostate. Sleep apnea, hypertension, left ventricular hypertrophy, valvular dysfunction, cardiomyopathy, arrhythmias, atherosclerosis, and congestive heart failure contribute to higher mortality rates in patients with acromegaly. Other

endocrine abnormalities such as thyroid dysfunction, hypoadrenalism, hyperinsulinemia, insulin resistance, and abnormal bone metabolism are also prevalent.

Diagnosing acromegaly

Once clinically suspected, acromegaly is diagnosed by an elevated IGF-1 level. Follow-up testing will reveal inadequate GH suppression in response to an oral glucose load and the diagnosis is confirmed by a pituitary mass found on MRI. GH levels are affected by sleep, stress, exercise, and food intake. Normal GH levels demonstrate considerable hour-to-hour variation because of its pulsatile and diurnal secretion. IGF-1 levels are not subject to significant daily fluctuation and reflect cumulative GH secretion, making it the preferred screening test for acromegaly (although it should be noted that IGF-1 values are normally elevated in pregnancy).

The formal diagnosis of acromegaly is made by administering a 2-h 75-g oral glucose tolerance test. Normal patients should suppress serum GH concentrations to less than 1 ng/mL while those with acromegaly will fail to suppress. Patients with suspected acromegaly should undergo a MRI of the head with gadolinium contrast to evaluate for pituitary, and less commonly hypothalamic, tumors. If the head MRI is negative, a CT scan or MRI of the chest and abdomen should be performed to evaluate for ectopic GH-secreting tumors. Most cases of acromegaly will demonstrate a persistently elevated serum GH level, but a "normal" random GH level does not exclude the diagnosis of acromegaly.

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40% of GH-secreting adenomas have an activating mutation in the α subunit of the guanine nucleotide stimulatory protein-α (Gs-α) resulting in constitutive activation of adenylate cyclase and enhanced GH secretion.

Treating acromegaly

Given the increased morbidity and mortality associated with acromegaly, all patients, even when asymptomatic, should undergo treatment. The goals of therapy are to normalize the excess secretion of GH and IGF-1, and to surgically remove or debulk large tumors before these can further compromise pituitary and parasellar structures. Effective treatment is associated with regression of tissue overgrowth and improvement of metabolic abnormalities, although bony abnormalities and associated joint symptoms often persist. Surgical cure rates as high as 80–90% are observed with trans-sphenoidal surgery performed by experienced neurosurgeons for microadenomas, while less than 50% of patients with acromegaly undergoing surgery for macroadenomas will demonstrate biochemical criteria of a cure. Surgical complications include hypopituitarism, central diabetes insipidus, cerebrospinal fluid rhinorrhea, and meningitis. Recurrence rates of up to 10% have been reported postoperatively.

When surgery fails to achieve adequate disease control, or when it is contraindicated or declined, patients can be offered radiotherapy and/or pharmacological treatments. The majority of GH-secreting tumors express somatostatin receptors, making treatment with somatostatin agonists an attractive option. Two-thirds of patients display normalization of IGF-1 levels and up to one-half will have documented tumor shrinkage when treated with somatostatin agonists such as intravenous or subcutaneous octreotide (50–400 μg three times daily), intramuscular octreotide (10–40 mg monthly) or subcutaneous lanreotide (60–120 mg monthly). Although subcutaneous administration of the GH receptor antagonist pegvisomant (10–40 mg) effectively normalizes IGF-1 levels in 95% of patients, it does not cause tumor shrinkage or allow resumption of gonadal function. Therefore, the use of pegvisomant is reserved for patients who fail, or do not tolerate, somatostatin agonists. Dopamine agonists such as cabergoline have also been used to treat acromegaly, but these medications are less effective than other treatment modalities and require high doses which often produce untoward side effects. Adequate biochemical remission of acromegaly is established using age- and sex-adjusted IGF-1 levels and by producing a corrected GH level (<1.0 ng/mL) after oral glucose challenge.

Conventional radiotherapy is reserved for patients with medication-resistant disease or recurrent disease after surgical therapy. Radiotherapy is associated with a high incidence of side effects, with more than 50% of patients experiencing hypopituitarism. Rare complications of radiotherapy include local nerve damage, secondary brain tumors, visual disorders, and cerebrovascular accidents. Limited long-term outcome data are available for newer techniques such as stereotactic radiosurgery (gamma knife).

CAUTION

Surveillance of IGF-1 levels, not growth hormone (GH) suppression after an oral glucose tolerance test, is used when monitoring acromegalic patients' response to therapy. Some patients with suppressed GH levels after glucose challenge will still have clinically active disease and will demonstrate elevated IGF-1 levels when tested. IGF-1 levels also correlate better with measures of insulin sensitivity than do GH levels.

Acromegaly and infertility

The pituitary mass effect commonly observed in acromegaly, combined with associated hyperprolactinemia, can disrupt gonadotropin secretion and often leads to menstrual irregularities, infertility, and decreased libido. The hyperprolactinemia (with or without galactorrhea) occurs because GH is a prolactogen and because many poorly differentiated tumors will cosecrete GH and prolactin. Also contributing to hyperprolactinemia may be the mass effect of some GH adenomas which can disrupt dopamine-mediated suppression of pituitary prolactin secretion.

Women with acromegaly wishing to become pregnant should undergo treatment to prevent tumor expansion prior to pregnancy, normalize serum IGF-1 and GH levels, and maximize their fertility potential. Medical treatment should be stopped upon confirmation of pregnancy. Levels of GH normally rise in pregnancy and GH is produced by the placenta. Elevated GH levels are not believed to be harmful to the fetus. Visual field testing to monitor for disease progression should be performed during pregnancy in patients with known macroadenomas. MRI evaluation and surgery may be performed if visual impairment or mass effects develop during pregnancy.

Thyroid disorders: hyperthyroidism and hypothyroidism

Regulation of thyroid hormone production

The hypothalamic–pituitary–thyroid axis is tightly regulated through negative feedback by thyroid hormone. TRH from the hypothalamus signals the anterior pituitary to produce TSH. TSH, in turn, stimulates thyroid gland production and release of thyroxine (T_4) and, to a lesser extent, the more potent triiodothyronine (T_3). In the circulation T_3 and T_4 are heavily protein bound to thyroxine-binding globulin, albumin and prealbumin. T_4 is a precursor to the active hormone T_3. T_3 is produced from T_4 in the periphery as needed. T_3 and, to a lesser extent T_4, exerts negative feedback at the levels of the hypothalamus and anterior pituitary to suppress TRH and TSH, respectively. Perturbations in thyroid hormone homeostasis can lead to states of over- or underproduction of thyroid hormone. Thyroid dysfunction is more prevalent in women than in men and both hyperthyroidism and hypothyroidism can have significant effects on the menstrual cycle, fertility, pregnancy, and fetal development.

Hyperthyroidism

Presenting symptoms of hyperthyroidism

The most common cause of hyperthyroidism is Graves disease, an autoimmune condition caused by thyroid stimulating immunoglobulins/anti-TSH receptor antibodies. Women with hyperthyroidism may present with menstrual dysfunction, but some may remain ovulatory and will present during pregnancy. Oligomenorrhea and amenorrhea are frequently observed, although some patients present with polymenorrhea. Other symptoms may include weight loss, fatigue, increased frequency of bowel movements, heat intolerance, anxiety, dyspnea, palpitations, diaphoresis, lid lag, tremor, hyperreflexia, and supraventricular tachycardia. Rarely hyperthyroidism can present as acute life-threatening

thyrotoxicosis or "thyroid storm" with severe tachycardia, hypertension, heart failure, agitation, delirium, psychosis, or coma.

Diagnosis of hyperthyroidism

While a goiter may be present (or absent) in conditions of hyper- and hypothyroidism, clinical thyroid exam may also reveal the presence of single or multiple nodules in some cases. Diagnosis of Graves disease begins with laboratory testing that demonstrates a low serum TSH and high free T_4 and T_3 and may be associated with a diffuse goiter. While clinical findings consistent with Graves disease may be present, thyroid antibody testing and a 24-h thyroid radio-iodine uptake scan should be pursued in order to differentiate Graves disease from other causes of hyperthyroidism such as a toxic adenoma or toxic multinodular goiter. Additional diagnostic testing, such as thyroid gland ultrasound, fine-needle aspiration, or thyroid biopsy may be warranted to rule out malignancy. For this reason, the evaluation and treatment of hyperthyroidism is often pursued in collaboration with a medical endocrinologist.

CAUTION

Care should be taken when interpreting thyroid function studies obtained during pregnancy. Serum thyroxine-binding globulin (TBG) nearly doubles in pregnancy secondary to the effects of placental estrogens. Sialylation of TBG also results in decreased clearance of TBG which contributes to the elevated levels in pregnancy. Consequently, total serum T_3 and T_4 will be increased in pregnancy while free T_3 and T_4 should remain normal. In addition, hCG has weak thyrotropin (TSH) activity. The markedly elevated hCG levels observed near 10 weeks of gestation can result in mild increases in free T_3 and T_4 with a compensatory decrease in serum TSH in the first trimester. This mild hyperthyroidism of early pregnancy may be exaggerated in conditions with excessive levels of hCG, such as cases of multiple gestation or gestational trophoblastic disease, but rarely requires medical treatment.

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Rarely, hyperthyroidism can be of pituitary (secondary) or hypothalamic (tertiary) origin. In these cases, testing will reveal *elevated* TSH associated with elevated free T_4 levels, indicating a failure of the thyroid hormone to suppress pituitary TSH production.

Treating hyperthyroidism

The goal of treatment is to restore a euthyroid state. Treatment modalities include pharmacotherapy, radio-iodine (contraindicated in pregnancy), and surgery. Medications commonly used to threat hyperthyroidism include carbimazole, methimazole, and propylthiouracil (PTU). Beta-blockers may be used to reduce symptoms associated with hyperthyroidism and will reduce the conversion of T_4 to T_3. Successful treatment is associated with the resumption of regular ovulatory menstrual cycles and the ability to conceive. Furthermore, hyperthyroidism should be controlled prior to conception to decrease the risk of miscarriage, premature labor, low birth weight, stillbirth, pre-eclampsia, and heart failure.

CAUTION

Thyroid stimulating antibodies, as well as the medications used to treat hyperthyroidism, can cross the placenta and affect the developing fetus. The goal of therapy during pregnancy is to use the lowest effective dose of medication to maintain maternal serum free T_4 concentration in the high-normal range. Because of a possible association between methimazole and the scalp defect aplasia cutis, propylthiouracil is generally first-line therapy in the first trimester. Both agents can produce considerable side effects and require titration and monitoring for adverse effects. Patients with hyperthyroidism should be referred to the high-risk maternal fetal medicine specialist for preconceptual counseling in order to coordinate the treatment of maternal hyperthyroidism during pregnancy and later to monitor the fetus for signs of thyroid toxicity during

gestation (from either maternal antibodies or antithyroid medications).

Hypothyroidism

Presenting symptoms of hypothyroidism

The most common cause of hypothyroidism in the United States is chronic autoimmune thyroiditis/Hashimoto's thyroiditis. Less common causes of hypothyroidism are indicated in Table 10.2. Women with hypothyroidism may present with polymenorrhea, irregular menses, anovulatory cycles, menorrhagia, or infertility. Other common symptoms at presentation include weight gain, fatigue, constipation, cold intolerance, hair loss, dry skin, periorbital edema, hoarseness, sluggish reflexes, and anemia. Hypothyroidism may also have direct affects on the ovary and the endometrium. Hypothyroidism has been associated with decreased ovarian steroidogenesis and luteal-phase insufficiency.

Diagnosis of hypothyroidism

The guidelines regarding screening for thyroid dysfunction in pregnant women and in those attempting to conceive remain inconsistent. Screening is generally recommended for symptomatic patients and for patients with infertility or recurrent pregnancy loss. An elevated serum TSH is suggestive of primary hypothyroidism. This is confirmed with a repeat study demonstrating an elevated TSH and a low free T_4. Mildly elevated TSH (>2.5 mIU/L but <10 mIU/L) associated with normal free T_4 indicates subclinical hypothyroidism. The clinical relevance of subclinical hypothyroidism in the general population remains controversial and it is uncertain whether subclinical hypothyroidism affects fertility. Testing for antithyroid peroxidase (TPO) and antithyroglobulin (TG) antibodies is warranted in infertile patients with subclinical hypothyroidism, where the detection of antibodies is beneficial in counseling and directing treatment. The presence of antithyroid antibodies is associated with a 2–4% risk of developing overt hypothyroidism each year and has been associated with an increased risk of miscarriage and preterm birth even in patients who are euthyroid prior to conception.

Table 10.2 Causes of hypothyroidism

Primary	Chronic autoimmune thyroiditis (Hashimoto thryoiditis) Radioactive iodine ablation Thyroidectomy External neck irradiation Postpartum thyroiditis Subacute thyroiditis Iodine deficiency Infiltrative disease (hemochromatosis, scleroderma, amyloid goiter) Infectious (Myobacterium tuberculosis, Pneumocystis carinii) Medications (thionamides, lithium, amiodarone)
Secondary and tertiary	Tumors of the pituitary or hypothalamus Pituitary necrosis (Sheehan syndrome) Craniopharyngiomas Infiltrative diseases Trauma Radiation therapy Infection (hypophysitis) Inactivating mutations of TSH, TRH, TSH receptor, TRH receptor Generalized resistance to thyroid hormone (mutation in T_3 receptor)

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Patients with hypothyroidism have reduced levels of sex hormone binding globulin (SHBG) and therefore have reduced levels of circulating estrogens and testosterone which may have consequences for sex steroid actions on reproductive tissues. In addition, primary hypothyroidism is associated with increased thyrotropin releasing hormone (TRH) which causes the release of pituitary TSH and prolactin. Excess prolactin can interfere with GnRH pulsatility and LH/FSH secretion, contributing to the menstrual irregularities seen in patients with primary hypothyroidism.

CAUTION

Rarely, hypothyroidism can be of pituitary (secondary) or hypothalamic (tertiary) origin. In these cases, testing will reveal *low* TSH levels associated with low free T_4 levels, indicating a failure of the pituitary or hypothalamus to up-regulate TSH or TRH, respectively, in the setting of low circulating thyroid hormone levels.

Treating hypothyroidism

The mainstay for the treatment of infertile or pregnant patients with hypothyroidism, subclinical hypothyroidism, or euthyroid patients with antithyroid antibodies is replacement with synthetic thyroid hormone (levothyroxine). Treatment for hypothyroidism and subclinical hypothyroidism is usually started at a dose of 1 µg/kg per day with repeat thyroid testing performed at 4-week intervals until the dose is titrated to achieve a TSH level not greater than 2.5 mIU/L. For patients with positive antithyroid antibodies, treatment is initiated and maintained at 0.5–1.0 µg/kg per day. Repeat laboratory testing for those with hypothyroidism is performed until adequate suppression of TSH has been achieved, and then as clinically indicated.

Producing a euthyroid state prior to conception is highly recommended. Notably, levothyroxine requirements will increase (25–50%, on average) during ovarian hyperstimulation with purified FSH (assisted reproductive technologies) and in pregnancy. Importantly, for women with antithyroid antibodies, adequate thyroid hormone replacement in pregnancy has been shown to reduce the risks of congenital malformations, miscarriages, preterm births, and preterm labor. Adequate replacement is also necessary to optimize intellectual development of the fetus and children born to mothers with thyroid hormone deficiency have, on average, IQ scores four points below those of matched controls. The increased metabolic demands of pregnancy, the elevated TBG in pregnancy, and the fetal requirements for maternal thyroid hormone in early pregnancy each contribute to the increased levothyroxine requirements during pregnancy. Women with hypothyroidism should increase their levothyroxine dose by 30–50% when pregnancy is initially confirmed.

TIPS & TRICKS

To maximize absorption of levothyroxine, the drug should be taken first thing in the morning on an empty stomach. Other drugs, particularly iron and calcium, may interfere with the absorption of levothyroxine. In addition, patients on estrogen therapy often require higher doses of levothyroxine because estrogens stimulate the production of TBG.

Adrenal disorders: Cushing syndrome and congenital adrenal hyperplasia

Adrenal disorders

The adrenal gland is composed of the outer cortex which is responsible for the production of mineralocorticoid (aldosterone), glucocorticoid (cortisol), and adrenal androgens; and an inner medulla which produces the catecholamines epinephrine (adrenaline) and norepinephrine (noradrenaline). Cortisol and dehydroepiandrosterone sulfate (DHEAS) production are primarily regulated by the hypothalamus and anterior pituitary through CRH and ACTH, respectively. Aldosterone is subject to regulation by the renin–angiotensin system. Both cortisol excess (Cushing syndrome) and the inability to effectively produce glucocorticoids (congenital adrenal hyperplasia, CAH) can disrupt normal reproductive function and may complicate pregnancy.

Cushing syndrome

Hypercortisolemia may be caused by an adrenocorticotropin (ACTH)-producing tumor (Cushing disease), ectopic ACTH secretion by a nonpituitary tumor, ectopic secretion of CRH, or primary excess cortisol secretion by an adrenal adenoma or carcinoma. Cushing syndrome is associated with excess androgen production from the adrenal gland and can be manifested by hirsutism, acne, male pattern hair loss, easy bruising, decreased libido, and virilization. Cushing syndrome is often associated with menstrual irregularities, anovulation, and infertility. Other

features include dermatologic findings including skin atrophy and striae, central obesity, proximal muscle wasting, bone loss, hypertension, and glucose intolerance/diabetes.

None of the signs or symptoms of Cushing syndrome is pathognomonic of the syndrome, and diagnosis is often delayed for years. There is considerable phenotypic overlap between Cushing syndrome and other diseases of reproductive-aged women including polycystic ovary syndrome (PCOS) and CAH, a feature which contributes to delayed diagnosis. After ruling out exogenous glucocorticoid intake, a common iatrogenic cause of glucocorticoid excess, screening for hypercortisolemia can be performed using a 24-h urinary free cortisol assessment which may need to be repeated twice for confirmation. Alternatively, a low-dose dexamethasone suppression test or a late evening salivary or serum cortisol level may suggest hypercortisolism (normally, cortisol peaks in the early morning and reaches its nadir around midnight; salivary cortisol is in equilibrium with serum cortisol). Once the diagnosis of hypercortisolism is secured, further testing to distinguish between ACTH-dependent (Cushing disease) and ACTH-independent (Cushing syndrome) secretion is recommended.

CAUTION

Because of the significant diurnal and minute-to-minute variation in cortisol secretion, a random daytime serum or "spot" urinary cortisol level is not a sensitive assay for hypercortisolism. Further, patients with pseudo-Cushing's states such as alcoholism, severe illness, anorexia nervosa, and clinical depression may screen positive for hypercortisolism and will warrant further testing.

Congenital adrenal hyperplasia

A variety of mutations in the enzymes necessary for adrenal cortisol biosynthesis have been described. More than 90% of cases of congenital adrenal hyperplasia are due to classical 21-hydroxylase deficiency which usually causes ambiguous genitalia (in females) and salt-wasting in the newborn. A smaller proportion of cases of 21-hydroxylase deficiency have a milder genetic mutation which may go unrecognized until adulthood. These women go through normal or premature pubertal development and may exhibit mild virilization (contrasexual sexual development). Impaired adrenal synthesis of cortisol results in cortisol deficiency and shunting of the steroidogenic pathway in the direction of excessive adrenal production of androgen precursors such as DHEA and DHEAS, leading to hyperandrogenism, menstrual irregularities, and anovulation. Additional signs and symptoms of CAH will mimic those of polycystic ovarian syndrome. A serum 17-hydroxyprogesterone level will screen for CAH in women with symptoms suggestive of androgen excess such as acne, hirsutism, and menstrual irregularities. Although somewhat controversial because of its suboptimal sensitivity, it is reasonable to screen women with hyperandrogenism for ovarian and adrenal androgen-producing tumors by testing serum testosterone and DHEAS levels, respectively. Serum testosterone greater than 200 ng/dL or DHEAS greater than 700 µg/dL will warrant further evaluation for an androgen-producing tumor.

Treatment with glucocorticoids will supplement deficient cortisol production and will suppress adrenal production of androgen precursors, usually resulting in the resumption of normal ovulatory menstrual cycles and the ability to conceive. Preconceptual genetic testing and counseling is recommended for couples affected by CAH in order to evaluate for the risk of CAH to future offspring. A decision regarding preimplantation genetic diagnosis, or postconceptual glucocorticoid therapy to avoid risk of ambiguous genitalia in affected female fetuses, remains a preconceptual priority.

CAUTION

17-hydroxyprogesterone is produced by the corpus luteum after ovulation. In ovulatory patients, screening for CAH with 17-hydroxyprogesterone should be performed during the proliferative phase of the menstrual cycle.

Summary

This chapter has reviewed several of the common endocrine disorders of the pituitary, thyroid, and adrenal glands that may impact human fertility. Hyperprolactinemia, most commonly from a prolactin-secreting adenoma or secondary to diverse pharmacologic agents, is a frequently encountered source of hypogonadism and ovulatory dysfunction and is easily detected and treated. It is worth remembering that primary hypothyroidism will produce a secondary hyperprolactinemia that will correct when thyroid status is normalized. Though rare, acromegaly can produce hyperprolactinemia and may contribute to ovulatory dysfunction and infertility. Screening for acromegaly begins with a serum IGF-1 level. A more significant cause of decreased fertility and adverse pregnancy outcomes is hypothyroidism, screened for by testing a serum TSH. Hypothyroidism, most commonly secondary to autoimmune thyroiditis, should be aggressively detected and treated in the subfertile population. In addition, thyroid hormone requirements will increase in pregnancy. When detected, hyperthyroidism will require more extensive testing in order to rule out malignancy. A multidisciplinary approach with consideration of all treatment options is advisable prior to conception. Cushing syndrome, like acromegaly, often progresses in an insidious fashion and may be difficult to recognize in the early clinical stages. When suspected, screening using a 24-h urinary free cortisol may be followed by further provocative testing as warranted. CAH is a common masquerader for PCOS and should be screened for in all conditions of oligo- or anovulation associated with hyperandrogenism. The treatment for CAH differs from that for PCOS and the heritable consequences of the two disorders are distinct and warrant counseling prior to conception.

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11

Physiologic Basis of Ovulation Induction

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Introduction

This chapter describes the way in which the commonly used fertility drugs work to induce ovulation or to augment ovulation in the case of assisted reproductive technologies. In order to understand clearly the mechanism of action of these drugs, and the problems that may be associated with their use, it is important to first review the physiology of the normal menstrual cycle. The rest of this review then briefly describes how injectable fertility medications work and then focus mainly on the use of oral agents, clomiphene citrate (CC) and aromatase inhibitors, to induce ovulation.

Physiological basis of ovulation

Prior to puberty and menarche, there is a progressive and rapid atresia of ovarian follicles starting at about 5 months of intrauterine gestation. At the time of puberty, the ovarian cortex still contains hundreds of thousands of primordial follicles, each consisting of an oocyte arrested at the diplotene stage of the first meiotic division, surrounded by a few flattened cells that will develop into granulosa cells. Independent of pituitary gonadotropins, and in response to as yet unknown factors, a cohort (hundreds) of primordial follicles is recruited to grow each month. This process may be inhibited to some degree by antimüllerian hormone (AMH). Furthermore, it is likely that each cohort develops in a series of one to three waves, since recent elegant ultrasound studies by Roger Pierson and his colleagues in Saskatchewan have demonstrated a dominant and up to two subordinate follicle waves during each normal menstrual cycle. During this early follicle development, the oocyte enlarges and the granulosa cells proliferate to form a preantral follicle. Theca cells begin to differentiate from the surrounding stroma. It is believe that follicle and oocyte growth over the course of 3–6 months is required for the granulosa cells in the follicle to develop follicle stimulating hormone (FSH) receptors, and for the theca cells to develop luteinizing hormone (LH) receptors. At this stage, the follicle forms a fluid-filled space called an antrum within the granulosa cell layers and antral follicles become acutely dependent on FSH for further growth and development. A rise in FSH levels occurs in natural cycles just prior to menses, when falling estrogen and inhibin levels during involution of the corpus luteum result in withdrawal of negative feedback centrally and are accompanied by an increase in gonadotropins levels, predominantly FSH. FSH stimulates granulosa cell proliferation and differentiation, with the development of more FSH receptors and the production of the enzyme aromatase. LH stimulates the theca cells to secrete androstenedione and testosterone. The androgens diffuse into the granulosa cell layer and provide the substrate for estrogen secretion by FSH-induced aromatase (the so-called two-cell, two-gonadotropin theory). Brown postulated that FSH concentrations must exceed a certain level (the FSH "threshold") before follicular development will proceed. The time in which the threshold is exceeded (the FSH "window"), results in stimulation of the growth of antral follicles and

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the secretion of estradiol. The number of follicles that become dominant in each cycle is limited by a gradual decrease in FSH back below the threshold in response to negative feedback of rising estrogen levels on FSH secretion in the early–mid follicular phase. Generally, one follicle, probably by chance, may grow faster, have a higher number of granulosa cells, and therefore a higher concentration of FSH receptors. More FSH receptors lead to an increased sensitivity to FSH and a different follicular microenvironment, and this follicle continues developing despite falling FSH levels. In addition, FSH also induces LH receptors in larger antral follicles above 1.0 cm in diameter and at this point, endogenous LH may synergize with FSH to select out the preovulatory follicle. In fact, LH may substitute for FSH in stimulating follicle growth and aromatase activity in follicles larger than 1.2 cm. Smaller follicles, with fewer FSH receptors and no LH receptors on the granulosa cells, are no longer stimulated to grow by FSH levels that are below the FSH threshold and undergo atresia. Therefore, in the usual situation, only one follicle reaches the stage of ovulation each cycle, despite the fact that hundreds of primordial follicles may have begun development in the same cohort 3–6 months earlier.

Because the initial stage of follicle recruitment and growth is independent of gonadotropin stimulation, each month a new cohort of a few hundred follicles will begin growing even in women who have suppressed gonadotropins, for example women who are pregnant or taking combined hormonal contraceptives. In both cases, since gonadotropin secretion is suppressed by the elevated levels of circulating estrogen and progesterone or progestins, the appropriate signal for selection and development of a dominant follicle and ovulation is missing, and all of the follicles in the cohort undergo atresia. As a result, each month a fixed number of follicles is depleted. Therefore, menopause appears to be programmed to occur at around the age of 50 years and is not delayed by long-term anovulation induced by serial pregnancies or years of hormonal contraceptive use.

Rapidly increasing circulating levels of estradiol produced by the mature preovulatory follicle precede the mid-cycle LH and FSH surge that will initiate ovulation. The actual trigger of the surge is unclear but there is the suggestion that it may be a response to a rise in both estrogen and progesterone. The surge turns on a gene cascade in granulosa cells that initiates completion of oocyte meiotic maturation with extrusion of the first polar body, and digestion and rupture of the follicle wall with release of the oocyte. Angiogenesis and luteinization of granulosa and theca cells occurs to form the corpus luteum in which an alteration of the steroidogenic pathway results in progesterone as the primary steroid hormone produced after luteinization. We have recently demonstrated that follicular fluid contains a large number of $CD56^{bright}$, $CD16^{-ve}$ natural killer (NK) cells that are distinct from the cytotoxic $CD56^{dim}$, $CD16^{+ve}$ NK cells found in the circulation. Instead, the follicular NK cells are similar to the recently described decidual NK cells that appear to be angiogenic. We also determined that these cells seem to be unique in that they express CXCR3 receptors whose ligand (CXCL10) is expressed by granulosa cells. These observations suggest that granulosa cells attract angiogenic NK cells to the follicle where they may play a role in follicle, and subsequent corpus luteum, vascularization. Further research into this area is required to delineate the physiologic role of these cells.

The corpus luteum retains the ability to produce estrogen, and during the luteal phase both estrogen and progesterone concentrations in the circulation are increased. In addition, as discussed earlier, the demonstration of multiple waves of follicular growth in the majority of cycles by Baerwald and Pierson also suggests that some estrogen in the luteal phase may be contributed by growing follicles that will subsequently undergo atresia. LH pulses maintain corpus luteum steroid production. Early work by Crowley and colleagues demonstrated that LH pulses decrease in frequency from one pulse per hour during the mid-cycle LH surge to about one pulse every 6 h in the mid-luteal phase, and about one pulse every 12–24 h near the end of the luteal phase. Yen's laboratory, using naloxone (narcotic antagonist) infusions, demonstrated that increased central endogenous opioid peptide activity is the cause of the decreased pulse frequency in the luteal phase. It has also been shown that progesterone is the steroid hormone

responsible for the increased opioid peptide secretion leading to inhibition of the frequency of gonadotropin releasing hormone (GnRH) neuronal secretion. In the absence of pregnancy, LH levels in the second week of the luteal phase likely become too low to sustain the corpus luteum, as a result of very infrequent LH pulses and the short circulating half-life of LH. The corpus luteum undergoes regression with a fall in progesterone and estrogen and the onset of menses. FSH levels rise with withdrawal of estrogen negative feedback and the next cohort of follicles begins to develop. With implantation and pregnancy, hCG production by the trophoblast cells results in rescue and maintenance of corpus luteum function. Luteal progesterone is required until about 8 weeks gestation when the placenta takes over the production of adequate amounts of this steroid to support pregnancy.

In summary, normal follicular development culminates in ovulation of a mature oocyte, followed by the development of a corpus luteum producing adequate amount of progesterone. This sequence of events is orchestrated by the interaction of local ovarian factors and endocrine factors from the pituitary and hypothalamus. In addition, adequate functioning of other endocrine glands such as the thyroid and adrenal glands, is important in maintaining a normal interaction between the hypothalamus, pituitary, and ovaries resulting in ovulation. Any derangement may result in dysfunctional ovarian follicular development or even the complete failure of ovulation. The presence of subtle abnormalities despite the occurrence of ovulation may be responsible, at least in part, for unexplained infertility in some women, and perhaps for endometriosis-related infertility. On the other hand, overt anovulation or oligo-ovulation has traditionally been classified into three groups: World Health Organization (WHO) type I, II, and III anovulation.

WHO classification of anovulation

Women in WHO group I suffer from a defect at the level of the hypothalamus/pituitary. They are estrogen deficient with normal or low FSH or prolactin levels and have no space-occupying lesion in the hypothalamic pituitary region. They typically have amenorrhea and do not bleed in response to progestin challenge. Women in this group with infertility generally require pulsatile GnRH infusions or injectable gonadotropins in order to ovulate.

Women in WHO group II are not estrogen deficient. Their FSH and prolactin levels are normal. They typically experience oligomenorrhea, but they may have anovulatory cycles or amenorrhea with bleeding in response to a progestin challenge. This is the most common type of anovulation and includes women with polycystic ovary syndrome (PCOS). For women in this group with infertility, oral agents such as insulin sensitizers, selective estrogen receptor modulator (SERM) agents such as CC or, more recently, aromatase inhibitors (AI) are useful for ovulation induction.

Women in WHO group III include those with elevated gonadotropins secondary to primary ovarian failure mainly due to diminished ovarian reserve and the loss of ovarian follicles. These women are resistant to various methods of ovarian stimulation and the best approach for their anovulation-associated infertility is oocyte donation.

