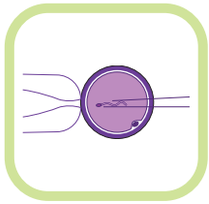

Assisted Reproductive Technologies

31

INTRODUCTION

Assisted reproductive technologies (ART) encompass all techniques involving direct manipulation of oocytes outside the body. The first and still most common form of ART is in vitro fertilization (IVF), but other related techniques also reside within the realm of ART. The success of modern ART has completely revolutionized both the evaluation and the treatment of infertility. Some traditional diagnostic methods and treatments have been rendered obsolete, and others have only limited applications because ART is simply more effective. The trend is clear and certain to continue.

IVF involves a sequence of highly coordinated steps beginning with ovarian stimulation with exogenous gonadotropins, followed by retrieval of oocytes from the ovaries under the guidance of transvaginal ultrasonography, fertilization and embryo culture in the laboratory, and transcervical transfer of embryos into the uterus. The first pregnancy resulting from IVF was reported in 1976 and was ectopic.[1] The first child resulting from IVF was born in 1978.[2] Over the subsequent 40 years, ART has been greatly refined and expanded, resulted in millions of births worldwide, and now accounts for 2% of all births in the United States.[3] ART includes methods for assisted fertilization by intracytoplasmic sperm injection (ICSI) using sperm isolated from the ejaculate or obtained by microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE), assisted embryo hatching, and preimplantation genetic testing (PGT) for aneuploidy (PGT-A) and/or PGT for monogenic disorders (PGT-M). In most cases, IVF is used to help an infertile couple conceive their own biologic child; however, donor sperm, donor oocytes, and gestational surrogates also play an important role in modern ART. Medically indicated or elective fertility preservation (Chapter 32) is another area where ART is increasingly being used.

Other forms of ART include tubal transfer of oocytes and sperm (gamete intrafallopian transfer, GIFT), zygotes (zygote intrafallopian transfer, ZIFT), or embryos (tubal embryo transfer, TET) via laparoscopy. Whereas these more invasive techniques once had certain advantages over traditional IVF for some infertile couples, they are no longer part of clinical practice.

A truly comprehensive discussion of ART is well beyond the scope of any single book chapter. The objective here is to provide an overview of the indications for ART; the most common methods for ovarian stimulation, oocyte retrieval, sperm recovery, fertilization, and gamete/embryo transfer (ET); and the results and complications of ART, with emphasis on newly developing technologies and areas of controversy.

INDICATIONS FOR IVF

IVF was first developed as a method to overcome infertility resulting from irreparable tubal disease but is now applied much more broadly for the treatment of almost all causes of infertility. IVF is most clearly indicated when infertility results from one or more causes having no other effective treatment; severe tubal disease relating to previous infection or advanced endometriosis and severe male factor infertility are the most obvious examples. IVF is also often the best treatment for couples with multifactor infertility because it can address or overcome all contributing causes at the same time. IVF is a legitimate treatment option for women with age-related or otherwise unexplained infertility and also represents the treatment of last resort when other treatments fail.

In women with premature ovarian insufficiency or reproductive aging and healthy women beyond normal reproductive age, IVF using oocytes from a young donor is highly

successful. For women with normal ovaries but no functional uterus (müllerian agenesis, severe intrauterine adhesions, previous hysterectomy) and those with medical disorders that preclude pregnancy due to serious health risks, IVF with ET to a gestational surrogate still offers the possibility of genetically related offspring. IVF using oocytes from a donor and embryos transferred to a gestational carrier also allows male same-sex couples to pursue parenthood. In couples who carry autosomal or sex-linked genetic disorders or balanced chromosomal translocations, IVF with PGT can avoid the risk of delivering an affected child.

Tubal Factor Infertility

Before the advent of IVF, women with irreparable bilateral tubal obstruction were essentially sterile, and the prognosis for those with less-severe distal disease was only fair. In the modern era of ART, surgical treatments are declining in importance, and the prognosis for women with tubal factor infertility has improved dramatically. Approximately 10% of patients using ART have a primary diagnosis of tubal factor infertility.[4] The relative advantages and disadvantages of surgery and IVF for the treatment of tubal factor infertility and the factors bearing on a choice between the two are discussed in depth in Chapter 28 and summarized here.

Reconstructive surgery remains a viable option for young women with mild distal tubal obstruction or peritubular adhesions (because postoperative live birth rates can exceed 50%),[5–7] but IVF is the treatment of choice for women with severe distal disease. Results achieved with surgery have varied, but success rates (10–35%) are generally lower than with IVF, and the risk of ectopic pregnancy is higher (5–20%).[4,8–11] In 2017, the overall IVF live birth rate (per cycle start) for US women with tubal factor infertility (all ages) was 31.2%; however, this rate increases with decreasing age.[12] IVF is also the best treatment for women who remain infertile for more than a year after tubal surgery (the likelihood for success diminishes progressively with time after operation), for older women with significant distal tubal disease (cycle fecundity is low after distal tubal surgery and time is limited), and for women with recurrent distal tubal obstruction (repeated attempts to correct distal tubal occlusive disease are rarely successful).

Although not candidates for reconstructive surgery, women with severe distal tubal disease can still benefit from surgery prior to IVF. **A substantial body of evidence indicates that the presence of communicating hydrosalpinges (characterized by proximal tubal patency and distal occlusion) is associated with approximately a 50% reduction in pregnancy, implantation, and live birth rates, as well as an increased risk of miscarriage.** The mechanism for the adverse effect of hydrosalpinges on IVF outcomes could involve mechanical interference with implantation or toxic effects on the embryo or endometrium.[13–18] A 2020 systematic review including four randomized controlled trials involving 455 women observed that the probability of a clinical pregnancy was twice as great after laparoscopic salpingectomy for hydrosalpinges before IVF (RR = 2.02, 95% CI = 1.44–2.82).[19] Laparoscopic occlusion of the fallopian tubes also increased the probability of clinical pregnancy, compared to no intervention (RR = 3.21, CI = 1.72–5.99), and neither surgical procedure was superior.[19] These data demonstrate clearly that laparoscopic salpingectomy or tubal occlusion improves IVF pregnancy rates in women with hydrosalpinges. This holds true even when only one tube is affected.

Proximal tubal occlusion observed during tubal patency assessment with hysterosalpingography or hysterosalpingo-contrast sonography is often a reaction to the procedure itself and results from "cornual spasm" or other technical pitfalls of the procedure (Chapter 28). **Efforts to confirm the diagnosis are justified; otherwise, many women may needlessly undergo IVF.** Common methods include repeated hysterosalpingography or hysterosalpingo-contrast sonography[20] and laparoscopic "chromotubation."[21–23] Fluoroscopic or hysteroscopic selective tubal cannulation both establish the diagnosis and provide the means for successful treatment.[20,21,24–28] Microsurgical segmental resection and anastomosis is another proven treatment for true proximal tubal obstruction[29–32] but requires uncommon technical expertise. IVF is the obvious alternative when cannulation is contraindicated (salpingitis isthmica nodosa) or technically unsuccessful and when infertility persists for more than 6 to 12 months after the procedure.

According to the National Survey of Family Growth, tubal sterilization is the most commonly used contraceptive method among women aged 15 to 49 years.[33] Approximately 700,000 US women have an elective **tubal sterilization** procedure each year; up to 30% regret the decision, and about 1% later request its reversal.[34–37] The most commonly cited reasons for regret include new relationships, changes in family planning goals, and death of a child. Regrets are more common in younger women, those who were unaware of the spectrum of contraceptive options, women whose decision for sterilization was influenced by a third party (partner, other family member, friend, or physician), and in those sterilized postpartum or after an abortion.[35,36,38,39] Women 30 years old or younger are twice as likely as older women to express regret, 3.5 to 18 times more likely to request information about reversal of the procedure, and approximately 8 times more likely to have a sterilization reversal or IVF.[36,37,40]

Young women sterilized using rings or clips and women having no other infertility factors have the best surgical prognosis; success rates are lower for older women, those sterilized by cautery (particularly multiple-burn techniques), and women with other infertility factors.[41–48] Although conception rates are quite good (45–82%) after microsurgical tubal anastomosis in properly selected candidates, IVF is a legitimate alternative to surgery, particularly for older women, those with a poor surgical prognosis or preferring to avoid surgery, and women who desire only one additional pregnancy.

Endometriosis

The association between endometriosis and infertility and the pathogenic mechanisms involved are considered at length in Chapter 35. In summary, 20% to 40% of infertile women have endometriosis, and accumulated evidence indicates that fertility decreases with the severity of the disease. Endometriosis may cause infertility by distorting adnexal anatomy and interfering with ovum capture[49] or probably by impairing oocyte development, early embryogenesis, or endometrial receptivity.[50–57] IVF should be expected to overcome any anatomic obstacles, and data from IVF cycles suggest similar performance of oocytes from patients with endometriosis in terms of fertilization, blastulation, euploidy, and overall similar live birth rates, lending little credit to other mechanisms of endometriosis-associated infertility.[58–61] Endometriosis is the primary diagnosis in approximately 6% of patients using ART, although this number is likely an underestimate as not all patients with infertility undergo a diagnostic test for endometriosis.[4]

Treatment options for infertile women with advanced stages of endometriosis include conservative surgical treatment and IVF. For those with severe pain symptoms, surgery is the most logical treatment. However, whether surgical treatment of advanced endometriosis improves IVF outcome beyond symptom relief is controversial.[62] Data from uncontrolled case series suggest that cumulative pregnancy rates 1 to 3 years after surgical treatment are approximately 50% for women with endometriomas and about 30% for women with complete cul-de-sac obliteration.[63–65] Patient selection and careful surgical technique are important because ovarian function can be compromised by excision of excessive tissue or damage to hilar vessels[66,67]; the risk of ovarian failure after excision of bilateral ovarian endometriomas is approximately 2.5%.[68] Ablation of the cyst wall with plasmajet or sclerotherapy have been suggested as alternative methods with a lesser impact ovarian reserve in preliminary studies, but more studies are needed.[69] Oocyte or embryo cryopreservation should be considered prior to surgery in women at high risk of ovarian failure—for example, those with bilateral endometriomas and already low ovarian reserve. **After surgical treatment, the choice between expectant management, empirical treatment, and IVF should be based on age, surgical findings, and the severity of any other coexisting infertility factors. The endometriosis fertility index, an externally validated staging system, incorporating female age, duration of infertility, history of a prior pregnancy, tubal and ovarian status at conclusion of surgery, and American Fertility Society score can aid in the identification of couples with a poor prognosis for non-IVF conception and avoid loss of time without effective treatment.**[70–74]

Asymptomatic infertile women with advanced endometriosis, including those with ovarian endometriomas, can be treated surgically or proceed directly to IVF. Even though endometriomas can grow during ovarian stimulation and prevent access to some follicles during oocyte retrieval, there is no evidence to indicate that endometriomas have any important adverse effect on the response to ovarian stimulation or IVF outcomes.[75–77] Consequently, endometriomas can be left untreated before IVF. Aspiration of endometriomas before ovarian stimulation or at the time of oocyte retrieval has been associated with an increased risk for developing an ovarian abscess and is not recommended,[78–81] although the risk appears quite low.[77,82]

Treatment options for asymptomatic women with known or suspected minimal or mild endometriosis and no other infertility factors include expectant management, surgical treatment, empiric treatment with clomiphene or letrozole and intrauterine insemination (IUI), and IVF. In older women, those with other coexisting infertility factors, and women who have failed other forms of treatment, IVF is often the best overall choice.

Male Factor Infertility

Poor semen quality is the sole cause of infertility in approximately 20% of infertile couples and an important contributing factor in another 20% to 40%.[83,84] Many infertile men have disorders than can be corrected medically or surgically if properly diagnosed and treated, allowing them to achieve natural conception with their partners. In others, mild but important semen abnormalities can be overcome by IUI. **When treatment is not possible or fails, IVF and ICSI, using sperm isolated from the ejaculate or extracted from the epididymis or testis, offer realistic hope for success.** The evaluation and treatment of male factor infertility are the focus of Chapter 29. Discussion here is limited to the indications for ART.[84]

The likelihood of male factor infertility is increased in men whose ejaculates consistently exhibit a sperm concentration under 16 million sperm/mL, less than 42% progressive motility, or fewer than 4% morphologically normal sperm (strict criteria, WHO III standard).[85] The overall odds of male infertility increase with the number of abnormal parameters in the subfertile range; the probability is 2 to 3 times higher when one is abnormal, 5 to 7 times higher when two are abnormal, and approximately 16 times greater when all three parameters are abnormal.[86] **Additional genetic evaluation is indicated for men with severe oligospermia (sperm concentration <5 million/mL)** (Chapter 29).

Medical or surgical treatment to improve or normalize poor semen quality is always the first and best option, when that is possible. When treatment is not feasible or proves unsuccessful, timely IUI can help improve cycle fecundity in some couples with male factor infertility. **Best results with IUI are achieved when the number of total motile sperm in the insemination specimen exceeds a threshold of approximately 10 million.**[87–89] Higher counts do not further increase the likelihood of success,[87] and IUI is seldom

successful when fewer than 1 million total motile sperm are inseminated.[90,91] The effect of sperm morphology on IUI success is controversial, and predictive value of percentage of morphologically normal sperm is low, yet most studies suggest lower pregnancy rates with less than 4% sperm with normal morphology.[92–98] However, a low percentage of morphologically normal sperm alone does not necessitate omitting IUI in favor of IVF. The likelihood of success with IUI also decreases with increasing female partner age and with coexisting infertility factors (ovulatory dysfunction as well as uterine and tubal factors).

When IUI is not possible, the prognosis for success with IUI is poor, or IUI proves unsuccessful, IVF is the logical alternative. Approximately 28% of patients using ART have a primary diagnosis of male factor infertility.[4]

Conventional fertilization rates in IVF cycles are decreased when the total motile sperm count is less than 2 to 3 million (postwash).[99,100] Similarly, conventional fertilization rates are also decreased when less than 4% of sperm are morphologically normal (also called teratospermia).[99,101–104] Although severe teratospermia is widely accepted as an indication for assisted fertilization by ICSI, some, after observing no differences in fertilization, pregnancy, and live birth rates achieved with ICSI, compared with conventional fertilization, isolated teratospermia is not regarded as an indication for ICSI.[105–108]

Ovulatory Dysfunction

For women with ovulatory disorders (hypogonadotropic hypogonadism, polycystic ovary syndrome [PCOS], thyroid disorders, hyperprolactinemia), ovulation induction alone generally restores fertility (Chapter 30), but for some who require exogenous gonadotropins, ovulation induction proves difficult to achieve or consistently results in excessive ovarian stimulation and cycle cancellation for undue risk of ovarian hyperstimulation syndrome (OHSS) and high-order multiple gestation. For these difficult patients, IVF is an obvious treatment alternative, making their high sensitivity to gonadotropin stimulation an asset instead of a liability. Ovulatory dysfunction is the primary diagnosis in approximately 14% of patients using ART.[4]

Unexplained Infertility

The incidence of unexplained infertility ranges from 10% to as high as 30% among infertile populations, depending on diagnostic criteria.[109,110] For women with unexplained infertility, treatment options (cycle fecundability in parentheses) include expectant management (2–4%),[111] combined treatment with IUI and clomiphene (5–10%)[112–114] or letrozole (7–10%),[112,115,116] and IVF (25–45%).[4,117] As might be expected, success rates with all forms of treatment decline progressively with increasing age of the female partner.

Among couples with unexplained infertility, IVF is the preferred treatment for some but the treatment of last resort for others. **In either case, there is no question that IVF is the most effective treatment for couples with unexplained infertility.** A higher incidence of fertilization failure has been observed in several, but not all, studies of IVF outcomes in couples with unexplained infertility,[118–121] prompting many to recommend ICSI when IVF is planned. However, whether ICSI improves fertilization rate in the absence of male factor infertility remains controversial. It also seems unlikely that ICSI improves clinical outcome over that achieved with IVF in unexplained infertility.[122–126] Approximately 11% of patients using ART have a diagnosis of unexplained infertility.[4]

Diminished Ovarian Reserve and Ovarian Insufficiency

An increasing number of women undergo ART due to diminished ovarian reserve (DOR). In the United States, DOR was the primary diagnosis for 10% of ART cycles in 2003, 19% in 2015, and 26% in 2022 (**Figure 31.1**).[4]

IVF using oocytes from a known or anonymous young donor was first developed for women with premature ovarian failure or menopause.[127] Now, oocyte donation is most commonly performed in women over age 42, those with grossly abnormal ovarian reserve test results, and women whose IVF cycles consistently yield poor-quality embryos. Similar to the trend observed with DOR, the number of women undergoing oocyte donation in the United States almost doubled in the past decade.[4]

Other Indications for IVF and Related Technologies

Although less commonly encountered, there are a number of other legitimate indications for IVF and related ART procedures.

Fertility preservation is fast becoming a common indication for ART. Women with cancer or other illnesses requiring treatments (chemotherapy, radiation therapy) that pose a serious threat to future fertility may be candidates for urgent IVF and cryopreservation of embryos before treatment begins, if time and health allow.[128] Oocyte cryopreservation is a viable option for women in similar circumstances having no male partner[129] and is an option for young women at risk for premature ovarian failure, healthy aging women, and others who anticipate delayed childbearing.[130–132]

For women with normal ovaries but no functional uterus, due to a developmental anomaly (müllerian agenesis), advanced disease (multiple myomas, severe intrauterine adhesions), or a previous hysterectomy, and for women with medical conditions that preclude pregnancy due to serious health risks, **gestational surrogacy** offers the opportunity to have their own genetic offspring.[133,134]

Male same-sex couples utilize egg donation and gestational surrogacy to achieve parenthood. IVF may also be utilized by female same-sex couples to achieve pregnancy using

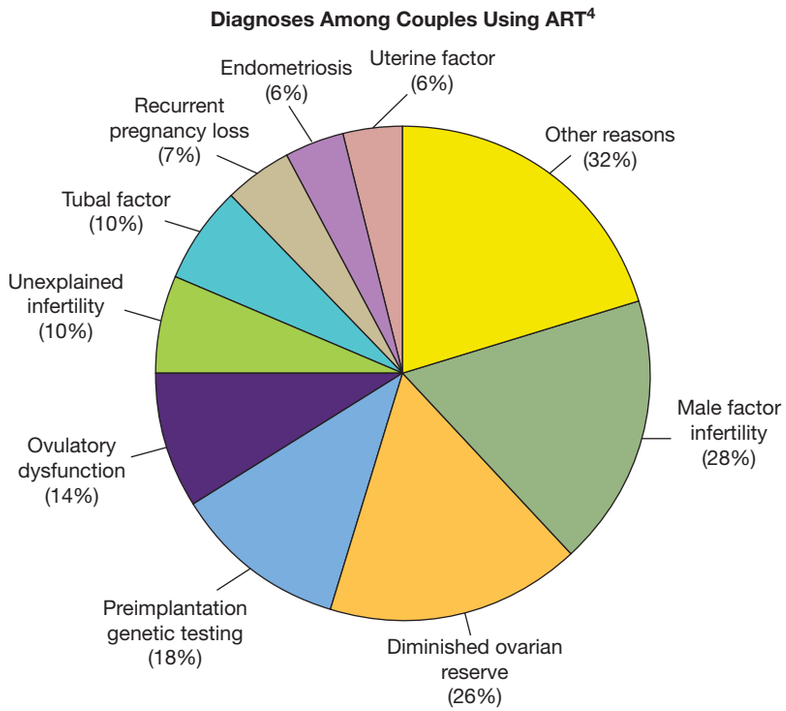

FIGURE 31.1 Total percentages are greater than 100% because more than one diagnosis can be reported for each cycle. Fresh and frozen eggs or embryos from patients and donors are included. Banking cycles are excluded. (Modified from National ART Summary by the Centers for Disease Control and Prevention. 2022 assisted reproductive technology national summary report. Accessed December 21, 2024. https://www.cdc.gov/art/php/national-summary/index.html)

eggs retrieved from one partner, fertilized by donor sperm, and transferred to the other partner, in a process termed reciprocal IVF.

For couples at risk for transmitting a specific genetic disease or abnormality to their offspring, IVF with **preimplantation genetic testing for monogenic disorders (PGT-M)** provides the means to identify and exclude affected embryos and thereby avoid that risk. PGT-M is applied most commonly in couples who carry autosomal recessive and sex-linked disorders. Individuals with a balanced chromosomal translocation benefit from **preimplantation genetic testing for structural rearrangements (PGT-SR)**.[135] Women who carry a genetic disorder not amenable to diagnosis by PGT-M or who decline PGT-M may be candidates for oocyte donation. More recently, maternal spindle transfer to enucleated donor oocytes have been employed to prevent mitochondrial disease.[136] **Preimplantation genetic testing for aneuploidy (PGT-A)** applies the same technology in couples having no known chromosomal or genetic abnormality in efforts to identify and exclude aneuploid embryos.[135]

PROGNOSTIC FACTORS

The probability of success with IVF relates to several factors, many of which are, unfortunately, not known until the treatment cycle is well underway (response to stimulation) or even nearing completion (number and quality of embryos). Before an IVF cycle begins, the primary prognostic indicators are maternal age, ovarian reserve, body mass index (BMI), diagnosis, and past reproductive performance.