Injectable gonadotropin preparations

All of the injectable gonadotropin preparations on the market for fertility use contain either urinary-derived or recombinant human FSH with or without the addition of LH/hCG activity. The mechanism of these injections as fertility drugs is very simple. For anovulatory patients with WHO group I or II classifications, the administration of exogenous FSH allows FSH concentrations to reach the threshold FSH level in the circulation, and this level of FSH will stimulate the growth of follicles that have the most FSH receptors. Since estrogen negative feedback is irrelevant if exogenous FSH is administered, care needs to be taken to prevent more than the desired number of follicles from growing to the preovulatory stage. Hence, serial ultrasound monitoring is required to track follicle growth. In assisted reproductive technology (ART) cycles where more than one follicle is required, the administration of exogenous FSH is able to prolong the time FSH levels are above the FSH threshold and extend the FSH window. This addition of exogenous FSH overrides the negative

feedback effect of increasing estrogen levels and allows multiple ovulation by slightly increasing FSH and rescuing smaller follicles that would otherwise have undergone atresia because of a decreased serum level of FSH. Usually, injectable gonadotropins are started on day 2 or day 3 of the cycle at a dose ranging from 50 IU to 300 IU per day, or higher in some cases. In women who are normal responders, generally the larger the dose of FSH, the higher the FSH concentrations are in the circulation and the higher above the FSH threshold. Consequently, the higher the dose, the smaller and more numerous are the follicles that will be rescued and continue to grow to the preovulatory size.

Based on the earlier observation that larger antral follicles develop LH receptors following FSH stimulation, Fillicori et al. suggested that exogenous LH/hCG could be used as a substitute for FSH to continue follicular maturation prior to ovulation. The advantage of this approach of completely replacing FSH with LH (or microdose hCG) is that only follicles large enough to have LH receptors will respond, while smaller follicles are unable to keep growing and will undergo atresia. We have used this technique successfully in our clinic to prevent ovarian hyperstimulation in women who are suspected to be at risk as a result of ultrasound detection of too many follicles growing in the early follicular phase of gonadotropin-stimulated cycles.

Clomiphene citrate

For the last 40 years, the first line of treatment for WHO type II anovulation in infertile women has been CC. The choice of CC was appropriate because the drug was highly effective in inducing ovulation in appropriately selected patients, it was orally administered, relatively safe, and inexpensive. In contrast, alternative treatments usually involved parenteral gonadotropin preparations that were significantly more complicated and uncomfortable to administer, were very expensive and were associated with more frequent and more serious complications. However, CC was also found to have adverse effects, especially in the form of relatively common antiestrogenic endometrial and cervical mucous changes, that could prevent pregnancy in the face of successfully induced ovulation as discussed below. Since the early 1960s, pregnancy rates with CC treatment have not changed appreciably, despite the advent of modern immunoassays for steroid hormones, advances in ultrasound technology for cycle monitoring, and the introduction of commercial ovulation predictor kits that allow accurate identification of the mid-cycle LH surge. It is also interesting to note that CC is considered pregnancy risk category X.

Pharmacokinetics of clomiphene citrate

Like other SERM compounds (e.g., tamoxifen), CC exhibits both estrogen agonist and antagonist properties. CC is a racemic mixture of two distinct stereoisomers, enclomiphene and zuclomiphene, having different properties. Available evidence indicates that enclomiphene is the more potent antiestrogenic isomer and the one primarily responsible for the ovulation-inducing actions of CC. Levels of enclomiphene rise rapidly after administration and fall to undetectable concentrations after a few days. Zuclomiphene is cleared far more slowly; levels of this less active isomer remain detectable in the circulation for more than 1 month after treatment and may actually accumulate over consecutive cycles of treatment. The SERM activity of CC depends on the prevailing levels of endogenous estrogen. Estrogen agonist properties are manifest only when endogenous estrogen levels are extremely low. Otherwise, CC acts mainly as an antiestrogen.

Mechanism of action of clomiphene citrate

CC binds to estrogen receptors (ER) throughout the body because of its structural similarity to estrogen. With its long tissue half-life, extended binding of CC depletes ER concentrations by interfering with the normal process of ER replenishment. The antiestrogenic effect on the hypothalamus, and the pituitary is believed to be the main mechanism of action of CC for ovarian stimulation. Depletion of hypothalamic ER leads to inability of circulating estrogen concentrations to impact central feedback mechanisms, leading to reduced estrogen negative feedback on gonadotropin releasing hormone (GnRH) production by the hypothalamus and subsequent increased gonadotropin (FSH and LH) secretion by the pituitary. The rise of FSH

promotes growth of ovarian follicles and ovulation in anovulatory women. It is believed that the hypothalamus is the main site of action because in normally ovulatory women CC treatment was found to increases GnRH pulse frequency. However, actions at the pituitary level may also be involved since CC treatment increased pulse amplitude, but not frequency in anovulatory women with PCOS, in whom the GnRH pulse frequency is already abnormally high.

During CC treatment, levels of both LH and FSH undergo a prolonged rise compared to a natural cycle because of the prolonged ER depletion by CC in the brain. Supraphysiologic levels of estrogen can occur without central suppression of FSH, since the normal ER-mediated feedback mechanisms are blocked as a result of prolonged ER depletion (Figure 11.1). This perturbation of normal negative feedback is reflected in the FSH window being extended leading to multiple follicle growth and a higher multiple pregnancy rate with CC than occurs in natural cycles.

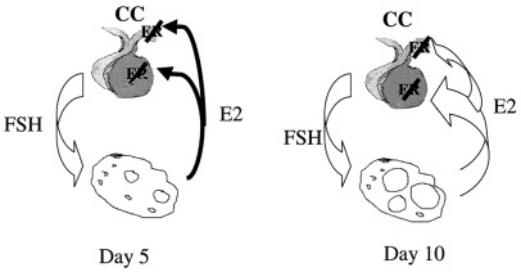

Figure 11.1 Day 5: Administration of CC from days 3 to 7 results in ER depletion at the level of the pituitary and mediobasal hypothalamus. As a result, estrogen negative feedback centrally is interrupted and FSH secretion increases from the anterior pituitary leading to multiple follicular growth. (Day 10) By the late follicular phase, because of the long tissue retention of CC, there continues to be ER depletion centrally and increased E2 secretion from the ovary is not capable of normal negative feedback on FSH. The result is multiple dominant follicle growth and multiple ovulation. CC, clomiphene citrate; E2, estradiol; ER, estrogen receptor. Reproduced from Casper 2003, with permission from Elsevier. Copyright American Society for Reproductive Medicine, Inc.

Indications for clomiphene citrate treatment

The two main indications for CC treatment are induction of ovulation in anovulatory infertility and for stimulating multifollicular ovulation or enhancing ovulation in ovulatory infertile women (i.e., unexplained infertility). For anovulatory infertility, CC treatment will likely be effective only in conditions in which adequate levels of circulating estrogen exist to exert estrogen negative feedback on gonadotropin production, which in turn could be antagonized by the antiestrogenic effect of CC as explained above. CC is the initial treatment of choice for most anovulatory or oligo-ovulatory infertile women who are euthyroid and euprolactinemic while having adequate circulating levels of estrogen (WHO type II anovulation, e.g., PCOS). On the other hand women with very low circulating estrogen levels such as (WHO type I and III) or women with a defective hypothalamic–pituitary axis, such as in Sheehan syndrome and Kallmann syndrome, will not respond to CC treatment. For women with ovulatory infertility, CC is believed to enhance chances of achieving pregnancy by stimulating multifollicular development as well as alleviating possible subtle endocrine dysfunction, although there is a risk of multiple-gestation pregnancy.

Regimens of clomiphene citrate administration

CC is administered orally, usually starting on day 2–5 after the onset of spontaneous or progestin-induced menses. Treatment typically begins with a single 50-mg tablet daily for five consecutive days, increasing by 50-mg increments in subsequent cycles until ovulation is induced. After ovulation is achieved, the same dose is repeated until pregnancy is achieved or a maximum number of around six cycles is reached. Once the effective dose of CC is established, there is no indication for further increases in dose unless the ovulatory response is lost. Higher doses will not improve the probability of conception, but the risk of hyperstimulation and multiple-gestation pregnancy may increase.

Outcome of clomiphene citrate treatment

Classically, CC treatment has been reported to successfully induce ovulation in 60–80% of properly selected candidates. More than 70% of those

who ovulate respond at the 50-mg or 100-mg dosage level. Cumulative conception rates between 60% and 70% are observed after three successfully induced ovulatory cycles, and 70–85% after five. Overall, cycle fecundity is approximately 15% in women who ovulate in response to treatment. It is important to realize that these figures apply to young women in whom anovulation is the sole reason preventing them from conceiving. In the reality of daily clinical practice, such a group of patients does not frequently exist, particularly in a subspecialty referral infertility practice, in which much lower pregnancy rates are observed with CC induction of ovulation. Age, presence of other infertility factors, treatment history, and duration of infertility are important factors affecting treatment outcome. Amenorrheic women are more likely to conceive than oligomenorrheic women, probably because those who already ovulate, albeit inconsistently, are more likely to have other coexisting infertility factors. Generally speaking, failure to conceive within six CC-induced ovulatory cycles should be regarded as a clear indication to expand the diagnostic evaluation to exclude other factors or to change the overall treatment strategy when evaluation is already complete.

EVIDENCE REVISITED

Using clomiphene citrate for ovulation induction, cumulative conception rates of 60–70% are observed after three successfully induced ovulatory cycles, and 70–85% after five cycles.

Adverse effects and risks of clomiphene citrate treatment

CC is generally well tolerated. Adverse effects are generally divided into the side effects related to CC itself and the adverse effects related to induction of ovulation in general. Hot flashes due to the antiestrogenic property of CC are dose-dependent and occur in approximately 10% of CC-treated women. Symptoms are transient and typically resolve soon after treatment ends. Visual disturbances, including blurred or double vision, scotomata, and light sensitivity, are generally uncommon (<2% prevalence) and reversible. Less specific side effects include breast tenderness, pelvic discomfort, and nausea, all observed in 2–5% of CC-treated women. In addition, we have noted relatively common reports of premenstrual syndrome-type symptoms such as irritability and depression in women on CC. Another side effect of CC that must be considered is the risk of multiple gestation, mainly twins, estimated to be between 8% and 13% of pregnancies. This side effect is related to the prolonged action of CC in the brain with delayed suppression of FSH by rising estrogen concentrations as outlined above.

CAUTION

The risk of multiple gestation, mainly twins, is a complication of clomiphene citrate that must be considered. Multiple gestation is estimated to occur in 8–13% of pregnancies resulting from clomiphene citrate treatment.

Adverse antiestrogenic effects associated with clomiphene citrate treatment

Because of the relatively long half-life of CC isomers, CC may exert undesirable and unavoidable antiestrogenic effects on peripheral estrogen targets (endocervix and endometrium) that likely explain the absence of pregnancy despite ovulation observed in some CC-treated patients. Numerous studies in women and in various model systems have described adverse effects on the quality or quantity of cervical mucus, and endometrial growth and maturation. It is generally believed that these effects have distinct clinical consequences that are most apparent at higher doses or after longer durations of treatment. The endometrium is believed to be one of the most important targets of the antiestrogenic effect of CC and may be important for the lower pregnancy rate with CC. A reduction in endometrial thickness below 6 mm, the level thought to be needed to sustain implantation, was found in up to 30% of women receiving CC for ovulation induction or for unexplained infertility.

EVIDENCE REVISITED

It is thought that to sustain implantation the endometrium needs to be at least 6 mm thick. In up to 30% of women receiving clomiphene citrate treatment for ovulation induction or for unexplained infertility the endometrial thickness was less than this.

Pregnancy outcome with clomiphene citrate

There is no evidence that CC treatment increases the overall risk of birth defects or of any specific malformation. Several large series have examined the question and have drawn the same conclusion. A small study of pregnancy outcome in women inadvertently exposed to CC during the first trimester also found no increase in the prevalence of congenital anomalies. However, CC is classified as a pregnancy category X drug and this is particularly important when considering its relatively long clearance half-life of about 5 days to 3 weeks (depending on the isomer), and that CC may be stored in body fat. A more recent study by Tulandi et al. found a significantly increased risk of cardiac anomalies in newborns following ovulation induction or augmentation with CC.

Aromatase inhibitors

In our tertiary referral center for infertility, we frequently identified women, especially with unexplained infertility, who had failed to conceive with up to 1 year of CC treatment cycles prior to referral. Almost universally, we discovered an endometrial thickness less than 6 mm on transvaginal ultrasound. As a result of the antiestrogenic effects of CC on the endometrium in these and other women, we attempted development of a novel oral ovulation method that would be free of these antiestrogenic side effects. Aromatase was identified as a potential target for suppressing estrogen negative feedback centrally and the availability of new specific AI led to initial studies with these drugs.

Pharmacokinetics of aromatase inhibitors

Aromatase is a microsomal cytochrome P450 hemoprotein-containing enzyme (P450arom, the product of the *CYP19* gene). Aromatase catalyzes the rate-limiting step in the production of estrogens, that is, the conversion of androstenedione and testosterone via three hydroxylation steps to estrone and estradiol respectively. Aromatase activity is present in many tissues, such as the ovaries, the brain, adipose tissue, muscle, liver, breast tissue, and malignant breast tumors. The main sources of circulating estrogens are the ovaries in premenopausal women and adipose tissue in postmenopausal women.

The third-generation AIs commercially available include two nonsteroidal preparations, anastrozole and letrozole, and a steroidal agent, exemestane. These AIs are available for clinical use in North America, Europe, and other parts of the world for treatment of postmenopausal breast cancer. Letrozole and anastrozole are triazole (antifungal) derivatives that are reversible, competitive AIs and at doses of 1–2.5 mg/day they inhibit estrogen levels by 97–99%. AIs are completely absorbed after oral administration with mean terminal half-life of approximately 45 h (range 30–60 h) with clearance from the systemic circulation mainly by the liver. Mild gastrointestinal disturbances account for most of the adverse events, although these have seldom limited therapy. Other adverse effects—asthenia, hot flashes, headache, and back pain—were based on studies in postmenopausal women. Exemestane is a steroidal, suicide inhibitor of aromatase with a circulating half-life of approximately 9 h but potentially a longer effect to inhibit aromatase because it is irreversible.

Induction of ovulation with aromatase inhibitors

We postulated that it would be possible to block estrogen negative feedback, without depletion of ER as occurs with CC, by administration of an AI in the early part of the menstrual cycle. Both circulating estrogen (produced mainly by the ovarian follicles and peripheral conversion of androgens in fat and other tissues) and locally produced estrogen in the brain exert negative feedback on the release of gonadotropins. Inhibition of aromatization will block estrogen production from all sources and release the hypothalamic–pituitary axis from estrogenic negative feedback. The resultant increase in

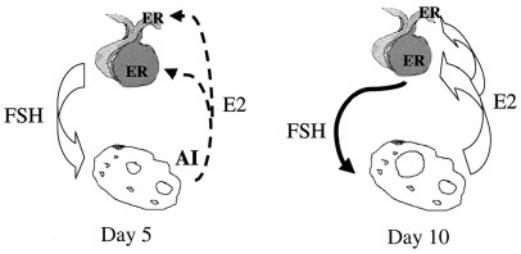

Figure 11.2 Day 5: Administration of an AI from days 3–7 results in suppression of ovarian E2 secretion and reduction in estrogen negative feedback at the pituitary and mediobasal hypothalamus. Increased FSH secretion from the anterior pituitary results in stimulation of multiple ovarian follicle growth. (Day 10) Later in the follicular phase, the effect of the AI is reduced and E2 levels increase as a result of follicular growth. Because AIs do not affect ER centrally, the increased E2 levels result in normal negative feedback on FSH secretion and follicles less than dominant follicle size undergo atresia, with resultant monofollicular ovulation in most cases. AI, aromatase inhibitor; E2, estradiol; ER, estrogen receptor. Reproduced from Casper 2003, with permission from Elsevier. Copyright American Society for Reproductive Medicine, Inc.

gonadotropin secretion will stimulate growth of ovarian follicles (Figure 11.2).

The selective nonsteroidal AIs have a relatively short half-life (approximately 45 h) compared to CC, and would be ideal for ovulation induction since they are eliminated from the body rapidly. Because AIs do not deplete ERs, as does CC, normal central feedback mechanisms remain intact. As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally resulting in suppression of FSH and atresia of the smaller growing follicles. A single dominant follicle, and mono-ovulation, should occur in most cases (Figure 11.2).

In women with PCOS, relative oversuppression of FSH may be the result of excessive androgen produced from the ovary being converted to estrogen by aromatization in the brain. The AIs suppress estrogen production in both the ovaries and the brain. In the case of PCOS, therefore, AIs should result in a robust increase in FSH release and subsequent follicle stimulation and ovulation. Since aromatase inhibition does not antagonize ER in the brain, the initiation of follicle growth accompanied by increasing concentrations of both estradiol and inhibin results in a normal negative feedback loop that limits FSH response, thereby avoiding the risk of high multiple ovulation and ovarian hyperstimulation syndrome (OHSS). We demonstrated the efficacy of the AI letrozole to induce ovulation in women with PCOS for the first time in 2000.

Peripheral mechanism of action of aromatase inhibitors

AIs may also increase follicular sensitivity to FSH. This could result from temporary accumulation of intraovarian androgens, since conversion of theca cell androgen to estrogen is blocked by aromatase inhibition. Recent data support a stimulatory role for androgens in early follicular growth in primates. Testosterone was found to augment follicular FSH receptor expression in primates suggesting that androgens promote follicular growth and estrogen biosynthesis indirectly by amplifying FSH effects. Also, androgen accumulation in the follicle stimulates insulin-like growth factor 1 (IGF-1) which may synergize with FSH to promote folliculogenesis. It is likely that women with PCOS already have a relative aromatase deficiency in the ovary leading to increased intraovarian androgens that leads to the development of multiple small follicles responsible for the multifollicular morphology of the ovaries. The androgens, as described above, may also increase FSH receptors making these PCOS ovaries exquisitely sensitive to an increase in FSH either through exogenous administration of gonadotropins (hence the high risk of OHSS), or through endogenous increases in FSH as a result of decreased central estrogen feedback induced by aromatase inhibition. In the latter case, a relatively small rise in FSH generally leads to monofollicular ovulation, thus avoiding the occurrence of OHSS.

Indications for aromatase inhibitors in induction of ovulation

As a result of the proposed mechanisms of action of the AIs described above, we proposed that AIs could be used alone for induction of ovulation, or

as an adjuvant in conjunction with exogenous FSH or other medications to improve the outcome of ovulation induction. A major advantage of an AI is the ability to achieve restoration of monofollicular ovulation in anovulatory infertility, PCOS for example, as a result of the intact estrogen negative feedback loop as described above. An AI could also be used in conjunction with FSH injections to increase the number of preovulatory follicles that develop and to improve the outcome of treatment in cases of ovulatory infertility such as unexplained or endometriosis-associated infertility. An increase in intraovarian androgen concentrations during aromatase inhibition could improve ovarian response to the addition of exogenous gonadotropins in poor responders by increasing ovarian sensitivity to FSH (peripheral mechanism of action described above).

To summarize, the AI when used alone should result in a predictable response with the development of one or two mature follicles and a significantly reduced risk for ovarian hyperstimulation and multiple gestation. To achieve multiple ovulation, the addition of FSH to the AI is likely necessary.

Optimal dose of aromatase inhibitor for repeated administration

The optimal dose of each AI is not yet clear. In most of the studies to date, the dose of letrozole (2.5 mg) or anastrozole (1.0 mg) typically used for breast cancer treatment in postmenopausal women has been chosen. A randomized study comparing 2.5 mg and 5.0 mg of letrozole in women with unexplained infertility suggested that the higher dose might be associated with more follicles developing. However, the study was not large enough to demonstrate a significant advantage. Based on current data, it is likely that the optimal dose of letrozole for a 5-day course of treatment is between 2.5 and 5.0 mg, with doses higher than 5 mg resulting in persistence of aromatase inhibition and estrogen levels too low for normal endometrial development by the time of ovulation. For anastrozole, there are not yet enough data to determine the preferred dose although it appears that the standard 1-mg dose may be too low for optimal follicle recruitment and ovulation. Another potential option for AI administration is a step-up protocol as described by Mitwally et al. In this protocol letrozole was given in an escalating dose from 2.5 mg on day 3 to 10 mg on day 6. This protocol seems to be associated with two or more follicles in many patients and may be a novel approach to mild stimulation for intrauterine insemination (IUI) cycles.

☆ TIPS & TRICKS

The optimal dose of letrozole or anastrozole to be used for ovulation induction is not yet clear. In most studies to date, the chosen dose is the one typically used for breast cancer treatment in postmenopausal women: letrozole 2.5 mg or anastrozole 1.0 mg.

Adverse effects and concerns about using aromatase inhibitors for induction of ovulation

Aromatase inhibition is associated with significantly lower serum estrogen levels at mid cycle than seen with CC. Markedly reduced to absent intrafollicular concentrations of estrogen are known to be compatible with follicular "expansion," retrieval of fertilizable oocytes, and apparently normal embryo development. The rapid clearance of the AIs, the reversible nature of enzyme inhibition, and elevated levels of FSH, which induces new expression of aromatase enzyme, are factors that limit accumulation of androgens and likely result in increasing estrogen production that should be relatively normal at the time of ovulation. Interestingly, in keeping with this hypothesis, intrafollicular estrogen levels associated with aromatase use for augmentation of ovulation in IVF were found to be similar to those with exogenous FSH treatment alone.

Safety concerns in aromatase inhibitors

Recent safety studies have found anastrozole to have no teratogenic or clastogenic effects in animal embryo development, whereas there have been some concerns regarding teratogenic effects of letrozole. Because of the short half-life of each drug, administration in the early follicular phase should result in clearance of the agents before implantation takes place. Nevertheless,

care should be taken in all cases of ovulation induction with these drugs to ensure that the patient is not pregnant prior to administration. We recommend a blood beta-hCG level be ascertained before using letrozole or anastrozole in premenopausal women.

Pregnancy outcome with aromatase inhibitors

We recently reported the clinical outcome of pregnancies obtained through the use of AIs for ovulation induction or controlled ovarian stimulation for intrauterine insemination. Pregnancies conceived after AI treatment were associated with comparable miscarriage and ectopic pregnancy rates compared to all other groups including the spontaneous conceptions. In addition, letrozole use was associated with a significantly lower rate of multiple gestation compared to CC (4.3% versus 22%), consistent with our hypothesis of an intact negative feedback loop centrally with aromatase inhibition.

Is there an increased risk of birth defects in babies born after use of aromatase inhibitors?

The question of whether letrozole use for ovulation induction is safe was raised by an abstract presented at the 2005 ASRM meeting in which a significant increase in birth defects was observed in 150 babies born after letrozole for ovulation induction compared to over 36 000 babies delivered after spontaneous conception. There were many flaws in this study which was not accepted for peer-reviewed publication. Nevertheless, Novartis issued a letter to all physicians stating that letrozole use for ovulation induction was contraindicated because of an increased risk of fetal and maternal toxicity and of birth defects.

Tulandi and colleagues followed up this issue by examining pregnancy outcome of 911 babies conceived after CC or letrozole treatment in infertile women. Overall, congenital malformations and chromosomal abnormalities were found in 14 of 514 newborns in the letrozole group (2.4%) and in 19 of 397 newborns in the CC group (4.8%). The major malformation rate in the letrozole group was 1.2% (6/514) and in the CC group was 3.0% (12/397). One newborn in the letrozole group was found to have a ventricular septal defect (0.2%) compared to 7 newborns with cardiac anomalies in the CC group (1.8%). The authors concluded that there was no difference in the overall rates of major and minor congenital malformations among newborns from mothers who conceived after letrozole or CC treatments. However, it appears that congenital cardiac anomaly is less frequent in the letrozole group. The concern that letrozole use for ovulation induction could be teratogenic appeared to be unfounded based on this data.

A second study used the Motherisk database in Toronto to select a control group of spontaneous conceptions. This comparison of the outcome of babies conceived after the use of letrozole with matched controls conceived after use of CC and a control group of babies whose mothers conceived spontaneously found that the birth weight of babies in the CC group was significantly lower than the birth weight of babies in both the letrozole group and the control group, even after controlling for maternal age and gestational age at birth, and excluding multiple gestations. A possible mechanism for the observed intrauterine growth restriction when CC is used for ovulation induction is the long half-life of the CC isomers (elimination time 5–7 days) and the possible presence of antiestrogenic effects in the endometrium and uterus causing reduced blood flow or affecting embryogenesis.

Use of aromatase inhibitors for IVF in breast cancer patients

Another reason to avoid elevated estrogen levels during ovarian stimulation is the presence of breast cancer. With the recent success of oocyte cryopreservation, some women are opting to freeze oocytes or embryos prior to chemotherapy for later use by themselves or a gestational carrier. Oktay et al. studied 33 ovarian stimulation cycles with either tamoxifen 60 mg/day alone (Tam-IVF) or in combination with low-dose FSH (TamFSH-IVF) or letrozole 5 mg in combination with FSH (Letrozole-IVF). Compared to women receiving tamoxifen there was a significant increase in the number of mature oocytes retrieved in the group receiving letrozole. However, peak estradiol levels were significantly lower with Letrozole-IVF than with TamFSH-IVF. After almost 2 years of follow-up, the cancer recurrence rate was similar in IVF patients and control patients. The authors concluded the

letrozole protocol may be preferred for IVF stimulation in women with breast cancer because it resulted in lower peak estradiol levels.

Summary

Preliminary studies have demonstrated that AIs are effective for ovulation induction or augmentation of ovulation in infertile women. Based on the evidence reviewed above, these oral agents seem to be efficient and safe and have many advantages compared to CC. We believe that a major advantage of AIs for ovulation induction is mono-ovulation. Especially in PCOS patients, who are often hyper-responsive to gonadotropins, a drug that consistently results in a single ovulation is very desirable. In addition, because negative effects peripherally on the endometrium are absent, a second advantage of AIs for ovulation induction is that minimal (if any) ultrasound monitoring needed for endometrial thickness. Both of these advantages allow primary care providers or community-based gynecologists, without ready access to ultrasound monitoring, to participate in the management of PCOS and other anovulatory women.

In IUI cycles, AIs alone are probably not the optimal choice since, generally speaking, it is preferable to see two or three mature follicles developing, depending on the patient's age. In order to ensure multiple ovulation in IUI cycles, the addition of a low dose of FSH to the AI is required although FSH can be delayed (sequential protocol) until estradiol levels begin to rise and when the exogenous FSH administration would override negative feedback occurring centrally. This type of protocol does not accelerate follicle development and ovulation occurs normally around cycle day 14. There is adequate time for endometrial growth to occur normally and a small dose of gonadotropin is required, generally in the range of 50–75 IU daily. These advantages make AIs a viable option to replace CC in the future as the new primary treatment for ovulation induction and in combination with FSH for assisted reproduction procedures.

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12

Intrauterine Insemination

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Introduction

Intrauterine insemination (IUI) is one of the simplest and most commonly performed assisted conception methods that can be used to alleviate infertility in selected infertile couples.

It is performed by using a very thin catheter through the uterine cervix to inject washed sperm directly into the uterus. The rationale behind the use of IUI is to increase the number of highly motile, morphologically normal-appearing spermatozoa near the site of fertilization in the upper reproductive tract of the female partner. Several different techniques have been used for IUI. The original technique used for over a century was intravaginal insemination, where semen sample that did not involve any preparation or processing (unprocessed) was placed high in the vagina. In the later 20th century, it was discovered that placing the semen sample into the endocervix (intracervical insemination) resulted in superior pregnancy rates when compared with vaginal insemination. The effectiveness of IUI has been clearly established in specific subsets of infertile patients such as those with idiopathic infertility, or mild male factor infertility. IUI is less invasive and less expensive when compared with other more invasive assisted reproductive technology (ART) procedures. Despite extensive research over the last two decades, there is a lack of well-designed randomized controlled trials (RCTs) in this area. Nevertheless, the best available evidence concerning the different subsections of IUI will be presented in this chapter.

Indications for IUI see Table 12.1

IUI is utilized in treating a variety of infertility causes, as long as at least one patent fallopian tube is present. Diagnoses in patients undergoing IUI generally include:

Cervical factor infertility

The cervix serves as an effective barrier against sperm, so that even slight changes in cervical mucus quality may rapidly transform the cervix into a "hostile" environment which may prevent conception. A postcoital test (PCT), performed immediately after intercourse, determines the vitality of spermatozoa under the influence of cervical secretions. Although PCT is a common tool for investigation of subfertile couples, it has limited diagnostic and prognostic characteristics. Additionally, with the increasing number of cervical operations performed for cervical dysplasia, a significant quantity of cervical glands are removed and its impact on subsequent fertility is unclear. IUI appears as a logical treatment for cervical factor hostility as it bypasses the cervix by directly depositing sperm into the uterine cavity. However, the available evidence concerning the efficiency of IUI as treatment for cervical hostility is controversial.

Male factor infertility

Forty to fifty per cent of infertility causes are attributed to male subfertility, which is therefore a major indication for IUI. Male subfertility has been defined over time with various thresholds

Infertility, 1st edition. Edited by Emre Seli.

Table 12.1 Mechanisms of infertility and possible IUI solutions

Infertility factor	IUI procedure	Possible mechanism of action
Male factor	Sperm washing and preparation	Enhance sperm motility Enhance sperm concentration Select normal sperm and increase its concentration Decrease sperm migration work Decrease sperm attrition
Female factor Cervical factor	Intrauterine insemination	Bypass cervical barrier: Mechanical barrier Immunologic barrier Bypass need of cervical mucus: Capacitation site Migration medium Increase number of sperm available for fertilization in the ampulla of the tube
Ovulatory factor	Ovarian stimulation Monitoring (ultrasound monitoring of follicular count, LH kits)	Increase number of fertilizable oocytes (mature follicles) Improve timing of insemination
Unexplained infertility	All of the above	Possibly all of the above

for various prognostic factors such as sperm concentration, total progressive motility, morphology, and total motile sperm count, with the last of these having the most predictive value when using postwash sperm. The lack of standardization of routine semen analysis renders it a poor predictor of male fertility potential. Although IUI is proposed as a first-line therapy for most oligozoospermia, asthenozoospermia, and teratozoospermia, there is unfortunately a lack of high-quality RCT studies with clinical pregnancy rates as the main outcome of interest, but results are inconclusive. It has been suggested that IUI in male subfertility would only be superior to other assisted reproductive techniques when a certain threshold value of motile sperm count is present. Most studies estimate this threshold to be equal to 1×10^6 spermatozoa after preparation, but consensus is lacking. A retrospective study of 1636 IUI cycles, by Pasqualotto et al., investigated the relationship of postwash total motile sperm count (TMSC) and percentage motile sperm to successful pregnancy rates. The relationship between successful pregnancies and postwash TMSC greater than 1 million was not statistically significant. Furthermore, there was no significant difference in successful pregnancy rates even with 20 million sperm inseminated. However, the postwash motility was found to be predictive of IUI success at a cut-off value of 40% regardless of the TMSC. A more recent prospective observational study associated IUI success to the total number of motile sperm and to normal sperm morphology. There is a lack of RCTs in this area, and evidence is weak.

Sexual and ejaculatory dysfunction

Sexual dysfunction, such as severe vaginismus in the female or erectile dysfunction in the male, is more frequent than once thought and should be specifically inquired about in the clinical evaluation of infertility. Fortunately, medical and psychological treatments available for these conditions may reduce the need for ART. Ejaculatory dysfunction interferes with the capacity to deposit semen into the vagina during intercourse. In patients suffering from retrograde ejaculation, when antegrade ejaculation cannot be obtained, artificial insemination using sperm

recovered from previously alkalinized urine is indicated. Anejaculatory men with spinal cord injury can also be treated effectively with electroejaculation. Hypospadias can be severe enough to interfere with deposition of semen in the vagina. If this cannot be treated, IUI using washed sperm can be proposed to the couple.

Unexplained infertility

According to the United Kingdom National Institute for Health and Clinical Excellence (NICE), as a general rule, 10% of infertile couples end up with a diagnosis of unexplained infertility after a complete infertility work-up. Theories of etiology include subtle ovulatory disorders, cervical factors, and male factors not identified by the usual work-up. According to the NICE guidelines, these couples should be offered IUI treatment before resorting to other ART techniques. Live births rate in couples with unexplained infertility has been shown to significantly improve following ovarian hyperstimulation.

Endometriosis

Patients with mild or minimal endometriosis (stages I and II) and infertility may benefit from IUI. In more severe cases, especially when the tubes are involved, going directly to other ARTs should be considered.

Work-up before IUI

The work-up is similar to the one offered to any infertile couple for investigating the etiology of infertility. It comprises of semen analysis for the man, and baseline ultrasound, ovarian reserve follicle stimulating hormone (FSH), estradiol monitoring of ovulatory function, and hysterosalpingogram for the woman. Laparoscopy with chromotubation retains certain indications: although it is frequently bypassed, it can sometimes reveal and treat certain abnormalities such as tubal adhesions or minimal endometriosis, thus increasing the chances of achieving spontaneous pregnancy. Other factors attributing to infertility or reducing the chances of IUI success (such as endometrial polyps or ovarian cysts in females or genitourinary infections in males) might be discovered and treated during this evaluation, thus increasing the chances of spontaneous pregnancy.

Contraindications

Some of the contraindications related to pregnancy outcome are:

- Rhesus incompatibility
- Medical conditions which could endanger the patient's life
- Partner with a hereditary disease which could result in an offspring with a significant disability

Other contraindications related to the IUI technique are:

- Cervical neoplasia
- Active cervical, intrauterine, or pelvic infection, or evidence of a sexually transmitted disease at the time of IUI in either partner

Natural cycles or controlled ovarian hyperstimulation?

Natural cycles

Natural or unstimulated cycles combined with IUI present clear advantages of low cost and absence of additional risk(s) from drug treatment. They are an option for patients with ejaculatory dysfunction, vaginismus, or cervical factors. Ovulation is documented by detecting the urinary luteinizing hormone (LH) surge with over-the-counter ovulation predictor kits starting on day 10 of the cycle. Ovulation usually occurs 24–36 h after the urine LH surge, indicating the right moment for IUI. These dipstick LH kits have more than 80% positive predictive value in predicting ovulation. Both basal body temperature measurement and assessment of the cyclical changes of cervical mucus have been found unreliable for predicting timing of insemination. Ultrasonography, scanning for ruptured follicles, is the most precise method of confirming ovulation.

Controlled ovarian hyperstimulation

Controlled ovarian hyperstimulation (COH) is suitable for couples with unexplained infertility, male subfertility, cervical factors, or early stage endometriosis. Clomiphene citrate (CC), an antiestrogenic agent, with its low cost and lesser side effects, is popular as the standard first-line therapy, particularly in oligo-anovulation, such as in polycystic ovary syndrome (PCOS), espe-

cially since it does not require extensive monitoring and is associated with lower incidence of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy than gonadotropins. CC is started at day 2–5 of the cycle with urine LH testing starting at day 10–12. IUI is performed 12–48 h after documenting the LH surge. Almost 75% of the pregnancies achieved with CC occur within the first three cycles of treatment.

In a recent systematic review, CC and IUI proved to be an effective early treatment option in couples with ovulatory infertility, resulting in a significantly four to fivefold higher pregnancy rates in comparison to natural cycles and timed intercourse. Clomiphene-resistant patients are identified after a course of three cycles and shifted to injectable gonadotropins.

Aromatase inhibitors have been suggested as an alternative treatment and are under investigation (see Chapter 11). Letrozole, the best known aromatase inhibitor, has been shown to be effective in early trials, in inducing ovulation and pregnancy in women with anovulatory PCOS and inadequate clomiphene response. However, further studies are needed to confirm its efficacy and safety. Injectable gonadotropins are started at day 2 after documenting ovarian suppression. Human chorionic gonadotrophin (hCG) is given as a subcutaneous or intramuscular injection to trigger ovulation when at least one follicle reaches 15–18 mm diameter, as detected by serial transvaginal ultrasonography. hCG or recombinant LH has replaced the physiological LH surge and ovulation occurs 34–36 h after injection, indicating the timing of insemination. Human menopausal gonadotropin (hMG), human urinary FSH (hFSH) with or without LH, or recombinant FSH (rFSH) are all possible protocols when it comes to injectable gonadotropins. Recent studies have shown that low-dose regimens of gonadotropins retain the same efficacy for clinical pregnancy rate as classical regimens, with the advantage of reducing the incidence of multiple pregnancies. The available data failed to demonstrate an increase in pregnancy rate with the adjunction of gonadotropin releasing hormone (GnRH) agonists or antagonists. The latter are reserved to women who showed evidence of premature luteinization in previous cycles.

Counseling of patients before starting COH in IUI cycles is of paramount importance and has to clarify that the higher pregnancy rates to be expected are counter-balanced by the increased cost and with potential complications such as OHSS and multiple pregnancy. On the other hand, these complications might be prevented by close monitoring of follicular growth and endometrial development once COH is started by serial pelvic ultrasound and regular dosage of plasma estradiol, LH, and progesterone.

EVIDENCE AT A GLANCE

Natural cycles versus controlled ovarian hyperstimulation

For all pooled infertility causes, randomized controlled trials clearly show that controlled ovarian hyperstimulation (COH) combined with IUI generally improved the probability of conception compared with IUI in the natural cycle. In couples with unexplained infertility, IUI combined with COH results in significantly higher conception rates compared to IUI alone, independent of the drug used. While some studies demonstrated better cumulative pregnancy rate (CPR) with rFSH as compared to urinary FSH and human menopausal gonadotropin (HMG), other failed to show a significant difference between rFSH + IUI, CC + IUI, letrozol + IUI or HMG + IUI in couples with unexplained infertility. In contrast, conception rates were only significantly increased when IUI was combined with gonadotropins instead of clomiphene citrate (CC) in couples with male subfertility. Furthermore, conception rates were only higher in those subgroups with total motile sperm count of more than 10×10^6. In couples with ovulatory infertility, CC combined with IUI is an effective first-line therapy, causing a four to fivefold increase in CPR compared to natural cycles and timed intercourse, but comparison of gonadotropins with IUI to CC with IUI yielded a three-fold increase in CPR.

A systematic review of randomized trials of women undergoing IUI noted significantly higher pregnancy rates when gonadotropin rather than CC was used in combination with IUI (OR = 1.8,

CI = 1.2–2.7). This review did not demonstrate any efficacy of one gonadotropin over another, nor did it show the usefulness of adding a GnRH agonist or antagonist to a gonadotropin +IUI cycle. The latter are reserved for women who show evidence of premature luteinization in previous cycles.

A meta-analysis comparing the outcome of natural cycle IUI to IUI combined with ovarian stimulation for male subfertility showed that there is limited evidence to support the use of gonadotrophins, especially in case of severe semen defect. In other causes of subfertility, higher pregnancy rates may be achieved when IUI is combined with gonadotrophins. However, the benefit of increasing pregnancy rates has to be weighed against the additional concomitant risk of multiple pregnancies. Large RCTs comparing natural cycle IUI with stimulated IUI for all causes of infertility are needed.

Semen parameters and processing

Predictive semen parameters

Identifying individual semen characteristics that are useful in predicting positive pregnancy outcome after IUI is difficult. This can be explained by a lack of standardization of routine semen analysis as well as by the other variables implicated in the technique, such as patient selection, type of ovarian induction, etc. However, it is clear that semen quality influences the effectiveness of IUI, and it seems certain that there is a threshold below which IUI may not be successful.

Following sperm preparation, total motile sperm (TMS) count per insemination and sperm morphology using strict criteria seem to be the two most important sperm parameters influencing IUI outcome. However, a significant overlap of sperm characteristics among fertile and infertile men has recently been reported and sperm motility and concentration were shown to be more accurate than sperm morphology (both WHO morphology and Tygerberger's strict criteria). Currently, there is no universal threshold level below which IUI cannot be performed with acceptable pregnancy rates. Nevertheless, IUI success is seriously impaired with less than 5% normal spermatozoa and a TMS of less than 1×10^6. A significant improvement in pregnancy rates has been reported for strict criteria morphology threshold of 4% and total sperm motility cut-off between 30% and 50%. Hypo-osmotic swelling test (>50%) and sperm DNA fragmentation (<12%) are two other parameters influencing pregnancy rates after IUI. Furthermore, DNA damage by sperm chromatin structure assay and the measurement of reactive oxygen species provide potential fertility markers in clinical practice. Antisperm antibodies (ASA) are another factor affecting fertility by obstructing penetration of spermatozoa into the cervical mucus or by interfering with sperm binding and penetration of the zona pellucida. It has been suggested that ASA be tested routinely in infertility work-ups. Biochemical tests, electron microscopy, microarray analysis of transcriptome, proteomics, and metabolomics are all emerging technologies that promise future advances in the targeted diagnosis and management of male factor infertility (see Chapter 8).

Normal semen parameters continue to be defined by the WHO criteria, which have shown little prognostic value, and a number of authors have proposed revising the WHO cut-off values. New WHO reference values for human semen characteristics have been published recently. The data presented characterizes global semen characteristics of fertility in a number of countries and is meant to provide an appropriate tool in conjunction with clinical data to evaluate a patient's semen quality and prospects for fertility. According to this report, lower reference limits are:

- Total sperm number — 39 million/ejaculate
- Sperm concentration — 15 million/mL
- Progressive motility — 32%
- Total motility — 40%
- Morphologically normal forms — 4%

Studies are now necessary to determine the predictive value of these thresholds.

Sperm preparation techniques

Semen preparation is necessary to separate the normal sperm from the rest of the sample comprising of debris of the ejaculate and obtain as many normal motile sperm as possible. Washing procedures remove seminal plasma, prostaglandins, infectious agents, and antigenic proteins, and eliminate nonmotile spermatozoa, leukocytes, and immature germ cells, which in turn reduces the production of reactive oxygen species and release of cytokines. These substances negatively influence the ability of normal spermatozoa to fertilize the egg. The final result is subsequent improvement in fertilizing capacity of the sperm both in vitro and in vivo. RCTs comparing prepared sperm to unprepared first-split ejaculates showed that semen preparation significantly increased the probability of conception after IUI.

Four main groups of sperm preparation procedures are available. First of all, the selection procedure may be based on the ability of the spermatozoa to swim. A culture medium is layered over the liquefied semen from which motile spermatozoa are allowed to swim up into the culture medium. The upper part of the layered medium is then removed for further use. This is known as the "swim-up technique".

Second, spermatozoa can be selected by use of density gradients. A semen sample (1–2 mL) is carefully layered on top of the upper phase of the density gradient. The gradients are prepared by carefully layering 2 mL each of lower phase (90–95% gradient) and upper phase (45–47% gradient). The tube is then centrifuged at 1600 rpm for 20 min, which separates spermatozoa according to their density and motility. The supernatant and the gradients are carefully removed and the pellet containing the highly motile, morphologically normal sperm is washed again by adding sperm wash medium and centrifuged again for 7 min at 1600 rpm. The clean pellet is resuspended in 0.5 mL of sperm wash medium and used for insemination.

The third method is the conventional wash method where the semen sample is diluted with a medium, then centrifuged. The pellet is then resuspended in medium and incubated until the time of insemination. The fourth method relies on filtration of sperm through a glass wool column.

There is no general consensus as to the best sperm preparation technique for IUI. In general, all the previously described techniques effectively produce adequate sperm samples. The preparation techniques most commonly used today are the double density gradient centrifugation and the glass wool filtration sperm washing techniques. These techniques have been shown by some studies to improve the number of morphologically normal spermatozoa with "grade A" motility and normal chromatin condensation in the prepared sample, as compared to the swim-up technique. However, a meta-analysis considering the clinical outcomes after IUI concluded that the available data from RCTs was insufficient to recommend any one of the semen preparation techniques over another. Advanced sperm selection methods according to their ultrastructural morphology, surface charges (by electrophoresis), or molecular characteristics are currently being developed. A number of in-vitro additives aimed to stimulate sperm function and protect them from reactive oxygen species have been recently studied such as pentoxifylline, xanthines, bicarbonate, metal chelators, prostaglandins, kinin-enhancing drugs and platelet activating factor, but further clinical studies are needed to prove their clinical value in the IUI setting.

☆ TIPS & TRICKS

Semen collection

A semen specimen is best produced in the morning after 48–72 h of abstinence. Ejaculate should be collected into a sterile, wide-mouthed cup, to minimize the risk of subsequent uterine infections. The donor should be instructed that lubricants should not be used as most lubricants are toxic to sperm. The sperm donor should be aware of the ethical and legal implications of his donation before sperm is collected.

SCIENCE REVISITED

Infection transmission and IUI

In conditions where insemination with the partner's sperm is not possible, insemination with donor sperm is recommended. This implies thorough evaluation of all potential sperm donors to avoid unintentional transmission of sexually transmitted diseases or known genetic syndromes. Medical records, personal history, and family history should be reviewed for all donors and they should be submitted to a physical examination. It is of paramount importance to determine normal semen parameters and to perform blood grouping and karyotyping. Screening for risk factors and clinical evidence of communicable diseases should be performed. These include HIV types 1 and 2, human T-lymphotropic virus types I and II, hepatitis B and C (HBV, HCV), cytomegalovirus, human transmissible spongiform encephalopathy (including Creutzfeldt–Jakob disease), *Treponema pallidum*, *Chlamydia trachomatis*, and *Neisseria gonorrheae*. All donor semen samples are cryopreserved and quarantined for 6 months. The donor is retested before his sample is used for insemination, and then his eligibility is determined.

It is also recommended that couples receive systematic screening before undergoing an assisted reproductive technique (ART), so that serodiscordant couples are managed accordingly, knowing that it has been suggested by the ethics committees of both the American College of Obstetrics and Gynecology and the American Society for Reproductive Medicine that infection with HIV should not deprive from the right to be treated for infertility.

Despite some evidence that sperm washing techniques reduce the risk of contamination, few cases of contamination have been reported and there is no unanimous agreement on the ability of sperm to serve as a vector for HIV, which is why safety remains a concern. The Centers for Disease Control and Prevention recommends against insemination with semen from HIV-infected men. Little is known about HBV and HCV, for which techniques of preparation and postpreparation are under investigation.

IUI in practice: description of the procedure

Patient preparation

Proper identification of the patient in the clinic with the processed semen sample collected from the laboratory is essential, as are the markings on the test-tube. Ideally, the procedure should be carried out in a dedicated area. The patient should be undressed from the waist down and lie on an examination table in dorsal lithotomy/modified lithotomy position with her feet in stirrups to provide adequate exposure for insemination. The vulva is cleaned with normal saline and sterile drapes put in place. The cervix is exposed by inserting a bivalved speculum and the cervix is in turn cleansed with normal saline.

Technique

The processed sperm and its suspension media are aspirated into a 1-mL syringe with the attached insemination catheter. It is important to attach the catheter before loading the sample to avoid the problem of dead space in the catheter, which could be significant compared to the insemination volume. Then the catheter is inserted through the cervical os and into the uterus to a depth of approximately 6–7 cm so that the tip of the catheter rests just short of the fundus. Touching the fundus would result in cramping or bleeding, both toxic to embryo development. In difficult inseminations, rigid stylets, tenaculum or abdominal ultrasound guidance should be considered. The catheter is withdrawn after slow injection of the sperm over 60–80 s.

Postprocedure care

Patients lie supine (or in reverse Trendelenburg position) for 10 min, after which they can resume their normal activities. They are then instructed to do a serum beta-hCG evaluation 14 days from the first insemination. Clinical pregnancy is then confirmed by transvaginal ultrasound.

IUI protocols

Single versus double insemination

An important issue concerning IUI is whether one or two inseminations should be done in each IUI cycle. The rationale behind it is that ovulation of oocytes does not occur in a synchronized pattern after hCG administration, but rather in waves of release. Single insemination is usually performed 34–36 h after the LH surge or after the hCG injection. Double insemination protocols are less standardized, with studies reporting inseminations at 12 then 36 h, 18 then 42 h, or 18–24 then 36–48 h. The double insemination increases sperm availability during the periovulatory window, which is still to be determined. Although many studies showed no difference between single and double insemination, others showed a significant increase in pregnancy rates in couples with mild male factor subfertility (with sperm counts <10–20 million), but not in couples with unexplained subfertility; a Cochrane meta-analysis revised in 2008 conclude that the data was insufficient. Double insemination also seems to be more effective when more dominant follicles are available (three dominant follicles >15 mm compared with a mean of 1.7 dominant follicles in the studies that did not report a significant difference between single and double insemination) but this may also imply more aggressive ovarian stimulation leading to more side effects such as multiple pregnancies and OHSS.

IUI versus timed intercourse

Most of the current available data show that IUI is more effective than timed intercourse (TI) either in couples with unexplained infertility or with male subfertility. Despite its low invasiveness and cost, IUI should only be used if the probability of conception is improved significantly in comparison to the natural chance of conceiving. In a review by Cohlen et al. that included 3662 completed cycles, IUI was shown to improve the probability of conception in natural cycles compared with TI (OR 2.43) regardless of the type of infertility. Similarly, in cycles with COH, IUI demonstrated significantly higher pregnancy rates than TI (OR 2.14). Additionally, the comparison of IUI in COH cycles with TI in natural cycles showed the odds of conception to be 6.23. These conclusions were further supported by a more recent review.

Number of cycles

Most (97%) of the cumulative pregnancy rate (CPR) reported in most studies was obtained in the first four treatment cycles. No evidence from the available data shows benefit beyond the sixth cycle. The recommendation is therefore to offer four to six cycles of IUI with or without COH to the infertile couples who are eligible for IUI (see indications above). If pregnancy is not achieved by the fourth to sixth cycle, the couple should then be offered IVF.

Intrauterine versus other forms of artificial insemination

Intracervical insemination

Intracervical insemination (ICI) involves deposition of sperm at the external cervical os. In contrast with IUI, in which the sperm to be used needs preparation in the laboratory, leading to a loss of sperm during the process, ICI does not require in-vitro sperm processing, making it cheaper. ICI is also thought to be less invasive and safer, as it does not involve manipulation with an intrauterine catheter, possibly leading to the introduction of foreign material to the uterus. ICI could potentially be a better alternative if its effectiveness is proven. On the other hand, IUI may be more effective than ICI as the sperm bypasses the cervical mucus (attrition phenomenon) and is deposited closer to the fallopian tubes, which may increase the number of sperm reaching the site of fertilization. Few true RCTs comparing the efficacy of these two techniques are available. Nevertheless, the available evidence shows that pregnancy rate outcomes remain significantly better for IUI than for ICI. This is true for both natural and stimulated cycles. Additionally, there is no evidence of significant differences for any adverse outcomes of IUI as compared to ICI.

Direct intraperitoneal insemination

Direct intraperitoneal insemination (DIPI) has been suggested by some authors for infertile couples after failure of IUI + COH and before

going to IVF. The technique consists in injecting the processed sperm directly into the cul-de-sac at both tubal fimbriae where they will reach the fertilization area by their own motility and with peritoneal movements and will be picked up with the extruded oocyte. The procedure is done under transvaginal sonographic guidance. However, the procedure is very invasive and most of the available data did not show significant differences in CPR by DIPI as compared to IUI in the different categories of subfertility. It could still be useful in certain cases of severe cervical stenosis.

Fallopian tube sperm perfusion

The number of spermatozoa declines progressively along the length of the female reproductive tract. In normal fallopian tubes, a maximum of only 200 spermatozoa are present in the ampulla and the number of spermatozoa in the pouch of Douglas after IUI is even lower. The rationale behind the development of fallopian tube sperm perfusion (FSP) is to increase the sperm density in the site of fertilization at the time of ovulation and it was thought that the number of spermatozoa could be significantly increased with uterotubal flushes. FSP is based on three essential criteria: (1) a pressure injection (70–200 mmHg) to achieve perfusion and spill in the tubes which should have minimal adherences; (2) the injection of higher volumes of sperm suspension (4 mL as opposed to 0.2–0.5 mL in regular IUI), while (3) attempting to seal the cervix to prevent semen reflux. However, the large volume of inseminate may flush the ova out of the tubes directly or by inducing abnormal myosalpingeal contractions, thus leading to failure of fertilization. Although some studies support the efficacy of FSP compared to IUI, there is a lack of evidence that FSP results in higher pregnancy rates in couples suffering from nontubal subfertility than with IUI. A meta-analysis involving a total of 595 couples failed to give practical advice on the optimal treatment of nontubal subfertility due to heterogeneity in patient population, techniques of sperm preparation, artificial insemination performance, and type of catheter used. It is noteworthy in this context that the lowest FSP results were associated with the use of Foley catheters.

Additional techniques

Additional techniques include intrafollicular insemination, because the stable follicular fluid environment is thought to contain a substance beneficial to sperm function; transuterotubal and transvaginal intrafallopian insemination under ultrasonographic guidance; and intrauterine tuboperitoneal insemination, which differs from FSP in the increased volume of the inseminate (10 mL), ensuring peritoneal delivery of sperm. Because of their increased invasiveness and risks, and the lack of evidence concerning their efficacy, further investigation of these techniques is needed before they are offered in standard clinical practice.

Risks and complications of IUI see Table 12.2

Early complications

Risks of procedure

The risks of the actual IUI procedure itself are minimal. The most frequent and severe

Table 12.2 Risks and complications of IUI

Early complications	Risks of procedure	Bleeding or spotting Infection Pain Sensitization/anaphylaxis Vasovagal response Identification errors
Late complications	Ovarian stimulation	OHSS Multiple births
	Treatment of infertility	Childhood abnormalities

OHSS, ovarian hyperstimulation syndrome.

complications are mostly caused by the drugs used in induced cycles or by the etiology of the infertility itself.

CAUTION

Povidone iodine should not be used to cleanse the external cervical os because it is toxic to sperm.

Bleeding or spotting

IUI is associated with vaginal bleeding or spotting after the procedure. The bleeding could originate from the cervix, especially if a cervical tenaculum was used, or from the uterine cavity. Bleeding after an IUI has been associated with a lower CPR.

Infection

Risk of infection after an IUI procedure has rarely been reported. Theoretically, the risk could be caused by instrumentation in the sterile uterine cavity, but in practice, patients are screened for sexually transmitted disease before undergoing IUI, the sperm preparation process is thought to eliminate germs, and the whole procedure is performed in a sterile manner. The bacteria most commonly reported after IUI is *Escherichia coli*. The incidence of pelvic inflammatory disease has not been reported to be higher after IUI than in the general population.

Pain

Although the procedure is mildly uncomfortable, pain rarely results in interruption of the procedure. Patients in whom more manipulation is needed—straightening of the cervix with a tenaculum or dilation of the cervix because of stenosis—usually experience more intense pain, and local anesthesia should be considered in those patients (lidocaine jelly or cervical block). Delayed cramping or lower abdominal discomfort can be caused by prostaglandin release from trauma to the endometrium in difficult cases.

Sensitization

The literature remains unclear on the possible increase in titers of antisperm antibodies in the serum and cervical mucus of women after insemination by sensitization. The clinical significance of this issue as to future fertility is also ambiguous. Anaphylactic reactions have rarely been reported after IUI in specimens where penicillin was added to the media, in specimens containing potential allergens such as bovine serum albumin, or if the seminal fluid was not thoroughly washed from the inseminate.

Vasovagal response

Vasomotor symptoms or occasionally, syncope may rarely occur after IUI. Personnel capable of proper resuscitation should be readily available, although the treatment is mostly psychological support.

Identification errors

Labeling is crucial at each time of the procedure and it should include the names of the patients and the time of sampling and processing. Matching of names should always be verified and a time-out should precede an insemination in the same way as in the operating room.

TIPS & TRICKS

IUI technique

- Women with an anteverted uterus can be asked to maintain a full bladder to facilitate straightening of the uterus. This is not useful for women with retroverted uteri.
- Antibiotic prophylaxes is unnecessary for IUI, and complete scrubbing as for an operative procedure is not mandatory. However, the clinician should wash his/her hands and forearms with an antiseptic soap and wear a cap/mask and powder-free gloves for the insemination. The instruments to be used should be kept sterile.
- There should be no delay between loading the syringe with the processed sperm and its placement into the uterus because this would affect sperm motility.
- Injected volumes of greater than 0.4 mL are needed to reach the uterus and tube. Volumes larger than 1 mL could be expelled or refluxed from the cervix and

could cause uterine contractions after insemination.

- The material of the catheter used for IUI should not affect sperm motility, should be easy to use, made of a nontoxic material, and should have minimal dead space from the intrauterine tips. Multiple randomized trials have compared flexible to rigid catheters, hard or soft-tip catheters, and none demonstrated a difference in live birth rates. Stiffer catheters are easier to insert in the uterine cavity but are more uncomfortable for the patient and are associated with more postprocedure bleeding, due to endometrial trauma.
- Different types of insemination exist: single bolus technique, pulsatile IUI which uses an automated pump, with its disadvantage being restricted patient's activity for 4–6 h and slow-release IUI by which a persistent low concentration of spermatozoa is maintained at the oviduct, thereby prolonging the period of potential fertilization.

Late complications

Abnormal children

All advanced reproductive procedures carry the risk of augmenting the risk of birth of abnormal children. The theoretical explanation for this is that these techniques allow fertilization by a sperm which would otherwise not have fertilized, possibly because of genetic problems in the sperm. The same applies for anovulatory women. On the other hand, there is growing evidence that exogenous gonadotropins increase the rate of aneuploidy and that ovarian stimulation can cause developmental toxicity. Fortunately, that risk is not proven to be substantial by the available data.

Multiple births

Multiple births are associated with an increased risk of maternal morbidity, preterm birth with its accompanying neonatal morbidity and mortality, as well as social and economic burden. The risk of multiple births remains the major complication of all ART. Careful monitoring of the patients undergoing ovarian hyperstimulation and IUI may identify patients at higher risk of developing this complication. Risk factors appear to be a maternal age less than 30 years, the presence of more than six preovulatory follicles and a peak serum estradiol level greater than 1000 pg/mL. Actions that can be triggered to prevent the risk of multiple births in patients with great risk are to cancel the cycle when there are three or more mature follicles and start another at a lower dose of gonadotropins; to use soft stimulation protocols in the first place; and possibly to convert to IVF in which limiting the number of embryos transferred can decrease the incidence of multiple births. Other measures like aspiration of supernumerary follicles or multifetal pregnancy reduction have been proposed, but should not be considered as a routine practice. Additionally, patients with a good chance of spontaneous pregnancy should not be subjected to unnecessary fertility therapies.

Ovarian hyperstimulation syndrome

OHSS is a potentially life-threatening iatrogenic condition and is the most serious complication of medical infertility treatments. Diagnosis and treatment are respectively clinical and symptom-directed, as prevention and prediction remain elusive to the clinician. OHSS can cover a spectrum from mild ovarian multicystic enlargement to severe multisystem failure. Mild OHSS is associated with some degree of abdominal discomfort, occurs in nearly all patients treated with gonadotropins, and can be considered as a mere consequence of ovulation induction. Severe and critical OHSS may lead to intravascular fluid loss into the third space with two groups of complications: intravascular depletion can lead to hemoconcentration, hypercoagulability state and thromboembolic complications as well as to renal failure. Massive ascites and/or hydrothorax can have disastrous respiratory consequences, evolving to acute respiratory distress syndrome (ARDS) in the most severe situations. The underlying pathophysiological mechanisms behind OHSS can be summarized as increased vascular permeability, angiogenesis, and vasodilatation. Predictive factors of OHSS appear to be young age, high estradiol levels, and follicle

numbers. PCOS and ongoing pregnancy appear to cause more severe cases of OHSS. None of the available preventive measures has been proved to completely avoid the occurrence of the syndrome, but using very gentle ovarian stimulation protocols or withholding the administration of hCG and abandoning the treatment cycle were both proved to be efficient. Most importantly, correct classification of OHSS when it appears and prompt and aggressive therapy can dramatically improve the outcome of the disease.

Outcomes, benefits, and limits

The overall success rate of IUI varies widely between international studies, with a mean pregnancy rate per IUI cycle around 9%, ranging from as low as 5% to as high as 70%. Many variables could explain this difference, such as variability of IUI and ovarian stimulation protocols, as well as the heterogeneity of the patient population and other factors. The lack of prospective randomized trials renders the estimations approximate and unsatisfactory. Nevertheless, there are few unambiguous determinants of success that the clinician should be aware of to be able to provide couples with appropriate counseling. The most important of these by far is female age, because of its negative effect on ovarian reserve and oocyte quality. A significant decline in conception rates is noted beyond the age of 32. The impact of increased male age, although not as manifest as female age, still exists. Another influencing factor is the duration of infertility, which negatively correlates with the conception rate after IUI, especially in couples with unexplained infertility. Moreover, the type of infertility can negatively affect the pregnancy rates with endometriosis and pelvic infections being the most deleterious and anovulation and unexplained infertility being the less detrimental. Another important factor is the elevated number of leading follicles in the IUI cycle, which is associated concomitantly with higher pregnancy rates and increased multiple birth rates. Finally, during the IUI procedure itself, the presence of trilaminar endometrium on the day of insemination is a good predictor of success, and sperm parameters impact on IUI outcome as discussed above.

In a retrospective review of 1728 cycles, Hendin et al., aiming to help clinicians in predicting IUI outcome based on criteria available in preliminary patients evaluation, assessed 38 variables, related to female patients as well as semen characteristics, with live birth rates as a primary outcome. Their results confirmed female age as a major influencing factor, but their original finding was that a history of previous surgical correction for anatomic pelvic abnormalities, as well as the percentage of postwash motile sperm (rather than the number of motile spermatozoa) are significantly correlated with pregnancy rates. Any combination of those factors would seriously affect IUI success rates, but success is close to 0% with poor postwash sperm motility associated with any other risk factor. It is necessary to counsel the couple about their poor success rate with IUI in the presence of any of those factors, even when ovarian stimulation is used, in order to advise them to consider IVF/ICSI. As mentioned earlier, the actual recommendation is to offer four to six cycles of IUI with or without COH to infertile couples who are eligible for IUI, and then to shift to IVF, which provides the highest per cycle pregnancy rate (20–40% per cycle, versus 9–16% for gonadotropin injection plus IUI) in the shortest time interval, but is also the most costly intervention and has a high rate of high-order multiple pregnancy. When it comes to cost-effectiveness, it has been demonstrated that three cycles of IUI offer the same CPR rate as IVF and remain more cost-effective for unexplained infertility as well as moderate male factor subfertility.

Summary

In conclusion, IUI is clearly an efficient treatment for several indications such as cervical factor, sexual dysfunction, male factor subfertility, and unexplained infertility. The method that leads to the highest pregnancy rates—nearly one-third of couples—has been shown to be IUI combined with COH, particularly gonadotropin injection, but this advantage is paralleled with increased cost and potential risk of OHSS and multiple pregnancies. The risk of multiple pregnancy is considerable, especially if gonadotropins are employed, but there are minimal other risks in the procedure itself. With well-timed and

monitored insemination, most pregnancies occur within three to six cycles, after which the couples should be directed to IVF. The number of cycles proposed must take into consideration the diagnosis, age of the female patient (the variable that mostly affects IUI success), and the financial situation of the couple.

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13

In-vitro Reproductive Technologies

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Introduction

The treatment of infertility has been a technological challenge as far back as the 14th century when there are accounts of Arab peoples using artificial insemination on horses. As early as the 3rd century CE, records show that Jewish thinkers were discussing the possibility of accidental or unintentional human insemination by artificial means.