Maternal Age

The relationship between maternal age and fertility and the physiology of reproductive aging are discussed in detail in Chapter 28, but only briefly summarized again here, where the focus is on the relationship between maternal age and IVF outcomes.

The average age of women using ART in the United States is 36.3 years.[4] **Maternal age is the most important factor in determining the likelihood of success with IVF.** Although IVF can overcome most causes of infertility in younger women, it cannot negate or reverse the age-related decrease in biologic fertility in older women.[137] The success rates achieved with IVF, like natural fertility rates, decline as maternal age increases.[4] **The pattern reflects a progressive decline in response to ovarian stimulation, resulting in fewer oocytes and embryos, and a decreased embryo implantation rate, due to increasing embryo aneuploidy mainly caused by meiotic errors in the aging oocytes.**[138–142] In 2022, the percentage of cycles that resulted in a live birth from fresh nondonor oocytes, by maternal age, was 43% for women under age 35, 40% for ages 35 to 37 years, 35% for

ages 38 to 40 years, 28% for over 40 years.[4] The pattern of decreasing success rates achieved with IVF parallels that associated with other, less complex forms of treatment for infertility.[143]

Evidence from numerous lines of investigation indicates that the age-dependent decrease in success rates achieved with IVF relates primarily to an increasing prevalence of aneuploidy in aging oocytes,[144–147] which is reflected in the incidence of miscarriage in pregnancies achieved with ART without PGT-A: 14.9% in women younger than 35 years, 20.8% in 35 to 37 years, 26.3% in 38 to 40 years, 39.2% in 41 to 42 years, and 46.9% in older than 42 years (**Figure 31.2**).[4] Consistent with this trend, in a case series of women aged 45 to 49 undergoing IVF, 70/231 cycles (30%) were cancelled before oocyte retrieval and 34/161 retrievals (21%) resulted in a pregnancy, but only 5/34 pregnancies (15%) and 5/231 cycles (2%) resulted in a live birth.[148]

Ovarian Reserve

Cumulative live birth rate continuously increases with number of eggs collected.[149,150] The major determinant of the number of oocytes collected is the ovarian reserve. The concept of ovarian reserve, generally defined as the size and quality of the remaining ovarian follicular pool, and the various methods for its measurement are discussed in detail in Chapter 28. In brief, the total number of oocytes in any given women is genetically determined and inexorably declines throughout life, from approximately 1 to 2 million at birth to about 300,000 at puberty, 25,000 at age 40, and fewer than 1,000 at menopause.[151–153] The rate of follicular depletion is not constant but increases gradually as the number of follicles remaining decreases.[154–157] As the size of the remaining follicular pool decreases, circulating inhibin B levels (derived primarily from smaller antral follicles) decrease, resulting in lower levels of feedback inhibition and a progressive increase in serum follicle-stimulating hormone (FSH) levels, most noticeably during the early follicular phase.[158–166] Increasing intercycle FSH concentrations stimulate earlier follicular recruitment, resulting in advanced follicular development early in the cycle, an earlier rise in serum estradiol levels, a shorter follicular phase, and decreasing overall cycle length.[167–169]

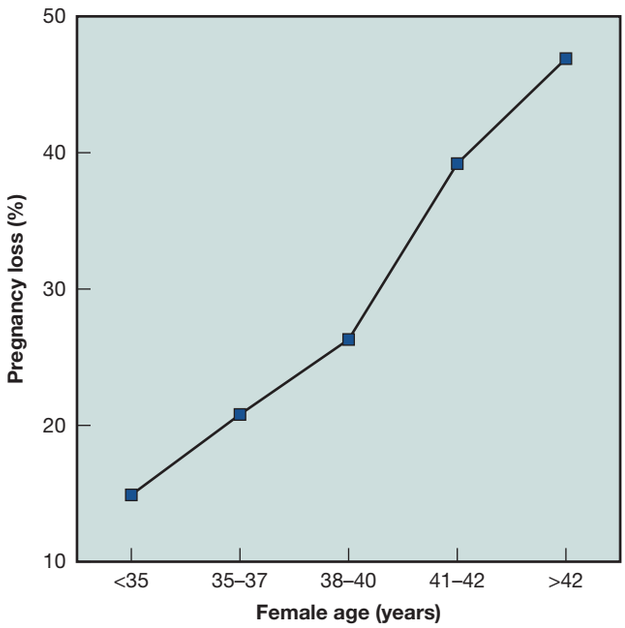

FIGURE 31.2 Risk of pregnancy loss in ART pregnancies without PGT-A. (Modified from National ART Summary 2022, Centers for Disease Control and Prevention. 2022 assisted reproductive technology national summary report. Accessed December 21, 2024. https://www.cdc.gov/art/php/national-summary/index.html)

The physiology of reproductive aging provides the foundation for all contemporary tests of ovarian reserve. Traditionally, the basal early follicular phase (cycle days 2–4) FSH level was the most common test; however, AMH and antral follicle count (AFC) are being used increasingly more often in contemporary practice.

As basal FSH levels increase, peak estradiol levels during stimulation, the number of oocytes retrieved, and the probability of pregnancy or live birth decline steadily.[170–176] **With current assays (using IRP 78/549), FSH levels greater than 10 IU/L (10–20 IU/L) have high specificity (80–100%) for predicting poor response to stimulation, but their sensitivity for identifying such women is generally low (10–30%) and decreases with the threshold value.**[175] Although most women who are tested have a normal result, including those with a DOR, the test is still useful because those with abnormal results are very likely to have DOR. Moreover, even when serum FSH levels are within the "normal" range (ie, <10 IU/L), they are independently associated with ovarian response and inversely proportional to the number of oocytes collected.[177]

The basal serum estradiol concentration, by itself, has little value as an ovarian reserve test[178–181] **but can provide additional information that helps in the interpretation of the basal FSH level.** An early elevation in serum estradiol reflects advanced follicular development and early selection of a dominant follicle (as classically observed in women with advanced reproductive aging) and will suppress FSH concentrations, thereby possibly masking an otherwise obviously high FSH level indicating DOR. When the basal FSH is normal and the estradiol concentration is elevated (>60–80 pg/mL), the likelihood of low response to stimulation is increased, and the chance for pregnancy is decreased.[182–185] When both FSH and estradiol are elevated, ovarian response to stimulation is likely to be very low.

AMH derives from preantral and small antral follicles. The number of small antral follicles correlates with the size of the residual follicular pool, and AMH levels decline progressively with age, becoming undetectable near the menopause.[186–189] Overall, lower AMH levels have been associated with low response to ovarian stimulation and low oocyte

yield, pregnancy rates, and cumulative live birth rates,[190–194] but studies correlating mean AMH levels with IVF outcomes have not yielded threshold values that can be applied confidently in clinical care.[178,191,192,195–204] **In the general IVF population, low AMH threshold values (0.2–0.7 ng/mL) have had 40% to 97% sensitivity, 78% to 92% specificity, 22% to 88% positive predictive value (PPV), and 97% to 100% negative predictive value (NPV) for predicting low response to stimulation (<3 follicles or <2–4 oocytes) but have proven neither sensitive nor specific for predicting pregnancy.**[190,205–207] In women at low risk for DOR, values of 2.5 to 2.7 ng/mL have had 83% sensitivity, 82% specificity, 67% to 77% PPV, and 61% to 87% NPV for clinical pregnancy.[179,208] A study in women at high risk for DOR (involving older women, those with an elevated FSH, or history of low response to stimulation) observed that an undetectable AMH had 76% sensitivity, 88% specificity, 68% PPV, and 92% NPV for three or fewer follicles.[191]

A large prospective cohort study of more than 2,000 women younger than 38 years undergoing their first IVF cycle found that the odds of a given fertilized oocyte developing to a blastocyst being aneuploid or leading to a live birth after euploid transfer were no different if the oocyte was retrieved from a cycle with AMH in the less than 10th percentile (corrected for age) compared to an oocyte retrieved in a cycle with those parameters in the 25th to 75th percentiles. The AMH level in the less than 10th percentile did more commonly result in cycle cancellation prior to retrieval and after retrieval prior to transfer due to global arrest of embryos. These findings suggest that while young women with evidence of quantitative depletion of ovarian reserve have lower live birth rates per stimulation cycle, this is not simply attributable to poor oocyte quality.[204] Similar findings have been reported from other groups, further supporting the absence of a strong association between quantitative and qualitative aspects of ovarian reserve.[209,210]

The AFC is the total number of antral follicles measuring 2 to 10 mm in both ovaries during the early follicular phase and is a useful measure of ovarian reserve because it quantifies the number of follicles at the stage of development that responds to FSH stimulation.[211–215] Several studies have observed a relationship between the AFC and the response to ovarian stimulation in IVF cycles. In the general IVF population, including women at low and high risk for DOR, an AFC threshold value of three to four follicles has high specificity (73–100%) for predicting low response to ovarian stimulation and failure to conceive (64–100%) but relatively low sensitivity for both end points (9–73% for poor response and 8–33% for failure to conceive).[180,216–222] The PPV and NPV of AFC have varied widely in studies. **A low AFC has high specificity for predicting low response to ovarian stimulation and treatment failure, making it a useful test, but its low sensitivity limits its overall clinical utility.**

In summary, both AFC and AMH level are useful tools to identify patients who are likely to have a low, normal, or hyperresponse to exogenous gonadotropin stimulation.[219,223–225] **However, none of the ovarian reserve tests currently in use are an accurate predictor of pregnancy in IVF cycles, unless extreme abnormal threshold values are applied, which results in very low sensitivity for identifying women having a poor prognosis.**[226] The tests are adequate for predicting low response, which does have prognostic value, although not as great in young women as in older women.[227–229] Although ovarian reserve tests have become a routine element of pretreatment evaluation for couples planning IVF, it can be argued that routine testing has limited clinical utility in the large majority of patients and can be misleading, especially in women at low risk for having a DOR.[230] Ovarian reserve tests should always be interpreted with caution. Rigid application of test results risks inappropriate recommendations for treatment or for no treatment, and both must be avoided. An abnormal test result does not preclude the possibility of pregnancy. **Except perhaps at the extremes, test results should not be used to deny treatment but only to obtain prognostic information that may help guide the choice of treatment and best use of available resources. Although the probability of pregnancy may be low, many with abnormal test results will achieve pregnancy if afforded the chance.**

Combinations of laboratory parameters, AFC, age, and past response to stimulation (if applicable) have been proposed as diagnostic criteria to identify women with expected low (or poor) response to stimulation and to establish a diagnosis of DOR or poor ovarian response (POR). The Bologna Criteria by the European Society of Human Reproduction and Embryology (ESHRE)[231] diagnoses POR based on two out of three of the following criteria: (1) AMH less than 0.5 to 1.1 ng/mL and AFC less than 5 to 7, (2) age (≥40 years), and (3) prior poor response (≤3 oocytes). Subsequently introduced, the POSEIDON (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) classification system for POR[232] subdivides predicted poor responders into four groups, based on age and ovarian reserve markers, as well as the presence of prior poor response.[233] While these diagnostic systems have not been widely adopted, a recent retrospective analysis of 6,889 cycles showed that the Bologna and POSEIDON criteria (with the exception of POSEIDON Class I, which was not different than normoresponders) predict low response. In the studied population, the POSEIDON Class II, III, and IV had a 77%, 70%, 45% chance of obtaining at least one euploid blastocyst in a stimulation cycle, respectively, compared to 90% in patients predicted to have a normal response. Patients who met the Bologna criteria, which are more stringent, had the worst outcome: approximately a 30% chance of obtaining at least one euploid blastocyst.[232] However, it is noteworthy that both the Bologna and POSEIDON criteria suffer from using strict age cutoffs (40 for Bologna and 35 for POSEIDON), which makes it difficult to use them as personalized diagnostic systems.

Body Mass Index

A large retrospective study, using the United States Center for Disease Control and Prevention (CDC)'s National ART Surveillance data, analyzed 196,916 women who underwent autologous ART and 25,831 donor oocyte recipients treated between 2016 and 2018.[234] The study identified a nonlinear association between BMI and cumulative live birth rates. Live birth rates remained relatively constant between a BMI of 17 and 25 kg/m² but declined significantly with a BMI of more than 25 kg/m² in adjusted analyses.

Two additional retrospective studies examined the effect of BMI on live birth rates following euploid embryo transfers using Society of Assisted Reproductive Technology (SART) Database.[235,236] These studies covered overlapping periods, one from 2014 to 2017 and the other from 2016 to 2019, with varying inclusion criteria and patient populations. Both studies reported a similar nonlinear relationship, where the probability of pregnancy and live birth was highest within the normal BMI range of 23 to 24.99 kg/m². However, increasing BMI was associated with lower chances of clinical pregnancy and live birth following frozen-thawed PGT-A-tested blastocyst transfers. Notably, comparable effect sizes were observed in autologous and donor recipient cycles, suggesting that uterine factors, rather than oocyte quality, are primarily affected by high BMI. Supporting this, multiple studies have reported that blastocyst euploidy rates are not associated with BMI.[237–240]

A 2024 European study also explored the effect of oocyte donor and recipient BMI on the outcomes of 1,394 first single blastocyst transfers.[241] The study found no association between donor BMI and the probability of live birth. However, recipient BMI of more than 30 kg/m² was negatively correlated with live birth rates in adjusted analyses. Furthermore, both recipient and donor BMI of more than 30 kg/m² significantly increased the risk of clinical pregnancy loss. Similarly, another retrospective study reported an elevated risk of pregnancy loss with a BMI of more than 30 kg/m² following euploid blastocyst transfers.[242]

Although retrospective studies provide valuable insights, they have inherent limitations that make definitive conclusions challenging. For example, a prospective study of over 2,000 infertile couples did not find significant associations between male or female BMI, percent body fat, and key IVF laboratory or clinical parameters.[237] Nevertheless, consistent findings across multiple large retrospective studies suggest a relationship between high BMI and negative ART outcomes. Importantly, while live birth rates tend to decline with a BMI of more than 25 or 30 kg/m², they remain sufficiently high to warrant treatment.

Encouraging a normal BMI is advisable, given the anesthetic, procedural, and obstetric risks associated with high BMI. However, in the absence of robust evidence showing that weight reduction interventions improve ART outcomes, it is inappropriate to deny treatment solely based on BMI.[243] Considering the time required for weight loss and the primarily uterine impact of high BMI, freezing oocytes or embryos and postponing ET may be a prudent approach. This is particularly relevant for patients with advanced age, decreased ovarian reserve, and high BMI, where time-sensitive factors are critical.

Diagnosis and Past Reproductive Performance

Although the average overall IVF live birth rate per cycle is approximately 38% for all women in the United States, success rates vary, to some extent, with the cause of infertility. In 2022, the success rates for women with tubal factor infertility, ovulatory dysfunction, endometriosis, male factor, and unexplained infertility were above average, and those for women with multiple infertility factors, a uterine factor, and DOR were below average.[4] Whereas these data are useful, it is important to note that criteria for the different diagnoses are not standardized and likely vary among treatment centers.

Women with a previous live birth are more likely to succeed with IVF than nulliparous women. Success rates for women having one or more previous live births are modestly higher than for women with no previous live births, while women with previous unsuccessful ART cycles attain lower live birth rates beginning after two previous failures. A history of an earlier unsuccessful pregnancy has no effect on success rates.

Other Prognostic Factors

As discussed earlier in the section focused on indications for ART, there is substantial evidence indicating that hydrosalpinges adversely affect IVF outcomes and that salpingectomy or proximal tubal occlusion before IVF increases the likelihood for achieving a live birth by 2-fold.[19] Laparoscopic salpingectomy before IVF is generally recommended for women with hydrosalpinges, even when only one tube is affected. A study evaluating the cost-effectiveness of preliminary salpingectomy concluded that the procedure decreases the average cost per live birth, compared to no treatment.[244] One concern with salpingectomy or even tubal occlusion or ligation is its impact on ovarian reserve. Despite contradictory results in early reports, salpingectomy does not impact ovarian reserve, except perhaps when it is done for ectopic pregnancy. Salpingectomy does not significantly decrease serum AMH concentration, ovarian response to stimulation or clinical pregnancy rates with IVF.[245–247] Ovarian stimulation parameters, implantation, and clinical pregnancy rates are similar in women who underwent salpingectomy or laparoscopic proximal tubal occlusion.[248]

Ultrasound-guided aspiration of hydrosalpingeal fluid at the time of oocyte retrieval has been suggested as an alternative treatment.[249] The procedure is effective in improving IVF outcome as compared to no intervention, simpler and less costly than surgical options. However, salpingectomy or proximal tubal occlusion improves success rates more than fluid aspiration with regard to ongoing pregnancy, clinical pregnancy, ectopic pregnancy, and miscarriage rates.[250]

Moreover, the fluid reaccumulates rapidly.[251] Aspiration of hydrosalpingeal fluid should be reserved for women who are likely to have severe intra-abdominal adhesions prone to complications with pelvic surgery.

The effect of uterine myomas on IVF outcomes depends on their location. They alter global endometrial receptivity through the production of cytokines rather than simply a mechanical effect.[252–254] There is a clear consensus that submucous myomas have a significant adverse effect on clinical pregnancy rates (OR = 0.3, CI = 0.1–0.7) and delivery rates (OR = 0.3, CI = 0.1–0.8).[255–260] Available data also support the conclusion that submucous myomas increase the risk for miscarriage by more than 3-fold,[259,260] while the effect of intramural myomas that do not distort the cavity on the risk of miscarriage is less clear.[261] Results of early studies examining the effect of intramural myomas on IVF outcomes are inconsistent, with some finding adverse effects[262–266] and others not.[259,267–272] Large-scale prospective studies report significantly lower implantation, pregnancy, ongoing pregnancy, and live birth rates in women with intramural myomas.[265,266] A 2023 systematic review on the effect of noncavity distorting fibroids less than 6 cm on IVF outcomes included five studies in which women with fibroids were age matched with women without fibroids and reported significantly decreased clinical pregnancy rates, live birth rates, and increased miscarriage rates in the 2 to 6 cm fibroid group.[273] The differences were short of statistical significance in subgroup analyses for fibroids less than 2 cm, which included only one study.[274] The effect appears to be related to both myoma size and proximity to the endometrium. All of the evidence concerning the effects of subserosal myomas is consistent in finding no evidence of adverse effects on IVF outcomes. **In sum, the accumulated body of evidence indicates that submucous myomas reduce IVF success rates by approximately 70% and intramural myomas by approximately 20% to 40%, while subserosal myomas have no adverse impact on outcomes. Submucous myomas increase risk for miscarriage after successful IVF at least 3-fold and intramural myomas by more than half. Submucous and large proximal intramural fibroids should be removed prior to IVF.**[275]

Tobacco Use

All smoking women should be strongly encouraged to stop smoking before IVF because smoking decreases the likelihood of success.[276–278]

EVALUATION BEFORE IVF

Individuals and couples planning IVF require additional specific evaluation before a treatment cycle begins. At a minimum, evaluation generally includes a test of ovarian reserve, a current assessment of semen quality, infectious disease screening, and imaging of the uterine cavity.

The American College of Obstetricians and Gynecologists (ACOG)[279] recommends that all women considering pregnancy should be offered carrier screening for cystic fibrosis and spinal muscular atrophy, as well as a complete blood count and screening for thalassemias and hemoglobinopathies. Women with a family history of fragile X–related disorders or intellectual disability suggestive of fragile X syndrome or women with premature ovarian insufficiency should be offered fragile X permutation carrier screening.[279] Additional screening may also be indicated based on family history or specific ethnicity; an example is the Ashkenazi Jewish population, where an expanded carrier screening for a number of recessive disorders is indicated. Couples with consanguinity should be offered genetic counseling to discuss the increased risk of recessive conditions being expressed in their offspring and the limitations and benefits of carrier screening. More recently, the American College of Medical Genetics recommends an ethnic and population neutral paradigm and a tiered approach to carrier screening, which is supported by the ACOG. This implies screening for conditions with a career frequency of more than 1/200 in the population to all women planning a pregnancy.[280] Indeed, career screening is increasingly offered, particularly to patients starting ART cycles. When a woman is found to be a carrier for a specific condition, her reproductive partner should be offered screening to provide accurate genetic counseling regarding risk of an affected child if he tests positive and reproductive options (eg, donor gametes, PGT, prenatal diagnosis).

Ovarian reserve tests (basal FSH, AMH, AFC) have value for predicting response to gonadotropin stimulation and can therefore be helpful in planning treatment. If a threshold value with high specificity for detecting DOR is applied, the test can accurately identify women at high risk for low response and treatment failure.