The first attempts at human artificial insemination by John Hunter are believed to have occurred in 1785, with a baby born the same year. Early reports of donor insemination were published in the *British Medical Journal* in 1945, and in 1955 four successful pregnancies using previously frozen sperm were reported.

In the 1960s a great increase in the different aspects of what we now have come to know as the different aspects of in-vitro fertilization (IVF) came to bear as the knowledge of ovarian stimulants, how eggs mature, ovulation, fertilization and the growth of the embryo in vitro, and better laparoscopy all increased. In 1973, the first IVF pregnancy in the world was reported by a team in Melbourne, Australia, which resulted in early embryo death. Subsequently, in 1977 another IVF pregnancy was reported but it was an ectopic pregnancy.

In 1978, the culmination of knowledge in the technologies associated with IVF led to the first IVF birth in the world. The pioneering work in the United Kingdom and Australia resulted in the birth of Louise Brown, the first "test tube baby" born as a result of IVF. Further improvements in the early 1980s by the groups in Australia led to births after drug-induced superovulation in the mother, the world's first frozen embryo baby, and the first donor egg baby. The Melbourne team achieved the world's first birth in a woman without ovaries, using donor eggs, the creation of an artificial menstrual cycle, and a special hormone schedule for the first 10 weeks of pregnancy.

In 1986, the IVF technologies were adapted to assisting infertile men by achieving the world's first pregnancy and birth from a sperm retrieval operation performed on a patient with vasectomy. In addition to the challenging aspects of the technological nature the advances in IVF have also brought forward bioethical challenges. Today the most apparent challenge is one that has led to the possibility of the first human clone.

This chapter gives a broad review of the laboratory technologies that have allowed IVF to be a routine clinical procedure.

Laboratory procedures after stimulation

Routine IVF

Stimulation protocols have been reviewed previously in Chapter 11. Once satisfactory stimulation is achieved the retrieved oocyte–cumulus complexes are collected from follicular aspirates (Figure 13.1).

The retrieved oocyte–cumulus complexes are rinsed, graded, and placed in a holding medium normally containing HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) or sometimes MOPS 3-(*N*-morpholino)propanesulfonic acid. HEPES is a zwitterionic organic chemical buffering agent which is widely used in cell culture,

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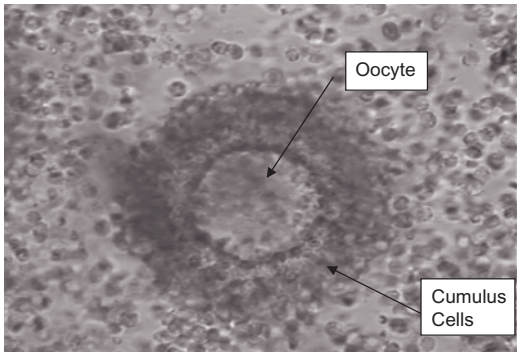

Figure 13.1 An oocyte–cumulus complex is harvested from the follicular aspirate once egg retrieval commences.

largely because it is better than bicarbonate buffers, which are also commonly used in cell culture, at maintaining physiological pH despite changes in carbon dioxide concentration. This property allows HEPES to act as a better buffering agent, in particular, when the oocytes are maintained outside an incubator and at 37 °C. An example of a holding medium containing HEPES is shown in Table 13.1. For example, a commonly used medium to maintain the oocyte cumulus complexes is HEPES-buffered human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA) overlaid by 5 mL of paraffin oil. (HTF is a widely used fertilization medium developed by Quinn in the 1980s.) Many clinics also perform egg retrieval directly into HTF medium with bicarbonate. In these practices the retrieval is performed in a mobile incubator where the eggs are collected and harvested in an enclosed environment at 37 °C in 5.0% CO_2, 5% O_2, 90% N_2, or 5% CO_2, in air. This avoids any additional manipulation of the oocyte–cumulus complexes.

Once the oocyte–cumulus complexes are all collected they are rinsed again, to remove any blood remaining from the follicular aspirate, transferred into drops of fertilization medium such as HTF supplemented with albumin, and placed in an incubator. A common fertilization media is HTF, developed by Quinn in the 1980s (Table 13.1). The oocyte–cumulus complexes are then placed in an incubator at 37 °C in 5.0% CO_2, 5% O_2, 90% N_2, or 5% CO_2, in air at 98% humidity for 3–5 h prior to insemination.

Table 13.1 The basic constituents (mM) of human tubal fluid (HTF) and modified HTF containing HEPES

	HTF	Modified HTF
NaCl	95	95
KCl	4.7	4.7
KH_2PO_4	1.2	1.2
NaH_2PO_4	–	–
$CaCl_2$	2.0	2.0
$MgSO_4$	1.2	1.2
$NaHCO_3$	25.0	55.0
Ca lactate	2.54	2.54
Lactate	24	24.0
Pyruvate	0.33	0.33
Glucose	5.6	5.6
HEPES	–	20.0

Preparation of semen samples

Semen samples are obtained by masturbation into a sterile container. The man should generally have not ejaculated for the previous 2–3 days. A diagnostic semen analysis is carried out according to the World Health Organization (WHO) guidelines. Briefly, for the purposes of an IVF treatment cycle, a complete semen analysis is not performed as the aim is to prepare the sperm for treatment rather than for diagnosis. A diagnostic semen analysis should be performed before treatment of the couple begins. Once collected, the semen can be prepared using a number of methodologies including swim-up or, more commonly, by density gradient centrifugation (Figure 13.2). These procedures are described in detail in Chapter 12.

Insemination and intracytoplasmic sperm injection

IVF fertilization and sperm preparation are performed in HTF with albumin. Oocytes can be inseminated in groups or individually in droplets of HTF with albumin containing 250 000–500 000/mL motile sperm overnight (Figure 13.3a). Insemination is routinely performed between 1300 and 1500 hours. The culture media, timing, and numbers of spermatozoa used for insemination can vary depending on the clinic.

In preparation for intracytoplasmic sperm injection (ICSI), oocytes are first placed in HEPES-buffered HTF media with hyaluronidase for under 2 min for partial removal of the cumulus cells. The remaining cumulus cells are removed by mechanical manipulation of the oocyte during further rinsing in HEPES-buffered HTF with albumin. Mechanical manipulation consists of passing the oocyte–cumulus complex through a series of pipettes decreasing in size from 250 to 135 µm. The final aim is to denude the oocytes of the cumulus cells to allow complete visualization of the oocyte and polar body so that the ICSI procedure can proceed.

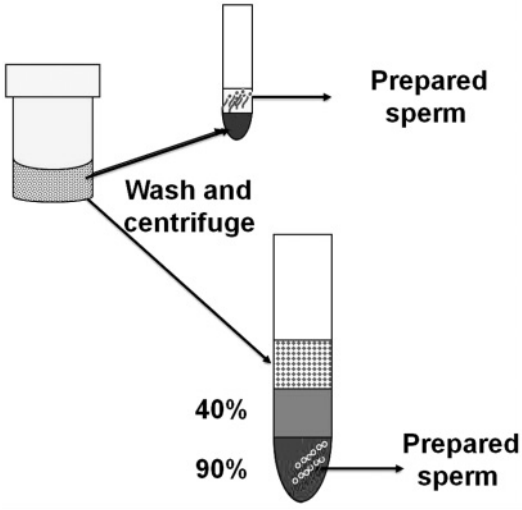

Figure 13.2 Preparation of semen samples by swim-up where the sample is centrifuged and clean media is overlaid on top. The motile sperm then swim up into the clean fraction and are collected after 20–30 min. Density gradient preparations involve overlaying the 40% and 90% fractions with semen and then collecting the motile spermatozoa in the 90% pellet after centrifugation.

Assessment of nuclear maturity

Each egg is carefully assessed, noting the presence or absence of a germinal vesicle or the first polar body. Only those eggs that have extruded the first polar body (metaphase II) and are morphologically intact are suitable for microinjection. Eggs that are yet to extrude the first polar body (metaphase I) are returned to culture for several hours and re-examined for extrusion prior to the injection. Germinal vesicles are not injected.

Microinjection procedure

Ready-made micropipettes for injection and holding the oocyte are now readily available from commercial vendors. They are prepared from borosilicate glass tubing. The holding pipettes have an external diameter of approximately 100 µm and an internal diameter of 30 µm. The injection pipettes have an external diameter of 7 µm and an inner diameter of 5 µm. Both holding and injection pipettes can be angled or straight (Figure 13.3b).

The ICSI procedure is carried out at ×400 magnification on a heated stage using an inverted microscope equipped with a modulation contrast system. The microscope is also equipped

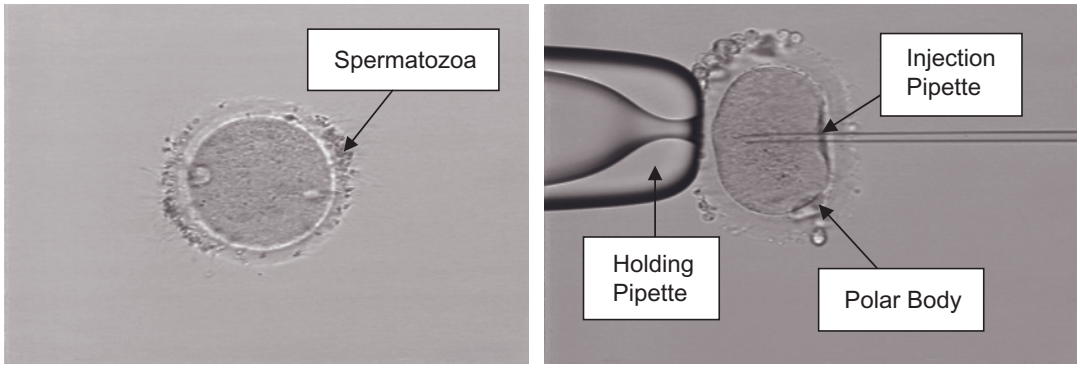

Figure 13.3 Left: An oocyte surrounded by spermatozoa in an insemination droplet. Right: A denuded oocyte undergoing the process of intracytoplasmic sperm injection (ICSI).

with two coarse positioning micromanipulators and two three-dimensional hydraulic remote control micromanipulators.

The injection pipette is fitted to a tool holder connected to an air- or oil-driven syringe. The holding pipette is fitted to a tool holder connected to a micrometer syringe driven by a hydraulic system filled with light liquid paraffin oil. All micromanipulation procedures are carried out in plastic Petri dishes.

Microinjection technique

Prior to injection a Petri dish is prepared with a central 5–7-μL droplet of HEPES modified HTF which contains dissolved polyvinylpyrrolidone (2 μL) to which is added 1–2 μL of sperm suspension. This is surrounded by a 4–5-μL droplet of modified HTF medium and covered with sterile liquid oil. Oocytes are transferred to these microdrops at the time of injection.

A single sperm is selected from the central droplet and is aspirated into the tip of the injection pipette. It is then gently blown out and immobilized by gently pressing the pipette on to the tail at a right angle to the head. The tail is pressed onto the bottom of the Petri dish until the sperm stops moving. The sperm is then aspirated into the injection pipette. The Petri dish is then positioned in order to visualize an oocyte in a microdroplet. With the holding pipette the oocyte is immobilized using slight negative pressure. The egg is manipulated on the holding pipette until the polar body is at the 12 o'clock or 6 o'clock position. The injection pipette is held touching the oocyte at the 3 o'clock position. The sperm is gently positioned at the tip of the injection pipette. The pipette is then firmly pushed through the zona pellucida and oolemma until it is in the centre of the ooplasm.

Prior to releasing the sperm, gentle suction is applied to the pipette until the oolemma can be seen to rupture and ooplasm streams into the pipette. (This stage is very important for a good fertilization rate as it ensures the oolemma has been properly breached and the sperm is deposited into the cytoplasm and not into the perivitelline space.) Immediately after the oolemma has been ruptured, the sperm is carefully injected into the cytoplasm, and the injection pipette is withdrawn gently. The injected egg is then released from the holding pipette. This procedure is continued until all the mature eggs have been injected. Following the injection procedure the eggs are washed through two droplets of HTF medium and returned to culture. Continued culture, assessment of fertilization, and embryo cleavage now proceed as in routine IVF.

The inseminated or ICSI oocytes are cultured overnight at 37 °C in 5.0–6.0% CO_2, 5% O_2, 90% N_2, or 5% CO_2, in air.

Embryo culture

At 16–18 h after insemination (day 1), each oocyte is examined for evidence of fertilization and placed into groups or individual droplets of a cleavage medium for culture to the cleavage stage. The general constituents of the cleavage culture medium are shown in Table 13.2. Embryos can remain in the cleavage medium until the morning of day 3 when they are changed over to blastocyst cleavage medium (Table 13.2). Embryos are maintained in blastocyst cleavage medium until day 5 or 6, depending on the day of transfer decided for the treatment of the patient. Numerous commercial sequential culture media exist which follow the above protocol; however, some culture media are able to support development from day 1 through to day 5 and it is not necessary to use two different media. Various amino acids are common con-

Table 13.2 Major constituents of day 1–3 cleavage stage and day 3–5 blastocyst stage media

Cleavage culture media	Blastocyst culture media
Pronuclear to eight-cell stage	**Eight-cell to blastocyst stage**
• Low or no glucose	• High glucose
• High pyruvate and lactate	• Low pyruvate and lactate
• EDTA	• Essential amino acids
• Nonessential amino acids	• Nonessential amino acids
• Taurine	

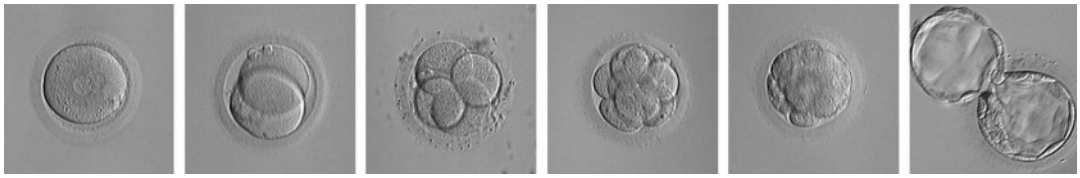

Figure 13.4 Embryo development. From left to right: day 1, two-cell (pronuclear) stage; day 2, four-cell stage; day 3, eight-cell stage, day 4, compacted; day 5, hatching blastocyst stage.

stituents of modern embryo culture media. Amino acids are important regulators of many cellular functions: they act as chelators, osmolytes, phi buffers, antioxidants, regulators of energy metabolism, biosynthetic precursors, and energy substrates.

Assessment of the embryo

Numerous methods have been adopted to assess the embryo as it develops from the pronucleate through to the blastocyst stage. The many transformations that take place during the fertilization process make the pronucleate stage a dynamic one to assess. The human oocyte contains most of the developmental materials, maternal mRNA, for ensuing that the embryo reaches the four- to eight-cell stage. The quality of the oocyte therefore plays a crucial role in determining embryo development and subsequent viability. Features assessed include the orientation of pronuclei relative to the polar bodies, alignment of pronuclei and nucleoli, the appearance of the cytoplasm, presence of nucleolar precursor bodies (NPB), and the timing of nuclear membrane breakdown.

The most widely used criteria for selecting the best embryos for transfer have been based on cell number and morphology. It is believed that by using strict embryo criteria to select "top-quality" embryos a high implantation potential can be achieved. Top-quality embryos have the following characteristics:

- Four or five or blastomeres on day 2 and at least seven blastomeres on day 3 after fertilization
- Absence of multinucleated blastomeres
- Less than 20% of fragments on day 2 and day 3 after fertilization.

When these criteria were utilized in a small prospective randomized clinical trial comparing single and double embryo transfers it was found that in single embryo transfers where a top-quality embryo was available an implantation rate of 42.3% and ongoing pregnancy rate of 38.5% was obtained (Figure 13.4).

However, assessment of the embryos at either the pronuclear or cleavage stages can at best be considered as an assessment of the oocyte. The quality of the oocyte is undoubtedly important, as the quality of the developing embryo is ultimately dependent on the quality of the gametes from which it is derived, but it provides limited information regarding true embryo developmental potential. Furthermore there is the potential of a paternal effect on development that is mainly evident after the eight-cell stage, when the embryonic genome is activated. Only by culturing embryos past this stage up to the blastocyst does it become possible to assess true embryonic development.

The time of blastocysts development is evidently important, but forming a blastocyst per se is not the criterion most strongly associated with pregnancy outcome (Figure 13.5). In animal models it has been determined that total cell number, inner cell mass (ICM) cell number, and glycolysis have the strongest correlation with viability. An alphanumeric scoring system that takes into account three aspects of blastocyst morphology—degree of expansion, ICM development, and trophectoderm development (see Figure 13.2)—has therefore been found to be more effective in scoring blastocyst development. Using this system it is possible to identify those blastocysts with the highest probability of implanting and giving rise to a pregnancy.

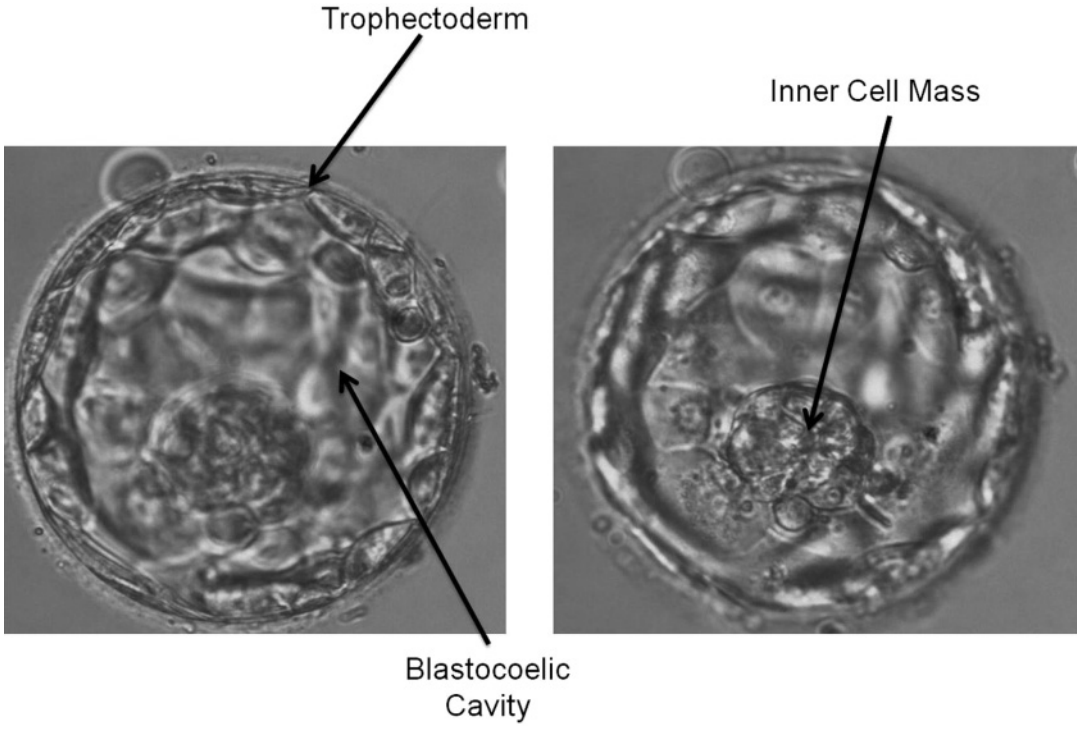

Figure 13.5 Blastocyst scoring characterizes the expansion of the blastocoelic cavity, the quality of the trophectoderm, and tightness of the inner cell mass.

EVIDENCE AT A GLANCE

A lot of controversy still exists in relation to the best day to transfer an embryo. The latest Cochrane review examining this question provides evidence that there is a significant difference in pregnancy and live birth rates in favor of blastocyst transfer, with good-prognosis patients with high numbers of eight-cell embryos on day 3 being the most favored in the subgroup for whom there is no difference in cycle cancellation. There is emerging evidence to suggest that, in selected patients, blastocyst culture maybe applicable for single embryo transfer.

Embryo freezing

Cryopreservation is now an integral part of many IVF clinics throughout the world, enabling patients to utilize the maximum number of embryos generated from any one oocyte retrieval, while protecting against ovarian hyperstimulation syndrome (OHSS) and the risk of higher-order multiple births.

A number of different cryoprotectants are currently used in freezing and thawing of embryos, such as dimethylsulphoxide (DMSO), ethylene glycol (ETG), glycerol and propan-1,2-diol (PROH), together with numerous different methodologies, such as slow freeze–rapid thaw, rapid freeze–rapid thaw, and vitrification. In addition, embryo freezing is applied at all stages of embryo development and to the oocyte. The most widely utilized freezing protocol is PROH with slow freeze–rapid thaw (Figure 13.6a), which produces consistently good frozen embryo transfer (FET) success rates. Freezing is classically performed in a closed straw system (Figure 13.6b) after passing the embryo through a gradient of increasing cryoprotectant solutions. The aim of this gradient is to remove water from the cell (embryo) and replace it with cryoprotectant so that there is no crystallization during the freezing procedure.

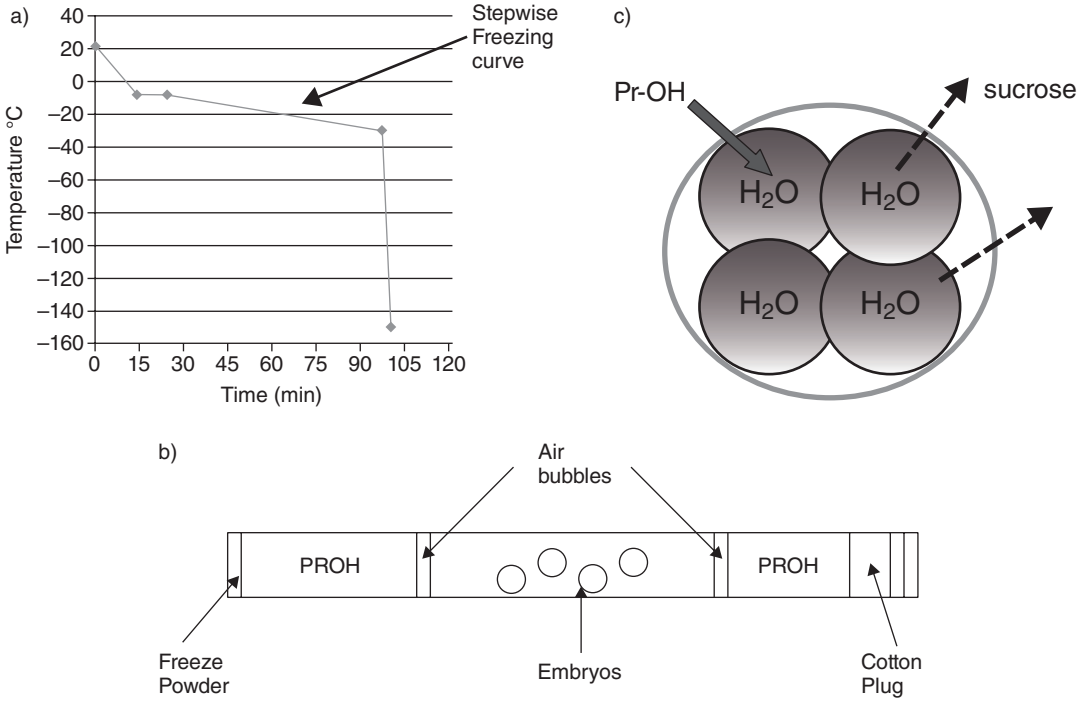

Figure 13.6 Freezing of embryos using (a) the slow freezing procedure, (b) straws where the embryo sits in a final PROH cryoprotectant and (c) the removal of water by cryoprotectants to minimize crystal formation.

Formation of crystals within the cell will lead to unrepairable damage to the embryo and drastically decrease its viability (Figure 13.6b).

SCIENCE AT A GLANCE

The methodology available to assess the embryo prior to transfer is now becoming more complex. Numerous technologies are now being assessed so that the embryo chosen for transfer is more likely to be viable. These technologies use either a genomic, proteomic or metabolomic approach to assessing a cell biopsied from the embryo or sampling medium that the embryo is being cultured in.

In recent years a number of advances have been made in freezing embryos. These include the introduction of new freezing tools to store the embryo, and vitrification. The new tools replace the straws used classically and are more beneficial as they limit the amount of cryoprotectant surrounding the embryo hence allowing the freezing process to be achieved much faster and with less damage. Vitrification is defined as "the instant solidification of a solution brought about by an extreme elevation in viscosity during cooling, without ice crystal formation." In other words, vitrification is faster and lacks some of the typical disadvantages of traditional slow freezing. Initial data indicate that vitrification in IVF can allow freezing of spare embryos with better post-thaw survival rates and higher pregnancy and live birth rates from FET cycles. It appears particularly beneficial when freezing blastocyst stage embryos.

SCIENCE AT A GLANCE

The types of embryo freezing technologies now available are rapidly changing. Slow freezing has been the staple technology for many years to freeze embryos, but

vitrification is now becoming more widely used and is providing equivalent or improved results, in particular for oocyte and blastocyst freezing. Vitrification is an ultrarapid IVF embryo freezing technique and is the process of converting something into a glass-like solid that is free of any crystal formation. The main benefit of vitrification is that it is a very fast process.

Summary

IVF reproductive technologies have come a long way since the first birth in 1978. The incorporation of techniques to treat more difficult infertility patients and the availability of spare embryos also means that this technology is constantly also pushing the barriers of bioethical questions. Treatment of men with limited spermatogenesis by injection of round spermatids has already been attempted, but many concerns over the possibility of paternal imprinting anomalies in offspring have led to a moratorium on this technique in many countries. Numerous other treatment technologies have also caused concern, including that of injecting cytoplasm from young eggs into older eggs to attempt to make them more viable. Finally, the availability of spare embryos has led to questions about the ability to perform cloning and also about the creation and use of embryonic stem-cell lines.

Currently in many countries between 1–6% of children born are from IVF technologies, so aside from the bioethical concerns, IVF reproductive technologies have brought immense happiness to millions of couples worldwide.

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14

Egg Donation and Surrogacy

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Introduction

Egg and embryo donation first drew the attention, and scrutiny, of the world over a quarter century ago, as headlines in the early 1980s announced the birth of a baby to a recipient of a donated embryo. In the early 1990s, sensational births to menopausal women again attracted worldwide interest, engendering both praise as a breakthrough medical technology and criticism as a dangerous and unnatural method of conception. Regardless of one's personal view, most would agree that egg and embryo donation remain one of the most successful of all techniques used in the ever-increasing armamentarium of assisted reproductive technologies (ART).

The United States remains out front in the provision of egg donation services. Typically today, 10,000–12,000 attempts at donor in-vitro fertilization (DIVF) take place annually. This is approximately 2–3 times the number reported per year in Europe or in Asia, where more stringent restrictions on the practice are in place. It can be assumed from reviewing the annual reported data to the Centers for Disease Control and Prevention (CDC) that well over 100,000 cycles of egg donation have been performed in the United States during the past decade.

Surrogacy services can be manifested in a variety of ways, although the "gestational carrier" model is currently the most commonly employed approach. These "carriers" may be implanted with embryos created by couples in whom the woman cannot (e.g., posthysterectomy; serious medical disorders precluding pregnancy), or chooses not, to carry the baby, or in other cases may be an option for gay men needing a uterus to gestate their intended child. In the United States, such treatment cycles are reported to occur approximately 1,000 times per year, and tend to be concentrated in states where local laws enforce surrogacy contracts (e.g., California, Connecticut, Florida, Maryland, Pennsylvania). Services are expensive, requiring the interaction of cooperative parties in law, medicine, social work and/or psychology.

This chapter reviews practical aspects of the method of egg and embryo donation as seen from the perspective of an American clinician who provides this service to patients approximately 150–200 times per year. A brief discussion of surrogacy follows, as it uses similar techniques for the screening and synchronization of participants. However, as mentioned above, gestational surrogacy is much more legally cumbersome and in many states is either outlawed altogether or difficult to practice because there is a lack of enforceable law to ensure the validity of the contracts.

Recipients

Initially, and for nearly 10 years, egg donation was an infertility treatment applied largely to younger women with ovarian failure or other poor prognostic profiles. This was forever changed once egg donation was adapted to treat older women whose infertility was a natural result of reproductive aging. Eventually, many of these recipients were frankly menopausal and brought with them the additional health concerns that accompany advancing age.

Infertility, 1st edition. Edited by Emre Seli.

Currently, DIVF recipients generally fall into one of four major diagnostic categories:

- Advanced reproductive age with diminished ovarian reserve (most common), with or without a history of multiple IVF failures in the past
- Premature ovarian failure
- Heritable genetic disease not preventable by preimplantation genetic diagnosis
- Severe pelvic adhesive disease and/or other anatomic disease precluding safe surgical access to the ovaries

Of note, there is some evidence to suggest that patients with a diagnosis of recurrent pregnancy loss will increase their chance of an ongoing pregnancy by using donor eggs; this does not seem to be the case, however, if the patient demonstrates an inherited thrombophilia or the antiphospholipid antibody syndrome.

DIVF recipient candidates are initially screened in an identical manner to those patients undergoing nondonor IVF (see Table 14.1), including a thorough medical history, physical examination with cervical cytology, transvaginal ultrasound, laboratory tests including a complete blood count with blood type, serum electrolytes, thyroid stimulating hormone (TSH), and an infectious screen consisting of cervical cultures for gonorrhea and chlamydia, HIV types 1 and 2, rapid plasma reagin (RPR), hepatitis B and C, and rubella antibody. All DIVF candidates should also undergo a uterine cavity evaluation via sonohysterogram, hysterosalpingogram, or hysteroscopy (the choice of modality should be based on patient-specific characteristics and patient/provider preference).

Additional screening specific to DIVF recipients includes general medical and social/psychological/legal evaluation.

Table 14.1 Required medical screening for DIVF recipients

All patients: • CBC • Blood type • Serum electrolytes • TSH • Pap smear • Cervical cultures for gonorrhea and chlamydia • HIV 1 and 2 • RPR • Hepatitis B and C • Rubella antibody • Uterine cavity evaluation
If age ≥40: • ECG • Chest radiograph • Mammogram • Glucose tolerance test • Cholesterol/lipid profile
If age ≥45: • Exercise tolerance test
If age ≥50 and/or menopausal or premature ovarian failure: • Bone densitometry

General medical evaluation

In cases of advanced maternal age (often defined as 40 years of age or more, for these purposes), prior to initiating treatment, a concerted effort should be made to ascertain the state of the recipient's general medical wellbeing and fitness for pregnancy, and to optimize any comorbid conditions which may be present. This applies equally, if not more so, to younger patients who are known to carry underlying medical conditions. Patients 40 years of age or over should undergo screening ECG, chest radiograph, mammogram, glucose tolerance test, and cholesterol and lipid profile, and patients 45 years of age or more should undergo an exercise tolerance test, as cardiovascular and metabolic disorders (specifically manifested in the hypertensive disorders of pregnancy and gestational diabetes) are the medical complications most often encountered in an older obstetric population. If specific medical concerns exist, consultation with (at a minimum) an internist and maternal–fetal medicine specialist are necessary to ensure that the patient fully comprehends the potential risks inherent to pregnancy.

Social/psychological/legal evaluation

The decision to proceed with DIVF is fraught with emotional complexity. Sadness surrounding the loss of potential for genetic parentage, confusion regarding feelings toward the donor

(particularly if known) and their relationship with the partner/spouse, along with concerns and attitudes about disclosure to future offspring, can all contribute to uncertainty and a fragile psychosocial state. It is imperative that the DIVF recipient and her partner have an adequate opportunity to explore and discuss any of the above issues, or any other hesitation or doubts they may harbor, before proceeding with treatment. This component of screening may be performed by a physician (reproductive endocrinologist or psychiatrist), nurse, social worker, psychologist, or most often, some combination of these providers. Finally, in order to clarify and concretize all possible legal ramifications of more complicated DIVF arrangements (e.g., the use of sibling donors), consultation with an attorney is often recommended.