Semen quality should be assessed shortly before the treatment cycle is scheduled to start, even when earlier diagnostic evaluation revealed normal semen parameters, to ensure there has been no appreciable change that might affect the choice between conventional fertilization and ICSI. Sperm cryopreservation is prudent when semen quality is severely abnormal or when there is reason to anticipate difficulty with obtaining a fresh specimen on the day of oocyte retrieval. Although fertilization rates achieved with frozen-thawed sperm may be somewhat lower than when fresh sperm are used, pregnancy rates are comparable.[281–283]

Infectious disease screening recommended for both partners includes human immunodeficiency virus (HIV), hepatitis B (hepatitis B surface antigen), hepatitis C (hepatitis C antibody), and syphilis (rapid plasma reagin), for the protection of medical and laboratory staff, protection of any fetus that may result from IVF, and protection against the risk for cross-contamination of cryopreserved embryos in storage. Whereas some advocate routine testing for chlamydia and gonorrhea in the female partner, others choose to limit evaluation to women with tubal factor infertility or

other risk factors. Testing for antibodies to rubella and varicella in women undergoing IVF should be performed for timely immunization, if indicated.

Imaging of the uterine cavity before a cycle of treatment identifies submucosal myomas or endometrial polyps that may interfere with implantation or have an adverse effect on pregnancy outcome. An hysterosalpingography (HSG) performed earlier during the diagnostic evaluation may suffice if entirely normal and relatively recent (within ~6 months), but sonohysterography and hysteroscopy are the more sensitive and preferred methods. Routine office hysteroscopy before IVF was expected to identify potentially significant abnormalities such as polyps, myomas, adhesions, or septa in 10% to 20% of patients without symptoms.[284–286] However, two multicenter randomized controlled trials suggest that routine hysteroscopy prior to an IVF cycle does not improve outcomes over routine imaging.[287,288] Many prefer sonohysterography to hysteroscopy because it is easier to perform, is highly sensitive, and can also detect hydrosalpinges and unsuspected ovarian pathology.[289–291]

Some clinicians would also perform a **trial transfer** to determine the technique required to achieve an atraumatic ET and to identify women whose transfer may be difficult to accomplish, although the orientation of the uterus can change when the ovaries are enlarged after stimulation.[292–295]

OVARIAN STIMULATION REGIMENS

The ideal ovarian stimulation regimen for IVF should have a low cancellation rate; minimize drug costs, risks, and side effects; require limited monitoring for practical convenience; and maximize cumulative live birth rate per oocyte retrieval procedure—that is, cumulative chances of one or more live births after exhausting all fresh and frozen embryos generated from a single oocyte retrieval. Singleton pregnancy delivered at term is the ultimate goal of ART, but family completion through one ovarian stimulation cycle can also be considered a goal in women with sufficient ovarian reserve.[296]

There are three components of a typical ovarian stimulation protocol for IVF; the first is exogenous gonadotropins to stimulate multifollicular growth, the second is pituitary suppression to prevent spontaneous ovulation before oocyte retrieval, and the third is luteinizing hormone (LH) activity to induce oocyte maturation. The source and type of gonadotropin—for example, endogenous or exogenous, human menopausal or recombinant gonadotropin, the pituitary suppression agent (ie, an agonistic or antagonistic gonadotropin-releasing hormone [GnRH] analogue) or, more recently, a progestin—vary according to the selected protocol and the intention to do a fresh ET or not. The LH activity required for final oocyte maturation can be mimicked by exogenous human chorionic gonadotropin (hCG), or an endogenous LH surge can be induced by a single dose GnRH agonist (GnRHa) in cycles without pituitary desensitization.

Numerous stimulation protocols have been defined, and new ones are being developed with increasing experience to render ovarian stimulation more efficient and patient-friendly according to changing practice patterns.

Stimulation of Follicular Growth

Natural Cycle

The first birth resulting from IVF derived from a single oocyte collected in a natural ovulatory cycle.[2] A pure natural cycle (NC) IVF involves only monitoring the spontaneous cycle and retrieving a single oocyte before the midcycle LH surge occurs. It is physically less demanding, requires little or no medication, decreases costs,[297,298] and all but eliminates risks for multiple pregnancy and OHSS. The chief disadvantages of NC IVF are high cancellation rates due to premature LH surges and ovulation and the comparatively low success rate of approximately 7%.[299]

When oocyte retrieval is based on detection of the midcycle rise in LH, careful and frequent monitoring is required, and procedures are difficult to schedule efficiently. Alternatively, exogenous hCG can be administered when the lead follicle reaches a size consistent with maturity, thereby better defining the optimum time for oocyte retrieval.[298] Adjuvant treatment with a GnRH antagonist can also be used to prevent a premature LH surge but requires "add-back" treatment with exogenous FSH. With these additions, the protocol is dubbed a "modified natural cycle"; however the success rates remain still quite low, ranging up to 14% per cycle in nonrandomized trials.[300–303] In one large cohort study involving 844 treatment cycles in 350 good prognosis patients, the cancellation rate was 13%, the pregnancy rate was 8% per cycle, and the cumulative pregnancy rate after three "modified natural IVF cycles" was 21%.[304] In a cohort of infertile couples with male factor infertility, success rates in modified NCs have reached as high as 13% per cycle, with a cumulative pregnancy rate of 44% after six treatment cycles.[305] A retrospective study compared modified NC with daily GnRH antagonist and 75 IU human menopausal gonadotropin from the day when the leading follicle reached 14 mm with 300 IU daily gonadotropin stimulation in advanced aged women with low ovarian reserve and reported significantly lower oocyte yield and live birth rates.[306] Since the number of oocytes retrieved is positively correlated with pregnancy rates,[149,307] natural or modified NCs are not the first choice for women who can produce multiple follicles with gonadotropin stimulation. However, they can be a reasonable compromise for women who are expected to grow one or two follicles despite high-dose gonadotropin stimulation.

Clomiphene Citrate

Clomiphene citrate was the first method of ovarian stimulation used in IVF[308,309] but has now been almost entirely replaced by more effective stimulation regimens using exogenous gonadotropins, in combination with pituitary suppression.[310]

Clomiphene (100 mg daily) is usually administered for 5 to 8 days, beginning on cycle day 3, and induces development of two or more follicles in most normally ovulating women,[311–313] although egg yields (1–3) are only slightly greater than in unstimulated cycles and substantially lower than in cycles stimulated with exogenous gonadotropins.[313–315] Cycle cancellation rates are somewhat lower than in NCs, and the numbers of oocytes retrieved, embryos transferred, and pregnancy rates are greater. As in NCs, exogenous hCG is administered when the lead follicle reaches mature size and a gonadotropin-releasing hormone antagonist (GnRH) can be used to prevent a premature endogenous LH surge.

Sequential treatment with clomiphene (100 mg daily for 5 days) and modest doses of exogenous gonadotropins (150–225 IU daily beginning on the last day of clomiphene treatment or the day after) stimulates multifollicular development more effectively than treatment with clomiphene alone.[316–318] Drug costs and monitoring requirements are moderately higher but still substantially less than in standard stimulation regimens involving higher-dose gonadotropin treatment.[319,320] In one comparative trial, higher cancellation rates and lower pregnancy rates were observed in sequential clomiphene/gonadotropin compared to gonadotropin/GnRHa cycles.[320] In another, the sequential stimulation regimen yielded fewer oocytes and embryos, but pregnancy rates were similar and the risks of OHSS were lower.[319] Adding a GnRH antagonist to the treatment regimen can prevent premature LH surges and improve outcomes but also increases costs. In a randomized trial, sequential clomiphene/gonadotropin stimulation and GnRH antagonist treatment yielded a pregnancy rate comparable to that achieved with a more aggressive standard treatment protocol,[321] confirming the results of two earlier retrospective studies,[322,323] although contrasting with those of another observing lower pregnancy rates.[324] A 2021 systematic review and meta-analysis included three randomized controlled trials (RCTs) comparing a combination of clomiphene and gonadotropins with exogenous gonadotropin stimulation alone and reported similar live birth rate (RR = 0.88, 95% CI = 0.69–1.12) but a lower risk of OHSS with the use of CC combination (RR = 0.12, 95% CI = 0.03–0.51).[325]

Apart from its common use as a stimulant for ovaries, clomiphene can help prevent premature LH surges in IVF cycles. Clomiphene may be used continuously until the day of ovulation trigger; this simplifies the protocol and reduces the cost of medication, as GnRH analogues are not needed. Large retrospective case series of minimal ovarian stimulation with clomiphene reported premature ovulation rates around 2.5%, attesting to the effectiveness of clomiphene in preventing premature LH surges.[326] Overall, protocols that use clomiphene both to stimulate ovaries and to inhibit LH surge are reported to result in an average number of oocytes collected between 1.5 and 2.5, cycle cancellation rates between 49% and 66%, clinical pregnancy rates in the range of 15% to 17%, and live birth rates in the range of 9.5% to 12.2%.[326–329] Regardless of the details of the protocols, studies that use mainly CC for ovarian stimulation yield a relatively lower number of oocytes with comparable clinical pregnancy rates (albeit being ~10% lower) than conventional stimulation cycles.

Use of Gonadotropins to Stimulate Multifollicular Growth

The general principles of gonadotropin choice and dosing are similar for different pituitary suppression protocols. The initial dose of exogenous gonadotropins is tailored to the needs of the individual woman. Typical starting doses range between 150 and 450 IU of urinary FSH (uFSH), recombinant FSH (rFSH), or urinary menotropins (hMG) daily, depending on age, the results of ovarian reserve testing, body weight, and the response observed in any previous stimulation cycles. However, there is an ongoing discussion regarding individualized dosing versus administering a standard dose of 150 IU/d for all women undergoing ovarian stimulation for IVF.[330–333] A multicenter trial conducted in the Netherlands categorized 1,032 women according to anticipated ovarian response as assessed by AFC and compared cumulative live birth rates over 18 months in women who received the standard starting dose of 150 IU/d and those who received 450 IU/d (women with AFC ≤8), 225 IU/d (women with AFC 8–10), or 100 IU/d (women with AFC >15).[330,332] Women with AFC ≤10 had similar cumulative live birth rates (43.2% vs 45.6%) and time to ongoing pregnancy (212 vs 197 days) regardless of FSH dosing, while women receiving higher doses had significantly more oocytes collected (7.6 vs 6.4), significantly lower incidence of poor response (35% vs 50%), and significantly higher incidence of hyperresponse (8.6% vs 3.4%) and treatment costs. Likewise, cumulative live birth rates (66.3% vs 69.5%) and time to ongoing pregnancy (185 vs 191 days) were similar between women with an AFC of more than 15, who received 100 or 150 IU/d doses, respectively. Critics suggest the use of more complex models for dose selection, which include other markers of ovarian reserve; for example, AMH can improve outcomes or decrease the risk of OHSS.[334–336] A 2024 Cochrane systematic review included RCTs that compared ovarian reserve testing-based algorithm-selected gonadotropin dosing with no algorithm-based dosing and reported similar live birth/ongoing pregnancy (OR = 1.12, 95% CI = 0.98–1.29) and clinical pregnancy rates (OR = 1.04, 95% CI = 0.91–1.18), based on seven studies involving 4,400 women. Likewise, the risk of OHSS was similar between the groups (OR = 0.74, 95% CI = 0.42–1.28).[337]

Numerous clinical trials and meta-analyses have compared outcomes in cycles stimulated with uFSH, rFSH, or hMG, concluding that there is no compelling evidence to indicate the superiority of one gonadotropin preparation over others.[338–341] However, a 2011 systematic review including 11 trials comparing outcomes in cycles stimulated with rFSH or

hMG, involving 3,179 patients, observed a significant difference in live birth rate (OR = 0.84, CI = 0.72–0.99) favoring hMG.[342] A 2019 systematic review and meta-analysis comparing rFSH and highly purified hMG similarly reported significantly lower live birth rate with rFSH (RR = 0.90, 95% CI = 0.81–1.00, seven RCTs, 3,397 women); however, cumulative live birth rates, including frozen embryo transfers (FETs) from the stimulation cycle, were similar with both gonadotropins (RR = 0.91, 95% CI = 0.80–1.04, three RCT, 2,109 women).[343] Another randomized trial, published after the systematic review, compared equal doses of highly purified hMG and rFSH with the GnRH antagonist protocol in anticipated high responders and reported similar cumulative live birth rates per cycle (absolute difference = 0.8%, 95% CI= −8.7 to 7.1%) and live birth rates with fresh ETs (52.2% vs 48.7%) or FETs (63.4% vs 50.8%). The risk of OHSS was significantly lower with hMG compared to rFSH (9.7% vs 21.4%).[344] It should be noted that the differences observed in live birth rates between rFSH and hMG seem to derive mainly from fresh ETs and are probably mediated by higher serum progesterone levels attained during the late follicular phase of rFSH stimulated cycles. Elevated late follicular phase progesterone levels cause endometrial advancement and shifting of the receptive window, leading to asynchrony between embryo developmental stage and endometrium, decreasing live birth rate following a fresh ET.[345] Late follicular phase serum progesterone levels are positively associated with the number of growing follicles, and asynchrony increases with ovarian response, and the 2019 meta-analysis reports a significantly higher number of oocytes being collected from rFSH-stimulated cycles than from hMG-stimulated cycles.[343,345] A 2024 randomized controlled trial involving oocyte donors who received 225 IU/d hMG or rFSH in a GnRH antagonist protocol reported significantly lower serum progesterone levels in the hMG arm than in the rFSH arm (0.46 ± 0.27 vs 0.68 ± 0.50, respectively, $P = 0.01$), despite a similar number of oocytes being retrieved (16.5 ± 7.5 vs 17.5 ± 7.9, respectively).[346] A 2013 systematic review and meta-analysis, including 55,199 fresh ET cycles from 63 prospective and retrospective studies, reported that serum progesterone levels above 0.8 ng/mL on the day of hCG administration was associated with significantly decreased odds of live birth/ongoing pregnancy rate, in a dose-dependent manner.[345]

Recombinant DNA technology has been used to develop a longer-acting form of rFSH. Corifollitropin alfa comprises a α-subunit identical to that of FSH and a β-subunit produced by the fusion of the C-terminal peptide from the β-subunit of chorionic gonadotropin (hCG) to the β-subunit of FSH. Corifollitropin alfa has a half-life 3 times longer than standard rFSH (95 vs 32 hours).[347,348] A single dose (100 µg for women ≤60 kg, 150 µg for those >60 kg) can induce and sustain multifollicular growth for a week in women receiving ovarian stimulation for IVF. Ovarian stimulation with corifollitropin yields similar or significantly higher numbers of oocytes and good quality embryos, as well as similar ongoing pregnancy rates compared to women stimulated with daily rFSH injections. Stimulation characteristics, embryology, and clinical outcomes are also similar, and multiple pregnancy or OHSS rates are not increased over daily FSH regimen. Corifollitropin alfa molecule does not seem to be immunogenic and does not induce neutralizing antibody formation. Drug hypersensitivity and injection site reactions are not increased. Incidence and nature of adverse events, serious adverse events, and rate of congenital malformations are reported to be similar to daily FSH injections.[349,350] This product is not available in the United States.

The low levels of LH secretion remaining after downregulation with a GnRHa are sufficient to support normal follicular development in most women stimulated with uFSH or rFSH alone[351] because only about 1% of LH receptors must be occupied to sustain normal levels of steroidogenesis.[352] However, in some women treated only with FSH, LH levels are markedly suppressed (<1 IU/L) and may be inadequate.[353,354] In such cycles, the dose and duration of gonadotropin stimulation required is higher, peak estradiol levels are lower, and the numbers of oocytes and embryos may be reduced.[355,356] Extremely low LH levels may also adversely affect fertilization, implantation, and pregnancy rates[357–361] and have been associated with a higher incidence of biochemical pregnancy and early pregnancy loss.[362,363] Whether LH activity improves clinical outcome of ovarian stimulation remains controversial. A 2014 meta-analysis comparing rFSH alone and rFSH with recombinant LH concluded that the addition of rLH did not increase the number of oocytes collected.[364] However, consistent with the aforementioned Cochrane review,[342] rLH was associated with a small but statistically significant improvement in clinical pregnancy rate (rate ratio [RR] = 1.09, 95% CI = 1.01–1.18).[364,365] Even though the authors concluded that addition of rLH was associated with a more prominent increase in clinical pregnancy rates in low responders, as well as significantly higher number of oocytes collected, this was not confirmed in several subsequent RCTs.[366–370] In sum, the evidence indicates **there may be a subgroup of women who could benefit from supplemental rLH or hMG during ovarian stimulation.** In the absence of any reliable method for identifying such women, and in light of evidence suggesting that use of hMG may increase live birth rates,[371] many clinicians favor combined stimulation with FSH and hMG or rLH over stimulation with FSH alone.

Protocols for Pituitary Suppression to Prevent Premature LH Surge During Ovarian Stimulation

GnRH Agonist Downregulation Gonadotropin Stimulation—The "Long" Protocol

The introduction of long-acting GnRHa in the late 1980s revolutionized the approach to ovarian stimulation in ART by providing the means to suppress endogenous pituitary gonadotropin secretion and thereby prevent a premature

LH surge during exogenous gonadotropin stimulation. Adjuvant treatment with a GnRHa eliminated the need for frequent serum LH measurements and assuaged fears of premature luteinization, which had previously required cancellation of approximately 20% of all IVF cycles before oocyte retrieval.[372–374] Because fewer than 2% of cycles are complicated by a premature LH surge after downregulation with a GnRHa,[292] stimulation could continue until follicles were larger and more mature. Numerous clinical trials subsequently demonstrated that egg yields and pregnancy rates were significantly higher than in cycles stimulated with exogenous gonadotropins alone.[375,376] Moreover, GnRHa treatment offered the welcome additional advantage of scheduling flexibility, allowing programs to coordinate cycle starts for groups of women simply by varying the duration of GnRHa suppression. Not surprisingly, the "long protocol" quickly became the preferred ovarian stimulation regimen for all forms of ART. Its only disadvantages are that GnRHa treatment sometimes blunts the response to gonadotropin stimulation and increases the dose and duration of gonadotropin therapy required to stimulate follicular development. The combined costs of the additional gonadotropins and the agonist itself increase the total cost of treatment substantially. **Nevertheless, because GnRHa have more advantages than disadvantages, the long protocol became and has remained the standard ovarian stimulation regimen in IVF cycles until more patient-friendly protocols utilizing GnRH antagonists gained widespread acceptance.**

In the typical cycle, GnRHa treatment begins during the midluteal phase, approximately 1 week after ovulation, at a time when endogenous gonadotropin levels are at or near their nadir and the acute release of stored pituitary gonadotropins in response to the agonist, known as the "flare" effect, is least likely to stimulate a new wave of follicular development.[377,378] GnRHa treatment can be scheduled to begin on cycle day 21 (assuming a normal cycle of ~28-day duration), but some prefer to first confirm that ovulation has occurred by measuring the serum progesterone concentration. In women who do not cycle predictably, oral contraceptives (OC) can be used to control the onset of menses, starting GnRHa treatment 1 week before their discontinuation. In the United States, leuprolide acetate (administered by subcutaneous [SC] injection) is the most commonly used GnRHa. In Europe and elsewhere, buserelin acetate (administered by SC injection or intranasal spray) and triptorelin (administered subcutaneously) are more commonly used[379]; all work equally well. For leuprolide, the usual treatment regimen begins with 1.0 mg daily for approximately 10 days or until onset of menses or gonadotropin stimulation, decreasing to 0.5 mg daily thereafter until the ovulation trigger. A single dose of a longer-acting depot form of GnRHa (leuprolide, goserelin) offers greater convenience. While the evidence indicates that the total dose (on average 26 IU) and duration of gonadotropin stimulation (on average 0.65 day) required are slightly increased when depot forms of the agonists are used, the differences are clinically insignificant.[380]

Gonadotropin stimulation begins after confirming that effective pituitary downregulation has been achieved (serum estradiol level <30–40 pg/mL, no follicles >10 mm in diameter). Some women require longer durations of treatment to achieve suppression or may develop an ovarian cyst.[372] The significance of an ovarian cyst has been controversial. Whereas some investigators have observed that baseline cysts are associated with a lower response to gonadotropin stimulation, decreased numbers of oocytes and embryos, and lower overall IVF success rates,[381–383] others have not.[384–388] **Overall, the weight of available evidence suggests that women who develop cysts or require longer durations of GnRHa treatment to achieve suppression are more likely to respond poorly to gonadotropin stimulation and less likely to achieve pregnancy.** Cyst aspiration immediately before stimulation does not appear to effect rates of live birth, clinical pregnancy, number of follicles recruited, or number of oocytes collected. Since cyst aspiration requires anesthesia, extra cost, psychological stress, and risk of surgical complications, it is probably not warranted in the absence of another indication (**Figure 31.3**).[389]

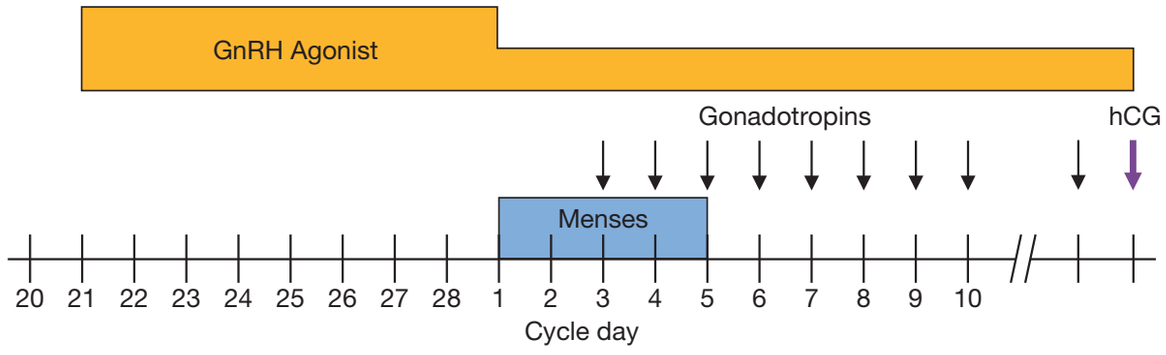

FIGURE 31.3

GnRH Agonist "Flare" Gonadotropin Stimulation Protocol

The "short" or "flare" protocol is an alternative stimulation regimen designed to exploit both the brief initial agonistic phase of response to a GnRHa and the suppression that results from longer-term treatment.[390,391] In a typical standard short protocol, leuprolide acetate (1.0 mg daily) is administered on cycle days 2 to 4, continuing thereafter at a reduced dose (0.5 mg daily), and gonadotropin stimulation begins on cycle day 3.