The importance and utility of a "mock" or "preparatory" cycle to ensure the potential for adequate endometrial response is controversial and overall not well supported in the literature. In our program we do not routinely perform a mock cycle except in patients who are at especially high risk for an inadequate endometrial response (e.g., history of severe uterine adhesive disease, history of pelvic irradiation, history of multiple failed DIVF, etc.).

Donors

In the United States, anonymous egg donors have always been financially compensated for their participation. Although they are screened in much the same way as their male counterparts serving as sperm donors, egg donors typically receive significantly higher reimbursement, corresponding to the increased time, effort, and discomfort entailed in oocyte donation (as compared with sperm donation). It is instructive to consider that in many countries other than the United States, regulations regarding financial compensation and/or mandated disclosure have severely hampered donor recruitment efforts, and hence the supply of egg donors in these jurisdictions is exceedingly limited.

With the expansion of services to include older women, even in the United States the demand for eggs has far outpaced the supply of available donors. Consequently, free market competition for services has progressively driven up the fees paid to these young women in hope of securing their services. In the 1980s the typical egg donor was paid $500 for each cycle of participation. Today this fee more commonly ranges from $5,000 to $8,000.

The party responsible for the matching process can be the fertility clinic providing the DIVF services itself or an independent "donor agency." Of note, the Society for Assisted Reproductive Technology (SART) regularly updates a listing of egg donor agencies (currently numbering approximately 70 agencies nationwide) that have signed an agreement to abide by the American Society for Reproductive Medicine (ASRM) Ethics Committee guidelines for managing financial incentives in the recruitment of egg donors; actual adherence to the guidelines is, however, self-reported rather than verified by SART itself. Highlights of these guidelines include:

- Given that compensation is being made for the donor's time, effort, and discomfort rather than a true "purchase" of the eggs themselves, payment should be consistent and not vary according to quantity or perceived quality of eggs retrieved.
- Payments in excess of $10,000 are inappropriate.

The process of matching an egg donor can incorporate some of the recipient's basic desires regarding race, physical appearance, and education. However, the need to ensure donor anonymity, combined with the limitations of the donor pool in general, may constrain the clinic or agency's ability to take into account very specific donor characteristics in the matching process.

Guidelines for the management of egg donors have been published by ASRM and revised several times over the years. Landmark changes were introduced by the U.S. government when implementing oversight control over donor gametes in 2005. Additionally, several states have enacted legislation that either limits or provides tight control over the practice. The New York State Department of Health requirements regarding donor gametes are even more stringent than regulations imposed by the federal Food and Drug Administration (FDA).

Table 14.2 Required medical screening for DIVF donors

CBC
Blood type
Toxicology panel
HIV types 1 and 2
RPR
Hepatitis B surface antigen and core antibody
Hepatitis C
Ovarian reserve testing (e.g., müllerian inhibiting substance)
Pap smear
Cervical cultures for gonorrhea and chlamydia
Cystic fibrosis
Fragile X
Spinal muscular atrophy
Other genetic screening depending on ethnicity (e.g. hemoglobin electrophoresis, Tay–Sachs)

The screening of egg donors must address the woman's eligibility to participate in a variety of ways, including her overall medical health, reproductive status, mental health, genetic background and social history (see Table 14.2). Several areas in particular merit special attention, as reviewed below.

Infectious disease

In contrast to well-established protocols in sperm donation, the currently experimental status of oocyte cryopreservation precludes using a period of quarantine as definitive protection against the inadvertent transmission of infectious diseases in DIVF. The DIVF provider must therefore be extremely vigilant to ensure the appropriate safeguards are in place, including rigorous assessment of sexual and substance history and habits, as well as an appropriately timed laboratory screen (no more than 30 days prior to egg retrieval, according to FDA regulations).

Medical suitability

Every effort should be made to maximize the chance that the donor will produce eggs of sufficient quantity and quality to lead to a healthy pregnancy. Accordingly, ASRM recommends that donors be no more than 34 years of age, and many programs exclude anonymous donors over the age of 30. To complement age restrictions, in our program we routinely perform ovarian reserve testing in all donors via day 2 follicle stimulating hormone (FSH)/estradiol and/or müllerian inhibiting substance. We also perform a transvaginal ultrasound to screen for undiagnosed pelvic pathology, including polycystic ovarian syndrome (PCOS), because of the increased risk of hyperstimulation as well as increased risk of miscarriage. Furthermore, donors who carry a known genetic disorder or a major congenital malformation of polygenic/multifactorial inheritance (e.g., cardiac anomaly, cleft lip or palate) should be excluded. A significant family history of diseases such as diabetes, cardiovascular disease, and familial cancers should be carefully considered as possible grounds for exclusion, although some recipients might be willing to accept a donor with one of these conditions after appropriate counseling regarding the risk of heritability. ASRM endorses universal screening for cystic fibrosis; other genetic testing (Tay–Sachs, sickle cell, etc.) should be customized to the ethnicity of the potential donor.

Counseling and informed consent

The DIVF provider should ensure that the prospective donor fully comprehends the investment of time, energy, and discomfort that egg donation entails. The risks of medical complications of controlled ovarian hyperstimulation and egg retrieval include ovarian hyperstimulation syndrome (OHSS; estimated at less than 1% risk of moderate–severe OHSS requiring intervention) and the largely theoretical link to an increased risk of malignancy in the long term. It should be made clear that the donor can opt to discontinue treatment at any time, and that the DIVF provider is serving equally as the donor's (not just the recipient's) physician. The donor should be aware she may seek independent legal counsel in order to fully understand the legal implications of the arrangement to which she is a party. All donors should undergo psychological evaluation to explore motivations for donation and ensure adequate emotional and psychoso-

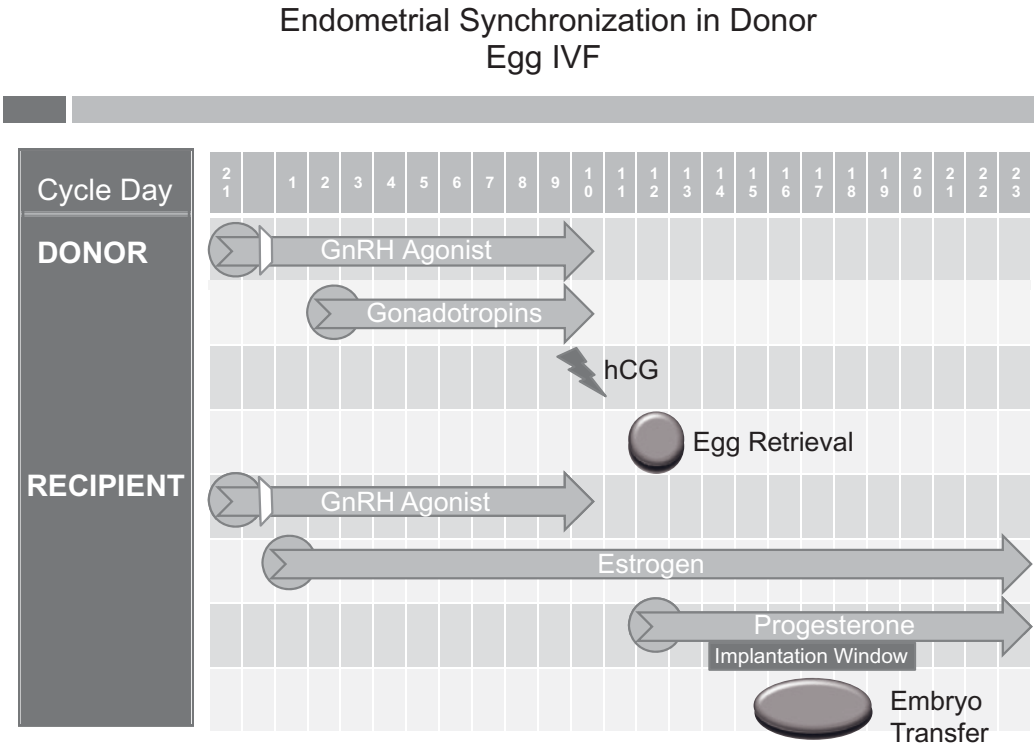


Figure 14.1 Schematic of synchronization of donor and recipient achieved using GnRH agonist down-regulation.

cial stability prior to initiating treatment. Some countries (e.g., the United Kingdom) have instituted a policy of mandated disclosure to offspring resulting from DIVF, but in the United States the donor should be informed that every effort will be made to maintain donor anonymity. The outcome of the DIVF cycle is also not routinely revealed to the donor.

Cycle management

The success of DIVF rests on successful synchronization of the donor and recipient; specifically, eggs must be available for fertilization at a point in time leading to cleavage-stage embryos or blastocysts being available for transfer during the relatively narrow "implantation window" in the recipient (see Figure 14.1).

SCIENCE REVISITED

Implantation window

In normal reproductive physiology, ovulation sets in motion a delicately choreographed unfolding of synchronous events, starting with the formation of a corpus luteum and secretion of progesterone. Progesterone exposure transforms the proliferative ("estrogen-primed") endometrium to a secretory ("receptive") state. These events occur in concert with fertilization of the oocyte and migration of the early embryo to the uterine cavity, ultimately leading to trophoblast apposition (initial attachment) and endometrial invasion.

Studies performed in the 1950s using luteal-phase hysterectomy specimens suggested that implantation occurred no earlier than day 20 of a 28-day cycle; more recent literature generally confirms this observation. Wilcox et al. collected daily urine samples from 221 women

attempting to conceive for up to 6 months. In those women who conceived, human chorionic gonadotropin was first detected on cycle days 20–26. Interestingly, delayed implantation was significantly associated with increased risk of miscarriage (13% if implantation occurred by day 23 vs. 52% if implantation occurred day 25 or later), perhaps reflecting a deficiency in embryo-endometrial synchronization.

The advent of DIVF provided a particularly useful model to study the timing of implantation in relation to progesterone exposure. By transferring DIVF embryos on days 16–24 of the cycle, Navot and colleagues at the Jones Institute demonstrated in 1991 that implantation will not occur if a cleavage-stage embryo is transferred prior to day 17 (day 3 of progesterone exposure) or later than day 20 (day 6 of progesterone exposure). Later studies further narrowed the optimal timing for transfer of cleavage-stage embryos (day 4–5 of progesterone exposure) and blastocysts (day 7 of progesterone).

The molecular mechanisms underlying successful implantation are generally poorly understood. A large array of factors are thought to play a role, including multiple growth factors (e.g. insulin growth factor), cell adhesion molecules (e.g. integrins), and some proteins involved in mediating the immune response (e.g. glycodelin); many of these factors are progesterone-dependent and have been demonstrated to be most abundant in the endometrium during the implantation window. From a morphologic standpoint, "pinopods" are progesterone-dependent apical cell protrusions that are characteristic of endometrial secretory cells during the window of implantation. Their true importance in implantation is still uncertain.

Once screened and found to be acceptable, the donor is often started on oral contraceptives (OCPs) in order to leverage some control over the timing of her menses. When a match has been made with a recipient, OCPs are stopped, withdrawal menses occurs, and the decision is made to initiate medications to hyperstimulate the donor's ovaries; a gonadotropin releasing hormone (GnRH) agonist (e.g., leuprolide acetate) or antagonist (e.g., ganirelix acetate) is often administered simultaneously in order to prevent a premature and/or inadvertent luteinizing hormone (LH) surge.

Concomitant with the initiation of the donor's stimulation, the recipient, who is either menopausal or who had been previously "down-regulated" with a GnRH agonist, will begin estrogen treatment (oral or transdermal) for priming of the endometrium. Estrogen is usually started several days prior to commencement, and never later than the first day of, the donor's stimulation regimen.

☆ TIPS & TRICKS

Recipient monitoring

Monitoring of the DIVF recipient who is still ovulatory can be effectively minimized without compromising the chances of successful pregnancy. Prior to initiating GnRH down-regulation in the luteal phase, serum measurements of hCG and progesterone combined with a transvaginal ultrasound should be performed to ensure no pregnancy has recently occurred. By the time of initiation of estrogen therapy, an ultrasound and estradiol measurement should ensure adequate suppression has been achieved. At some point prior to scheduling embryo transfer another ultrasound should demonstrate an adequately proliferative lining (typically ≥7 mm). Monitoring serum levels of estradiol and/or progesterone during the periods of replacement of these hormones has not been shown to be of any clinical utility.

Of note, although a thin lining has been associated with decreased implantation, successful pregnancy has been reported in linings as thin as 4 mm. Thus, unless significant medication noncompliance is suspected or confirmed, one should proceed with embryo transfer even in the setting of a thin lining. If pregnancy does not occur, one can consider performing a mock cycle prior to repeat DIVF in such a patient.

In order to effect multifollicular development in the donor, usually a combination of gonadotropins (e.g., purified recombinant FSH or urinary gonadotropins) along with the GnRH agonist or antagonist mentioned above are administered. Drugs are used daily and are delivered parenterally (subcutaneously or intramuscularly) as they are fast acting and quickly metabolized. Most stimulations are completed within a 2–3-week window of time and require frequent visits (8–10 times per cycle of participation) for monitoring of the response. Adequacy of response is gauged by the daily transvaginal ultrasound report along with accompanying serum estradiol level. Most donors will produce 10–20 eggs per retrieval. In our center a response of less than five dominant follicles is typically cancelled.

Egg retrieval occurs under conscious sedation with propofol and fentanyl and is usually accomplished in less than 15 min. Bleeding is minimal and the procedure itself is largely painless. Relatively rare complications may occur at the time of aspiration including excessive bleeding (particularly true in undiagnosed von Willebrand disease), bladder atony, adverse anesthesia reactions, and pelvic hematoma. More often, delayed problems arise as a result of OHSS (1% incidence). Fluid and metabolic instability in these cases usually arise with the onset of nausea or difficulty breathing 3–7 days later and in severe cases require hospitalization to stabilize the patient.

Complications are not only unsettling to the donor; they also may present unique insurance coverage problems for payment of incurred medical expenses. To avoid conflicts over the payment of bills, many programs (including ours) contract with an insurance carrier to cover the cost of incidental expenses that may exist secondary to problems arising directly as a result of donor participation. The nominal charge per cycle to the program is a reasonable offset to the potential tens of thousands of dollars incurred by an emergency room admission or intensive care unit stay.

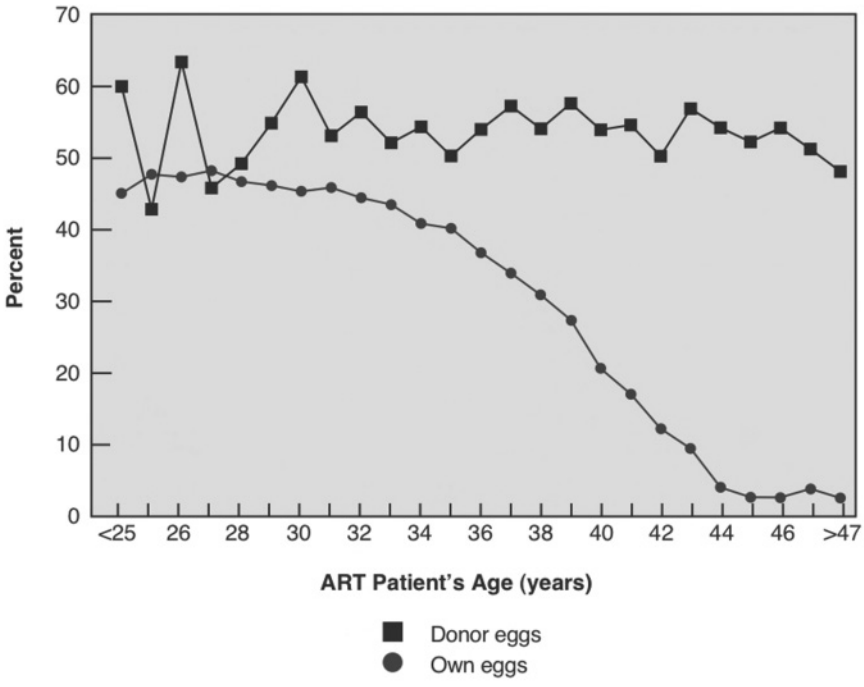
Adequate and well-timed progesterone exposure is ultimately the key to achieving an endometrium receptive to implantation of the embryo. The scientific literature suggests that the so-called "window of implantation" opens no sooner than 2 days after the start of progesterone exposure. The optimal time for transfer is thought to be approximately day 4–5 of progesterone exposure for cleavage-stage embryos and day 7 of progesterone exposure for blastocysts. Thus, progesterone administration is initiated on either the day before egg retrieval or the day itself. Because of the hepatic first-pass metabolism of oral progesterone, only parenteral (IM) or vaginal progesterone administration is acceptable.

As with all IVF cycles, a serum pregnancy test is checked 10–12 days after embryo transfer. Because of the absence of a functioning corpus luteum in the recipient to support a pregnancy, it is imperative to continue estrogen and progesterone replacement until at least 7–9 weeks of gestation, when the so-called luteo-placental shift will occur. Conventionally, for added safety, both hormones are administered until the end of the first trimester (12–14 weeks gestation).

Clinical results

The pregnancy rates of recipients of donated eggs and embryos remain as high as or higher than rates seen in younger women using autologous eggs and IVF. For example, CDC data indicate that in 2006, the live-birth rate per transfer using donor eggs was 54%, whereas live-birth rates per transfer for women aged 21–30 ranged from 35% to 43%. This surprising fact was first documented in the late 1980s and remains true today. It has been estimated that regardless of age, egg donation has the potential to lead to a cumulative live-birth rate of up to 90% in recipients who are willing and able to undergo multiple DIVF cycles. Most interestingly, this holds true even for patients in their fifties and older, who would literally have no success if attempting to conceive on their own. With the recent birth of babies to Indian women in their seventies, it is fair to say that there is no biologic barrier to embryo implantation and pregnancy due to advancing age, even in a geriatric population (see Figure 14.2).

As important as pregnancy rates are in reflecting the success of the method, it is ultimately the obstetric outcomes that matter most. As might have been expected, other than the increased rate of multiple births that also plague women undertaking conventional IVF, younger recipients of donated eggs and embryos have no known unique risks to them or their offspring.



Percentages of Transfers That Resulted in Live Births for ART Cycles Using Fresh Embryos from Own and Donor Eggs, by ART Patient's Age, 2006

Figure 14.2 Live birth rates for autologous IVF versus DIVF as a function of age (CDC 2006).

However, this is not the case for women of advanced reproductive age where additional morbidity has been reported. Complications occur in this population roughly 20% of the time and include hypertensive disorders of pregnancy, gestational diabetes, small-for-dates infants, placental abnormalities, and stillbirth. The higher incidence and prevalence of these problems has persisted despite greater surveillance prenatally in medical and obstetrical testing. With regard to pediatric and developmental outcomes, studies are overall still lacking, but at least one group has followed a cohort of DIVF children and their families for as long as 12 years without noting significant concerns.

Ethics

From the beginning, egg and embryo donation has been surrounded by controversy. Payment for "donation" has been criticized and has grown over the years as services have increased and become more commercially competitive. Initial payments in 1984 of $250 were considered excessive, yet certainly pale in comparison to the common charge of $8,000 per cycle seen in Manhattan today. Concern over the potentially coercive effect of large payments has fostered discussion on limiting reimbursement by law, although no statutes have yet been passed in any state to control free market spending on donor services. As mentioned above, ASRM guidelines discourage payment over $10,000 per cycle but carry no policing power and may be ignored largely without penalty. As a result, an impressive number of agencies have arisen to broker these services, typically charging recipients large fees to secure the participation of donors, especially in cases where women with specific traits (e.g., race, religion) or pedigrees (e.g., advanced degrees, artistic talent) are sought.

CAUTION

Egg sharing

In light of the controversy surrounding the payment of healthy young women to undergo the invasive process of egg donation, as well as the overall shortage of available donors, the concept of "egg sharing" was introduced as an alternative to "traditional" or "commercial" egg donation. In this model, infertile women who are already undergoing IVF agree to "donate" some proportion (usually half) of the eggs retrieved in exchange for a discount on the fees they incur for their treatment. On the surface, this arrangement accomplishes two goals:

- There is no explicit transfer of money to the egg donor, minimizing the commercial nature of egg donation (also referred to as "commodification").
- No healthy person is undergoing a treatment and procedure she would not otherwise undergo, hence minimizing the sense of financial inducement of the egg donor.

Given these perceived benefits, several countries have adopted legislation making "egg sharing" the only legally permissible form of egg donation.

One must, however, question the true ethical superiority of this approach. First, while there is no explicit payment for the donation, studies have demonstrated that the financial incentive is a major component of the motivation to donate. Secondly, the most compelling ethical justification for paying egg donors is that the payment serves as compensation for their time and effort rather than a purchase price for their eggs. An egg sharer is clearly not being compensated for the time and effort required for a procedure and treatment she would be undergoing anyway; thus, the financial incentive is more directly linked to the eggs as a commodity in this case. Furthermore, implantation rates of the recipient may be higher than that of the stimulated "donor," resulting in a discordant pregnancy rate between the parties. Finally, as opposed to healthy young volunteers, infertile women undergoing IVF should be the category of women *least* willing to donate eggs and *most* vulnerable to exploitation in the setting of financial inducement. All in all, egg sharing seems to be no less, and in many ways more, ethically problematic than "traditional" commercial egg donation.

Following the birth of a baby to a "menopausal" 43-year-old, many ethicists argued that DIVF was being used in "unnatural" ways and should be restricted to women of normal childbearing age. This argument gained more momentum after mothers in their fifties and sixties delivered babies. Perhaps the recent birth of twins to a 72-year-old woman in India seeking to provide a male heir for her family is the most poignant example of pushing the technology to its limit. The main criticisms of older women having babies relate to the danger of their carrying the fetus and the uncertainty of their longevity after delivery to safely ensure that the child grows up in a nurturing home environment. Although the pregnancy and delivery outcome data are largely favorable, it remains to be seen if the children of these older parents reach adulthood without peculiar experiences relating to their unique circumstances.

Mandating the open disclosure of donors' identity has been suggested as a means of allowing children an opportunity to know their biologic/genetic background. This practice was passed into law in the United Kingdom in 2005; unfortunately this has resulted in further depletion of the already shallow donor pool. It is also unclear whether or not families actually desire such opportunities, in light of the fact that many, if not most, recipients will not choose to disclose the nature of their conception to their offspring. Unlike adoption or surrogacy, processes in which public records and/or biologic parents and often siblings exist, in the United States the record of DIVF is protected from disclosure by the 1996 Health Insurance Portability and Accountability

Act (HIPAA) and may be destroyed after a number of years.

Surrogacy

In its purest form, surrogacy involves the simple insemination of a woman who will carry the pregnancy to term and allow the baby to be adopted by the contracting parents. This approach has been largely usurped by gestational carrier surrogacy, which is most often used today. In this scenario the surrogate is the gestating mother, but she most often carries a baby that was created in vitro using designated sperm and eggs (including donor gametes); thus, she is genetically unrelated to the child. Gestational surrogacy is an attractive treatment option for couples in whom there is no healthy uterus available to carry a pregnancy, including gay men and heterosexual couples in whom the female partner has an absent or unusable uterus (e.g., status post hysterectomy, congenital anomaly) or an absolute medical contraindication to pregnancy (e.g., significant cardiomyopathy).

Clinical management of gestational surrogacy is largely the same as that required for successful DIVF pregnancy. The physical and psychological fitness of potential carriers must be ascertained, again via extensive medical and psychosocial evaluation. Most programs will restrict the age of carriers to 21–40, and most will require the carrier to have successfully delivered at least one child in the past. This requirement not only "proves" the carrier's physical ability to carry a healthy pregnancy; perhaps more importantly, it is also the only way to ensure that her consent is fully "informed" as to the potential discomforts and inconveniences of pregnancy, labor, and delivery. The risks of multiple gestation in assisted reproduction must be clearly delineated and issues such as selective reduction, amniocentesis, and intended mode of delivery should all be discussed explicitly prior to initiating treatment.

Perhaps the most challenging aspect of successful gestational surrogacy is the navigation of potential legal pitfalls. Despite the best efforts of attorneys specializing in reproductive law, many states will not recognize the validity of surrogacy contracts in the event of misunderstanding or a change of heart. The most worrisome circumstance, which has been reported more than once in the United States, occurs when the carrier develops an emotional bond with the gestating fetus and refuses to transfer parental rights to the intended parents after birth. In states that do not recognize surrogacy contracts, these couples would be left with no helpful legal recourse, sometimes even if the gametes used to conceive the baby belong to them. Because of this potentially devastating scenario, in many areas of the country gestational surrogacy is largely absent from clinical practice.

Summary

Egg and embryo donation has evolved from a curious and sensational research project to a commonly practiced form of assisted reproductive care. From such modest roots it has continued to promise high rates of success in women for whom the prognosis of pregnancy is otherwise greatly compromised by disease or age. Demand for donor services has fueled commercial interest and impacted the price of professional care. As a result of competition, the payment of egg donors has escalated to prices which make DIVF unaffordable to many potential recipients. Occasionally combined with surrogacy, DIVF may be employed by men and women who otherwise would be incapable of having children. Unconventional applications, such as assisting gay men in their quest to have children, have further fueled debate. However, despite the attendant controversy, it is likely that DIVF will continue to be a popular choice for childbearing and remain in high demand and frequent use.

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15

Complications of Infertility Treatment

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Introduction

Thanks to assisted reproductive techniques (ART) such as vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), cases of infertility that once were untreatable are today easily resolved. Nevertheless, concerns have been raised about the safety of ART and the potential acute and even long-term problems.

Because infertility treatment is "elective" and infertile patients are in general in good health, clinicians must be aware of risks; counseling patients and minimizing complications is imperative.

Complications of infertility treatment can be divided in a) acute, occurring during the phase of ovulation induction, and b) late, manifesting during or after pregnancy (Table 15.1).

Acute complications

Medication-related complications

Multiple pregnancy

Multiple gestations are the most significant risk of ovulation induction and infertility treatment, with a relative risk as high as 20–30-fold compared to normal conception. For example, the 2007 US incidence of twins and high order multiple gestations (3 or more implantations) after IVF was 33% and 1.8% respectively, compared to 2–3% and 0.1% after spontaneous conception. These numbers may be an underestimate, since many multiple pregnancies may reduce spontaneously or as the result of medical intervention before delivery. In addition, the incidence of monozygotic twins after ART (1–5%) also appears to be higher than in nature (0.4%).

Many efforts have been attempted to curtail the incidence of multiple gestation after ART. Sweden demonstrated that single embryo transfer policy resulted in a stable pregnancy rate (26%) with reduced multiple pregnancy rates (<1%).

Multiple gestation increases both maternal and infant health risks. In 2007, the Centers for Disease Control and Prevention reported that the preterm delivery rate of higher-order multiples was 95% and for twins it was 63%. The respective percentages of infants born with low birth weight (LBW) were 90% and 57%. Both LBW and preterm delivery have been independently associated with other morbidities such as cerebral palsy and neurodevelopmental delay. Furthermore, the mortality rates for infants from multiple gestations are more than fivefold higher (31.5/1,000) compared to infants from singleton gestations (6/1,000).

Importantly, maternal risks are also increased as a result of multiple gestations. Walker et al. analyzed a large retrospective cohort of multiple gestations and reported the maternal morbidity. The relative risks for pre-eclampsia (2.78), gestational diabetes (1.12), myocardial infarction (3.70), heart failure (12.94), venous thromboembolism (2.65), pulmonary edema (7.13), postpartum hemorrhage (1.88), hysterectomy (2.29), and blood transfusion (1.67) were significantly

Infertility, 1st edition. Edited by Emre Seli.

Table 15.1 Complications of infertility treatment

Short term	
Medication-related	Side effects of medications Ovarian hyperstimulation syndrome Ovarian torsion Cyst rupture Thromboembolism Ectopic/heterotopic pregnancy Multiple pregnancy Psychological stress
Procedure-related	Bleeding Infection Injuries to other organs
Pregnancy complications	Vanishing twin Rupture of uterine surface veins Preterm delivery Low birth weight Premature rupture of the membranes Perinatal mortality Placenta praevia Gestational diabetes Pre-eclampsia Delivery induction Caesarean section
Long term *On the child*	Congenital malformations ? Increased blood pressure ? Altered body fat composition Imprinting disorders
On the mother	No evidence of increased cancer risk

Table 15.2 Risk factors for OHSS

Previous history of OHSS
Younger age
Low BMI
Polycystic ovary syndrome
Polymorphisms of FSH receptor
Blood group A
Elevated estradiol concentrations during stimulation
Steep slope of estradiol increase during stimulation
High number and size of follicles
High basal AMH levels
High inhibin A and B levels
High VEGF and low α_2-macroglobulin levels (an inhibitor of VEGF)

AMH, antimüllerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; OHSS, ovarain hyperstimulation syndrome; VEGF, vascular endothelial growth factor.

increased for women with multiple gestations compared to women with singleton gestations.

Monozygotic twinning is associated with unique and severe complications: twin-to-twin transfusion syndrome, growth discordance, and acardiac twinning.

ART physicians, programs and patients all share responsibility for decreasing the rate of multiple gestation after ART. In order to decrease the infant morbidity associated with ART, the incidence of multiple gestation must be reduced.

Ovarian hyperstimulation syndrome

Despite the many approaches in stimulation protocols that have been proposed to reduce the incidence of ovarian hyperstimulation syndrome (OHSS), the risk of excessive response to treatment remains one of the most common complications (0.2–1% of ovulation inductions) and second in importance and incidence only to multiple pregnancy.

The syndrome is characterized by ovarian enlargement and fluid shift from the capillaries to the third space as a result of increased vascular permeability, triggered by human chorionic gonadotropin (hCG).

All women undergoing ovarian stimulation should be considered at risk of OHSS, although a subset of patients is at increased risk (Table 15.2).

The symptoms include abdominal discomfort due to the increased size of the stimulated ovaries (that can become frank pain with increasing amount of ascites), nausea, vomiting, occasional diarrhea, and shortness of breath. In more severe forms fluid accumulation can occur in the pleura

and pericardiac space with relative worsening dyspnea and chest tightness. "Third-spacing" of fluids may induce haemoconcentration, hypoalbuminaemia, and electrolyte imbalance; hypovolemia and oliguria may follow. In the most severe form, life-threatening conditions such as deep venous thrombosis (DVT) and pulmonary embolism, adult respiratory distress syndrome (ARDS), renal failure, and liver dysfunction can occur.

The treatment depends on the severity of the condition. Milder forms can be treated in outpatient settings with close monitoring of fluid intake and urine output. More severe forms require hospitalization with close fluid monitoring, DVT prophylaxis, and paracentesis for symptom relief.

CAUTION

OHSS

- Prevention is mandatory through identification of risk factors.
- In the most severe form, life-threatening conditions could occur.
- Although there are patients who appears to be predisposed to this complication, all women undergoing ovarian stimulation should be considered at risk of OHSS.
- OHSS is a dynamic condition and has to be carefully monitored for evidence of transition to more severe grades of the syndrome.
- Treatment could range from outpatient management to hospitalization.

Interestingly pregnancies progressing after OHSS have a higher incidence of complications. Higher rates of gestational diabetes, pregnancy-induced hypertension (PIH), placental abruption, prematurity, and LBW have been reported following severe OHSS. In addition, a higher miscarriage rate in OHSS patients has been well documented in the literature. The etiology of these complications can be related to the ovarian hyperstimulation itself, maternal predisposition to certain pathologies, hemodynamic instability and exposure to high endogenous estrogens, cytokines, renin, and prostaglandins. This evidence suggest that OHSS is a detrimental clinical situation not only for the mother but also for the developing pregnancy. Severe forms should be thus considered as high-risk pregnancies, and followed as such.

Ectopic pregnancy

Tubal pregnancies occur more frequently after IVF (1.9–3.4%, compared to 1–2% in natural conception), because of the increased number of embryos transferred and the increased levels of both estrogen and progesterone levels that can alter the tubal motility. Importantly, interstitial pregnancy can occur also after bilateral salpingectomy.

Diagnosis is performed by transvaginal ultrasound examination with systematic and attentive examination of the adnexa. Since the ultrasound appearance of an ectopic pregnancy is often more difficult to ascertain, it is important always to monitor the adnexa also if an intrauterine pregnancy is noted. A delay in diagnosis could have catastrophic consequences.

TIPS & TRICKS

Ectopic pregnancy

- Infertile women have a higher risk of ectopic pregnancy.
- Stimulated ovaries are bigger than normal and can mask the ectopic implantation.
- An intrauterine pregnancy does not rule out a coexisting ectopic one (heterotopic pregnancy).
- Peritoneal ascites is often present due to hyperstimulation and independent of ectopic presence.
- Adnexal examination can be complicated by the existence of coexisting pathologic conditions such as endometriosis, hydrosalpinges, adhesions.
- Management includes repeated sonographic examinations, weekly follow-up, and close monitoring of clinical symptoms.
- Salvaging a viable intrauterine pregnancy and avoiding maternal morbidity and mortality requires a high index of suspicion, repeated ultrasound scans, and early intervention.

A unique complication related to ovarian stimulation is the possibility of heterotopic pregnancy, or the existence of one pregnancy in the uterus and one or more pregnancies in an ectopic location. In the past heterotopic pregnancy was an extremely rare event, but the rate of heterotopic pregnancy after IVF is estimated to be as high as 0.2–1%.

Reassuringly, the intrauterine pregnancy is often healthy. Monitoring serum hCG levels plays a marginal role in making the diagnosis since the hormone is secreted by the live intrauterine embryo. A high index of suspicion, repeated ultrasound scans, and early intervention are mandatory to salvage the viable intrauterine pregnancy and avoid maternal morbidity and mortality.

Ovarian torsion

Adnexal torsion is a true gynecologic emergency. It is characterized by the twisting of the ovary around its pedicle to such an extent as to occlude the ovarian vein and/or artery. Three large studies determined the incidence of adnexal torsion after IVF to be 0.08–0.13%.

Since an increase in ovarian volume renders the ovary more prone to rotation, the ovarian enlargement that follows ovulation induction is a predisposing factor to torsion. Clinical presentation is often complicated by a coexisting OHSS or pregnancy (or both). In the first case, the abdomen is already distended and tender because of the enlarged stimulated ovaries; with pregnancy, the ovarian enlargement secondary to the corpora lutea persists for a longer time, prolonging the period of increased risk of adnexal torsion.

Patients with ovarian torsion usually present with acute onset of unilateral lower abdominal or back pain, intermittent or constant in nature, frequently accompanied by nausea and vomiting. Signs of ovarian torsion include palpable pelvic mass, localized peritoneal irritation, a low-grade fever, and leukocytosis. A modest amount of free fluid could be present in the pouch of Douglas; this sign is similar to the small amount of ascites found in mild OHSS, increasing the difficulty of differential diagnosis. As a result of varying degrees of ovarian arterial, venous, and lymphatic occlusion with torsion, the ovarian parenchyma can assume different appearances; sonographic findings can therefore be heterogeneous, ranging from a normal appearing ovary, to an ovary with marked stromal edema and reduced or absent Doppler flow. Clinical suspicion must therefore guide the decision-making process. A clinical picture consistent with ovarian torsion should advise laparoscopic surgical exploration even in the context of normal Doppler flow. In fact, intermittent torsion could result in flow being detected.

☆ TIPS & TRICKS

Ovarian torsion

- Adnexal torsion is a true gynecologic emergency.
- The risk of ovarian torsion is increased with ovarian stimulation and further increased with OHSS and with pregnancy.
- The diagnosis is clinical.
- The differential diagnosis include ovarian torsion, ruptured ovarian cyst, and appendicitis.
- Doppler flow in the enlarged cystic ovaries can be helpful but occasionally could be normal.
- The macroscopic appearance of the adnexa is not a reliable indicator of tissue viability.
- Preserve the adnexa whenever feasible, despite the ovary's appearance.

Early surgical treatment includes laparoscopy to unwind the ovary. The variability in the degree of vascular compromise and the possible collateral vasculature may help to preserve the ovary despite a significant ischemic insult. Unwinding saves over 90% of ovaries, despite their necrotic, hemorrhagic, bluish-black appearance. Oophoropexy to fix the enlarged ovary because of ovulation induction is not appropriate, since the enlargement of the ovary is likely to regress, thus removing the underlying cause. Aspiration reduction of cystic fluid can be performed since drainage could facilitate the ovary to be mobilized, reducing trauma and the risk of subsequent torsion.

Table 15.3 Thromboembolic complications

	Arterial thrombosis	Venous thrombosis
Presence of OHSS	95%	70%
Sites	54% cerebrovascular 23% extremities	78% neck and upper extremities
Time lapse from embryo transfer	10.5 days	40.0 days
Time lapse from hCG in ovulation induction cycles	8.5 days	26.6 days
Presence of inherited thrombophilia	19%	41%

hCG, human chorionic gonadotropin; OHSS, ovarian hyperstimulation syndrome.

Thromboembolism

The reported incidence of thromboembolism (TE) following ovulation induction is similar to the reported incidence of venous thrombosis during pregnancy (0.08–0.11% of treatment cycles; Table 15.3). Thirty per cent of thromboembolic cases occur during the stimulation cycle itself when the patient is not yet pregnant. It is thought that the marked increase in estradiol level and the activation of both coagulation and fibrinolysis pathways following ovulation induction are responsible for the increased TE risk. The risk is significantly increased in the presence of OHSS.

Cyst rupture

Women experience ovarian cyst rupture every month at time of ovulation. This event is benign in nature and often unnoticed. However, a subgroup of women notices this "midcycle pain" (mittelschmerz). The pain is secondary to peritoneal irritation secondary to the release of follicular fluid into the peritoneum. In addition, bleeding into the cyst can occur, provoking pain because of stretching of the ovarian capsule.

The incidence of cyst rupture in women following ovulation induction or in patients who developed ovarian hyperstimulation is thought to be high; however, the incidence of clinically significant rupture requiring surgical intervention is lower (<1%).

Side effects of fertility medications

Multiple medications are available for ovulation induction, including clomiphene citrate (CC), aromatase inhibitors (e.g., letrozole), human menopausal gonadotropin (hMG), urinary or recombinant preparations of follicle stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH) agonists and antagonists. In addition, oral contraceptive pills (OCPs) are often used to prepare an IVF cycle. Progesterone is used to support the luteal phase and the early pregnancy.

Most drugs used for ovulation induction are considered safe, and there is no clear evidence of teratogenicity.

CC is probably the medication with more reported acute side effects. Use of CC has been associated with hot flashes and mood changes (10% of patients), abdominal bloating or pelvic pain (5%), nausea or vomiting (2%), breast pain (2%), and headache (1%). Rare side effects that should prompt the immediate cessation of the medication include changes in vision and signs of an allergic reaction.

Subcutaneous injection of a urinary gonadotropin product can be associated with pain and bruising at the injection site.

Psychological stress

Emotional lability is common in patients undergoing fertility treatment. Couples with a diagnosis of infertility experience feelings of distress, loss of control, stigmatization, anxiety, and depression. Furthermore, the mental stress associated with IVF treatments could exert a negative influence in terms of pain perception or ability to react to adverse outcomes. Particular attention should be paid to reducing stress and monitoring the couple's long-term emotional adjustment to unsuccessful IVF.

Procedure-related complications

Egg retrieval

Transvaginal ultrasound-guided oocyte retrieval, otherwise known as oocyte pick-up (OPU), is the "gold standard" procedure performed to retrieve the eggs from the follicles.

OPU is normally performed with medications that induce conscious sedation or occasionally with a simple paracervical block. The patient may experience side effects of the anesthesia itself (inadequate oxygen saturation, hypotension), although this is rare. More commonly a tendency to vagal syndrome is observed, secondary to the immediate pain of the procedure and to the anxious state of the patient undergoing the procedure.

OPU can be associated with several complications, including pain, bleeding, infection, and injury to other organs. Pain score following OPU is dependent on the number of oocytes retrieved, indicating the greater trauma proportional to the higher number of oocytes obtained.

The overall incidence of intra-abdominal bleeding following OPU ranges between 0.08% and 0.2%; more common are minor vaginal hemorrhages (2.8%). In case of vaginal bleeding, local compression is usually sufficient to stop the bleeding. If there is evidence of intra-abdominal bleeding, the patient should be managed according to the clinical scenario. Serial physical examinations and hematocrit will clarify if the patient can be treated with observation and blood transfusion alone, or will need an emergency laparoscopy or laparotomy.

The incidence of pelvic infections and tubo-ovarian abscesses following OPU is low (0.3–0.6%). Since these complications can occur despite intravenous antibiotic administration, the use of prophylactic antibiotics is still controversial, except in case of actual genital infection in the patient.

Finally, pelvic structures may be inadvertently traumatized by the aspiration needle (usually a 16-gauge single- or double-lumen needle). The risk of injury to the bowel appears to be more theoretical than actual, since ultrasound guidance allows visualization of the bowel while retrieving oocytes. It is surprising that ureteral injuries are very rare, given the anatomical location of the ureters, immediately antero-lateral to the upper fornices of the vagina.

Intracytoplasmic sperm injection

The ICSI procedure requires a spermatozoon to be introduced into the cytoplasm of the oocytes with a sharp needle. This procedure therefore bypasses the natural series of events that occur at time of fertilization. In particular, (1) the sperm that fertilizes the eggs is chosen by the embryologist and might not represent the "fittest spermatozoon" according to Darwinian selection. In addition, (2) epididymal or testicular spermatozoa that were never ejaculated can be used (azoospermic patients—i.e., patients with no sperm in the ejaculate—can have spermatozoa in the epididymis or might have foci of testicular spermatogenesis). Finally, (3) imprinting might be incomplete and chromosomal aberration might be higher in men with nonobstructive azoospermia. Because of this, ICSI has always been observed with suspicion and multiple studies have been performed on the health of ICSI children.

Overall, studies have been reassuring and neonatal outcomes and major malformations rates appear to be comparable after IVF or ICSI treatments. In addition, recent studies show similar pregnancy rates after IVF or ICSI. Indeed, at least 50% of ART cycles in the United States are ICSI cycles, a larger number than expected based on the frequency of male factor infertility. However, long-term follow-up studies are needed to assess the sexual development and fertility of ICSI children. It is the authors' opinion that IVF should always be performed when possible.

Long-term complications

Pregnancy complications

In addition to the complications of multiple gestations described above, it is apparent that even singleton gestations following ART have increased complications. Adverse perinatal outcomes of singleton IVF pregnancy include preterm delivery, LBW, premature rupture of the membranes and perinatal mortality. Placenta previa, gestational diabetes, and pre-eclampsia are also associated with IVF.

The reason for these finding could be multifactorial. Maternal complications could be related to (1) the couple's fertility problems, (2) the treatment received (ovulation induction or culture of the embryo in vitro) or (3) the obstetrician's biases in treating a "highly desired" pregnancy. Indeed, even if they conceive spontaneously, women affected by infertility are predisposed to a series of complications in pregnancy including preterm delivery, LBW, perinatal mortality, placental abruption, and pre-eclampsia. Furthermore, the obstetric IVF population is unique: the women tend to be older, nulliparous, and sometimes affected by other chronic conditions (such as diabetes or metabolic alterations correlated to both PCOS and obesity). Women undergoing IVF are more prone to seek medical help, thus introducing an important bias when later investigations are undertaken.

IVF pregnancies have a 4.5 times higher risk for early bleeding. One in 10 IVF singletons originates from a twin gestation, and spontaneous reductions (the so-called "vanishing twin" syndrome) could be a possible cause of the increased morbidity in IVF singletons.

Long-term complications in the child

In developmental biology, one of the most compelling scientific hypotheses of the last decade is the developmental origin of health and disease (DOHaD) hypothesis, because it highlights the cardinal role played by early development in shaping future health. This hypothesis proposes that the initial etiologic event can be tracked back in time, decades before the disease manifests itself, at a time when the developing individual is particularly sensitive to environmental changes. In fact, in-utero exposures to noxious stimuli may cause permanent functional changes that are not overtly or grossly teratogenic yet result in increased susceptibility to disease later in the lifespan. Mounting evidence suggests that exposure to an abnormal environment early in pregnancy, or even limited to the preimplantation period, is associated with long-term sequelae.

The underlying scientific hypothesis behind the fetal basis of adult diseases has been developed in epidemiological studies by Dr. David Barker. Dr. Barker has shown that, during development, fetuses respond to severe malnutrition by favoring the metabolic demands of the growing central nervous system and heart at the expense of other tissues. The long-term consequences of this response are that the fetus is protected from death, but is more prone to diseases. In fact, markers of malnutrition, such as LBW and intrauterine growth retardation (IUGR), predict the subsequent occurrence of hypertension, hyperlipidemia, insulin resistance, type 2 diabetes, ischemic heart disease, and breast or prostate cancer in adult life.

With the first children of IVF now reaching adulthood and reproductive age, attention has increasingly focused on the health of children beyond the immediate neonatal period. Three meta-analyses have shown a 30% increase in congenital malformations after ART (an incidence of 4–5% in the ART population vs. 3–4% in the general population). Recent evidence suggests that pubertal children conceived by IVF display increased blood pressure, and an altered body fat composition. These reports are not conclusive and may be clouded by detection and reporting bias. In fact, the IVF population is more prone to use the healthcare system for their child; furthermore, the parents' infertility status must be considered.

Recently attention has been directed towards epigenetic errors that might be associated with infertility itself or with infertility treatment (see box). Differential DNA methylation and histone modifications leading to expression of only one of two parental alleles is a mechanism of gene regulation known as imprinting. In particular, preimplantation embryo development is particularly sensitive to epigenetic regulation. An abnormality of this early process could have permanent consequences, and indeed abnormalities of imprinted genes are described following preimplantation embryo culture in animals. Furthermore, a series of reports in humans suggests an increase of two extremely rare imprinting disorders, Angelman syndrome and Beckwith–Wiedemann syndrome, in children born after ART. It is thought that there is a three- to fivefold increase in imprinted disorders following ART (1/4,000 liveborn, compared to 1/12,000 in the general population).

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Epigenetic regulation of gene expression

Gene expression in different cell types and tissues is regulated by differential DNA methylation and histone modifications. DNA methylation occurs via addition of a methyl group (CH_3) to a cytosine base, resulting in silencing of gene expression. Histone modification occurs via the addition of different organic groups (methyl, acetyl, phosphate, ubiquitin, etc.) to the tails of histones, the basic proteins around which the DNA coils. Histone modification can results in activation or repression of gene expression.

Culture of embryos in vitro can alter the epigenetic marks of embryos, resulting, potentially, in predisposition to diseases later in life.

Imprinted genes are a subgroup of approximately 100 genes that are unique because they are inherited in a silent state from one of the two parents and therefore the individual is a functional hemizygote.

Rare diseases resulting from abnormal epigenetic regulation of imprinted genes have been described in ART children.

Long-term complications in the mother

Since hormonal factors are known to be involved in the etiology of female cancers, a stimulating effect of fertility drugs on the risk of these cancers is theoretically possible.

A number of studies have attempted to ascertain whether infertility treatments have long-term effects on cancer risk, but most have had shortcomings. The most important confounding factor for the correct data interpretation is the infertile status of the patient.

Most studies are reassuring in not showing a strong association between the use of medications and risks of most cancers. Furthermore, an association between ovulation induction and female cancers does not necessarily mean a causal effect, since infertility alone could be an independent risk factor for the development of these cancers.

An association between infertility and an increased ovarian cancer risk has long been suggested, but so far no evidence for a carcinogenic effect of IVF treatments has been found.

In a recent review a combined analysis of 11 cohort studies showed no significant association of fertility medications and breast cancer (with a pooled relative risk of 1.06). However there may be a transient increase in the incidence of breast cancer in the first year due to earlier diagnosis.

Recognized risk factors for endometrial cancer are nulliparity, late-onset menopause, obesity, and PCOS. Many infertile women possess these characteristics and it is not surprising that the infertile population has an increased predisposition to endometrial cancer. However, it is difficult to ascribe additional risk to these women because of infertility treatment and indeed an independent increase risk because of treatment has not been demonstrated. A protective effect of CC was instead described for cervical cancer, probably due to its antiestrogenic effect. Finally the association between thyroid cancer and fertility drugs or CC and melanoma is still debated.

Summary

As technology advances the risk/benefit ratio is continuously shifting, and physicians need to continuously evaluate the best evidence to decide what treatment to offer to patients.

However the clinicians should not forget the principle of "first, do no harm", and long-term follow-up studies are needed both on the mother and the child to guarantee and reassure patients on the safety of ART. Particular care should be used when we consider the case of oocyte donors. In fact, when risks are incurred by young, healthy women undergoing procedures without medical benefit to themselves, it is imperative to work assiduously to safeguard their health.

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16

Preimplantation Genetic Diagnosis

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Introduction

Preimplantation genetic diagnosis (PGD) is the screening of embryos at the cleavage stage in order to select and transfer only the desired ones. The reason we use the term "desired" and not "normal" will become obvious when we present the PGD indications below. The European Society of Human Reproduction and Embryology (ESHRE) classifies PGD into two categories:

- High-risk PGD for patients at high risk of transmitting a genetic or chromosomal abnormality to their children, which includes single-gene defects, namely autosomal recessive, autosomal dominant and X-linked disorders, as well as chromosomal abnormalities (translocations, small deletions, etc.)
- Low-risk PGD (or preimplantation genetic screening, PGS) for sex selection, and for infertile patients undergoing in-vitro fertilization (IVF) with the aim of increasing the IVF pregnancy rates. Patients who fall into this category are those with advanced maternal age and repeated IVF failure, and couples with normal karyotypes who have experienced repeated miscarriages.

PGD is performed in very early stage embryos before implantation, and therefore requires the use of IVF despite the fact that a great proportion of patients are not infertile. For example, the great majority of patients seeking PGD for single-gene disorders are fertile and thus the pregnancy success rates after PGD are higher than for other groups of IVF patients.

CAUTION

Unlike prenatal diagnosis, which can reach confidence levels of 99%, PGD has a higher error rate and the confidence of diagnosis is approximately 90%, assuming a 10% error rate. For patients who are carriers of a monogenic recessive disorder, the chance of having an affected embryo is 25%. With PGD, the chance is reduced to 10%. This should be borne in mind when counseling patients.

Embryo biopsy

PGD can be performed either on one blastomere biopsied from the embryo 3 days after fertilization, or a group of cells removed from the blastocyst on day 5 after fertilization, or even in the polar bodies extruded from oocytes during meiosis. Polar body biopsy can only test for conditions inherited from the mother and can only speculate on the chromosomal complement of the oocyte, depending on what is found in the polar body. For example, if the polar body contains an extra copy of X chromosome, it is assumed that the oocyte lacks one X. This method is therefore not as accurate as blastomere biopsy.

Following IVF, the embryos to be tested are cultured for 3 days and are expected to have undergone 2–4 cell divisions and consist of 4–16 cells. At this stage all blastomeres are known to be identical and totipotent. The biopsy is performed by a trained embryologist, and is done in two steps: first, an opening is made in the zona pellucida surrounding the oocyte with a laser; a

Infertility, 1st edition. Edited by Emre Seli.

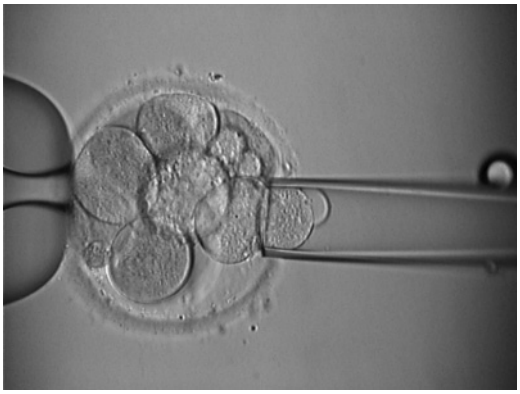

Figure 16.1 Embryo biopsy for PGD. Courtesy of Dr. Denny Sakkas, Yale University School of Medicine.

biopsy pipette is inserted through the opening and one blastomere is removed for analysis (Figure 16.1). The rest of the embryo is then returned to the incubator to continue its development.

CAUTION

Biopsy of two blastomeres rather than of one from an eight-cell embryo is contraindicated, because the viability of such embryos is reduced.

The biopsy procedure is the same regardless of the indication. However, further processing of the cell is dependent on the molecular technique used to diagnose the condition needed, as discussed below.

In most cases, fertilization of embryos to be tested by PGD is done using intracytoplasmic sperm injection (ICSI) and not regular oocyte insemination with sperm, in order to prevent the presence of spermatozoa in proximity to the oocyte. If present during biopsy, these spermatozoa could be transferred in the analysis tube together with the blastomere and confuse the analysis, especially in those methods involving the polymerase chain reaction (PCR). For the same reason, the oocyte has to be completely stripped of its cumulus cells prior to ICSI.

Ideally any embryo testing should be completed within 48 h from the biopsy, to allow direct transfer of the embryo to the mother without the need for embryo cryopreservation. More recently, improved embryo freezing methods have contributed to higher viability of cryopreserved biopsied embryos and have allowed PGD to be performed at later stages of embryo development, such as the blastocyst stage.

Embryo biopsy is an invasive method and should be only be done by a well-trained embryologist. Examination and medical follow-up of children up to 2 years of age have not shown any increase in congenital abnormalities due to PGD/PGS.

PGD for monogenic disorders

The first human pregnancy after PGD was achieved in 1990. The embryos were at risk for an X-linked disorder and male embryos were selected against, using markers on the Y chromosome. Since the first clinical application of PGD, the methodology has greatly improved and the approach to PGD for monogenic disorders has changed to direct detection of the causative mutation. PGD has been carried out for more than 100 monogenic disorders worldwide.

A PGD protocol can be created for literally any disease with a known causative gene and mutation. Each protocol is tailor made for the requesting family, since it is designed to detect their causative mutation. Certain mutations, such as ΔF508 in the cystic fibrosis gene, are very prevalent in the general population, and it is likely that in the near future there will be commercially available diagnostic kits that will be used by all laboratories without specific validation in every family. Currently, all PGD laboratories require set-up time prior to IVF/PGD.

Genomic DNA from the parents and any carrier or affected children is examined first to confirm the presence of the mutation. One of the most common problems of PGD is allele dropout (ADO), discussed in more detail below, which is failure to obtain information from one of the two alleles of a gene. ADO is common in single cells but very rare in genomic DNA, mainly because of the limited amount of DNA obtained from a single cell. For this reason, all protocols for monogenic disorders include the analysis of one or more linked polymorphic markers. The markers should be located very near or even

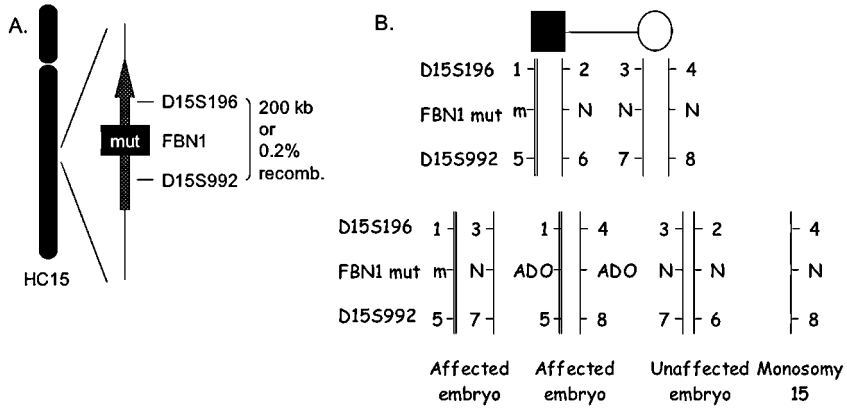

Figure 16.2 Schematic representation of the approach for PGD for monogenic disorders. (A) As an example, Marfan syndrome is caused by mutations on the *FBN1* gene on chromosome 15 (HC15). Two polymorphic markers flanking the *FBN1* gene were genotyped in combination with the *FBN1* mutation in blastomeres and the observed results and interpretation are shown in (B). (B) The parental haplotypes are shown at the top. Possible findings in PGD and the interpretation of the disease status of the embryos is shown on the lower panel. ADO, allele dropout.

within the gene of interest and cosegregate with the mutation during meiosis (Figure 16.2). They also need to be informative, which means that the father and mother have different alleles. For these reasons, most PGD centers test several markers prior to the IVF cycle and create a personalized PGD protocol for each couple that takes into consideration the mutation, the informative polymorphic markers, and the accuracy of the multiplex PCR when done in single cells. In order to establish the "phase" of the mutation, or which polymorphic allele is on the same chromosome as the mutation and which lies on the other chromosome, the investigator could examine the transmission on another affected or unaffected child of the family. In case of paternal mutations, analysis of single sperm cells can be done before IVF/PGD. Otherwise, the phase can be assessed directly on the embryos examined.

The blastomere biopsy is usually performed on day 3 and the blastomere is transferred in a PCR tube and lysed to expose its DNA. Multiplex PCR, including several primer sets for the markers that were optimized during the protocol set-up, is typically performed. Each fragment is labeled with a different fluorochrome, which is usually incorporated in the 5′ end of one of the primers. The PCR products can be detected after separation in a DNA sequencer which provides accurate determination of the amplicon size with 1-bp resolution. If the mutation is a deletion or insertion of one or more bases, such as the ΔF508 mutation in the cystic fibrosis gene, it can be directly detected on the sequencer. Otherwise, it may require further processing with restriction enzyme digestion selective for the normal or mutant alleles. Additional methods that involve real-time PCR or minisequencing have been developed in a gene-specific manner.

Pitfalls of PCR methods for PGD

The main problems faced by PGD providers using PCR are ADO, failed amplification (FA), and PCR contamination. ADO is defined as FA of one of the two gene alleles during the PCR, and therefore the absence of this allele in the final pool of PCR products (Figure 16.2). ADO is relevant when the embryo is heterozygous for a mutation or a linked marker and can be detrimental in cases of dominant disorders if the mutant allele is the one that fails to amplify to detectable levels. The embryo will appear normal although in reality it will be affected. FA is a severe case of ADO where both alleles fail to amplify. In this case a diagnosis is impossible. ADO and FA are influenced by the quality of the embryo, the lysis method, and the PCR conditions. Morphologically poor embryos

often contain degraded DNA in some or all of their blastomeres and can show ADO when analyzed. There have been extensive efforts to determine the best lysis solutions. Currently, most PGD centers use either a proteinase K-SDS lysis solution, or an alkaline lysis solution containing NaOH. The use of polymorphic markers residing near the gene of interest, described above, is designed to circumvent the problems of incomplete amplification.

PCR contamination is managed by physical separation of the pre-PCR and the post-PCR areas. Protective equipment such as gloves, masks, and sterile gowns have to be worn by the scientist who prepares the reactions and changed before moving between the post-PCR and pre-PCR areas. Moreover, primers, PCR enzymes and plastic consumables are handled only in the pre-PCR area, which is usually equipped with a UV lamp to eliminate any contamination after setting up the reaction. A negative control tube that includes all PCR materials except the DNA template should be included in every single reaction. In order to safeguard the optimal conditions for the performance of PGD, accreditation of the laboratory is recommended.

HLA typing

PGD for HLA matching is used to select an embryo who is immunologically matched with a sick sibling and can be used as a stem cell donor. The cord blood of the newborn child is to be used as a source of stem cells for the treatment of the older child. Often, the child who requires the transplant is a carrier of a known mutation. In this case the desired embryos are tested for the HLA loci and the causative mutation.

For HLA typing the biopsied blastomere is lysed in a PCR tube as described above for monogenic disorders and a multiplex PCR for all the tested genomic areas is performed simultaneously. Knowing the HLA type of the affected child is a prerequisite for the diagnosis.

The most common obstacle of this type of PGD is that the mother of the affected child may be of advanced maternal age when her child requires a transplant and her ovarian function may be declining. In this case the chances of producing healthy and HLA-matched embryos are low.

Chromosomal rearrangements

Chromosomal rearrangements include balanced translocations, inversions, and deletions. In balanced translocations, two chromosomes have exchanged fragments in a precise manner and therefore the carrier parent contains the correct amount of genetic material. During meiosis, upon separation of chromosomal pairs, a gamete, and subsequently the resulting embryo, can contain a mix of normal and translocated chromosomes that can result in a surplus or lack of genetic material (Figure 16.3). Depending on the genes carried by the affected genomic portion, these abnormalities are often incompatible with life, and result in miscarriage or elective pregnancy termination due to extensive fetal abnormalities. Approximately 1–2% of pregnancy losses are due to previously undiagnosed balanced translocations carried by one of the parents.

PGD can be performed to select the normal or balanced embryos. Traditionally these cases are investigated by fluorescent in-situ hybridization (FISH) with probes selected to detect the two affected chromosomes (Figure 16.3). The embryos are biopsied on day 3 after fertilization and the blastomere is fixed on a glass slide with methanol–acetic acid, which dissolves the cytoplasm and exposes the DNA of the nucleus. The fixed blastomeres are hybridized with probes chosen within the rearranged chromosomes. The protocol is made specifically for the type of translocation to be tested and requires set-up prior to IVF/PGD.

Several studies have shown the benefits of PGD for translocation carriers, including higher live birth rates and lower miscarriage rates. However, PGD for translocations is not as successful as theoretically predicted, because the numbers of normal or balanced embryos are lower than the expected 50% (25% normal and 25% balanced; Figure 16.3). This is most likely due to errors during meiosis, and in particular during the step of pairing and crossover (chiasmata) between homologous chromosomes.

More recently, the application of microarrays to whole-genome screening, discussed below, provides the possibility for testing for chromosomal imbalances in combination with a comprehensive chromosomal analysis.

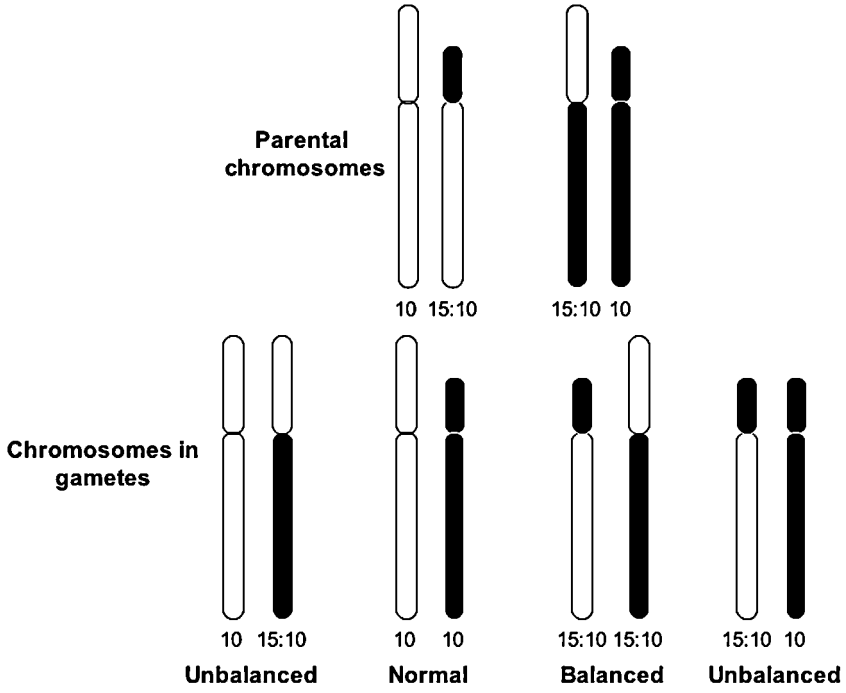

Figure 16.3 Schematic representation of a balanced translocation between chromosomes 10 and 15. The parental chromosomes are shown at the top. The chromosome 10 is white and the chromosome 15 is black. The possible segregation of these two chromosomes in the gametes is shown at the bottom.