Decreased scheduling flexibility is a distinct disadvantage of the flare protocol, unless the onset of menses is controlled by preliminary treatment with an OC or estrogen. The regimen can also result in a significant increase in serum progesterone and androgen levels, presumably resulting from late corpus luteum rescue.[392,393] This may adversely affect pregnancy rates if a fresh ET is attempted.[394–396]

The "OC microdose GnRH agonist flare" stimulation regimen is a variation of the standard short protocol involving 14 to 21 days of preliminary ovarian suppression with an OC (one pill daily), followed by microdose leuprolide treatment (40 μg twice daily) beginning 3 days after discontinuation of OC treatment and high-dose gonadotropin stimulation starting on day 3 of leuprolide therapy. Its primary advantage over the standard short protocol is that it does not induce any increases in serum progesterone or androgen concentrations,[397] possibly because the doses of GnRH administered are much lower but likely also because preliminary OC treatment all but eliminates the possibility that there may be a corpus luteum left to respond.[398,399]

Individual trials comparing stimulation protocols in women with poor response as well as meta-analyses reported conflicting results.[365,400,401] While the long protocol takes longer and requires higher total gonadotropin consumption, it better synchronizes the follicular cohort.[365,370,400] Given the absence of any clear benefit with regard to the number of oocytes collected or pregnancy rates, and some disadvantage with regard to gonadotropin consumption, the long protocol should not be the first choice for DOR, with the possible exception of patients with substantial asynchronous follicle development in repeated cycles.[370] Short GnRHa protocols seem to provide a significantly higher number of oocytes, albeit as small as 0.5 oocytes more on average, with similar gonadotropin consumption compared to GnRH antagonist protocols.[370] Hence, the preference between the two remains personal, taking into consideration the number of injections required and the cost of medication.

GnRH Antagonist Gonadotropin Stimulation Protocol

The introduction of GnRH antagonists into clinical practice provided another option for ovarian stimulation in ART. In contrast to the long-acting agonists, which first stimulate and later inhibit pituitary gonadotropin secretion by desensitizing gonadotrophs to GnRH via receptor downregulation, the antagonists block the GnRH receptor in a dose-dependent competitive fashion and have no similar flare effect[402,403]; gonadotropin suppression is almost immediate.

GnRH antagonists offer several potential advantages over agonists. First, the duration of treatment for an antagonist is substantially shorter than for an agonist. Since its only purpose is to prevent a premature endogenous LH surge and its effects are immediate, antagonist treatment can be postponed until later in follicular development (after 5–6 days of gonadotropin stimulation), after estradiol levels are already elevated, thereby eliminating the estrogen deficiency symptoms that may emerge in women treated with an agonist.[404] Second, because any suppressive effects that agonists may exert on the ovarian response to gonadotropin stimulation are also eliminated, the total dose and duration of gonadotropin stimulation required is decreased.[404,405] For the same reason, GnRH antagonist stimulation protocols may benefit women who are low responders when treated with a standard long protocol.[370,404,406] Third, by eliminating the flare effect of agonists, GnRH antagonists avoid the risk of stimulating the development of a follicular cyst. Finally, the risk of severe OHSS associated with the use of antagonists also appears lower than with agonists.[407–410] The risk of OHSS can be further diminished with the use of a GnRHa trigger in GnRH antagonist–stimulated cycles, an option that is not available when GnRHa are used for pituitary suppression.

GnRH antagonists have some potential disadvantages. When administered in small daily doses, strict compliance with the prescribed treatment regimen is essential.[404] Antagonists suppress endogenous gonadotropin secretion more completely than agonists. While initial studies suggested modestly lower pregnancy rates in antagonist treatment cycles than in cycles using agonists in the long protocol, this could have been due to patient selection or lack of experience with the use of antagonists, and recent evidence suggests similar live birth and pregnancy rates in GnRH antagonist cycles.[411–413] **Overall, due to their ease of use and comparable outcomes, antagonist protocols are used more commonly than long GnRHa protocols worldwide**.

The minimum effective dose of GnRH antagonists to prevent a premature LH surge is 0.25 mg daily, administered subcutaneously.[361,414] The treatment protocol may be fixed and begin after 5 to 6 days of gonadotropin stimulation[361,414,415] or tailored to the response of the individual, with treatment started when the lead follicle reaches a diameter of approximately 13 to 14 mm.[416,417] The individualized treatment regimen, also called "flexible" antagonist protocol, generally requires fewer total doses; however, a 2023 systematic review and meta-analysis including seven RCTs that compared fixed and flexible GnRH antagonist protocols reported a significantly lower ongoing pregnancy rate with the flexible GnRH antagonist protocol compared to the fixed protocol (RR = 0.76, 95% CI = 0.62–0.94).[418]

A common variation of the antagonist stimulation regimen uses preliminary treatment with an OC to control the onset of menses, typically ending at least 5 days before the scheduled start, which may also help synchronize the follicular cohort before stimulation begins. However, OC priming may be associated with a significant decrease in ongoing pregnancy and live birth rates.[419–422] It should be noted that the effect could vary with different contraceptives and for different durations and intervals, as well as whether a fresh transfer or freeze-all approach is taken.[423,424] Based on retrospective studies, micronized estradiol (2 mg 2–4 times daily, administered orally) starting from the second or third day of the menstrual cycle can be used to schedule the start of gonadotropins in GnRH antagonist cycles. Estradiol suppresses endogenous FSH, halting follicular growth, and is stopped on the day when gonadotropin is scheduled to start.[425–427] Follicular phase estradiol scheduling does not seem to increase gonadotropin consumption or hamper clinical outcome. Another variation advocated for poor responders uses micronized estradiol (administered orally, beginning in the midluteal phase of the preceding cycle) to suppress FSH during the late luteal phase for the same purpose, ending on the day before gonadotropin stimulation begins[428,429] or continuing through the first 3 days of gonadotropin stimulation.[430] Preliminary estrogen treatment prior to the initiation of stimulation could potentially lead to an improvement in follicular dynamics and cause a rebound increase in endogenous FSH levels that follows the discontinuation of estradiol treatment that could synergize with exogenous gonadotropins to promote multifollicular development. However, available evidence from randomized controlled trials does not suggest a benefit of estradiol priming with regard to oocyte yield, pregnancy, or live birth rates beyond scheduling the cycle[420,431–434] (**Figure 31.4**).[435,436]

Women with PCOS characteristically exhibit high tonic LH secretion and are predisposed to premature LH surges when treated with standard ovulation induction regimens. Women with PCOS are also at increased risk for developing OHSS when aggressively stimulated with exogenous gonadotropins. Whereas both GnRH agonists and antagonists can suppress elevated circulating LH concentrations, the smaller follicular cohorts observed in antagonist cycles may help reduce the risk of OHSS in women with PCOS who tend to be high responders. Indeed, a 2022 systematic review and meta-analysis including 10 RCTs and 1,214 women reported a significantly lower number of retrieved oocytes (weighed mean difference = −1.82; 95% CI = −3.48 to −0.15) and a significantly lower risk of OHSS (RR = 0.58, 95% CI = 0.44–0.77) with similar live birth rates.[437] **The use of antagonists for pituitary suppression, rather than agonists, provides the opportunity to use an agonist instead of hCG to induce final oocyte maturation, thereby further decreasing the risk of OHSS.**[438] Whereas a single bolus injection of an agonist (leuprolide 0.5 mg, triptorelin 0.2 mg) triggers a physiologic LH surge that lasts less than 24 hours, hCG levels remain elevated for several days and stimulate markedly higher estradiol and progesterone concentrations.[439]

Antagonist stimulation protocols are also advocated for low responders, primarily because they avoid the suppressive effects that agonists can have on follicular response and can prevent the premature LH surges observed commonly in women stimulated with gonadotropins alone.[231] However, evidence is insufficient to indicate they yield consistently better results than other stimulation regimens.[365,370,440]

Progestins

Progestins are the latest addition to the methods used for pituitary suppression in ovarian stimulation protocols. Inspired by the natural suppression of endogenous gonadotropins by progesterone during the luteal phase, a 2015 study

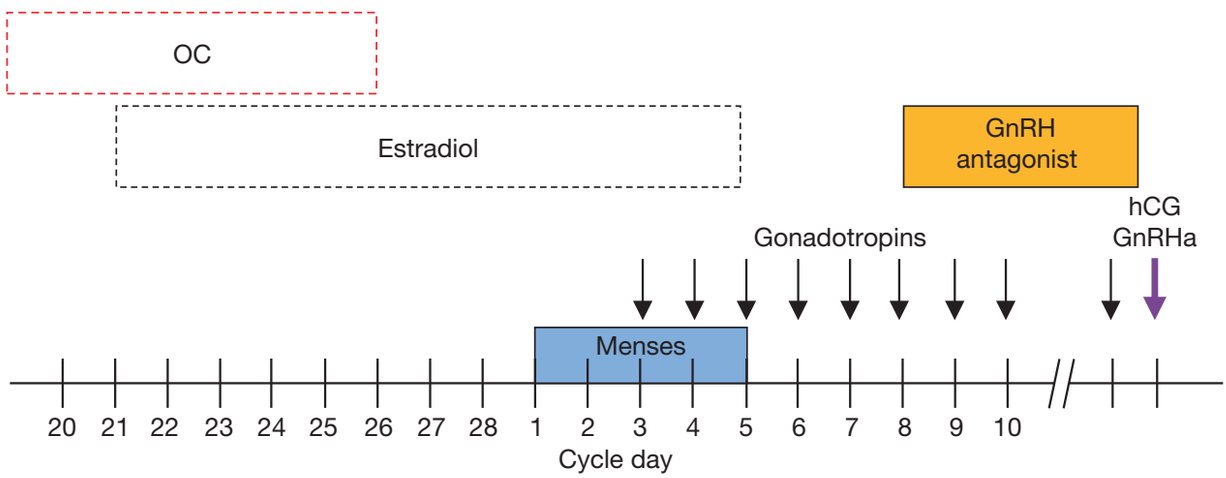

FIGURE 31.4

demonstrated that administering 10 mg/d of medroxyprogesterone acetate (MPA) alongside gonadotropins successfully suppressed premature LH surges and ovulation in 150 women[441] (**Figure 31.5**). While this was named progestin-primed ovarian stimulation, it is a misnomer because it involves no priming, and perhaps progestin-suppressed stimulation may be a more appropriate definition. Progesterone suppression of gonadotropin production is likely mediated by hypothalamic effects.[442] Although the continuous progesterone exposure during the follicular phase was suggested to be depleting LH reserves in the gonadotrophs, in a small retrospective study, the amplitude of the endogenous LH surge induced by a single dose of GnRHa was found to be significantly higher in flexible progestin-suppressed cycles compared to GnRH antagonist cycles.[443,444] Various progestins and micronized progesterone are being successfully used for pituitary suppression in stimulation cycles without an intent for a fresh ET. Commonly used progestins and doses include MPA at doses of 4 to 10 mg/d, dydrogesterone at 20 to 30 mg/d, micronized progesterone at 100 to 200 mg/d, but others have also been used with success.[445–448] Modified short versions involving commencement of progestins later in the cycle, on stimulation day 6 or 7 or when the leading follicle reaches 14 mm, similarly to fixed and flexible GnRH antagonist protocols, have also been reported to be effective.[449–455]

While the low cost and oral use of progestins make them an attractive choice, progestin suppressed stimulation protocol precludes a fresh ET due to the endometrial effects of progestins. Forfeiting an otherwise possible fresh ET brings about the additional cost of embryo freezing and thawing, as well as additional direct (ie, cost of endometrial preparation for the FET) and indirect (eg, loss of time from work) costs and can render progestin suppressed stimulation a more expensive approach.[456] However, if a fresh ET is not already the intent of the stimulation cycle, such as in oocyte donors, fertility preservation or PGT cycles or otherwise planned freeze-all cycles, progestins represent the less costly option, since the available evidence consistently suggests a similar response to ovarian stimulation with progestin and GnRH analog suppression.[445–448]

A 2023 systematic review of randomized controlled trials reported that progestin suppressed cycles may yield more oocytes than GnRH antagonist cycles, without significant differences in pregnancy or live birth rates.[448] While it should be noted that the vast majority of the evidence on progestin suppression cycles are from retrospective studies, their results are consistent among themselves and so far in agreement with the limited number of available RCTs.

Given the already high follicular fluid progesterone levels in the follicular phase, as demonstrated by Westergaard and colleagues,[457] who reported mean follicular fluid progesterone concentrations of 43 and 76 ng/mL from 6 mm or less healthy follicles in the early and late follicular phases, respectively, 153 and 57 ng/mL from more than 6 mm healthy follicles in the early and late follicular phases, respectively, of natural menstrual cycles, orally administered progestins in the doses used for progestin suppressed cycles would be unlikely to affect oocyte development potential. Indeed, the developmental potential of oocytes collected from progestin suppressed cycles seems to be similar with oocytes from GnRH analogue suppressed cycles with regard to fertilization, blastulation, and euploidy rates.[445,458–461] In sum, available evidence suggests that progestin suppressed stimulation can become an established protocol for the increasing number of freeze-all cycles.

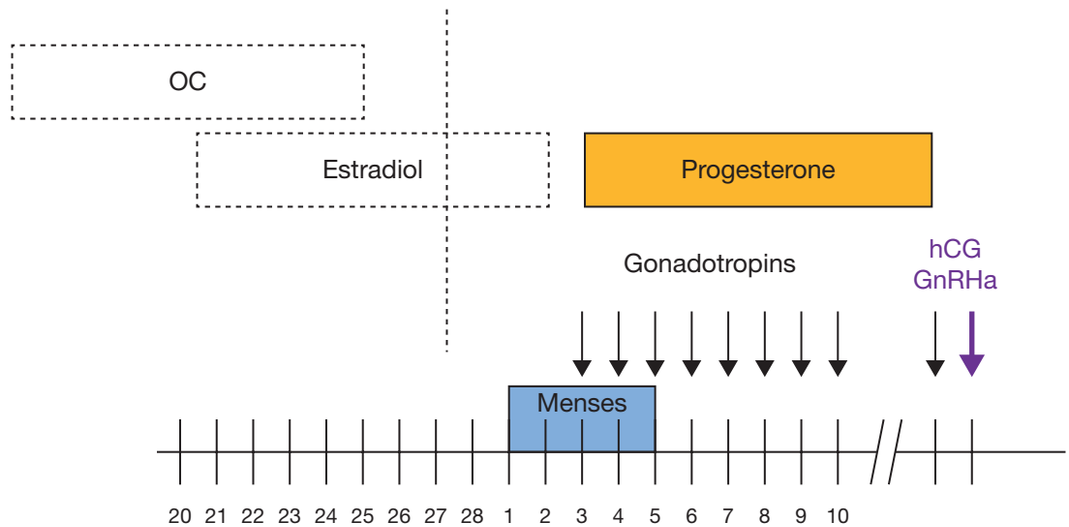

FIGURE 31.5

Monitoring Ovarian Stimulation

The response to stimulation is monitored with transvaginal ultrasonography and often serial measurements of serum estradiol. While randomized trials have failed to document an additional value of endocrine monitoring, it is commonly undertaken.[462,463] Given the dose-dependent negative effect of progesterone elevation in the late follicular phase of intended fresh ET cycles, it may be justified to monitor serum progesterone levels on the day of ovulation trigger.[345] In general, stimulation continues until at least two follicles measure 17 to 18 mm in mean diameter, when others typically measure 14 to 16 mm and the serum estradiol concentration reflects the overall size and maturity of the cohort. Most women require a total of 7 to 12 days of stimulation. It is important to emphasize that these parameters only approximate the goals of stimulation. In clinical practice, follicle measurements vary among observers, and estradiol assays vary in their performance characteristics.[464] Ultimately, each program must empirically establish its own thresholds, based on its own experience.

When fresh ET is intended, the endometrium is also monitored during stimulation by measuring the endometrial thickness or "stripe" (the sum thickness of the two layers, measured in the midsagittal plane). Numerous studies have suggested a prognostic value of endometrial thickness and pattern in ART cycles; however, the predictive value is modest at best. Many have suggested that results are better when endometrial thickness measures 8 to 9 mm or greater or appears "trilaminar" and poor when the endometrium is less than 6 to 7 mm in thickness or appears homogeneous on the day of hCG administration.[465–472] However, numerous others have failed to observe any absolute correlation between endometrial thickness or appearance and outcomes.[473–488] Some have suggested that excessive endometrial growth (>14 mm) is also a poor prognostic indicator,[489,490] but that too has been refuted.[491,492] Overall, although measurements of endometrial growth are routine, their utility remains unclear.[486–488] Consequently, changes in stimulation regimens and cycle cancellations based on endometrial thickness or appearance alone are difficult to justify except in the extreme.[486–488,493,494]

Triggering of Oocyte Maturation

When the cohort of ovarian follicles reaches maturity, urinary hCG (5,000–10,000 IU) is administered to stimulate the final stages of follicular development. The equivalent dose of the recombinant form of hCG now available is 250 μg. A 2016 systematic review, including 15 trials, comparing recombinant and urinary hCG observed no differences in clinical outcomes.[495]

Oocyte maturation can also be triggered with a single bolus of a GnRHa in cycles without pituitary downregulation. GnRHa injection triggers an endogenous LH surge, which is accompanied by an endogenous FSH surge as in the NC.[496] The LH surge triggered by a GnRHa bolus is of a shorter duration than the endogenous LH surge; is inadequate to support corpora lutea; and is associated with lower implantation, pregnancy, and live birth rates than the conventional hCG trigger in fresh ET cycles. Since the agonist trigger results in rapid luteolysis, it is either used alone as a means of preventing OHSS or in combination with the hCG trigger, dubbed as "dual trigger" in order to possibly improve oocyte quality by mimicking the simultaneous LH and FSH surges in the NC.[496,497]

The first report of GnRHa trigger in human IVF was in 1990 and involved 18 women who underwent ovarian stimulation without pituitary suppression and received GnRHa or hCG as the trigger to induce final follicular maturation. Similar numbers of oocytes were collected; fertilization rate, embryo development rates, and pregnancy rates were also similar with GnRHa as compared to hCG triggers.[498] However, soon after this report, pituitary suppression with GnRHa to prevent the LH rise prior to egg retrieval became the norm in ART, and the use of GnRHa trigger was abandoned until the introduction of GnRH antagonists into IVF practice.

When reintroduced, GnRHa trigger was initially used to prevent OHSS in GnRH antagonist cycles.[499] The LH surge following GnRHa trigger lasts even shorter than natural LH surge. In addition, the half-life of LH is much shorter than that of hCG.[439,496] These two characteristics serve to decrease the luteinizing stimulus on the granulosa cells, limiting the production of vascular endothelial growth factor, which is responsible for increased vascular permeability, the hallmark of OHSS.[500–502]

GnRHa trigger is associated with decreased size and number of corpora lutea, decreased expression of steroidogenic enzymes, and consequently lower estradiol and progesterone production during the luteal phase.[503,504] Initial clinical trials confirmed findings of similar oocyte yield and embryo quality following GnRHa trigger. However, significantly lower pregnancy rates accompanied by higher rate of pregnancy loss compared to conventional hCG trigger were alarming.[439,505,506] A randomized trial demonstrated similar implantation and pregnancy rates in oocyte recipients from donors triggered with hCG or GnRHa, suggesting the adverse effect being on the endometrium rather than the oocytes.[507] Two uncontrolled case series of oocyte and embryo freezing following GnRHa trigger reported cryosurvival, implantation, and pregnancy rates within expected range of hCG trigger. Altogether, these data provided convincing evidence for a suboptimal luteal phase impacting on birth rates following GnRHa trigger.[508,509]

Initial studies of rescuing luteal phase by "intensive luteal support" through administration of high doses of exogenous progesterone and estrogen yielded contradictory results.[510,511] High endogenous LH level is an important factor contributing to the observed success of intensive luteal support in some studies.[512] The magnitude of the LH peak induced with

GnRHa bolus, which is related to early follicular phase serum LH levels, is a determinant of pregnancy rates following GnRHa trigger and intensive luteal support.[513] Overall, intensive luteal support can be a viable option for women with PCOS, the very same patients who comprise the highest risk group for OHSS following hCG injection.

Rescuing the luteal phase with a small dose of hCG after GnRHa trigger is another option. A series of randomized controlled trials suggested equivalent ongoing pregnancy rates with GnRHa trigger, followed by 1,500 IU hCG injection 35 hours later and luteal phase support with vaginal progesterone and oral estradiol.[505,514–516] Not only luteal phase serum estradiol and progesterone levels but also ongoing pregnancy rates were statistically similar to hCG trigger, despite a trend toward lower rates with GnRHa + 1,500 hCG. The success of hCG rescue in maintaining pregnancy rates was confirmed in several uncontrolled case series, and it was suggested that severe early OHSS could be successfully prevented with this method.[517,518] However, several reports of severe early OHSS following GnRHa trigger with or even without any hCG rescue proved it to be an elusive goal to completely prevent OHSS.[519–521] Therefore, it seems prudent to completely avoid hCG support in women with predicted high ovarian response, although precise markers of ovarian reserve and threshold levels remain to be determined.

Another setting where GnRHa triggering could prove useful is oocyte collection cycles for the purpose of fertility preservation in patients with hormone-sensitive tumors, such as breast cancer. The rapid decline in serum estradiol and progesterone levels following GnRHa trigger limits tumor exposure to these hormones.[522] Clearly, GnRHa trigger should be the norm in fertility preservation cycles, as it does not affect oocyte yield or quality.

Overall, GnRHa trigger enables collection of a similar number of oocytes as that with the conventional hCG trigger. It is the ovulation trigger of choice for women at risk for OHSS and in fertility preservation cycles. The decision to proceed with a fresh or frozen transfer should be individualized, as well as the method of luteal phase rescue in fresh transfers. It should be noted that OHSS is not the only concern in stimulation cycles with a high ovarian response. Live birth rates following a fresh ET decline in cycles with high response, probably in a dose-dependent manner and possibly due to increasing serum progesterone levels in parallel with the growing number of follicles and a GnRHa trigger with a freeze-all policy may represent the better choice for both effectiveness and safety.[523]

Simultaneous administration of hCG and a GnRHa, dubbed as dual trigger, has been proposed as an approach that exploits the advantages of both—that is, the strong longer-lasting luteinizing effect of hCG and the endogenous FSH surge brought about by the GnRHa. While the function of the FSH surge is unclear, a 2023 meta-analysis of 10 RCTs reported significantly higher live birth rates per cycle with dual trigger as compared with hCG trigger alone (OR = 1.61, 95% CI = 1.16–2.25). The number of oocytes retrieved was also significantly higher with the dual trigger (mean difference = 1.05, 95% CI = 0.43–1.68), but the number of mature oocytes retrieved was similar (mean difference = 0.82, 95% CI = 0. 84–1.16).[524] However, the beneficial effect was limited to fresh ET cycles. While dual trigger was associated with a significantly higher clinical pregnancy rate per fresh ET (OR = 1.37, 95% CI = 1.05–1.79), the difference was short of significant for FET (OR = 1.15, 95% CI = 0.64–2.08). Arguably, fewer studies reporting FET cycle outcomes could have caused the difference being insignificant; alternatively, the benefit of dual trigger can be through its effects on luteal phase rather than oocyte developmental potential. While more studies are needed, given the low cost and lack of side effects with a dual trigger, it can be preferred, as it employs two different mechanisms for ovulation triggering, exogenous hCG and endogenous LH and FSH surges. In theory, in the rare case when one fails, the other can still ensure oocyte maturation.

Random Start Ovarian Stimulation

Traditionally, ovarian stimulation is started in the early follicular phase. The rationale for this timing includes stimulation of a synchronous cohort of antral follicles recruited during the luteofollicular transition and inducing timely endometrial development to synchronize blastocyst development with the implantation window. However, synchronizing endometrial and follicular development is not required if the aim of the cycle is oocyte or embryo cryopreservation without a fresh ET. This is particularly relevant when time is a constraint, such as in women with malignancy awaiting gonadotoxic chemotherapy or even personal scheduling needs.

Increasing evidence suggests that multiple waves of antral follicles develop during one menstrual cycle, challenging the concept of a single recruitment episode during the follicular phase.[525] Among different theories of follicular recruitment, the wave theory forms the basis of ovarian stimulation during the luteal phase. While the dominant follicle formed in the final wave of the interovulatory interval reaches ovulation, the preceding waves are anovulatory.[525] However, follicles that are in the anovulatory waves that precede and follow the ovulatory wave can reach ovulatory stage if exposed to FSH stimulation. Based on this concept, ovarian stimulation may be started at any time during the menstrual cycle, the so-called random start ovarian stimulation.[526,527]

Initially, the random start stimulation was used for fertility preservation in women with cancer.[528,529] More recently, encouraging results have been reported with late follicular phase and luteal phase start ovarian stimulation in women with normal or poor ovarian reserve,[530–532] leading to the wider application of this protocol in routine clinical practice in order to avoid delays in cycle start and improve patient experience.

Stimulating the ovaries twice during a single menstrual cycle, often called the DuoStim protocol, has been done as

an alternative strategy that aims at rapid accumulation of embryos in poor responders. The initial stimulation is commenced in the follicular phase with a GnRH antagonist or progestin protocol, usually followed by a GnRHa trigger, and another cycle of stimulation is initiated after egg collection.[530,533] Already elevated progesterone levels from the corpora lutea prevent the endogenous LH surge, and the luteal phase stimulation can be completed even without using GnRH antagonist in many cases. A 2016 meta-analysis reports that the luteal phase stimulation takes significantly longer (on average 1.5 days), despite consumption of more gonadotropins (on average 817 IU), but may yield a higher number of mature oocytes.[534,535] Based on limited data, oocytes collected in the luteal phase develop into blastocyst at a similar rate as oocytes from the follicular phase; euploidy rates of these blastocysts are similar, and, when transferred in an artificial cycle, yield similar pregnancy rates.[535,536] Limited data on obstetric outcome of and congenital anomalies in pregnancies derived from frozen-thawed transfer of embryos derived from luteal phase stimulation of ovaries suggest similar results with follicular phase oocyte retrievals.[537]

OOCYTE RETRIEVAL

Oocyte retrieval is generally performed approximately 34 to 36 hours after hCG administration. Slightly longer intervals do not substantially increase the risk of ovulation or adversely affect oocyte quality, fertilization rates, or overall results in GnRHa downregulated stimulation cycles,[538–541] but earlier retrievals yield fewer mature oocytes.[542] A 2023 systematic review and meta-analysis report similar oocyte yield and live birth rates with a longer than 36-hour (up to 41 hours) interval between trigger and oocyte collection procedure, based on low-quality evidence.[543]

Whereas oocyte retrieval was once performed via laparoscopy, transvaginal aspiration guided by ultrasonography under intravenous sedation is now the standard technique. Deep sedation (propofol) is most common, but most women tolerate the procedure very well with "conscious sedation" using short-acting narcotics (fentanyl) and benzodiazepines (midazolam), administered in small doses, as needed. There is no compelling evidence to indicate any difference in patient satisfaction or outcomes.[544] Continuous monitoring by automated blood pressure recordings and pulse oximetry is essential to ensure that the proper plane of sedation is maintained and not exceeded. Specific reversal agents for narcotics (naloxone) and benzodiazepines (flumazenil) should be readily available.

Prophylactic antibiotic treatment (doxycycline 100 mg or cefoxitin 2 g), administered intravenously 30 to 60 minutes before retrieval is common but controversial because of the low incidence of infectious complications following retrieval (0.3–0.6%).[545,546] Alternatively, oral antibiotics may be started immediately following the procedure (tetracycline, doxycycline), reserving prophylactic intravenous antibiotics for women at increased risk for infection (history of pelvic inflammatory disease, endometrioma).

Antiseptics (povidone-iodine) are toxic to oocytes, and limited evidence suggests their use may be associated with lower pregnancy rates.[547] When used to prepare the vagina before retrieval, thorough irrigation with sterile saline should follow, but repeated irrigation with saline alone is generally sufficient to cleanse the vagina. The bladder can become distended as a result of intravenous fluid administration but can be drained immediately before retrieval; an indwelling catheter is unnecessary.

A vaginal probe (5–7 MHz) in a sterile plastic sheath with an attached needle guide is used to image the ovaries and to align the guide with the follicles in their largest diameter. A specially designed disposable 16- to 17-gauge needle is used to enter each follicle, in turn, and to aspirate the follicular fluid and oocytes. At the proper vacuum pressure (~100 mm Hg), the follicle walls collapse but do not obstruct the needle lumen. Whereas some have observed that flushing and reaspiration of follicles using a double-lumen needle can increase oocyte yield,[548] the majority of evidence shows similar oocyte yield, fertilization, and pregnancy rates while increasing operating time and analgesic requirements.[549–551] This holds true even for in women with few follicles.[552] As such, follicular flushing seems unnecessary and has been largely abandoned. Efforts to minimize the arc swept by the needle within the ovary help limit discomfort and ovarian trauma. In general, all follicles within the ovaries greater than 10 mm in diameter can be aspirated, with no more than one to three separate entries on each side. Flushing the needle and attached tubing with media after each withdrawal helps to maximize oocyte yield. Abdominal pressure can sometimes stabilize a mobile ovary or move an ovary into a more convenient location for aspiration. Ovaries adherent to the posterior uterus are often more easily approached from the contralateral side but may be difficult to enter without traversing a portion of the uterus.[553] It may be more prudent to simply abandon some follicles, particularly when the number of oocytes already retrieved is sufficient.

The "empty follicle syndrome," characterized by a failure to retrieve oocytes despite apparently normal multifollicular development, occurs in up to 0.5% to 1% of cycles.[554–556] The phenomenon can be observed when hCG is administered later than scheduled[557] or forgotten altogether[555] and might rarely result from reduced biologic activity in some lots of commercially prepared hCG.[558–560] The serum hCG concentration 36 hours after injection generally ranges between 100 and 300 IU/L.[559] Despite increasing use of recombinant hCG and agonist trigger, empty follicle syndrome occurs at similar rate.[513,561] In one series of women who received agonist trigger, all cases of empty follicle syndrome occurred in women with serum LH levels lower than 15 IU/L measured 8 to 12 hours after agonist administration.[513]

Serious complications of oocyte retrieval are uncommon. Limited vaginal hemorrhage from a puncture site is relatively common (8%) and can usually be controlled with a brief interval of direct pressure but may require a suture.[546] Acute hemorrhage from the ovary and hemorrhage or hematomas resulting from injury to the uterine, ovarian, or iliac vessels are rare (0.04–0.07%).[546] The incidence of postoperative pelvic infections is quite low even without prophylactic antibiotic treatment (0.3–0.6%), and, of those, almost half present as tuboovarian abscesses, 1 to 6 weeks after retrieval.[81,545,546] Women with ovarian endometriomas and those with a past history of salpingitis are at highest risk.[77–79,81,562–564] Other risk factors are a higher number of oocytes retrieved, longer duration of the procedure and the mean time per oocyte retrieved, inexperience of the surgeon, and younger age with lesser BMI.[565] Other rare reported complications include the rupture of a dermoid cyst,[566] laceration of a sacral vein,[567] and lumbosacral osteomyelitis.[568,569]

Oocyte Maturation

Up to 20% to 30% of retrieved oocytes may be immature at the time of retrieval, reflecting the varying size and maturity of follicles in the cohort at the time hCG is administered.[570] An accurate assessment of oocyte maturity is important to the timing of fertilization, even more so when ICSI is to be performed.

Like the LH surge in NCs, hCG triggers the resumption of meiosis in primary oocytes previously arrested at prophase I of the first meiotic division. Oocyte maturity can generally be judged by the expansion of the cumulus mass, radiance of the corona cells, the size and cohesiveness of granulosa cells, and the shape and color of the oocyte. When the cumulus mass is removed, as it is in preparation for ICSI, the oocyte can be further evaluated according to the presence or absence of the first polar body and germinal vesicle (nuclear membrane).

A mature (metaphase II) oocyte has extruded the first polar body and is in the resting phase of meiosis II. The cumulus cells are typically expanded and luteinized, and the corona radiata exhibits a sunburst pattern. A metaphase I oocyte of intermediate maturity has no polar body and denser cumulus cells, but the germinal vesicle and nucleolus have faded. Metaphase I oocytes require additional time in culture before fertilization and must be examined periodically to document extrusion of the first polar body. A prophase I oocyte is grossly immature and exhibits a compact corona containing relatively few cumulus cells and a prominent germinal vesicle (GV) and nucleolus; dissolution of the germinal vesicle signals the resumption of meiosis I.

In Vitro Maturation

Human oocytes reach full size (100–120 μm) during the early antral stage of follicular development. The ability of an oocyte to resume and complete meiosis relates to follicular diameter.[571–573] Although immature oocytes collected from small antral follicles can mature with time in culture (the majority within 46–48 hours), even those that reach meiosis II do not necessarily acquire developmental competence, which requires synchronous maturation of both the nucleus and the cytoplasm. Consequently, although they frequently fertilize, immature oocytes yield embryos that often develop poorly and exhibit low implantation potential.[574,575] Nuclear maturation involves germinal vesicle breakdown, normally induced by the LH surge, followed by resumption of meiosis and, finally, extrusion of the first polar body. Cytoplasmic maturation is more difficult to define but involves a number of factors that prepare the cytoplasm for fertilization and subsequent embryonic development.[576] Epigenetic processes are involved in both nuclear and cytoplasmic maturation and influence development after fertilization.[577,578]

Despite the existence of different opinions among clinicians regarding what constitutes an in vitro maturation (IVM) cycle, technically, the term IVM describes the maturation of immature oocytes in culture after their retrieval.[579] The conventional definition implies collection of GV oocytes from small follicles **not** exposed to exogenous LH or hCG in vivo. In efforts to improve the relatively low efficiency of classical IVM, "follicular priming" has been developed.[580–582] One method involves FSH treatment for 3 to 6 days, followed by retrieval on cycle days 9 to 10. Another involves a single injection of hCG (10,000 IU), administered when the largest follicle reaches 10 to 12 mm in size and 36 hours before retrieval. A third method combines the two techniques, involving sequential treatment with FSH and hCG before oocyte retrieval.

Numerous studies have explored methods for IVM using oocytes obtained from normal women[583–588] and from women with PCOS.[583,584,589–595] Studies examining the effects of follicular priming in vivo have yielded inconsistent results, but embryos derived from primed oocytes have generally yielded higher implantation and pregnancy rates than those derived from oocytes collected from unstimulated antral follicles.[591,593,595] In one large trial examining the efficiency of IVM in women with normal ovaries, 400 women were randomly allocated to receive no priming or priming with hCG, FSH, or FSH followed by hCG.[596] The overall maturation rate and total number of available metaphase II oocytes were significantly higher in the groups receiving hCG than in those not receiving hCG. The overall clinical pregnancy rate per transfer was 18.3%, and the implantation rate was 10.6%. Among the groups, the clinical pregnancy rate was higher in the group receiving both FSH and hCG priming (30%) than in all others.[596] However, a 2016 meta-analysis shows that hCG priming alone does not seem to improve live birth rates in IVM cycles.[597]

The best timing and method for efficient retrieval of immature oocytes from small follicles (<10 mm in diameter) have not been established.[593,598,599] Aspiration of follicles greater than 13 mm in size has generally yielded fewer

oocytes, possibly because such follicles are already atretic.[600] A variety of aspiration vacuum pressures (80–300 mm Hg) and needles (16–20 gauge) have been described.[598,601] Whereas evidence is insufficient to warrant recommendation of any one method, extremely high vacuum pressures appear to adversely affect oocyte development in vitro.[602] The culture media composition that best supports IVM[532] and the best method for fertilization of oocytes subjected to IVM also remain to be established; ICSI has achieved similar or higher fertilization rates, and embryos derived from oocytes fertilized by conventional methods have exhibited similar or higher implantation rates and yielded higher clinical pregnancy rates,[584,603] suggesting that ICSI is not required.

Although the clinical pregnancy rates achieved in IVM trials have been reasonably good, they do not approach those of standard IVF and have been achieved by transfer of a larger number of embryos. Implantation rates for embryos derived from IVM oocytes (5–22%) are also lower than those expected in similar women (age <35 years) receiving treatment with conventional IVF (34%).[604] A limited number of studies report similar aneuploidy rates of embryos derived from in vivo matured and in vitro matured oocytes; therefore, factors other than aneuploidy seem to be responsible for lower efficiency of IVM.[605–607] A retrospective study including 178 cycles in 121 women compared live birth rates following fresh ETs and FETs in IVM and IVF cycles. While live birth rate was significantly lower following fresh blastocyst transfers in IVM cycles, live birth rate was similar between the two groups following frozen blastocyst transfers.[608] This suggests that embryo–endometrium asynchrony can be a factor limiting the effectiveness of IVM. Although the number of children derived from oocytes subjected to IVM is still quite small, preventing confident conclusions, the incidence of malformations and developmental abnormalities has thus far not differed from those in children resulting from traditional IVF or ICSI.[609]

In summary, the results achieved thus far with IVM after follicular priming in vivo suggest the methods have clinical promise. However, numerous questions must be answered before IVM can be recommended for wider clinical application.[610] Women with PCOS having large numbers of antral follicles and the greatest risk for developing OHSS represent a population that could benefit from IVM, because purposeful retrieval of immature oocytes would require fewer days of gonadotropin stimulation. A retrospective study of women with an AMH level of more than 3.25 ng/mL compared 463 hMG-primed IVM cycles without hCG trigger and 1,244 conventional ovarian stimulation cycles.[611] The study reported overall lower cumulative ongoing pregnancy rates with IVM. However, in women with AMH levels of more than 10 ng/mL, IVM met the arbitrarily defined noninferiority margin of 10%, with pregnancy rates of 51.1% for IVM versus 60.4% for conventional IVF. It is noteworthy that the widespread adoption of GnRHa trigger has significantly reduced the risk of OHSS in women with PCOS, thereby decreasing the consideration of IVM in this population. Women with cancer represent another group that could potentially benefit from IVM, as it allows for immediate treatment. However, the increasing use of random-start stimulation protocols, which may yield better results, has further limited the application of IVM in this context.

FERTILIZATION

Fertilization can be achieved by conventional microinsemination or by ICSI when there is a known or suspected male factor and concern for poor or failed fertilization. In fact, male factor infertility is the most common diagnosis among couples who undergo IVF. In the US national ART summary for 2015, 35% of all cycles were performed for male factor indications, and a male factor was one of multiple infertility factors in another 18% of cycles.[4]

A semen sample should be obtained by masturbation immediately before or after retrieval. The two methods most commonly used for sperm preparation before fertilization, the "swim-up" procedure and density gradient centrifugation, are described in detail in Chapter 29. Whereas both methods can successfully isolate a population of highly motile sperm for insemination, density gradient centrifugation also appears to select sperm with normal morphology and is widely regarded as the better choice when semen parameters are abnormal.[612–613] The isolated sperm are then incubated in media supplemented with a high concentration of protein for 0.5 to 4.0 hours to achieve capacitation. A combination of both methods, density gradient centrifugation, followed by swim up, is reported to decrease the number of sperm with ultrastructural abnormalities in the head and tail.[616] Recently, microfluidic chips have been introduced for sorting motile sperm from unprocessed semen sample. While some studies show decreased DNA fragmentation in sperm selected by microfluidic chips, convincing evidence demonstrating improved live birth rates with their use is lacking.[617–619]

In general, each oocyte is incubated with 50 to 100 thousand motile sperm for an interval of 12 to 18 hours at 37°C in 5% to 20% oxygen and 4% to 7% carbon dioxide in air at 94% to 98% relative humidity. The acrosome reaction, which enables sperm to penetrate the zona pellucida, is initiated by contact between the sperm and the zona. In turn, sperm penetration triggers the cortical reaction, which involves exocytosis of cortical granules from the ooplasm and renders the zona pellucida relatively refractory to penetration by more than a single sperm (block to polyspermy). Conventional IVF typically achieves fertilization rates ranging between 50% and 70%.

Sperm penetration also activates the oocyte and stimulates the second meiotic division, resulting in segregation of chromatids between the fertilized oocyte and the second polar body. Oocytes are evaluated for evidence of fertilization at approximately 18 hours after insemination. A normally

fertilized oocyte exhibits two distinct pronuclei, one derived from the oocyte and the other from the sperm, and two polar bodies in the perivitelline space. The zygotes must be carefully inspected for the presence of extra pronuclei because polyploid embryos may cleave normally and go unrecognized at later stages of development. Polyploidy can be observed in up to 5% to 10% of embryos overall but is far more prevalent in immature oocytes (up to 30%) than in mature oocytes (1–2%).[620,621] Besides polyspermy, polyploidy may result from digyny (fertilization of a diploid oocyte), due to meiotic spindle errors or failure to extrude a polar body, which are more commonly associated with immature, aging, or postmature oocytes.[622,623] The fertilization process requires approximately 24 hours and ends with the first mitotic division (cleavage).