Preimplantation genetic screening

Aneuploidy screening using FISH

Approximately 20% of all pregnancies result in miscarriage. Losses due to aneuploidy depend on the maternal age: 57% of abnormal karyotypes found in fetuses of women less than 35 years of age, and 82% in women 35 and older. The success of IVF is also heavily dependent on maternal age. For these reasons, it seemed reasonable to use PGS in women with advanced maternal age (AMA) and repeated IVF failure.

PGS is performed using FISH on a blastomere from a day 3 embryo which is fixed on a glass slide, as is also done for translocations. DNA probes for the 9–12 chromosomes to be tested are labeled with different fluorochromes and hybridized to the slide in two or three successive hybridizations. The signals are visualized using a microscope. Most PGS protocols target specifically the nine most common aneuploidies seen in spontaneous abortions or birth (X, Y, 13, 15, 16, 17, 18, 21, 22).

Significant improvement of IVF success and pregnancy rates were reported in AMA and poor prognosis patients, although these were not randomized controlled trials. Most PGS cases worldwide until 2007 were testing embryo aneuploidy. However, a number of randomized control trials showed that this method did not fulfill the initial hope of improving pregnancy rates, and the number of cases in the last 3 years has dropped dramatically.

It is now believed that FISH for nine chromosomes is unsuccessful for two main reasons. Only a limited number of chromosomes can be tested, while several whole-genome studies have shown that aneuploidy can occur for all chromosomes. FISH is a technically difficult method and is prone to many errors. Whole-genome screening is currently the method of choice, and the different methods and limitations are discussed below.

Whole-genome screening for aneuploidy

Comparative Genome Hybridization

The first successful attempt to characterize all 23 pairs of chromosomes of a cleavage-stage embryo was done using comparative genome hybridization (CGH). In this method, the biopsied blastomere is lysed in a PCR tube and the DNA is amplified and labeled with a fluorochrome. Another DNA sample from a normal individual is labeled with a different color and the two are mixed and applied to a glass slide carrying chromosomes in metaphase. Any color intensity difference indicates the loss or gain of genetic material. CGH has been successfully done in polar bodies and cleavage stage embryos. Recently, a pilot study of blastocyst screening using CGH has shown very high pregnancy rates and is discussed further in "Advances in PGD and future perspectives" below.

Array CGH

Array CGH (aCGH) is defined as comparative genome hybridization done in a microarray format. Instead of chromosomes in metaphase, nucleic acid fragments covering the whole genome are dotted (arrayed) on a glass slide. In a manner similar to traditional CGH, the amplified DNA from the embryo is labeled with one fluorochrome and the control DNA with another. The mix is applied to the array and hybridized. Information is gathered and analyzed using computer software that calculates the probabilities of a chromosomal area being over- or underrepresented. Several commercial or custom-made microarray platforms have been tested for prenatal diagnosis and PGD. There are two main array platforms according to the type of DNA dotted on the array (bacterial artificial chromosomes and oligonucleotides). These arrays can detect copy number differences and imbalances of the tested cell. Therefore, they can also be applied in the detection of chromosomal rearrangements such as translocations or microdeletions, but they cannot distinguish a balanced from a normal sample.

Single nucleotide polymorphism (SNP) arrays

Another type of microarrays for genome screening is SNP arrays, originally developed for linkage analysis. They contain several thousand of the single nucleotide polymorphisms (SNPs) found in the human genome and therefore provide extensive coverage of the chromosomes. The analysis of the results requires powerful computer hardware and software, but it generates considerably more information than any other type of array. The DNA from the parents is also tested, and it is possible to follow which allele is inherited from which parent. In addition to aneuploidy this method can also detect uniparental disomy and the parental origin of each abnormality.

All microarray platforms require sophisticated statistics to analyze the results of the hybridization and can provide a vast amount of genomic information about the preimplantation embryos. It remains to be determined how much of this information is helpful for the clinician and the patient, and whether in some cases it may complicate treatment.

Pitfalls of whole-genome amplification

The amount of DNA present in one cell is not sufficient for any of the CGH methods, either conventional, aCGH, or SNP array. Ideally the DNA needs to be amplified to approximately 1 µg with equal specificity and sensitivity for all genomic regions, in order for their ratio to be kept identical with the starting material. Several whole-genome amplification methods have been evaluated and multiple displacement amplification (MDA) is currently the preferred method despite the higher than desired ADO rate.

Whole-genome amplification has also been used instead of nested PCR in several examples of PGD for monogenic disorders. In the future whole-genome amplification will be used to provide sufficient DNA for combined aneuploidy screening with aCGH and PGD for a monogenic disorder.

Ethical aspects of PGD

PGD can be performed for any known gene, mutation, or polymorphism. Ethical dilemmas arise from the potential use of PGD in predisposition to diseases, such as mutations in the *BRCA1* gene that predispose carriers to breast cancer. Opponents argue that a carrier who is aware of the mutation can take specific action to ensure early diagnosis, but the quality of life of a woman

who lives constantly with the fear of such a severe disease is greatly compromised. Another group of diseases that requires special management is the late-onset neurodegenerative disorders, such as Huntington's, that are currently incurable. From our experience and that of other clinical teams, couples requesting PGD for Huntington's often do not know if they are carriers of the disease but have an affected relative. Usually they do not desire to know if they carry the mutation, but want to undergo IVF/PGD to select embryos without the disease. In 50% of such cases, the pre-PGD set-up reveals that the family does not carry the mutation, and they have no need for PGD, but they go ahead because they do not want to know the result.

HLA typing is another use of PGD that presents ethical dilemmas. Only a minority of embryos are HLA-matched and the rest are discarded although some of them are normal and viable.

Currently the most controversial use of PGD is gender selection, also termed "social sexing" by ESHRE, and "family balancing" by the American Society of Reproductive Medicine (ASRM). This selection is not done to prevent an X-linked disease but only to select male or female embryos to satisfy the wish of the family or of their social surroundings.

As the use of PGD becomes more widespread and more knowledge becomes available about the function of genes and the relation between genetic and phenotypic variation, it is not unthinkable to speculate that requests for certain physical characteristics or intelligence will reach the PGD laboratory. Regulatory bodies such as ESHRE and ASRM should be prepared to deal with such requests and delineate what is acceptable and what not in terms of PGD indications.

Advances in PGD and future perspectives

Blastocyst biopsy and embryo vitrification

Embryo mosaicism, which is more prominent in the early stages of development, is one of the factors contributing to the PGS error rate. Embryos that reach the blastocyst stage have a higher chance of being euploid. In fact, 70% of the embryos that develop to blastocyst are euploid and 30% are aneuploid. However, not all blastocysts are normal, and therefore simply reaching the blastocyst stage is not enough the diagnose this embryo as normal.

SCIENCE REVISITED

Embryos at the cleavage stage (4–16 blastomeres) show a high degree of mosaicism and some of their cells may be abnormal while the rest are normal. Blastocyst-stage embryos are more homogeneous.

Recently a pilot study to assess the success rate of CGH in blastocyst screening showed 50% improvement in implantation rates. This study employed a first selection of potentially viable embryos by their conversion to blastocysts and then tested their whole genome using CGH. Since CGH requires several days to do, the embryos were frozen until analysis was complete. The survival of biopsied embryos after freezing with conventional slow freezing methods is low. Recently, vitrification has been shown to result in very high embryo survival. Vitrification will also be useful in the case of patients seeking PGD for monogenic disorders, who often have several normal embryos from one IVF cycle.

Another advantage of blastocyst biopsy for the PGD cases that require PCR is that the amount of DNA will be greatly increased from biopsying a group of cells and therefore, ADO rates will be lower and the accuracy of the results will be higher.

SNP arrays to detect single-gene mutations

There are several available SNP arrays that can be used for PGD as well as linkage analysis. Their design is constantly expanding to include more SNPs that are validated through the Human Genome Project. Some of the SNPs that are currently included in the SNP database of the National Center for Biotechnology Information (NCBI) are known gene mutations with high prevalence. For example the cystic fibrosis ΔF508 is classified as rs332 (http://www.ncbi.nlm.nih.gov/SNP). SNP arrays will be able to detect monogenic mutations on the same time as screening the rest of the genome for aneuploidy,

and there will be no requirement for set-up prior to PGD.

Summary

PGD is the screening of embryos at the cleavage stage in order to select and transfer only the desired embryo. The main indications for PGD are monogenic disorders, chromosomal rearrangements, and aneuploidy. Embryos can be biopsied at the cleavage or blastocyst stage, and therefore PGD requires the use of IVF despite the fact that a large proportion of the patients do not face infertility problems. This chapter presents the clinical indications for PGD, the recent advances in PGD methodology, their advantages, disadvantages, and challenges.

PGD for monogenic disorders remains the most successful indication, but is technically more challenging. A customized protocol to detect the specific mutation(s) of each family is designed, and the main method employed is PCR. The DNA from one blastomere needs to be amplified several times to become detectable and this amplification is the source of the two main challenges of PGD for monogenic disorders, which are ADO and PCR contamination.

Chromosomal rearrangements are tested using FISH with probes specific for the affected areas of the chromosomes. In addition to the technical aspects of FISH, the main challenge here is that only a small proportion of the gametes produced from a parent with the translocation are euploid due to complications that arise during meiosis.

PGD for aneuploidy (also known as preimplantation genetic screening, PGS) was offered mainly to patients with poor prognosis due to AMA or previous failed IVF attempts, but was proven inefficient. A limited number of chromosomes were tested using FISH. PGS has passed through a period of intense reconsideration and is now re-emerging using methods that offer whole-genome screening. This method can employ different types of microarray platforms and may have a much wider use than PGS. Chromosomal imbalances can be detected with arrays instead of FISH, offering additional whole-genome screening for aneuploidies.

Finally, this chapter discussed the ethical considerations arising from more widespread use of PGD to select for certain traits that do not cause a disease or a physical or psychological defect. One such example is gender selection. Moreover, as the knowledge of the gene contribution to the physical traits or intelligence is increasing, so is the demand for a designer child that possesses certain characteristics. Institutional ethics committees will soon have to decide on the appropriate use of such a technology.

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17

Fertility Preservation

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Introduction

Cancer is not uncommon among younger women. In the United States, 408.5 per 100,000 (>600,000) women were diagnosed with cancer yearly between 2002 and 2006, and one-tenth of these women were under the age of 45. Approximately 80% of teenage girls and young women diagnosed with cancer will survive, and it is estimated that survivors of childhood cancer constitute 1/250 of the adult population as of 2010.

The treatment required for most of the common cancer types occurring in younger women may involve removal of the reproductive organs, and/or cytotoxic treatment that could partially or definitively affect reproductive function. Therefore, women diagnosed with cancer prior to or during their reproductive period often have to deal not only with the uncertainty of long-term survival, but also with the partial or total loss of fertility as a result of cancer treatment.

In addition, women in Western countries have been increasingly delaying childbearing. In the United States, the birth rate in women 20–24 years of age decreased by 9% between 1990 and 2007. During the same time period, birth rates in women of 35–39 and 40–44 years of age increased by 33% and 42% respectively. Furthermore, from 1990 to 2007, first birth rate increased by 38% in women 35–39 years of age and by 100% in women 40–45 years of age. In other words, an increasing number of women in their late thirties and early forties are seeking their first pregnancy. As the incidence of most cancers increases with age, delayed childbearing results in more female cancer survivors who may be interested in fertility preservation.

In this chapter, we describe strategies for fertility preservation in women diagnosed with cancer. Most available options may also be applicable to women who face gonadotoxic treatment due to nonmalignant disorders such as systemic lupus erythematosus (SLE), or those who plan to delay fertility for personal reasons.

EVIDENCE AT A GLANCE

In Western countries, more women than ever before are seeking their first pregnancy in their late 30s to early 40s. As the incidence of most cancers increases with age, delayed childbearing results in more female cancer survivors who may be interested in fertility preservation.

Fertility preservation options involving cryopreservation of embryos or oocytes

Embryo cryopreservation

The most widely available option for fertility preservation in female patients who need chemo- and/or radiotherapy is the cryopreservation of embryos. Cryopreservation of embryos (as well as oocytes) involves an initial exposure to cryoprotectants, cooling to subzero temperatures, and storage. Upon demand, thawing occurs, and finally, a return to physiological conditions.

Embryo cryopreservation is routinely performed in most assisted reproductive technologies (ART) centers worldwide. Consequently, the

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Table 17.1 Comparison of embryo, oocyte, and ovarian tissue cryopreservation as strategies for fertility preservation

Characteristics	Embryo cryopreservation	Oocyte cryopreservation	Ovarian tissue cryopreservation
Appropriate in reproductive-age women	Yes	Yes	Yes
Requires sperm	Yes	No	No
Issues regarding ownership of reproductive material may arise	Yes	No	No
Requires ovarian stimulation	Yes	Yes	No
Requires delay in chemotherapy	Yes	Yes	No
Appropriate in prepuberty	No	No	Yes
Resumption of endocrine function	No	No	Yes
Requires surgery	No	No	Yes
Risk of reseeding cancer	No	No	Yes
Human live birth	>50,000 yearly worldwide	>300	5

methods used for embryo cryopreservation are well established and their risks and success rates have been investigated. In the United States, approximately 24,000 ART cycles using frozen/thawed embryos were performed in 2007, achieving a live birth rate of 34.0% in women less than 35 years old. During the same year, women less than 35 years old achieved a live birth rate per transfer of 46.1% when fresh embryos were transferred. In older age groups and in women using thawed embryos generated using donor oocytes, the difference in success rates between fresh and frozen cycles was even less marked. Therefore, storage of human embryos by cryopreservation is associated with a predictable but modest decrease in pregnancy and live birth rates in women undergoing infertility treatment. However, it is noteworthy that the effects of different types of malignancies upon reproductive potential is not yet known, and these statistics may not predict the outcome in women undergoing embryo cryopreservation for fertility preservation due to malignancy.

Despite well-defined success rates, embryo cryopreservation has important pitfalls (Table 17.1). First, it requires the patient to have a partner or to accepts the use of donor sperm. Second, ovarian stimulation precedes oocyte retrieval for ART, necessitating a delay in the initiation of chemo- or radiotherapy. Such a delay may not be acceptable in many cases. Finally, the high serum estrogen concentrations associated with ovarian stimulation may be contraindicated in women with estrogen-sensitive malignancies.

Recently, alternative strategies for ovarian stimulation prior to ART have been proposed for women with breast cancer, with the aim of retrieving more oocytes than would be available in a natural cycle without causing a significant increase in serum estrogen. Women with breast cancer constitute a special group because there is a 4–6-week hiatus between surgery and chemotherapy in most treatment protocols. The alternative approaches proposed for stimulation of follicle growth in women with breast cancer incorporate tamoxifen (an estrogen antagonist) or aromatase inhibitors. These two classes of medications, which have been used to promote follicle growth in anovulatory women, have been approved as adjunct chemotherapeutic agents for women with breast cancer because of their antiestrogenic effects.

Tamoxifen and its metabolites compete with estrogen in the body for binding to the estrogen receptor. Tamoxifen-bound estrogen receptor interacts with DNA and recruits other proteins known as corepressors to stop genes

from being switched on by estrogen. Aromatase inhibitors, on the other hand, work by inhibiting the action of the enzyme aromatase, which converts androgens into estrogens by a process called aromatization.

Oktay et al. first tried tamoxifen to control estrogen production while stimulating follicle growth in women with breast cancer. Using a dose of 40–60 mg/day, for a mean duration of 7 days, beginning on day 2 or 3 of the menstrual cycle, they obtained a higher number of oocytes (1.6 vs. 0.7, $p < 0.05$) and embryos (1.6 vs. 0.6, $p < 0.05$) per cycle compared to a retrospective control group consisting of breast cancer patients attempting natural cycle ART. However, the mean peak estradiol (E2) level in the tamoxifen group was significantly higher than in natural cycle ART patients (442.4 vs. 278 pg/mL) and the numbers of oocytes and embryos obtained were less than desired.

More recently, Oktay et al. reported improved follicle growth and increased number of embryos obtained using a combination of follicle stimulating hormone (FSH) with tamoxifen or letrozole (an aromatase inhibitor). They obtained a mean number of 5.1 mature oocytes and 3.8 embryos per cycle, with a mean peak estradiol of 1182 pg/mL using a combination of FSH and tamoxifen. When they used letrozole instead of tamoxifen, in combination with FSH, they obtained a mean number of 8.5 mature oocytes and 5.3 embryos per cycle, with a mean peak estradiol of only 380 pg/mL. They also reported the first pregnancy from cryopreserved embryos generated after tamoxifen stimulation.

The alternative ovarian stimulation protocol proposed by Oktay et al. is initiated by oral administration of 5 mg of letrozole starting on day 2 or day 3 of the menstrual cycle. After 2 days of letrozole administration, 150 U/day of FSH is added. Letrozole is continued until the day of hCG administration; the E2 measurement is repeated 3 days after the oocyte retrieval, and if the E2 level is more than 250 pg/mL, letrozole is continued until it decreases to less than 50 pg/mL. Although these findings are preliminary, the number of oocytes retrieved and embryos obtained using the protocol described by Oktay et al. are very encouraging for women diagnosed with breast cancer.

☆ TIPS & TRICKS

For women of reproductive age who have a partner or are willing to use donor sperm, embryo cryopreservation is the most established option for fertility preservation and has high success rates.

EVIDENCE AT A GLANCE

Using a combination of letrozole and FSH for ovarian stimulation, a high number of oocytes may be retrieved and embryos generated with only a modest increase in serum estradiol.

Oocyte cryopreservation

Cryopreservation of oocytes avoids the need for sperm and thus may be offered to a larger group of patients than embryo cryopreservation (see Table 17.1). In addition, oocyte cryopreservation may circumvent ethical or legal considerations associated with embryo freezing. However, although the first human live birth from cryopreserved oocytes was reported more than 20 years ago, success rates in ART using frozen oocytes have lagged behind those using frozen embryos, most likely as a result of the biochemical and physical properties of the oocyte.

Mostly due to a low efficiency of oocyte maturation in vitro, mature metaphase II (MII) oocytes are most commonly used for cryopreservation. MII oocytes are among the largest cells in the human body and contain the delicate meiotic spindle. As their cytoplasm contains a high proportion of water in comparison to other cells, damage due to ice crystal formation may be detrimental and affect oocyte viability after the freeze–thaw procedure. In addition, cryopreservation of mature oocytes results in hardening of the zona pellucida, adversely affecting fertilization. In recent years, protocols that include dehydration of oocytes before and during the cooling procedure have reduced ice crystal formation and led to much improved post-thaw survival rates. Significant improvements were also achieved in the fertilization of cryopreserved oocytes with the use of intracytoplasmic sperm injection (ICSI). Today, the two most common

freezing protocols used are referred to as slow cooling and vitrification.

The first protocol used to freeze oocytes was based on slow cooling, and applied a very slow rate of temperature decrease (<l°C/min) combined with rapid thawing, a protocol that had already been applied successfully for the cryopreservation of embryos. In 1986, the first pregnancy with frozen/thawed oocytes was reported using this protocol. Since then, much progress has been made, most of all in the optimization of cryoprotectant concentration and exposure time(s). The so-called "curve," or temperature versus time protocol, used for slow freezing has remained essentially unchanged from that used by Lassalle in the first embryo freezing protocol.

Vitrification is an alternative oocyte cryopreservation technique that aims to prevent ice formation and improve oocyte survival. Initially introduced by Rall and Fahy for mouse embryos, vitrification involves the use of highly concentrated cryoprotectant solution combined with a high cooling rate (nearly 1500 °C/min) in order to achieve a glassy, solid state without causing ice formation. First applied to human oocyte cryopreservation by Trounson, vitrification is easier, less expensive, and does not require the programmable freezer that is mandatory for the conventional slow cooling method. Recently, improved post-thaw survival and fertilization rates, and live births, have been achieved by vitrification of mature oocytes using potent cryoprotectants.

In 2006, a meta-analysis by Oktay et al. calculated the combined outcome(s) of a total of 26 reports using slow freezing, mature oocytes, and ICSI, published prior to June 2005. In these studies, the outcomes of a total of approximately 4,000 thawed oocytes were reported. Clinical pregnancy per thawed oocyte was 2.4%, and implantation rate per transferred embryo was 13.1%. When a separate analysis of seven studies using slow freezing published between June 2005 and March 2006 was done, clinical pregnancy per thawed oocyte or implantation rate per transfer were not improved. More recently, Bianchi et al. reported further optimization of the slow cooling–rapid thaw procedure and achieved 4.9% live birth rate per thawed oocyte and an implantation rate of 13.4%. In women under 38 years old, implantation rate was further improved at 16.6%.

In the meta-analysis by Oktay et al. only four reports of vitrification published prior to June 2005 were included. These studies reported the outcome of 503 thawed oocytes, with a 2.0% live birth rate per thawed oocyte and a total of 10 live births. However, the five more recent reports discussed separately (n = 636 thawed oocytes), published between June 2005 and March 2006, showed significant improvement, with 4.6% live birth rate per thawed oocyte and an implantation rate of 20.5%.

These results demonstrate a significant improvement in the outcome of oocyte cryopreservation in terms of pregnancy and implantation rates using both slow freeze and vitrification approaches. Indeed, the recently reported clinical outcomes are comparable to embryo cryopreservation where a 4–5% live birth rate per oocyte used to generate the cryopreserved embryos has been calculated.

Concerns regarding oocyte cryopreservation

There are concerns regarding the risk of chromosomal aneuploidy following oocyte cryopreservation due to the known effects of cryopreservation on the meiotic spindle of the oocyte, necessitating further studies to determine safety. The potentially detrimental effects of high cryoprotectant concentrations used in vitrification also need to be investigated. Moreover, while outcomes seem to be improving, the current pregnancy rates are significantly less than those seen with standard ART. Overall, as suggested by the Practice Committee of the American Society for Reproductive Medicine (ASRM), oocyte cryopreservation may be offered to women facing infertility due to cancer treatment following appropriate informed consent in an institutional review board (IRB) approved investigational protocol.

SCIENCE REVISITED

Using both slow freezing and vitrification, oocyte cryopreservation has become significantly more efficient with approximately 5% live birth per frozen thawed oocyte reported.

CAUTION

Despite its widening use and increasing success, oocyte cryopreservation is considered experimental by the American Society for Reproductive Medicine (ASRM). Obtaining an informed consent using an institutional review board (IRB) approved investigational protocol is suggested.

Ovarian tissue cryopreservation

Cryopreservation of ovarian tissue containing immature oocytes within primordial follicles has potential advantages over both embryo and oocyte freezing (see Table 17.1). A large number of primordial follicles may be cryopreserved without the necessity for ovarian stimulation or delay in the initiation of cancer treatment. In addition, primordial follicles are significantly less susceptible to cryoinjury compared to both mature and immature oocytes because of their smaller size, slower metabolic rate, and the absence of zona pellucida. Most importantly, ovarian tissue cryopreservation may be the only available option for most prepubertal girls, as well as women who cannot delay their cancer treatment.

In the ovary, most of the primordial follicles are located within the outer cortical layer. Therefore, cryopreservation of ovarian cortical tissue is a logical approach to storing the highest number of oocytes within the smallest tissue volume. Laparoscopy or laparatomy may be performed to remove the ovarian cortex, which is then cut into strips of approximately 1–3 mm in thickness and up to 1 cm^2 in total area, in order to enable adequate penetration of cryoprotectants. Ovarian tissue cryopreservation has traditionally been performed using a slow cooling technique, quite similar to that used for the cryopreservation of embryos and oocytes. More recently, vitrification has also been successfully applied to ovarian tissue cryopreservation. When ovarian tissue cryopreservation is performed, a piece of the cortical tissue should be analyzed to confirm the presence of follicles and rule out malignant metastasis. Once the ovarian tissue is cryopreserved, future options include transplantation of the tissue back to the donor (autotransplantation), or to nude mice (xenotransplantation), or in-vitro follicle culture.

Autotransplantation

Currently the most promising approach seems to be the transplantation of the ovarian tissue back to the donor, also termed autotransplantation. In animal models, autotransplantation has resulted in the return of ovarian function as well as pregnancies and live births. In humans, two different surgical approaches have been used for autotransplantation: orthotopic (pelvic) or heterotopic.

Orthotopic (pelvic) transplantation

Orthotopic transplantation places thawed cryopreserved ovarian cortical tissue at close proximity of the infundibulo-pelvic ligament with the hope that natural pregnancy may occur.

The fact that orthotopic transplantation is a feasible approach for fertility preservation has been suggested by studies involving monozygotic twins discordant for ovarian function. In a series of eight cases involving orthotopic transplantation of fresh ovarian cortical tissue from young fertile females to their monozygotic twin sisters with premature ovarian failure, ovarian endocrine function was restored in all, and pregnancy and live birth was achieved in six.

In 2004, Donnez et al. reported return of ovarian endocrine function, followed by a spontaneous pregnancy and live birth following orthotopic transplantation of cryopreserved ovarian tissue in a woman treated for Hodgkin lymphoma. This study has been criticized for not providing definitive evidence of a pregnancy resulting from cryopreserved and transplanted ovarian tissue, as oopherectomy had not been performed and evidence of ovarian failure prior to transplantation was found questionable. More recently, Meirow et al. reported a live birth using ART following the transplantation of thawed cryopreserved ovarian cortical tissue into the ovaries of a 28-year-old woman who had ovarian failure after high dose chemotherapy for non-Hodgkin lymphoma. It is noteworthy that, while spontaneous menstruation resumed after delivery, endocrine profile 22 months after transplantation indicated low ovarian reserve. To date, at

least five live births following orthotopic transplantation of cryopreserved ovarian tissue in women with cancer have been documented. While these case reports are quite encouraging, it is noteworthy that live births using this approach remain scarce, and the procedure is far from being widely utilized.

Heterotopic transplantation

Transplantation of the thawed cryopreserved ovarian cortical tissue to a site outside the pelvis is termed heterotopic transplantation, and has been proposed as an alternative to orthotopic approach. Transplantation to a heterotopic site such as the forearm or the abdomen is surgically less complicated and allows easier monitoring of follicle growth. However, it necessitates the use of ART to achieve pregnancy.

In 2001, Oktay et al. were first to report return of ovarian endocrine function and the development of a dominant follicle in two women using this approach. In one case, after blocking the patient's pituitary function with gonadotropin releasing hormone (GnRH) antagonist, and stimulating her with human menopausal gonadotropin for 11 days, they performed percutaneous oocyte retrieval from the forearm. Unfortunately, fertilization could not be achieved.

More recently, the same group of investigators transplanted the cryopreserved ovarian tissue beneath the abdominal skin and restored ovarian function in a woman previously treated for breast cancer. They subsequently performed controlled ovarian stimulation, and achieved egg retrieval, fertilization, and embryo transfer; however, pregnancy did not occur.

CAUTION

Concerns regarding ovarian tissue cryopreservation

The risk of transmission of metastatic cancer cells is a significant source of concern associated with autotransplantation. Blood-borne cancers such as leukemia and lymphoma are likely to be associated with the highest risk of ovarian metastasis and transmission through transplantation of thawed cryopreserved ovarian tissue. Histologic evaluation of ovarian samples has been suggested in order to prevent cancer transmission, although it is not possible to completely eliminate the risk of transmission in hematologic or disseminated malignancies.

TIPS & TRICKS

For prepubertal females who are not candidates for ovarian transposition, ovarian tissue cryopreservation is usually the only option for fertility preservation.

CAUTION

Despite significant advances, ovarian tissue cryopreservation has only resulted in five reported live births to date and carries the risk of transplanting neoplastic cells back to the patient.

Xenotransplantation

Xenotransplantation (from the Greek meaning "foreign") is the transplantation of living cells, tissues, or organs from one species to another. Mice with severe combined immunodeficiency (SCID) can accommodate tissues from foreign species without host-versus-graft response due to a deficiency in both T- and B-cell mediated immunity. Following xenotransplantation of cryopreserved ovarian tissue into SCID mice, healthy follicles were detectable in the graft more than 4 months after the initial transplantation. This approach eliminates the possibility of cancer cell transmission and relapse, as individual oocytes are retrieved from the host animal. Another advantage is the possible application in women in whom hormonal stimulation is contraindicated. Indeed, following subcutaneous placement of human ovarian cortical tissue into mice, follicular growth in response to exogenous gonadotropin stimulation, follicle maturation, and corpus luteum formation have been reported. Additional advantages of xenotransplantation

include convenient monitoring of follicular development, and easy access to follicle aspiration. However, possible transmission of zoonoses to humans is a serious concern, and this method is unlikely to be clinically available in the near future.

In-vitro maturation

The risk of transmission of metastatic cancer cells could potentially be eliminated by in-vitro maturation of follicles from cryopreserved ovarian tissue. Unfortunately, the production of live young from cultured primordial or preantral follicles has not yet been successful in humans.

While human in-vitro follicle maturation has not yet been optimized, studies within the past two decades using animal models have led to significant improvements. Several groups have achieved in-vitro culture and development of preantral follicles isolated from mouse ovaries. They obtained mature oocytes that resulted in pregnancy and live birth following in-vitro fertilization and embryo transfer in mice. Subsequently, in-vitro maturation, fertilization, and preimplantation embryo development was also achieved using preantral follicles obtained from cryopreserved ovarian cortical tissue.

Unfortunately, in-vitro culture and maturation of primordial follicles has been significantly more difficult. To obtain mature oocytes from primordial follicles, Eppig and O'Brien developed a two-stage culture system using the mouse model. First, primordial follicles were grown in organ culture to secondary follicles. Then the secondary follicles were isolated enzymatically and cultured further to mature oocytes. Routine in-vitro fertilization and embryo transfer was performed. The first mouse born was extremely obese, and postmortem examination revealed multiple malformations. To date, only 59 live offspring (5.7% of embryos transferred) have been obtained. Subsequently, live offspring was also produced following growth of oocytes obtained from primordial follicles of cryopreserved ovaries.

Human data on in-vitro maturation of follicles from ovarian tissue is scarce. At present, significant effort is being directed toward developing in-vitro systems that will allow derivation of mature oocytes from early stage follicles cryopreserved within human ovarian cortical tissue.

Cryopreservation of whole ovaries

Transplantation of fresh whole ovaries has been developed in animal models. While the duration of subsequent ovarian function was initially limited, as a result of ischemia resulting from thrombosis, graft survival time has since improved mainly due to the use of microsurgical techniques. Most recently, restoration of ovarian function followed by spontaneous pregnancy and live birth has been reported following microsurgical transplantation of an intact fresh ovary from a 38-year-old fertile woman to her monozygotic twin with premature ovarian failure.

Tissue survival in transplanted cryopreserved whole ovaries in animal models has also been significantly improved by meticulous surgical dissection of ovarian vessels during ovariectomy and perfusion of the ovary with cryoprotectants through these vessels. Similar rates of follicular viability and apoptosis using cryopreserved whole ovaries were achieved compared to ovarian cortical strips. Successful pregnancy was also reported following transplantation of frozen/thawed rat ovaries. Similarly, oocyte recovery, embryo development, and maintenance of ovarian function for 36 months after transplantation have been reported in sheep.