Past failure of fertilization or severe male factor infertility may require ICSI, which yields pregnancy rates in couples with male factor infertility that compare favorably with those in couples without a male factor.[624] In the absence of a male factor, ICSI offers no clinical advantage over conventional IVF[625,626]; in fact, evidence suggests that standard IVF yields higher implantation and clinical pregnancy rates.[4,625]

When there is no ejaculate (aspermia) or only rare or no sperm (azoospermia) in the ejaculate, a variety of methods can be used to retrieve sperm for fertilization. Donor sperm can also be used, either by design or as a contingency should efforts to retrieve sperm on the day of oocyte retrieval fail. Men with ejaculatory failure have either no ejaculate or retrograde ejaculation. Ejaculatory failure may result from neurologic dysfunction or injury to the sympathetic outflow tracts that control emission and ejaculation (spinal cord injury, diabetes mellitus, multiple sclerosis, retroperitoneal surgery) or can be psychogenic in origin. Azoospermia may relate to ductal obstruction (obstructive azoospermia) or result from Sertoli cell–only syndrome, maturation arrest, or hypospermatogenesis (nonobstructive azoospermia). The diagnostic evaluation for aspermic and azoospermic men is described in detail in Chapter 29.

Sperm Retrieval Techniques

In the past, men with nonobstructive azoospermia were considered sterile and untreatable by any means other than the use of donor sperm. However, testis biopsy specimens in such men often demonstrate sperm,[627] suggesting low-level production of sperm that do not survive epididymal transit to reach the ejaculate.[628] **Whereas conventional wisdom was that sperm must traverse the male reproductive tract to acquire the ability to fertilize an oocyte, success with ICSI using epididymal or testicular sperm has demonstrated otherwise.** Even grossly immature sperm (round spermatid nuclear injection [ROSNI]) have been used to achieve fertilization, albeit with limited success.[629]

It is important to reiterate that genetic evaluation and counseling are indicated for men with severe seminal abnormalities before their sperm are used for ICSI. Men with congenital bilateral absence of the vas deferens (CBAVD) or less severe forms of vasal aplasia, and their female partners, should be screened for cystic fibrosis gene mutations before any attempts at pregnancy via ART to determine the risk for transmitting cystic fibrosis or CBAVD to offspring.[630–632] Men with nonobstructive azoospermia or severe oligospermia (<5 million/mL) should be offered karyotyping and screening for Y chromosome microdeletions.[632]

Sperm Recovery in Men With Retrograde Ejaculation

Men with documented retrograde ejaculation may be treated with sympathomimetics directed at control of the internal sphincter (imipramine 25 mg twice daily or 50 mg at bedtime, pseudoephedrine 60 mg, ephedrine 25–50 mg 4 times daily, phenylpropanolamine 50–75 mg twice daily). When medical treatment proves unsuccessful, sperm can be recovered directly from the bladder after masturbation; best results are achieved when urine pH and osmolality (300–380 mOsm/L) are carefully controlled by alkalinizing the urine (sodium bicarbonate 650 mg 4 times daily, beginning 1–2 days before collection) and controlling fluid intake.[633,634] Alternatively, the bladder can be filled with buffered medium immediately before ejaculation.

Vibratory Stimulation and Electroejaculation

In men with psychogenic ejaculatory failure or spinal cord injuries below the T6 level, vibratory stimulation can often succeed in producing an ejaculate. Rectal probe electrical stimulation (electroejaculation) is recommended for men who fail vibratory stimulation and those with previous retroperitoneal surgery.[635,636] Induced ejaculations may be retrograde and further require the procedures described previously. Because electroejaculates frequently exhibit asthenospermia and teratospermia, ICSI is often necessary.

Epididymal Sperm Aspiration

Sperm can be obtained by MESA at the time of vasoepididymostomy or as an isolated procedure in men with CBAVD or uncorrectable obstructions. The technique involves incision of an isolated dilated tubule, gradually moving more proximally, if necessary, until sperm are obtained.[637,638] Sperm are collected into a micropipette by capillary action with gentle compression of the testis and epididymis and flushed into a container with a small volume of IVF culture medium. Recovered sperm are cryopreserved in multiple aliquots for use in IVF cycles, if required.[639]

Percutaneous epididymal sperm aspiration (PESA) using a fine needle has also been used successfully to obtain sperm and achieve pregnancy,[640,641] but the technique is less reliable, the small quantities of sperm obtained are sometimes inadequate to allow cryopreservation, and the pregnancy rates achieved have generally been lower than those with the open technique.

Testicular Sperm Extraction and Aspiration

In men with nonobstructive azoospermia and those in whom epididymal sperm aspiration techniques fail or do not apply, sperm can be retrieved using any of three other techniques. Open microsurgical TESE yields the greatest number of sperm with potential for cryopreservation. Percutaneous core biopsy or aspiration of the testis has also been described but is most applicable in men with normal spermatogenesis and obstructive azoospermia.[642,643]

Using the preferred open microsurgical technique, sperm can be retrieved from the majority of men, even those with nonobstructive azoospermia. Magnification minimizes the risk of injury to the testicular blood supply, increases the probability of retrieving a blood-free biopsy specimen, and allows identification of larger caliber tubules that are more likely to yield sperm.[644,645] Normal pregnancies have been achieved even in those with congenital or acquired testicular failure,[646] postchemotherapy azoospermia,[647] and Klinefelter syndrome.[648]

In men with nonobstructive azoospermia, TESE is best performed on the day of or the day before oocyte retrieval, when possible, and no earlier than approximately 6 months after any previous biopsy or TESE procedure, for several reasons. First, up to one-third of men with apparent nonobstructive azoospermia may exhibit sperm in their ejaculate on the day of planned retrieval and will not require TESE.[649] Second, sperm retrieved from men with nonobstructive azoospermia may not be motile or viable after cryopreservation and thawing; ICSI using immotile sperm may yield worse results than when performed with fresh motile sperm.[650] Finally, the likelihood of successful retrieval of viable sperm for ICSI is significantly reduced when TESE is performed soon after a testis biopsy or previous TESE.[650] Matched donor sperm should be offered and available as backup, because TESE yields viable sperm in only about half of men with nonobstructive azoospermia.[644,651,652] When TESE cannot be performed near the time of oocyte retrieval, elective TESE can be performed and the recovered sperm cryopreserved; the risk of having no viable sperm after thaw is real but relatively low, and donor sperm can be used if needed.[653–655]

Intracytoplasmic Sperm Injection

Assisted fertilization techniques were developed to circumvent the need for sperm to penetrate the zona pellucida. A variety of methods have been described, but the success of ICSI has rendered all others obsolete.[656,657] Earlier methods, including zona "drilling" (using a micropipette and acidified Tyrode solution or laser),[658,659] partial zona dissection (opening the zona with a microneedle),[660] and subzonal insertion or insemination (injection of sperm beneath the zona in the perivitelline space),[661] still required sperm to interact with the oolemma and did not prevent polyspermic fertilization, but ICSI solved those problems.[662]

In the ICSI procedure, a single selected sperm is first immobilized by compressing the sperm tail with an injection pipette (inner diameter 5–7 μm), then drawn into the pipette. The oocyte is stabilized with a holding pipette at the 9 o'clock position, usually with the polar body at the 6 or 12 o'clock position, and entered at the 3 o'clock position. The injection pipette pierces the zona and oolemma, and the sperm is injected directly into the ooplasm. ICSI does not require sperm to undergo the acrosome reaction or to fuse with the oocyte membrane, as occurs with natural fertilization. Instead, the mechanical disruption of the ooplasm and sperm membranes, facilitated by the sperm immobilization procedure and the gentle aspiration and reinjection of oocyte cytoplasm, triggers oocyte activation.[663–667] **In most cases, ICSI achieves fertilization rates comparable to those observed with conventional IVF in the absence of male factors (50–70%).**

ICSI can damage the meiotic spindle even if the area adjacent to the first polar body is avoided, because the second meiotic spindle varies in position and is not always located immediately beneath the first polar body.[668,669] A polarizing optical system that images the meiotic spindle can help reduce the risk of spindle damage.[670]

The principal indication for ICSI is male factor infertility. Threshold semen parameters vary among centers but typically include severe oligospermia (<5 million sperm/mL), asthenospermia (<5% progressive motility), or teratospermia (<4% normal forms by strict criteria). ICSI is also indicated when using surgically retrieved sperm (because the number of mature sperm is relatively limited) and for couples with previous failed or poor fertilization with conventional IVF. While it is not an absolute requirement, some clinics prefer ICSI for PGT cycles to prevent contamination of the sample for analysis by extra sperm attached to zona during IVF. Other circumstances where low fertilization efficiency or fertilization failure is anticipated may be viewed as an indication for ICSI. To guard against the potential consequences of an undiagnosed sperm function abnormality, some centers perform ICSI on at least a portion of the oocytes retrieved from women with unexplained infertility.[120,671,672] ICSI may also yield higher fertilization rates for oocytes matured in vitro[673–675] and in cryopreserved oocytes,[676,677] which often exhibit a hardened zona (resistance to digestion by proteases).[678–681]

EMBRYO CULTURE

Much attention has focused on culture media formulations. However, other components of the culture system are equally important, including the carbon dioxide (4–7%) and oxygen (5–20%) concentration, incubation volume (10–50 μL), embryo group size (1–4), and the type of protein supplement (human serum albumin, recombinant albumin, synthetic serum substitute).[682–684]

Although the first human birth after IVF resulted from transfer of a blastocyst,[2] most transfers in the following years have involved earlier cleavage-stage embryos (day 2 or 3 after fertilization), primarily due to the lack of culture media that could reliably sustain embryos during the compaction (morula) and blastocyst stages of development. However, the identification of key regulators and a greater understanding of the changing physiologic requirements of growing embryos have fostered the development of "sequential" media, which vary in composition with the stage of embryo development.[685] **Whereas precompaction embryos prefer pyruvate as a nutrient and nonessential amino acids (found in higher concentrations in the oviduct), postcompaction embryos favor glucose and essential amino acids (found in higher concentrations in the uterus).**[686,687] Later on, "single-step" culture media containing nutrients, which embryos will need at different stages of development until postfertilization day 5/6, were introduced, which yielded a clinical outcome similar to that obtained with sequential media.[688–692] Single-step media without the need to replenish the medium on the third day of culture allowed uninterrupted blastocyst culture in time-lapse monitoring systems. Commercially available media now provide the opportunity for any program to incorporate extended culture into its practice.

Extended culture and blastocyst transfer offer several potential advantages over the transfer of cleavage-stage embryos:

- better assessment of true viability, after activation of the embryonic genome, which occurs at the 4- to 8-cell stage in human embryos,[693]
- better synchronization between the stage of embryonic development and the endometrial environment,
- the opportunity to perform PGT, with higher reliability and lesser likelihood of damage to the embryo,[694,695] when it is indicated, and
- higher implantation rates, allowing transfer of fewer embryos, decreasing the risk for multiple pregnancy.[696]

Extended culture is a more reliable test of viability and developmental potential because few embryonic genes are transcribed before the eight-cell stage and early measures of quality relate almost exclusively to the quality of the oocyte.[693,697–699] Postcompaction embryos also possess a transporting epithelium and can therefore better regulate their intracellular physiology and adapt to their environment.[700–702] Although pronuclear and cleavage-stage embryos *can* adapt to relatively hostile environments, survive, and successfully implant, those demands generate stresses that may compromise viability.[687,703] Extended culture may also help minimize adverse effects of an abnormal hormonal milieu on uterine receptivity and contractility, in the aftermath of ovarian stimulation.[704–707]

The strongest argument in favor of extended culture is that the implantation rate for blastocysts (30–60%) is significantly higher than for cleavage-stage embryos (12–20%).[708–714] Almost certainly, the higher implantation rate of blastocysts merely reflects better selection of the most viable embryos, as there is no evidence that extended culture improves the intrinsic quality of embryos.[713]

The results of a 2022 systematic review of 32 randomized trials illustrates both the principal advantage and disadvantage of extended culture; a higher live birth rate (OR = 1.27, 95% CI = 1.06–1.51) and a higher cycle cancellation rate (2–4% compared to 1%) were observed after fresh blastocyst transfers compared to fresh cleavage-stage transfers.[713]

Although "omic" technologies (genomic, transcriptomic, proteomic, or metabolomic profiling) hold promise for helping to identify developmentally competent embryos, none has proven ready for application in clinical practice.[715,716] Evidence suggests that clinical measures (age, parity, AFC)[717,718] and laboratory parameters (fertilization method, number of blastomeres, and the degree of fragmentation observed on day 3)[711,719–721] can predict potential for blastocyst formation, but the ability to generate blastocysts in vitro varies widely among individuals,[722] and prediction models are not accurate enough to replace extended culture for identification of embryos that will develop into blastocysts. More recently, time-lapse imaging systems (TLS), which capture images of embryos at predetermined intervals, for example, every 5 to 15 minutes, while the embryos are still in the culture environment, have been introduced.[723] In these systems, the images are digitally compiled to create a time-lapse sequence of embryo development. This allows assessment of the dynamic morphology of embryos without removing them from the incubator. Morphokinetic parameters, including the timing of cell divisions, intervals between cell cycles, and other development factors, for example, dynamic pronuclei patterns, presence of multinucleation and fragmentation, and blastomere symmetry, which may not always be observed or calculated with standard morphologic assessment at wider time intervals, are used to identify the embryo(s) that will develop to a blastocyst or will have the highest implantation potential. Several algorithms, utilizing a variety of developmental milestones observed in TLS, have been developed to predict blastocyst development, euploidy status, and clinical outcomes.[724–728] However, their accuracy has not been fully established,[723,729,730] and they are not yet considered replacements for extended culture or blastocyst morphological grading.[731]

It is widely accepted that, in patients with good prognosis, elective single blastocyst transfer significantly reduces the incidence of twins without reducing the overall pregnancy rate.[732,733] A study in donor oocyte recipients found that single blastocyst transfer yields a somewhat lower overall pregnancy rate, compared to transfer of two blastocysts but reduces the twin rate dramatically.[733] Likewise, the US National ART Data for 2015 show that women with good prognosis—that is, younger than age 35 with surplus embryos to cryopreserve—had live birth rates of 50% and

58.3% with single and double blastocyst transfers,[734] while multiple pregnancy rates were 2.2% versus 46%, respectively.

A 2019 systematic review and meta-analysis report significantly increased risk of monozygotic twinning after blastocyst transfer in women younger than 35 years (OR = 2.16, 95% CI = 1.74–2.68).[735] The cause is unknown, but culture-induced changes in the zona pellucida or embryo hatching have been implicated.[736–738] Most, but not all,[739] have also observed that blastocyst transfer shifts the sex ratio, favoring males, compared to that observed in children conceived naturally,[740] or resulting from cleavage-stage ET.[709,741–745] The phenomenon may reflect the more rapid development of male embryos[746,747] and the tendency to select the most advanced embryos for transfer.

A 2022 meta-analysis found the risk of large for gestational age (LGA)–infants after fresh blastocyst transfer to be higher than after cleavage-stage transfer singleton pregnancies (RR = 1.14, 95% CI = 1.05–1.24).[748] Likewise, frozen-thawed blastocyst transfer resulted in higher risks of LGA (RR = 1.17, 95% CI = 1.08–1.27), preterm birth (PTB) (RR = 1.13, 95% CI = 1.03–1.24), and caesarean section (RR = 1.08, 95% CI = 1.03–1.13) but lower risks of small for gestational age (RR = 0.84, 95% CI = 0.74–0.95) and perinatal mortality (RR = 0.70, 95% CI = 0.58–0.86). A number of reports have raised concern that longer duration of embryo culture may predispose to a higher risk of epigenetic (imprinting) alterations,[749–753] although subsequent studies examining the question have been reassuring.[754,755] Evidence from animal studies showing that developmental programming during the preimplantation interval can be influenced by manipulations in vitro[756,757] suggests that efforts to define and standardize culture conditions are justified and that careful long-term studies of children resulting from blastocyst transfer are warranted.

Assisted Hatching

"Hatching" of the blastocyst from the zona pellucida is a natural process in which the embryo expands and emerges before implantation. Under culture conditions, the embryo erupts, leaving behind an empty zona, but in vivo, the mammalian zona normally dissolves. Evidence suggests that hatching in vivo results from embryo–uterine interactions, with the embryo secreting an activator of zona lysins in the uterine fluid.[758] Zona thickness and relative resistance to enzyme digestion correlate with embryo quality and implantation potential.[759–762]

"Assisted hatching" describes a variety of techniques for artificially thinning or opening the zona. The procedure is intended primarily to improve implantation potential. Assisted hatching also offers the opportunity to remove cytoplasmic fragments from the perivitelline space,[709] but evidence indicates that removing fragments has no impact on implantation, clinical pregnancy, or live birth rates.[763] A wide assortment of methods has been used for assisted hatching, including zona drilling with acidified Tyrode solution,[764–767] partial zona dissection with a glass microneedle,[768,769] laser photoablation,[770–772] enzymatic thinning,[773,774] and the use of a piezo-micomanipulator.[775] However, the current practice is almost exclusively full thickness laser photoablation of the zona pellicuda. Laser-assisted hatching is an absolute requirement for PGT to perform trophoectoderm biopsy through the opening in the zona pellicuda. The following discussion relates to non-PGT cycles, in the context of an optional procedure aiming to improve implantation potential of an embryo to be transferred.

The idea that assisted hatching might improve implantation and pregnancy rates arose from observations that embryos subjected to zona drilling during early experience with assisted fertilization exhibited increased implantation efficiency.[776] Results of subsequent clinical trials varied widely, with some suggesting that assisted hatching improved results in selected individuals having a relatively poor prognosis (advanced maternal age, previous failed IVF cycle, poor embryo morphology, thickened zona)[764,767,769–772,775] and others observing no demonstrable benefits, particularly when hatching was more broadly applied.[765,766,768,777] A 2012 systematic review and meta-analysis of combined data from 31 randomized trials involving 5,728 women found that assisted hatching increased clinical pregnancy rates (OR = 1.13, 95% CI = 1.01–1.27); however, the difference barely reached statistical significance in the overall analysis and did not remain significant in subgroup analyses limited to laser-assisted hatching, better quality trials, or patients with good prognosis.[778,779] The data on live birth are limited and do not suggest an improvement with assisted hatching (nine trials, OR = 1.03, CI = 0.85–1.26). While assisted hatching was associated with increased multiple pregnancy rates (14 trials, OR = 1.38, CI = 1.11–1.70), it had no impact on miscarriage rates (14 trials, OR = 1.03, CI = 0.69–1.54).[778] More recent studies and reviews yield conflicting results, even in subgroups of patients.

The varying results of clinical trials employing different techniques do not allow confident conclusions regarding the value of assisted hatching. **On balance, the available evidence is of limited quality and suggests that assisted hatching probably does not benefit patients undergoing a fresh ET, and the evidence is inconclusive for those undergoing transfer with thawed blastocysts. Routine or universal hatching is not warranted, particularly because the procedure also has potential risks. Hatching may cause damage to embryos and may increase the risk of multiple pregnancy and monozygotic twinning.**[780–787]

Preimplantation Genetic Testing

PGT broadly describes procedures involving the removal of one or two polar bodies from oocytes or cells (blastomeres, trophectoderm) from embryos to test for mutations or evaluate their chromosomal complement.[135] PGT for monogenic disorders (PGT-M) describes testing for a known genetic

abnormality carried by one or both parents to determine whether it has been transmitted to the oocyte or embryo. PGT for aneuploidy (PGT-A) describes testing for oocyte or embryo aneuploidy when the parents are known or presumed to be normal.[135] In the case of known parental balanced translocations, testing for affected chromosomal segments in the embryos is called PGT for structural rearrangement (PGT-SR). An emerging and controversial use of PGT is for polygenic disorders (PGT-P). PGT-P aims to assess the risk of the embryo for developing polygenic disorders, which are conditions influenced by genetic variants in multiple genes, such as cancer, heart disease, and diabetes.[788]

Techniques Used for Preimplantation Genetic Testing

The primary challenge in PGT is the small sample size, which varies between 1 and 10 cells depending on the type of biopsy. The past four decades witnessed extensive research that resulted in the rapid introduction of technologies to improve diagnostic efficiency of PGT. What follows is an introduction to the evolution of techniques and an overview of currently most relevant approaches. It is very likely that alternative technologies will be developed and have similar or better diagnostic accuracy in the near future.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) was introduced in ART in 1990. FISH utilizes probes labeled with colored fluorochromes that bind to specific DNA sequences unique to each chromosome. FISH can detect a large excess or missing piece of chromosomal material. After polar body or embryo biopsy, the cells are fixed on glass, the cytoplasm is dispersed, and the fluorescent probes are applied and allowed to hybridize with complementary DNA sequences on targeted chromosomes. The different colored fluorescent signals can be observed with microscopy using filters of the appropriate wavelength. The copy number for each chromosomal segment of interest is determined by the number of fluorescent signals detected. When FISH is utilized for a monogenic disease (PGT-M) or translocation (PGT-SR), the chromosomal region carrying the affected gene or chromosomal segments is targeted with the fluorescent-labeled complementary DNA probe. When the purpose is aneuploidy screening, FISH allows assessment of a maximum of 12 chromosomes. In addition to testing for a limited number of chromosomes, and only small sections of those chromosomes, hybridization failure, signal overlap, and splitting are other technical limitations of FISH for aneuploidy testing. Introduction of novel techniques that allow simultaneous assessment of all chromosomes almost in their entirety, having potential to assess monogenic disease/translocations together with aneuploidy, and failure to demonstrate a clinical benefit of PGT-A[789] with FISH has led to abandonment of the technology in favor of newer methods described below.

Array Comparative Genomic Hybridization

In array comparative genomic hybridization (aCGH), DNA from the biopsied cells is amplified, labeled, and hybridized on a DNA microarray together with a differently labeled control DNA. The array is then scanned, and the intensities of both hybridization signals are measured and plotted. Differences between the signals from the test and control DNA allow identification of quantitative deviations—that is, extra or missing DNA sequences—in the test DNA. While aCGH can detect aneuploidy and unbalanced translocations, balanced translocations, and inversions go undetected since there is no extra or missing chromosomal content, despite being mislocated. Likewise, aCGH cannot detect polyploidy since test DNA segments all still contribute proportionately equally to the hybridization process.

Single Nucleotide Polymorphism Array

Humans are diploid organisms as they have two **alleles** at each genetic locus, with one **allele** inherited from each parent. Single nucleotide polymorphisms (SNPs) are defined as loci with alleles that differ at a single base, with the rarer allele having a frequency of at least 1% in a random set of individuals in a population.

Following the amplification of the test DNA sample, analysis of the ratio of the intensities of both alleles at heterozygous loci allows detection of duplications in and deletions from whole chromosomes in small regions. SNP arrays also allow the determination of parental origin of abnormalities and the detection of uniparental disomy. However, these require genotyping the parents. Allowing the detection of parental origin and high resolution renders SNP array useful for PGT for monogenic disease and unbalanced translocation. SNP array also allows PGT for aneuploidy by assessing the copy number of all 24 chromosomes.

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) can be used for the diagnosis of monogenic disorders using primers targeting the mutation of interest. When applied for aneuploidy, qPCR involves amplification of at least two sequences from each arm of each chromosome by using high-order multiplex PCR. Then each product is rapidly quantified by qPCR, and the amounts of products are compared to each other to calculate total genomic content. An advantage of qPCR is running multiplex PCR directly on the test DNA, avoiding whole genome amplification.

Next-Generation Sequencing

Next-generation sequencing (NGS) is high-throughput sequencing and is the term used to describe a number of different sequencing technologies. Briefly, the test DNA is amplified and cleaved into short fragments, which are ligated to generic adaptors and annealed to a slide. The slide is flooded with fluorescent-labeled nucleotides and DNA polymerase, and depending on the particular technology, snapshots are

taken (Illumina and Roche 454) or pH changes are monitored (Ion Torrent Proton/PGM sequencing) after addition of each base. The process is rapidly repeated many times, and bioinformatics is utilized to construct the sequences with the optical information collected. Hundreds of thousands of fragments are sequenced simultaneously, until the desired sequencing depth—that is, the number of sequence reads for each given position in the genome—is reached. The generated sequence data from all chromosomes are compared with the reference genome and counted. Since the number of sequences from a particular chromosome should be proportional to the copy number of that chromosome, aneuploidy will result in greater or lower numbers of reads. NGS is currently the preferred method for most PGT platforms as it allows detection of whole chromosome aneuploidies, segmental chromosome imbalances, and single-gene disorders with high accuracy.

Timing of Biopsy for Preimplantation Genetic Testing

PGT can be performed on polar bodies removed from oocytes before and after fertilization (also called preconception diagnosis)[790] or on blastomeres (obtained from cleavage-stage embryos) or on trophectoderm biopsies (removed from blastocysts). The equipment and techniques required for embryo biopsy are the same as for ICSI and related procedures (assisted hatching). After creating an opening in the zona pellucida using a laser or acid Tyrode solution, the polar body, blastomere(s), or trophectoderm biopsy is extracted for genetic analysis.

Preconception diagnosis is cumbersome and often requires sequential removal of both the first and second polar bodies to decrease misdiagnoses.[791] Indeed, a recent study involving the biopsy of the first and the second polar bodies for aneuploidy analysis in women of advanced maternal age (mean age 40.0) revealed an approximately equal proportion of errors in both meiotic divisions. Interestingly, in about half of the cases where there had been an error in meiosis I, this error was balanced in meiosis II.[792] Therefore, in the zygote, there were more aneuploidies resulting from errors in meiosis II than in meiosis I. Even if both polar bodies are analyzed, mitotic errors in the embryo and any resulting from paternal inheritance cannot be detected. Moreover, when polar body analysis is inconclusive, embryo biopsy must be performed. The cumulative trauma likely exceeds that of a single cleavage-stage embryo or trophectoderm biopsy.

To detect abnormalities in cleavage-stage embryos, one or two nucleated cells are removed, typically on the third day after fertilization (the six to eight-cell stage), before compaction when the blastomeres become more tightly adherent.[793] After biopsy, the embryo can be placed in extended culture to develop to the blastocyst stage or cryopreserved until the results of genetic analysis can be completed. A potential advantage of blastomere biopsy is its compatibility with fresh ET on days 5 to 6 of embryo development, if the test results become available within 2 days of biopsy.[794] Extracting two blastomeres is more traumatic for embryo development and should be avoided, if possible.[795] A randomized controlled study showed that even single blastomere biopsy by an experienced operator in a high-volume laboratory harms the implantation potential of embryos, leading to a decrease in sustained implantation rates (50% implantation rate in unbiopsied vs 30% in biopsied sibling embryos in the same cycle).[694]

The risk of mosaicism—that is, the existence of different blastomeres with a different genetic constitution—is another drawback of blastomere biopsy. Mosaicism can lead to false-positive or false-negative results, when the biopsied blastomere is not representative of the embryo.[794,796]

A human blastocyst is 0.1 to 0.2 mm in size and has approximately 200 cells; typically, 5 to 10 trophectoderm cells are removed during the biopsy. The aforementioned randomized trial demonstrated that the developmental potential of embryos undergoing trophectoderm biopsy at the blastocyst stage was equivalent to the nonbiopsied control siblings.[694,797–799] Later sampling leaves little time for analysis before the embryo must be transferred or frozen. However, currently, trophectoderm biopsy is preferred over blastomere biopsy. Although biopsied embryos might be more sensitive to the rigors of freezing and thawing, technical modifications in cryopreservation techniques have largely overcome the limitation.[796,800,801] A 2017 RCT compared clinical outcome following fresh or vitrified-warmed transfer of euploid blastocysts and reported similar implantation (67% vs 75%, respectively) and live birth rates (59% vs 77%, respectively).[802] **Overall, trophectoderm biopsy is currently the most reliable and least traumatic option for PGT.**

Preimplantation Genetic Testing for Monogenic Disorders and Structural Rearrangements

PGT-M is indicated for couples at risk for transmitting a specific genetic abnormality to their offspring. The risk of transmission is 50% for carriers of autosomal dominant disorders (eg, Marfan syndrome), 25% for carriers of autosomal recessive disorders (eg, cystic fibrosis), and 25% (half of male embryos) for female carriers of X-linked disorders (eg, hemophilia A). PGT-M can also be used to detect genetic mutations that predispose to a disease (early-onset Alzheimer disease,[803] familial adenomatous polyposis coli,[804] p53 tumor suppressor gene mutations[805]), to detect an unbalanced chromosomal translocation in the embryos of a couple harboring a balanced translocation, and for human leukocyte antigen (HLA) matching of embryos to an existing child of the same parents (bone marrow transplantation).[806]

PCR was the method of choice for PGT-M, since customized PCR can detect particular mutations or changes in the

DNA sequence. Nevertheless, new technologies are being increasingly utilized for PGT-M. Parental SNP genotypes and Mendelian genetic analysis can be used to generate a karyomap for each chromosome or chromosome segment inherited by each embryo.[807] Although karyomapping is commercially available and theoretically applicable to all monogenic disease without the need for prior optimization of the assay for the disease concerned, relatively higher cost and the requirement for genetic information from an affected individual or embryos as reference limit the use of SNP for PGT-M.[808] Array comparative genomic hybridization (aCGH) is incapable of detecting single-gene mutations and can only be used to detect copy number variations. Recently, targeted NGS was shown to reliably detect monogenic disorders.[809] The potential for simultaneously screening for both monogenic disease and aneuploidy is noteworthy. In one study, approximately 50% of PGT-M unaffected embryos were aneuploid.[810] Accordingly, when the clinical outcomes of PGT-M/PGT-A versus PGT-M alone were compared, implantation rates were 75% versus 53%, live birth rates were 59.4% versus 37.5%, and miscarriage rates were 20% versus 40%, respectively. Therefore, although patients performing PGT-M/PGT-A may ultimately have fewer embryos for transfer, these embryos will potentially have a higher reproductive potential.[135]

Currently, aCGH, SNP microarray, or NGS with trophectoderm biopsy are used for PGT in balanced reciprocal and Robertsonian translocations. For couples harboring a balanced chromosomal translocation, whether PGT decreases the risk of miscarriage is debated. In a 2018 systematic review of studies reporting reproductive outcomes in couples with recurrent pregnancy loss due to structural chromosomal rearrangements, live birth rates after natural conception ranged between 25% and 71% among couples with a history of recurrent pregnancy loss (RPL) and translocations as opposed to 26.7% and 87% after IVF-PGT.[811] The miscarriage rate ranged between 25% and 55.6% after natural conception as opposed to 5.3% and 39% after IVF-PGT.[811] In two comparative studies including a total of 50 couples, the live birth rates were 66.6% and 67.6% in the IVF-PGT groups compared with 55.8% and 65.4% in the natural conception groups. Time to live birth varied greatly, being 12.4 and 23.3 months in the IVF-PGT groups compared with 11.4 and 17.5 months in the natural conception groups.[812,813] It should be noted that studies included in this review utilized suboptimal techniques of cleavage-stage biopsy and FISH for PGT, and whether trophectoderm biopsy and NGS yields the same result should be seen. Regardless, most couples with balanced translocations will ultimately achieve a successful pregnancy without IVF and PGT.[811,814–816]

PGT-M offers couples who carry serious genetic disorders the opportunity to have a healthy child without the practical and ethical problems associated with terminating an affected pregnancy after traditional prenatal diagnosis (chorionic villus sampling, amniocentesis). However, careful counseling is required and must include the following:

- The possibility of diagnostic error or inconclusive results
- The possibility that the chance of success may be reduced, compared to that expected when PGT-M is not performed, due to embryo trauma and the smaller number of embryos available after abnormal embryos are excluded
- The need for conventional prenatal diagnosis to confirm the accuracy of PGT-M

Preimplantation Genetic Testing for Aneuploidy

Aneuploidy is common in human embryos, most resulting from meiotic errors in the oocyte, which increase in prevalence with advancing age. Although aneuploidy is more common in morphologically abnormal embryos, even embryos with normal morphology and developmental progress may be aneuploid.[813,817] As a diagnostic test, PGT-A is not expected to improve total reproductive potential of an egg retrieval cycle—that is, cumulative pregnancy rate after exhaustion of all available embryos (fresh and frozen) from a single cycle. However, embryo biopsy, aneuploidy screening, and transfer of proven euploid embryos would be expected to improve implantation efficiency and to reduce the incidence of futile ETs and possibly miscarriage in pregnancies resulting from IVF. Indeed, PGT-A can be considered an embryo deselection method, to prevent transfer of embryos without the potential to result in a healthy live birth. One risk of employing PGT-A as a deselection method is erroneously categorizing euploid embryos as abnormal and preventing a possible live birth. In a seminal prospective blinded nonselection study, all usable blastocysts from 402 patients underwent PGT-A, but the findings were not revealed to the patients or physicians. Patients underwent 484 single frozen blastocyst transfers blinded for PGT-A outcomes. Of the 2,210 blastocysts biopsied, 24.6% were later diagnosed as whole chromosome aneuploid. While 64.7% of the 312 transferred euploid embryos resulted in an ongoing pregnancy or live birth, none of the 102 transferred embryos with whole chromosome aneuploidies had an ongoing pregnancy or live birth.[818] It is noteworthy that 40.2% of patients who received an aneuploid embryo had a positive pregnancy test but that only 23.5% reached a clinical pregnancy, all of which ended up in a spontaneous pregnancy loss. While the sample size was too small to suggest miscategorization risk to be zero, the confidence interval was calculated to be 0% to 2.43%, which can be considered reassuring for a deselection test. It should also be noted that this study was conducted at a high-volume PGT-A clinic, and that the generalizability of the results may be limited, since PGT is a multistep complex operator and platform-dependent procedure and studies have reported varying euploidy rates across female age between both IVF laboratories and genetic laboratories.[819–821] Indeed, in a case series of 76 aneuploid blastocyst transfers,

one live birth was reported.[822] Older women with a relatively higher rate of embryo aneuploidy and patients with good prognosis with several embryos available to choose from for ET are the most obvious potential candidates for PGT-A. Others include women with a history of recurrent pregnancy loss, those with repeated IVF failure despite transfer of morphologically normal embryos, and couples with severe male factor infertility.

Initial PGT-A utilized blastomere biopsy and FISH allowing the examination of a limited number of chromosomes. As such, all but one RCT of this initial approach failed to demonstrate a benefit.[789,823–825] Furthermore, two of these RCTs reported decreased live birth rates with PGT-A with blastomere biopsy and FISH.[789,825] As a result of these well-designed studies, the American Society for Reproductive Medicine (ASRM) and the European Society for Human Reproduction and Embryology released a statement advising against the use of PGT-A with FISH.

Since then, alternative techniques have become available (discussed previously), and new RCTs provided high-quality evidence regarding IVF pregnancy outcomes in select populations with PGT-A.[135] An RCT assessing the effectiveness of PGT-A utilized first and second polar body biopsy with aCGH in 396 women aged 36 to 40 years.[826] Fifty of the 205 (24%) participants in the chromosome screening group had a live birth with intervention within 1 year, compared to 45 of the 191 in the group without intervention (24%) (difference = 0.83%, 95% CI = −7.60 to 9.18%). In the comprehensive chromosomal screening (CCS) group, significantly fewer participants underwent an ET (relative risk [RR] = 0.81, 95% CI = 0.74–0.89), and fewer had a miscarriage (RR = 0.48, 95% CI = 0.26–0.90).[826] The rate of embryo development between the two groups was similar (53% vs 50% morphologically transferable), suggesting that the polar body biopsy procedure did not have a detrimental effect; however, the analysis was inconclusive for 8% of oocytes assessed by aCGH.[826] Due to the diagnostic limitations previously discussed, as well as the findings of this study, a polar body–based CCS approach is not commonly utilized.

Another multinational study randomized women with advanced maternal age (38–41 years old), prior to cycle start to routine blastocyst transfer versus a PGT-A group that had a biopsy of a single blastomere on day 3 with fresh blastocyst transfer on day 5 after the retrieval.[824] The live birth rates were significantly higher in the PGT-A group when analyzed per ET (52.9% vs 24.2%, $P < 0.0002$) and per started cycle (36% vs 21.9%, $P < 0.031$). Moreover, the miscarriage rate was significantly lower in the PGT-A group (2.7% vs 39%, $P = 0.0007$). When the outcomes for FET cycles performed during the 6 months after the completion of the study were added, cumulative live birth rates were similar (37% vs 33.3% in PGT-A and controls, respectively). However, the mean number of ETs needed per live birth was lower in the PGT-A group compared with the control group (1.8 vs 3.7), as was the time to pregnancy (7.7 vs 14.9 weeks).[824] The use of blastomere biopsy could be regarded as a factor limiting the generalizability of the results of this study.

Four other RCTs involved women with better prognosis—that is, younger than 35 years or having at least two blastocysts amenable to biopsy. The RCT including 112 women younger than 35 years, with tubal or male factor infertility and without any prior failed cycles, utilized trophectoderm biopsy on day 5, aCGH, and fresh transfer on day 6.[827] Blastocysts for transfer in the control group were selected by traditional morphologic assessment and also transferred on day 6. Array CGH group achieved significantly higher ongoing pregnancy rates (69.1% vs 41.7%, $P = 0.009$), while miscarriage rate was similar between PGT-A and control groups (2.6% vs 9.1%, in PGT-A and controls, respectively, $P > 0.05$). Another RCT, which included 175 women younger than 42 years old who had at least two expanded blastocysts on day 5 or 6, compared the clinical outcome of elective single euploid blastocyst transfer with the transfer of two morphologically selected blastocysts.[828] Trophectoderm biopsy and qPCR were used for PGT-A. Ongoing pregnancy rate beyond 20 weeks were similar in the two groups (60.7% vs 65.1%), with significantly lower multiple pregnancy rate in the PGT-A group (0% vs 53.4%, $P < 0.05$). The risks of preterm delivery, low birth weight (LBW), and neonatal intensive care admission were also lower with PGT-A.[829] Another RCT randomized 155 women with two or more blastocysts on day 5 to trophectoderm biopsy with qPCR-based PGT-A on day 5 and transfer on day 6 or morphologic grading and ET on day 5.[830] Patients in the control group had significantly more embryos transferred than the PGT-A group (2.0 vs 1.86, $P < 0.001$), which was due to 10 patients in the study group having only one euploid embryo for transfer while all patients in the control group underwent double ET. Clinical implantation rates were significantly higher in the PGT-A group (79.8% vs 63.2%, in PGT-A and controls, respectively, $P = 0.002$). Delivery rate per cycle was also significantly higher in the PGT-A group (84.7% vs 67.5%, $P = 0.01$). Based on the reported data, the calculated spontaneous abortion rates were 8.9% and 21.1%, in PGT-A and control groups, respectively. The fourth trial randomized 1,212 women between the ages of 20 and 37 years, who had three or more good quality blastocysts. Women underwent up to three elective single ETs, and the authors reported similar cumulative live birth rates in the PGT-A and morphology-based selection groups (77.2% vs 81.8%, absolute difference −4.6%, 95% CI = −9.2 to 0%). Cumulative pregnancy loss incidence was 8.7% versus 12.6% in the PGT-A and control groups, respectively (absolute difference −3.9%, 95% CI = −7.5 to −0.2%).[831] However, despite the large sample size, the trial has important limitations. First and foremost, only three blastocysts per participant, preselected based on their morphologic characteristics, underwent PGT-A even when more good quality blastocysts were present. This made it practically impossible to achieve higher cumulative pregnancy in the PGT-A group. In addition, blastocysts with intermediate

copy numbers (also called as mosaics) were excluded from transfer, despite having potential for healthy live birth, as discussed later.[832] Collectively, these RCTs suggest that elective single euploid blastocyst transfer using PGT-A may result in higher implantation, pregnancy, and live birth rates and lower pregnancy loss rate, following the first ET of an egg retrieval cycle in women with good ovarian reserve.

A 2017 trial randomized 323 women aged 25 to 34 years, 170 women aged 35 to 37 years, and 95 women aged 38 to 40 years to PGT-A or morphologic blastocyst assessment at 34 clinics in four countries. Women between 25 and 40 years of age, with less than three prior ART failures and less than two prior miscarriages, and who are not a known carrier of a single-gene disorder or chromosomal abnormality, were recruited. Decreased ovarian reserve, oligospermia, azoospermia, use of donor oocytes or gestational carrier, and undergoing PGT outside the study context were exclusion criteria. Trophectoderm biopsy and NGS were utilized for PGT-A. In both arms, blastocyst-stage embryos underwent vitrification for single ET in a later cycle. In women younger than 35, 20-week ongoing pregnancy rates were not different between PGT-A and control groups (53% vs 49%), while there was a significant improvement in women 35 to 40 years old (51% vs 37%, $P = 0.035$). Miscarriage rates ranged between 8% and 11% in age brackets and were not significantly different between the PGT-A and control groups.[833] Overall, this RCT failed to replicate the favorable results achieved with the aforementioned RCTs in women with good prognosis, while it showed a benefit in women over 35 years old.

A recurring theme in PGT-A is mosaicism and the complexity of determining the reproductive potential of human embryos diagnosed as mosaic. It must be noted that the diagnosis of "mosaicism" in trophectoderm cells is not made by direct observation of individual cells with different chromosomal numbers. The diagnosis is made when the NGS results suggest an intermediate chromosome copy number for one or more chromosomes, which is not consistent with monosomy, disomy, or trisomy.[834]

Given the rare occurrence of true mosaicism in human preimplantation embryos, it seems likely that the deviations observed in copy number of a chromosome can be due to test artifacts, in one or multiple steps of the PGT process, including the trophectoderm biopsy. In an analysis of 250 pregnancies following mosaic ETs, noninvasive prenatal tests (NIPTs), chorionic villus sampling, amniocentesis, and analysis of products of conception from eight pregnancy losses were conducted. Among these, 236 out of 250 pregnancies (94.4%) were found to have normal (euploid) results.[835] Of the 14 pregnancies with abnormal findings, eight resulted in infants without birth defects (they had either <10 Mb losses or gains, under the current PGT resolution, and/or in other chromosomes than the one called mosaic with PGT-A), one pregnancy loss was diagnosed with tetraploidy, two pregnancies showed mosaicism in chromosomes different from the one flagged as mosaic by PGT-A, and only three cases were confirmed to have mosaicism in the same chromosome identified by PGT-A. Overall, these observations suggest that "mosaic" diagnosis with PGT-A reflects more often a test artifact than true mosaicism in the embryo.