In humans, Bedaiwy and colleagues investigated the immediate post-thawing injury to the ovary that was cryopreserved as a whole with its vascular pedicle or as cortical strips. Bilateral oophorectomy was performed in two women (46 and 44 years old) undergoing hysterectomy. In both patients, one of the harvested ovaries was sectioned and cryopreserved as ovarian cortical strips. The other ovary was cryopreserved intact with its vascular pedicle. After thawing 7 days later, the overall viability of the primordial follicles was 75–78% in intact cryopreserved/thawed ovaries and 81–83% in ovarian cortical strips. Comparable primordial follicle counts, and absence of features of necrosis or apoptotic markers, led them to conclude that cryopreservation injury is not associated with significant follicular damage.

Although these results are encouraging, transplantation of cryopreserved whole ovaries

carries a potentially increased risk of returning metastatic disease to the patient compared to the handling of oocytes or even cortical strips.

Surgical approach to fertility preservation

Ovarian transposition

Transposition of the ovaries (oophoropexy) outside the pelvis to protect them from pelvic radiation is indicated in patients diagnosed with malignancies that require pelvic radiation, but not removal of the ovaries. The most common indications include Hodgkin disease, cervical and vaginal cancer, and pelvic sarcomas.

Initially described as a procedure performed through laparotomy, oophoropexy has more recently been performed laparoscopically. There are several approaches to laparoscopic oophoropexy, with small variations. In the most commonly described method, the ovaries are completely separated from the uterus and fallopian tubes by dividing the utero-ovarian ligament and incising the mesovarium. The peritoneum is also incised along the infundibulopelvic ligament and the ovaries are transposed laterally to the paracolic gutters and sutured. The left ovary is placed at the level of the aortic bifurcation and the right ovary is placed above the pelvic brim, between the level of the aortic bifurcation and the lower pole of the right kidney. Alternative approaches suggest less mobilization of the ovary by transecting the utero-ovarian ligament, but avoiding the separation of the ovary from the fallopian tube and complete dissection of the the infundibulopelvic ligament. These approaches suggest suturing the ovaries anteriorly and laterally at the level of the anterosuperior iliac spines.

During the past four decades, several reports of oophoropexy have documented a wide range of ovarian function preservation (15–90%) after radiation treatment. The variations could be due to the inability to calculate and prevent scatter radiation, concomitant use of chemotherapy, and different doses of radiation utilized.

In summary, laparoscopic ovarian transposition is a relatively simple, minimally invasive, and effective procedure that should be offered to reproductive-age patients who need pelvic radiation.

☆ TIPS & TRICKS

Ovarian transposition should be considered for prepubertal and reproductive-age women who are scheduled for pelvic radiation without the removal of their ovaries.

Medical approach to fertility preservation

Gonadotropin releasing hormone agonist cotreatment

Among all the options that are currently under investigation for the preservation of fertility in women diagnosed with cancer, the use of GnRH agonist (GnRHa) cotreatment with chemotherapy is probably the most controversial.

A GnRHa is a synthetic peptide modeled after the hypothalamic GnRH decapeptide with specific amino acid substitutions typically in positions 6 and/or 10. The GnRHa interacts with the GnRH receptor to initially elicit its biologic response, the release of the pituitary hormones FSH and luteinizing hormone (LH). However, after about 10 days, a profound hypogonadal effect (i.e., decrease in FSH and LH) is achieved through receptor down-regulation by internalization of receptors.

GnRHa cotreatment to preserve fertility in women undergoing gonadotoxic chemotherapy has initially been proposed based on the postulated role of gonadal suppression in the preservation of testicular function in men receiving chemotherapy, and the belief that the fertility of prepubertal girls is not affected by gonadotoxic treatment. In addition, animal studies have shown a protective role for GnRHa treatment against chemotherapy-induced gonadal damage. Indeed, in rhesus monkeys, GnRHa cotreatment resulted in approximately 50% decrease in the loss of primordial follicles in response to cyclophosphamide chemotherapy. Surprisingly, GnRHa cotreatment was ineffective in protecting against radiotherapy-induced gonadal damage.

Following encouraging findings in rodent and primate models, a multitude of nonrandomized

studies with short-term follow-up suggested a protective role for GnRHa cotreatment in women undergoing gonadotoxic chemotherapy for cancer or SLE. A recent meta-analysis based on the findings of these studies also reached the same conclusion. However, these studies were criticized for the lack of randomization, differences in follow-up periods in treatment and control groups, and the use of ovarian failure as endpoint which may not reflect the decrease in primordial follicle count in response to chemotherapy in young women.

Most recently, a randomized prospective trial performed in women diagnosed with nonmetastatic unilateral breast adenocarcinoma who had undergone modified radical mastectomy or breast-conserving surgery combined with full axillary lymph node dissection reported that GnRHa cotreatment before and during chemotherapy resulted in significant improvement of post-treatment ovarian function in women less than 40 years old. In this study, patients in the GnRHa cotreatment group received 3.6 mg subcutaneous goserelin 2 weeks prior to the initiation of chemotherapy and then every 28 days for 6 months. Of women treated with GnRHa and chemotherapy, 89.6% resumed spontaneous ovulation within 3–8 months following completion of treatment, compared to only 33.3% in women treated with chemotherapy alone. Mean serum FSH levels of those treated with GnRHa was 8.3 mIU/mL compared to 15.2 mIU/mL in the chemotherapy-alone group.

Despite these encouraging reports, the benefits and long-term effects of GnRHa cotreatment are unclear, and a consensus regarding the effectiveness of ovarian suppression is lacking. This is partly due to the fact that the mechanism by which GnRHa cotreatment may protect against chemotherapy-induced gonadal damage is still debated, as is the presence of FSH receptors in primordial follicles. Moreover, GnRH antagonists (unlike GnRHa) do not seem to be protective against cyclophosphamide-induced primordial follicle loss in mice.

At present, several randomized trials are under way to define the role and mechanism of GnRHa in ovarian function preservation. In the meantime, counseling of reproductive-age women facing chemotherapy about fertility preservation options should include the use of GnRHa therapy. However, until the results of large prospective randomized studies become available, GnRHa cotreatment for prevention of chemotherapy-induced gonadotoxicity should be offered to patients only with appropriate informed consent in an investigational protocol.

Individualization of fertility preservation

Different chemotherapeutic agents differ in their propensity to result in ovarian failure. In addition, ovarian failure is less likely in younger females using the same chemotherapeutic agent regimen. Therefore, the fertility preservation options that can be offered to an individual patient are determined by her age and prognosis, as well as the type and timing of gonadotoxic treatment planned. All these aspects should be emphasized during counseling.

Ovarian transposition should be considered for prepubertal and reproductive-age females who are scheduled for pelvic radiation but not the removal of their ovaries. Otherwise, for prepubertal females, ovarian tissue cryopreservation remains the only option. However, it is very important to inform these young females and their parents about the current limitations associated with this approach before embarking on a surgical procedure that may not be otherwise indicated.

For reproductive-age women, a multitude of options are available. For women who have a partner or are willing to use donor sperm, embryo cryopreservation is the most established approach and has predictable success rates. However, approximately 4 weeks is needed for the retrieval of oocytes, and the treatment is usually associated with a moderate and temporary increase in serum estrogen levels. Both these issues should be discussed with the patient and her oncologist, and ovarian stimulation for oocyte retrieval should only be initiated after their documented approval.

Oocyte cryopreservation is another viable option for reproductive-age women requiring fertility preservation, especially if they do not have a partner and they decline the use of donor sperm. Oocyte cryopreservation also circumvents legal concerns regarding embryo ownership. As stated for embryo cryopreserva-

tion, a delay in the initiation of chemo- or radiotherapy and a transient increase in serum estrogens are associated with this approach and should be discussed with the patient as well as her oncologist. It should also be emphasized that despite its widening use and increasing success, oocyte cryopreservation is considered experimental by many organizations including the ASRM, which suggests obtaining an informed consent using an IRB-approved investigational protocol.

In reproductive-age women, ovarian tissue cryopreservation should be considered only if there is not enough time to perform ovarian stimulation and/or an abdominal surgical procedure is independently planned. This is because there has been only a handful of live births reported using this approach, there is risk of transmitting neoplastic cells with cryopreserved tissue, and in-vitro maturation protocols are far from being successful in humans. For similar reasons, cryopreservation and transplantation of whole ovaries is an even less attractive option at the present time.

Finally, despite the controversy surrounding its benefit, GnRHa cotreatment is easy to use and has minimal side effects. Therefore, counseling of reproductive-age women facing chemotherapy should include the use of GnRHa therapy.

Logistics of fertility preservation

Fertility preservation in women diagnosed with cancer requires a well-coordinated team effort. The oncologic implications of the recent diagnosis as well as the timetable for chemo- and/or radiotherapy should be clarified immediately, in order to determine the options available to an individual patient. Because of the often limited time available for fertility-preserving interventions, it is crucial to treat fertility preservation as a medical emergency and expedite consultation with a fertility specialist.

It is also noteworthy that women recently diagnosed with cancer are often in a vulnerable psychological state. The inclusion of a social worker or psychologist in the care team is therefore very useful. This is especially true when adolescents are involved, making the situation even more challenging. At all times, despite the urgency of the situation, patients should be counseled in detail, explaining the limitations and potential benefits as well as risks of each approach.

Summary

Fertility preservation options for women facing gonadotoxic treatment has become an important area of investigation because of increasing cancer survival rates combined with delayed childbearing. In addition to embryo and oocyte cryopreservation, encouraging findings have recently been reported using ovarian tissue cryopreservation and GnRHa cotreatment. The approach to fertility preservation in women diagnosed with cancer requires a well-coordinated team effort, and a carefully planned treatment strategy tailored to meet their individual needs.

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18

Infertility Treatment: Varying Approaches Across Continents

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Introduction

Failure to conceive after 12 months of regular, unprotected intercourse is the most commonly used definition of infertility and it is a common problem estimated to affect approximately 10–20% of couples with a more or less similar incidence all around the world. The variation in reported figures seems to be caused by different definitions used for infertility as well as population characteristics. This notwithstanding, prevalence of factors causing and therefore clinical management of infertility vary in different regions/countries. Other determinants of treatment choices are the availability and affordability of services as well as varying social values and religious beliefs among different societies.

Treatment options for infertility can be categorized as rational (directed at the cause of infertility), empiric (not correcting a well-defined pathologic process leading to subfertility but commonly practiced to improve fecundity without robust data supporting their effectiveness), or symptomatic (aimed not at the cause but at increasing the per cycle fecundity). The major difference between empiric and symptomatic treatments is that the latter have proven efficiency, i.e., they are associated with a significant and clinically relevant increase in fecundity as compared to expectant management.

Commonly practiced rational treatments for infertility are few; they include ovulation induction for anovulation and gonadotropin treatment for male and female hypogonadotropic hypogonadism. As evident from the present guidelines across the world there are only minor variations, if any, in treatment choices for these obvious pathologies.

Surgical treatment of tubal obstruction and reversal of tubal ligation or vasectomy are also rational treatments, but they are being less commonly practiced because of the lack of trained physicians and also the overwhelming dissemination and relative simplicity of assisted reproductive techniques (ART). Intrauterine insemination (IUI) with or without ovarian stimulation for the treatment of unexplained subfertility or mild male factor infertility is an empirical treatment without robust data supporting its effectiveness. ART falls into the latter category of symptomatic treatment.

ART is the most successful treatment for infertility, but only a small percentage of those needing ART actually access it. Access varies greatly internationally owing to religious, cultural, and political factors, probably the most important factor being each society's perspective on the moral status of the embryo. Economic factors, the nature of the healthcare system, and public funding affect access both among and within countries. Regulations and guidelines are highly variable, but critical, in determining access and types of services.

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Currently ART represents the most effective treatment option for infertility, i.e., it provides the highest pregnancy/birth rate per attempt. Live birth rates have increased continuously over the decades and have reached approximately 20–40% per started cycle. However, ART requires expensive medical equipment and highly trained personnel, limiting its availability and utilization. As expected, public funding policies vary not only between countries but also between different jurisdictions within the same country. Besides being the most expensive treatment method, ART is also considered to be the most invasive and risky one. The major complications of ART are ovarian hyperstimulation syndrome (OHSS) and the increased incidence of multiple pregnancies associated with simultaneous transfer of multiple embryos. The latter has led regulatory authorities and professional societies to implement/suggest restrictions on number of embryos transferred. In this chapter we will give an overview of differences in regulations and practice of ART, with emphasis being on differences between European and North American practices.

Utilization and availability of ART

It is estimated that the need for ART cycles is 1500 per million population per annum (c.p.m. pa). This is a conservative underestimate, based on the assumption that only half of infertile couples actually take a consultation and use the provided treatment services. The real need is calculated to be at the level of 3000 c.p.m. pa. Regardless of this fact, it is only in a few countries that the utilization of ART services reaches even the conservatively estimated figure of 1500 c.p.m. pa.

In an review on health economics of ART published in the year 2002, Collins compiled data from over 40 countries and found that Greece, Finland, and Israel had the highest number of ART clinics per million population; 4.34, 3.88, and 3.68, respectively. Regarding treatment cycles, Israel was the leader with 1657 c.p.m. pa, being the only country exceeding the 1500 c.p.m. pa landmark at that time. Iceland and the Netherlands followed, with 899 and 829 c.p.m. pa respectively. In North America, annual numbers of ART centers and ART cycles per million population were much lower at respectivley 1.31 and 126 for the United States and 0.75 and 190 in Canada.

Although ART was utilized more extensively in most European countries than in North America, Middle Eastern countries with the exception of Israel had even lower utilization rates. The level of ART utilization in Australia was closer to the higher European figures, but Japan, Korea, and Malaysia had lower figures that were closer to those for North America.

Collins assessed the effects of various health and economic factors on the availability of ART services. Based on data from the late 1990s, infant mortality rate was the only factor significantly associated with the availability of ART services at a national level. Infant mortality and ART availability were inversely proportional, with one additional ART center being present for every four fewer infant deaths under the age of 1 year. Infant mortality is regarded as an indicator of overall quality of a national healthcare system, and it is plausible that countries with high infant mortality rates may have priorities for other basic healthcare services, rendering fewer resources available for ART.

However, the relation with infant mortality rates accounts for only some of the variability observed in the availability and utilization of ART services, especially in industrialized countries. First of all, the utilization of ART in most developed countries with low infant mortality rates was still below 1500 c.p.m. pa. Moreover, despite similar infant mortality rates, while the average figure of ART c.p.m. pa ranged between 1450 and 2209 in the year 2005 in Scandinavian countries, it was only 353 in Canada for the same year. The major difference between the Scandinavian countries and Canada was the ART reimbursement policies. In Scandinavia there was extensive reimbursement, but there was no federal reimbursement in Canada, and only one province (Ontario) provided partial reimbursement for its residents that excluded the cost of medications. Utilization of ART therefore seems to be also determined by the availability of public reimbursement for ART in the Western world.

In a more recent review on the economics of ART, Chambers and colleagues gathered information from selected developed countries; the costs as they were realized in the year 2003 were

expressed in 2006 USD. Similar to Collins's report in 2002, the average cost of an ART cycle including medications was the highest in the United States, at 12,513 USD (2006), followed by the cost in Canada at 8,500 USD. The U.S. figure was almost double the average cost across Europe. The average cost of a fresh ART cycle was 6,534 USD in the United Kingdom and 5,549 USD in the Scandinavian countries. The Japanese figure was lower, at 3,956 USD.

The actual cost of an ART treatment cycle per se is of limited value for understanding the economic implications of treatment costs on utilization of ART. Based on the latest data presented by Chambers and colleagues, although a single in-vitro fertilization (IVF) cycle costs more in North America where utilization is lower, the cost of a single treatment cycle is not the lowest in Scandinavian countries or Australia where the utilization of ART is the highest.

Chambers and colleagues adjusted the economic burden of an ART treatment cycle on a couple for the effect of reimbursement policy in practice in the countries analyzed. The cost of a standard IVF cycle was expressed as the percentage of annual disposable income before and after adjusting for government subsidization. Countries with more generous reimbursement policies minimizing the out-of-pocket expenses for the patients achieved higher utilization of ART. Scandinavian countries and Australia are examples, reaching ART utilization rates around 1500 c.p.m. pa with reimbursement policies that cut the cost of treatment for the couple by 50% or more.

In conclusion, the out-of-pocket cost to the patient is the key determinant of the utilization of ART services. Accordingly, ART utilization is much higher in Europe, where it is mostly an insurance-covered benefit or a service funded directly by the government, compared to North America and Japan, where no public reimbursement exists. With the exception of Israel, most Middle Eastern countries currently have limited utilization of ART services.

Reimbursement of ART

The regulation, provision, and funding of ART services differ among countries and at times between different jurisdictions in the same country. Opponents of public funding for ART held the opinion that ART was not a medically necessary treatment, or that ART procedures lacked effectiveness and the resultant cost-effectiveness was too low to be regarded as a valid treatment option. In fact, initial studies analyzing cost-effectiveness of ART in comparison to alternative conventional therapies, i.e., untreated observation, ovulation induction, and IUI, suggested that conventional treatments, where applicable, were more cost-effective, and the cost per additional live birth with IVF was too high to be considered feasible. However, the success rates of ART have improved consistently over the years and live birth rates per treatment cycle have reached the 20–40% range. Currently, ART is regarded as the most successful treatment modality in terms of pregnancy/live birth rates per attempt. On the contrary, the effectiveness of conventional treatments is once more being questioned.

These improvements have led to changes in perception of ART in both the medical and the general community, and an increasing number of countries are currently providing governmental reimbursement for ART services. However, the conditions and extent of reimbursement vary widely among countries. Some countries provide government funding for ART treatments performed in public centers only, whereas private centers remain the major service providers in others.

In the United States, where no publicly funded healthcare services are available for individuals in the reproductive age group, some states have mandates for fertility treatment coverage, including ART, by third party payers. As of 2006, only six states mandated insurance coverage. In Canada, where the majority of healthcare services are governmentally funded, at the time of writing only Ontario was providing a partial governmental reimbursement, excluding the cost of medication, for ART. The Quebec parliament has recently passed a bill for provision of public funding, but regulations regarding the extent of funding or conditions of eligibility had not been detailed at time of writing.

In contrast with North America, most European countries have some form of reimbursement for ART. Based on information provided by the European Society of Human Reproduction

and Embryology, among countries providing information regarding reimbursement policies in effect, Ireland, Ukraine, and Switzerland were the only countries that did not provide any public funding. Some countries, including Germany, Turkey, and Belgium had partial reimbursement policies. However, the extent of patient participation in treatment costs varies widely, from 5% in Belgium to 50% in Germany. Spain and Portugal are examples of countries which provide reimbursement only if treatment is performed in a public center. In most European countries with reimbursement schemes, funding is limited to a number of treatment cycles.

In Japan, government reimbursement of ART procedures did not exist before 2006, when a partial reimbursement system was introduced. Couples with low annual household income were eligible for funding up to two cycles per year for 2 years. The threshold for definition of low household income and the number of reimbursed ART cycles were changed in 2007.

Australia, like Israel, provides government funding for an unlimited number of ART cycles.

A common characteristic of governmental funding of ART in centers operated by the public sector is long waiting lists precluding couples from initiating the treatment cycle. Examples include the United Kingdom, Scandinavian countries, and Spain. Long waiting periods are one of the factors that promote cross-border reproductive tourism. Regulations governing several aspects of ART treatment, such as gamete and embryo donation, cryopreservation, limitations on numbers of oocytes that are allowed to be fertilized, or the number of embryos transferred are other factors leading patients to travel to other countries where legislation is more permissive or simply does not exist at all, thus allowing a more liberal approach to ART. These regulatory issues are discussed further below.

Regulation of ART

As with reimbursement policies, legislation and regulation governing ART are far from being homogenous across the world. In some countries regulations exist in the form of a detailed legal framework determined by parliamentary statute, but in others only professional guidelines exist, leaving the final decisions to the discretion of treating physicians. Furthermore, in some countries, no regulations exist in any form.

ART has been a rapidly changing and developing field, making previously unavailable treatment options possible in a relatively short period of 30 years. Examples include intentional production of a multiple embryo cohort among which the best one/s can be selected for transfer, cryopreservation of gametes and embryos for later use, oocyte donation/sharing, preimplantation genetic screening/diagnosis (PGS/D), and more recently the development of embryonic stem cells for research and possibly creation of gametes. Opinions vary widely regarding the use of these technologies; many ethical, legal, and at times religious questions have been raised in different societies, leading to at times over-restrictive legislative action. Similar to ART itself, legislation governing ART is evolving and changing rapidly, precluding a detailed overview. The most controversial subjects and major differences in relevant regulations are briefly discussed below.

Regulations governing the rights of the embryo

The moral status of a fertilized human egg—whether it should be considered as a human already in being—was the heart of the matter in most of the controversial issues including fertilizing several oocytes, discarding or freezing embryos, and embryo donation. Germany and Italy were two countries to adopt the view that the developing human embryo had such moral status and required protection. In February 2004, inseminating more than three oocytes was outlawed in Italy, and all embryos generated had to be transferred regardless of quality, prohibiting embryo cryopreservation. After several legal challenges on the grounds of individual rights had been declined, the requirement to fertilize a maximum of three oocytes was revoked and embryo freezing was permitted only if required for health reasons, i.e., risk of OHSS or another condition precluding pregnancy. In Germany, although the number of oocytes that can be fertilized is not limited, freezing cleavage-stage embryos after the two-pronucleus stage is forbidden. Croatia and Switzerland are the two other European countries that have banned embryo cryopreservation. The same opinion regarding the moral status of the embryo resulted in the

prohibition of PGD in Germany. Austria is the only other European country banning PGD, although cryopreservation of embryos and selection of embryos for transfer based on polar body biopsy and genetic testing was allowed. The rationale for permitting biopsy of the polar body but not the blastomere is based on regarding the latter as a part of the embryo.

Although most European countries allowed embryo selection, PGD, and embryo cryopreservation, almost half of them adopted a more conservative approach regarding embryo donation. In Denmark, Italy, Montenegro, Norway, Serbia, Sweden, and Turkey the law allows both embryo freezing and PGD, but all seven countries have banned embryo donation.

Production of multiple embryos, discarding, donating, and performing PGD on embryos are not prohibited at all in North America.

Regulations governing gamete donation

Gamete donation is yet another controversial issue. Gamete donation is naturally categorized as oocyte donation and sperm donation. Both can be undertaken anonymously, or gametes may be provided by a known donor. Oocyte donation requires utilization of IVF or intracytoplasmic sperm injection (ICSI), whereas sperm donation can be done in the context of IUI. The presence of so many categories and possible combinations has led regulations governing gamete donation be even more heterogeneous than regulations governing the rights of embryo. Some countries allow only sperm donation but not oocyte donation (e.g., Austria, Switzerland and Norway). Others allow donation of both gametes but only in an anonymous manner (e.g., Spain, France and Portugal), while still others allow only known but not anonymous donation (e.g., the United Kingdom, the Netherlands, and Sweden). Austrian legislation allows known, but not anonymous, sperm donation for IVF, but anonymous sperm donation is allowed for IUI cycles. Some countries, including Turkey and Italy, have banned all forms of gamete donation. Apparently almost every possible combination of possibilities has been adopted at least by one country in Europe.

The situation is not very different in America. While allowing altruistic oocyte donation, Canada has outlawed oocyte donation if the donor is paid in return. In the United States and some South American countries, gamete donation for a fee is allowed. In the United States there exist commercial services providing a catalogue of oocyte and sperm donors, including not only photographs of donors, but also personal information like pedigrees, educational background, occupational information, and short assays describing donors themselves. Most aspects of ART are regulated by professional guidelines, but eligibility criteria for gamete donors are regulated by the U.S. Food and Drug Administration (FDA).

Regulations governing gestational surrogacy, treatment of single women, and same-sex couples

Gestational surrogacy, the treatment of single women, and same-sex couples are perhaps among the most controversial issues in medicine. Although gestational surrogacy has been viewed as a means of restoration of an impaired natural function for women who present with treatment resistant severe intrauterine adhesions, who have had their uterus removed, or with a medical condition precluding pregnancy, treatment of women without a male partner with the use of donor sperm has been regarded outside the context of a medical treatment by some authorities. Ethical, religious, and legal opinions regarding these issues are numerous and subjective in nature. Discussion of these views is beyond the scope of this chapter, and only a brief description of the current practice around the world is given here.

In Europe, the countries banning gestational surrogacy outnumber countries prohibiting any other ART procedure. Only four European countries (the United Kingdom, the Netherlands, Greece, and Ukraine) endorse the practice of gestational surrogacy. While some states in the United States and some provinces in Canada explicitly allow gestational surrogacy, others prohibit it or even deem it a punishable act. Israel and India are among other countries allowing gestational surrogacy. Regulations regarding compensation of the surrogate and rights of parties involved are detailed and different in all the above-mentioned countries. Where surrogacy is not explicitly allowed by the law, adoption

of the child by the commissioning couple is regulated by judicial ruling on a case by case basis. India legally allows and regulates commercial surrogacy, and dedicated surrogacy clinics providing exclusive programs including identification of the surrogate, ART procedures, follow-up of surrogate's pregnancy, delivery, adoption processes, and even flight arrangements exist.

Similar to the case of surrogacy, treatment of single women and lesbian couples with sperm donation using either IUI or ART is a controversial issue, with varying opinions and regulations across the world. In the absence of clear legal regulations some clinics prefer to consult ethics committees and legal counselors for each case before initiating treatment.

At the other end of the spectrum, in some countries such as Canada ART treatment cannot be denied to same-sex couples or single women by legislation.

Regulations on number of embryos transferred

In the early days of ART, the transfer of a single embryo derived from a natural cycle was the norm. It subsequently became evident that ovarian stimulation resulting in the generation and transfer of multiple embryos was associated with higher pregnancy rates. The strategy of multiple embryo transfer was adopted rapidly throughout the world and led to an epidemic of twin and higher-order multiple gestations. It did not take long for the healthcare authorities to realize the burden of iatrogenic multiple pregnancies on the couple, the system, and society as a whole. This realization was followed initially by recommendations from leading societies to decrease the number of transferred embryos, and in some parts of the world led to the adoption of single embryo transfers. Despite these recommendations, there appears to be differences in the number of embryos transferred throughout the world and currently at least 20–30% of all IVF pregnancies are twin or higher-order multiple gestations. Multiple pregnancies are associated with a higher incidence of both maternal and neonatal complications and thus considerable morbidity and, although this is uncommon, mortality.

Transfer of more than two embryos does not appear to be reasonable except in women who are over 40 years of age or who have only poor-quality embryos available. Today the debate is between double and single embryo transfers. Single embryo transfer prevents all multiple pregnancies, other than monozygotic twinning, and is associated with comparable pregnancy rates in selected patients. However, randomized studies showed lower pregnancy rates in patients receiving a single embryo compared with patients in whom two embryos were replaced. Addition of a cryopreservation cycle on a single embryo transfer cycle yields similar cumulative pregnancy rates to double-embryo transfer. Furthermore, it has recently been shown that cumulative live birth rates with repeated cycles of fresh single embryo transfers compare favorably with those for double-embryo transfers. Health economics data also support the adoption of an elective single embryo transfer policy rather than double-embryo transfer when the number of deliveries with at least one liveborn child and all complications are considered.

There appears to be differences in the number of embryos transferred throughout the world. Most European and notably Scandinavian countries have passed legislation that limit the number of transferred embryos to one or two. In Sweden, a single embryo transfer strategy has been adopted without noticeable decrease in the pregnancy rates. In Finland, Norway, and Belgium the transfer of no more than one embryo is allowed for the first two or three IVF cycles. The legislated limitation in the number of embryos transferred caused a sharp decline in twin pregnancy rates in these countries. It is noteworthy that, despite the efforts made to promote single embryo transfers across the continent, most European countries allow transfer of up to three embryos depending on the reproductive history and the age of the female partner, and in Germany single embryo transfer is only allowed if no other embryos exist.

Single embryo transfer is less commonly practiced in the United States, and according to a recent report compiled using data from the Society for Assisted Reproductive Technology (SART) is mainly confined to women who are less than 30 years of age and in women presenting with a uterine factor. Although there is no legal limitation for the number of embryos transferred in North America at the time of writing, the Prac-

tice Committee of SART and Practice Committee of the American Society of Reproductive Medicine (ASRM) have published guidelines for the number of embryos to be transferred in ART cycles. These guidelines are far more flexible than the European legislation. Although the guidelines endorse elective single embryo transfer, the effect of the on day-to-day practice appears to be limited as the incidence of twin pregnancies has not declined in the United States.

ART outcome monitoring and success rates

The European IVF Monitoring Programme (EIM) and SART are the two organizations that compile, collate, and analyze data regarding the performance of ART in Europe and the United States respectively. The methods used to collect and collate data, however, have important differences. Gleicher and colleagues attempted to undertake a formal comparison based on the available data published by the U.S. Department of Health and Human Services and the European Society of Human Reproduction and Embryology. They outlined the major differences in data reporting and how these differences preclude direct comparisons and undermine the validity of indirect comparisons which have to be based on many assumptions.

Neither the United States nor Europe is homogenous with regard to ART practice and regulations governing it. In addition, data collection methods and formats also have significant differences preventing accurate direct and relevant comparisons in ART outcome between the two continents. Contrary to the more liberal practice of ART with fewer restrictions in the United States, data reporting is a federal mandate and each and every clinic has to report ART treatment outcomes in a predetermined format that is the same across the country. In Europe, regulations and requirements for treatment cycle and outcome reporting vary widely between countries. The United Kingdom, France, and Germany are examples of the few countries in Europe that introduced a centralized data collection system requiring all centers to report data from all started treatment cycles. Many European countries only require reporting of proportions of a number of defined events, such as pregnancy rate per started cycle or ectopic pregnancy rate per pregnancy, at certain intervals. Regardless of the regulations in individual countries, reporting to the EIM is voluntary, and reports from a country do not necessarily account for all clinics in that country, further limiting the validity of the data.

In the United States data collection is not only mandatory, but is also in a more detailed format, including etiology of subfertility, number of embryos transferred, and stratifying treatment outcome based on the age of the female partner, which is undoubtedly one of the most important factors effecting treatment outcome.

There is no doubt that developing and implementing a universal data monitoring and reporting system will benefit patients, health authorities, and medical professionals themselves. Complications can be better followed up and treatment efficacy can be better assessed. A reporting system that enables direct comparisons can help identification of possible reasons behind any differences in treatment outcome between different practices. Such information will facilitate learning from each other and improving practice all around the world. Such a system will undoubtedly provide policy-makers with more detailed information which could be used to estimate the actual need for ART, better plan allocation of resources, and hopefully increase couples' access to ART worldwide.

Summary

Currently ART utilization is higher in Europe than in the United States. More generous reimbursement systems in practice and relatively lower costs of treatment in Europe seem to be the major cause behind utilization rates. European governments not only reimburse ART treatment more, but also create a more rigid regulatory environment. Not only the more controversial issues of gamete donation, surrogacy, and treatment of single women and same-sex couples, but also less controversial practices such as embryo and gamete freezing or even the number of embryos transferred are subject to restrictions in many European countries. The North American regulatory environment is much more liberal. Substantial differences in collection and reporting data on ART outcome prevent reliable direct comparisons between two continents. It

is therefore difficult to quantify the effects of these different approaches on treatment outcomes. Implementation of a standardized universal monitoring system for ART outcomes will undoubtedly benefit patients, health authorities, and medical professionals.

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