In a retrospective analysis of 1,000 so-called mosaic and 5,561 euploid blastocyst transfers, ongoing pregnancy or live birth rates were 37% versus 52% in the mosaic and euploid groups, respectively. While the pregnancy loss rate was higher for pregnancies derived from "mosaic" blastocysts (20.4%) than euploid blastocysts (8.6%), 80% of "mosaic" pregnancies resulted in an ongoing pregnancy or live birth. The live birth rate decreased with increasing size of the affected chromosomal segment and the number of the affected chromosomes, suggesting that the diagnosis can reflect true chromosomal errors some of the time.[836] Other investigators reported minimal or no effect on pregnancy outcome after transfer of embryos diagnosed as mosaic, leading some clinical laboratories to exclude this diagnosis from their reports, unless specifically requested by the clinician.[818] These findings should be kept in mind when counseling women who undergo IVF resulting in mosaic embryos but no euploid embryos. Clinics should have policies for reporting intermediate copy numbers, providing genetic counseling, and whether or not and how to use such embryos.[837]

Techniques applied for PGT-A are rapidly evolving, and many of the key techniques used in published RCTs are no longer in use; qPCR and aCGH have been replaced by NGS-based analysis, fresh transfers have been replaced by thaw transfers of cryopreserved embryos. NGS is also being reassessed regarding the best way to amplify the genome and how to achieve the necessary depth of sequencing in a most cost-efficient way. The next decade will uncover whether these developments will further improve diagnostic accuracy of PGT-A and help determine the appropriate applications. While some express concern about the diagnostic accuracy, many view this approach for embryonic selection as an avenue to reduced time to live birth and increased live birth rate per transfer, at least in selected populations.

EMBRYO TRANSFER

Although embryos have been transferred successfully at any stage of early development, from zygote to blastocyst, transfer is most commonly performed 5 days after oocyte retrieval, while some programs continue performing day 3 transfers, at least for certain subsets of patients. The relative advantages and disadvantages of extended culture to the blastocyst stage have been discussed previously. Systems for grading the quality of embryos vary among programs, but the morphologic features on which grading is based are similar and depend on morphology and cleavage rate.

The essential features of ET have not changed significantly since the procedure was first described in 1984.[838] Although

the impact of transfer technique on results is difficult to study, most clinicians believe it is as important as embryo quality.[839] Indeed, a 2023 systematic review and meta-analysis reports significantly lower clinical pregnancy (RR = 0.71, 95% CI = 0.66–0.77), and live birth rates (RR = 0.68, 95% CI = 0.59–0.77) following a difficult ET defined with the use of a hard catheter, obturator, or tenaculum or with the opinion of the operator.[840] The success of the procedure is clinician dependent.[841–844] Yet a 2017 survey showed that all clinicians were allowed to perform ET, regardless of their skill level, without a standardized technique.[845] This has led the Practice Committee of the ASRM to prepare a guideline and a standard ET protocol template.[841,846] Further, the ASRM has developed ET simulators that can be used for training and standardization.

ET is typically a pain-free procedure. Thus, neither anesthesia nor analgesics are required routinely. Despite some theoretical advantages, evidence for an effect of massage, relaxation techniques, acupuncture, transcutaneous electrical acupoint stimulation, and Chinese medicine is limited and does not suggest an unequivocal benefit from these adjuvant interventions. Likewise, routine use of broad-spectrum antibiotics seems unnecessary.

A trial transfer before the cycle begins can identify women with cervical stenosis or an acutely angled cervicouterine junction that can make transfer technically difficult to perform.[847] Several studies have suggested that, when required, cervical dilation is best performed before the cycle begins[848–851]; shorter intervals of time between dilation and transfer may be insufficient to allow the endometrium to recover from the trauma or bacterial contamination and are associated with significantly lower pregnancy rates. Dilation with laminaria in advance of the treatment cycle can also be effective.[852] The notes from a prior mock or real ET, when available, should be reviewed before ET to prepare for an anticipated difficult transfer and to facilitate the procedure.

ET under transabdominal ultrasonography offers a number of advantages over a blind technique. **Ultrasonography facilitates the insertion of soft catheters, confirms correct positioning, and avoids inadvertent trauma to the fundal endometrium.**[853,854] Urine in the bladder may also help straighten the plane of the cervicouterine junction.[855,856] Uterine position and orientation often change in the interval between the mock transfer and the actual transfer, primarily due to the ovarian enlargement.[292] Two systematic reviews, both including 21 RCTs, reported significantly higher live birth rate after guided transfers[857,858]; the probability of a live birth was increased almost 1.5-fold with ultrasound guidance.

The use of sterile latex-free gloves is preferred as per common practice and standard expected occupational safety and health administration requirements. Two-thirds of physicians report using surgical masks during ET.[846]

An ET has a number of potential pitfalls. An appropriate size speculum should be used to expose the cervix easily, and any remnants of vaginal progesterone and secretions are gently cleaned with sterile saline or ET media. Mucus within the cervical canal may plug the catheter tip, resulting in retained embryos or improper placement.[848,859,860] Cervical mucus can also be a source of bacterial contamination of the endometrial cavity, adversely affecting results.[860,861] Any obvious or excess cervical mucus is best removed before transfer.[862–864] However, cervical trauma should be avoided, and excessive lavage is not shown to be helpful.[853]

Transfer catheters vary widely in design. Current catheters are comprised of an outer sheath and an inner catheter, the latter carrying the embryos. Definitions of "soft" and "firm" catheters vary in the literature. It is reasonable to categorize all catheters with a soft inner catheter as a soft catheter, since the aim is to dislodge the embryos without endometrial trauma.[841] Stiff catheters and those with a rigid outer sheath are easier to insert but more traumatic than soft catheters, which can better follow the contours of the endocervix and endometrium.[865] Controlled trials consistently show better clinical outcome with soft catheters, with 1.4-fold (95% CI = 1.2–1.6) higher pregnancy rates.[841] However, no soft catheter performs significantly better than another soft catheter, and commercially available soft catheters are regarded to perform similarly.[841,866] If a soft catheter with a firm outer sheath is used, pushing the tip of the outer sheath beyond the internal ostium can cause endometrial trauma and lower pregnancy rates.[867] When transfer proves difficult, a malleable stylet can be used to introduce the outer sheath of a soft catheter just beyond the internal cervical os, then replaced by the soft inner catheter containing the embryos ("afterloading"). Transmyometrial ET under ultrasound guidance has been described[868,869] but yields lower pregnancy rates,[870] probably relating to uterine contractions,[871] and should rarely be necessary.

The tip of the ET catheter is placed at the upper or middle area of the endometrial cavity before dislodging the embryos.[872–877] Transfers higher in the fundus may increase the risk of ectopic pregnancy,[878,879] and low transfers may result in cervical implantations.[880]

Larger volumes of transfer media (>20–50 μL) or air above the column of media may increase the risk that embryos may be expelled from the uterus or propelled into the fallopian tube.[881,882] The concentration of protein and the viscosity of transfer medium do not appear to affect results.[883,884] Whether adherence compounds containing hyaluronic acid in ET media improve clinical pregnancy rates is controversial.[885–890] Syringes that may be used with a transfer catheter also vary in design and performance characteristics. Even though quantitative analysis of injection speed is difficult, fast injection close to the fundus has been associated with increased ectopic pregnancy rates.[841,891–894] While the presence of mucus on the catheter tip is not associated with decreased pregnancy and live birth rates, results are mixed regarding the effect of the presence of blood.[841,848,859,895–904] Nevertheless, it is prudent to avoid cervical and/or endometrial trauma during ET.

Embryos adhering to the outside of the catheter after transfer can be relocated or removed inadvertently when the catheter is withdrawn.[853,881] Microscopic examination of the catheter immediately after transfer identifies retained embryos requiring a second transfer procedure; immediate retransfer of retained embryos does not seem to affect implantation, pregnancy, or abortion rates.[841,848,881,895,900–911]

There is good quality evidence to recommend against bed rest after ET.[841,912,913] Indeed, a 2016 meta-analysis suggests possible harm.[914] Thus, patients can resume normal daily activities; physical activity and diet have no demonstrable effect on outcomes. Mild intermittent cramping and bloating are normal symptoms, but moderate or severe pain requires evaluation to exclude infection, ovarian torsion, OHSS, and other causes of abdominal pain.

In summary, the goal of ET is to deliver embryos to the uterus in an accurate and atraumatic fashion. **A preliminary trial transfer can identify women who may benefit from cervical dilation before treatment begins, and transfers in small volumes using soft catheters guided by ultrasonography produce the best results.**[841] However, a universal trial transfer policy is low yield, costly, and time-consuming for both patients and health care professionals. A selective trial transfer policy for patients who are anticipated to have a difficult transfer may be a better option. A simple ultrasound examination of the cervix and the cervical canal may help with the prediction of such patients and provide multiple benefits such as adjusting patient expectations or taking measures in advance to facilitate the ET.[915]

Embryo Transfer Guidelines

The goal of IVF is to maximize pregnancy rates while, at the same time, minimizing multiple gestations, high-order multiple gestations in particular. The likelihood of success increases with the number of embryos transferred, to a point beyond which only the risk of *multiple* pregnancy increases.[916–918] Multiple pregnancy not only increases the risk of preterm birth and associated neonatal and infant morbidity and mortality but is also associated with maternal morbidity.[919] Moreover, preterm deliveries that result from ART-associated multiple pregnancies add a substantial burden to overall US health care expenditure annually.[920] Financial concerns affect couples' preferences for number of embryos to be transferred, and public reimbursement plans accompanied by strict regulations for number of embryos to be transferred seem to increase not only ART utilization rates but also the uptake of single ETs.[921–924] Strict regulations on the number of embryos transferred, as defined by law in some countries, reduce the number of multiple pregnancies and all but eliminate high-order multiple gestations[916]; however, they do not allow treatment to be individualized, considering unique patient characteristics and circumstances (age, the number and quality of embryos, the opportunity for cryopreservation, and the outcome of any previous cycles), or to be adjusted according to new clinical data.[925–927] Registry data from the United States strongly suggest that regulations ignoring the unique circumstances of individual women inevitably reduce the chance of pregnancy.[917]

In essence, elective single ET could be considered for almost all women, since cumulative live birth rate per egg retrieval cycle would be maintained following consecutive FETs with current success achieved in embryo cryopreservation. Indeed, clinics that perform higher rates of elective single ET in women aged less than 38 years have decreased rates of multiple gestation, with no significant impact on cumulative live birth rates.[928] The data generated by individual programs can guide the decision regarding the optimal number of embryos to transfer in women of varying age and clinical characteristics. In their absence, the SART and the ASRM have offered guidelines. First published in 1998,[929] the guidelines have been revised several times, based on new clinical data reflecting steady advances in ART indicating that fewer embryos can be transferred without adversely affecting the likelihood of success.[930,931] The guidelines issued in 2021 categorize patients as those with a favorable prognosis and those without.[932] The following characteristics have been associated with a favorable prognosis: (1) young age, (2) expectation of one or more high-quality embryos available for cryopreservation, (3) euploid embryos, and (4) previous live birth after an IVF cycle.[932] The recommended number of embryos to be transferred is based on the age of the woman, presence or absence of favorable characteristics, and stage of embryos—that is, cleavage or blastocyst. The recommendations are noted in the section that follows.

American Society of Reproductive Medicine Recommendations

Patients with a favorable prognosis:

- In patients of any age, transfer of a known euploid embryo should be limited to one.
- Patients under the age of 35 should be strongly encouraged to receive a single ET, regardless of the embryo stage.
- For patients between 35 and 37 years of age, strong consideration should be given to a single ET.
- For patients between 38 and 40 years of age, no more than three untested cleavage-stage embryos or two blastocysts should be transferred.
- Patients 41 to 42 years of age should plan to receive no more than four untested cleavage-stage embryos or three blastocysts.

Other scenarios:

- In all age groups, patients who do not meet the criteria for a favorable prognosis may have an additional embryo transferred according to individual circumstances (**Table 31.1**). The patient must be counseled regarding the additional risk of twin or higher-order multiple pregnancy.

TABLE 31.1 Recommendations for the Limit to the Number of Embryos to Transfer

	Age in Years			
Prognosis	**<35**	**35–37**	**38–40**	**41–42**
Cleavage-stage embryos[a]				
Euploid	1	1	1	1
Other favorable[b]	1	1	≤3	≤4
All others	≤2	≤3	≤4	≤5
Blastocysts[a]				
Euploid	1	1	1	1
Other favorable[b]	1	1	≤2	≤3
All others	≤2	≤2	≤3	≤3

Justification for transferring additional embryos beyond recommended limits should be clearly documented in the patient's medical record.
[a]See text for more complete explanations.
[b]Other favorable = Any ONE of these criteria: fresh cycle, expectation of one or more high-quality embryos available for cryopreservation or previous live birth after an IVF cycle; FET cycle, availability of vitrified day 5 or 6 blastocysts, euploid embryos, first FET cycle, or previous live birth after an IVF cycle.

- If otherwise favorable patients fail to conceive after multiple cycles with high-quality embryo(s) transferred, physicians and patients may consider proceeding with an additional embryo to be transferred.
- Patients with a coexisting medical condition for which a multiple pregnancy may increase the risk of significant morbidity should not have more than one embryo transferred.
- In the rare cases where the number of embryos or blastocysts transferred exceeds recommended limits, both the counseling and the justification must be documented in the patient's permanent medical record.
- In women ≥43 years of age, there are insufficient data to recommend a limit on the number of embryos to transfer when the patient uses her own oocytes. Caution should be exercised as the risk associated with multiple pregnancy increases dramatically with advancing maternal age.

In donor oocyte cycles, the age of the donor should be used to determine the appropriate number of embryos to transfer. For example, when the donor is less than 38 years old and other favorable criteria exist, single ET should be planned. Single ET should be strongly recommended in all gestational carrier cycles, given the health risks associated with multiple gestations for the gestational carrier. At a minimum, it is recommended to follow age-related limits on the number of embryos to transfer in gestational carrier cycles, on the basis of the age of the woman who produced the oocytes (either the intended parent or the oocyte donor).

In FET cycles, favorable characteristics should be based on the age of the woman when the embryos were cryopreserved and include the presence of high-quality vitrified embryos, euploid embryos, or previous live birth after a prior transfer with sibling embryos. ET numbers should not exceed the recommended limit on the number of fresh embryos transferred for each age group.

ESHRE suggests a different approach and recommends elective single ET across the board, regardless of patient characteristics such as age, prior IVF cycle outcomes, or embryo characteristics.[933] Given the higher obstetric and neonatal risks associated with multiple pregnancy and the difficulty of producing competent embryos in women with advanced age and low ovarian response, combined with the high success of current cryopreservation technology, such an approach may also be justifiable. ESHRE also recommends legislative and health insurance policies that promote the practice of elective single ETs to decrease financial pressure on the patients, which can be a motivating factor for them to consider multiple ET.

Overall, the weight of available evidence indicates that an optimal balance between pregnancy rates and the risk of multiple pregnancy can be achieved with a flexible ET policy based on maternal age, embryo quality, and the availability of surplus high-quality embryos but that single ET should be the norm and double transfer a medically justified exception.

LUTEAL PHASE SUPPORT

Controlled ovarian hyperstimulation with exogenous gonadotropins generally yields multiple corpora lutea that might well be expected to sustain supraphysiologic serum concentrations of estradiol and progesterone during the luteal phase of IVF cycles. Cotreatment with GnRH analogs for prevention of premature LH surge and luteinization effectively suppresses endogenous LH secretion, as intended. Unfortunately, even though agonist and antagonist treatment end abruptly on the day of hCG administration, residual suppression of endogenous LH does not. **Abnormally low levels of LH during the luteal phase may be insufficient to stimulate and maintain the level of luteal function required to promote timely endometrial maturation in preparation for implantation or to support an early pregnancy once established.** Endogenous LH secretion can remain suppressed for as long as 10 days after treatment with a GnRHa ends and luteal function is frequently inadequate in amount or duration.[934] Although antagonists have a much shorter duration of action, they often have the same consequence. Integrated estradiol and progesterone levels are abnormally low, and luteal phase duration is grossly short in GnRH antagonist treatment cycles, particularly when a GnRHa rather than hCG is used to stimulate the final stages of oocyte maturation.[935] Because there is no way to predict who may or may

not require luteal support in any given cycle, some form of treatment must be provided for all.

Progesterone supplementation generally begins on the day of oocyte retrieval or at the time of ET.[936–938] Numerous clinical trials have compared clinical, ongoing, or delivered pregnancy rates or spontaneous abortion rates between groups receiving treatment with different luteal phase support regimens, with varying results. Progesterone has been administered orally (300–800 mg daily); vaginally as a bioadhesive 8% gel (90 mg daily), cream, or tablet (100–600 mg daily); and by intramuscular (25–50 mg daily) or subcutaneous (25 mg daily) injection; supplemental doses of hCG have generally been administered every 3 days (1,500–2,500 IU). There is no evidence that any one treatment regimen is superior, although results achieved with oral progesterone have been inconsistent. However, dydrogesterone (30 mg daily), a retroprogesterone, which has higher oral bioavailability than micronized progesterone, has been shown to be as effective as vaginal progesterone gel in a 2016 meta-analysis and a 2017 RCT.[939–940] Even though stopping luteal phase support after a positive pregnancy test does not seem to be associated with lower live birth rates,[941–943] the majority of ART clinics continue luteal support until 8 to 10 gestational weeks.[944] Supplemental natural progesterone is not associated with any increased risks of birth defects.[937] Although supplemental estradiol is also commonly administered, there is no evidence that it improves outcomes, compared to those achieved with progesterone supplementation alone.[941,945,946]

EMBRYO CRYOPRESERVATION AND FROZEN EMBRYO TRANSFER

The first pregnancy resulting from transfer of a cryopreserved human embryo was reported in 1983.[947] In the years since, advances in cryobiology have made embryo cryopreservation an integral part of modern ART. Success with FET cycles significantly increases the overall cumulative pregnancy rate per retrieval. In addition, elective cryopreservation of all embryos can effectively manage the risk of OHSS in women with an excessive ovarian response to stimulation and may help prevent reduced live birth rates associated with fresh embryo transfers in such cases.[948–953]

The cryopreservation process has two distinct stages, freezing and thawing. The objective of freezing is to avoid ice crystallization of intracellular water, which can result in cellular damage. Freezing protocols vary with the stage of embryo development, which affects cellular permeability. There are two basic methods for embryo cryopreservation, the "slow-freeze" technique and "vitrification." In both, cellular water is gradually replaced by cryoprotectants (dimethyl sulfoxide, propanediol glycerol) via osmosis by passage through increasing concentrations of the cryopreservative. In the slow-freeze method, embryos are sealed in ampules or vials, cooled to temperatures between −30°C and −110°C in a programmed two-step process, and then stored in liquid nitrogen. The first phase of the freezing process is rapid in order to prevent ice crystal formation (more likely to occur with gradual cooling) and the second phase more gradual. In the vitrification method, embryos are flash frozen by immersion into liquid nitrogen, creating a solid glass-like state.[954,955] After thawing, the process is reversed, gradually passing the embryo through decreasing concentrations of the cryoprotectant, followed by an interval of culture before transfer.

Embryos can be frozen at any stage, from zygote to blastocyst, and remain viable for at least several years, perhaps indefinitely.[956] Numerous studies have compared embryo thaw survival, implantation, and pregnancy rates among embryos frozen at different stages of development. In general, postthaw survival rates after slow freezing range between 50% and 90% and are higher for zygotes than for cleavage-stage embryos and blastocysts.[957–962] Implantation rates (5–15%) and pregnancy rates (10–30%) after transfer of slow-frozen-thawed zygotes, cleavage-stage embryos, and blastocysts have varied among studies but not dramatically. In contrast to slow freezing, vitrification allows rapid cooling of the cells and the extracellular milieu into a glass-like state without ice crystal formation. This is achieved by high initial concentrations of cryoprotectants, low volumes, and ultrarapid cooling–warming rates. Vitrification is associated with consistently high survival rates (90–100%) and yields higher implantation and pregnancy rates.[954,955,963,964] Consequently, vitrification is the preferred method for embryo cryopreservation.

Although embryos of the highest quality are generally selected for fresh transfer, the overall success rates for vitrified embryo transfer cycles are approximately still the same as those observed in fresh transfer cycles. Results achieved with embryos derived from conventional IVF and ICSI are comparable, and cryopreserved sibling embryos derived from successful cycles yield higher success rates than those derived from unsuccessful cycles, probably reflecting overall better embryo quality.

Advances in vitrification have led to consistently high embryo survival rates, making FETs comparable in success to fresh ETs. This progress has expanded the use of FET from simply cryopreserving surplus embryos to include elective total embryo freezing for deferred transfers in specific scenarios. Increasing numbers of elective single ETs and PGT cycles have also significantly increased the number of FETs. In fact, FETs now constitute the majority of ETs in the United States, where 86.8% of ETs used frozen embryos in 2022.[4,965]

The endometrium can be prepared for FET in mainly three different ways: in a natural menstrual cycle, in an ovarian stimulation cycle, or in an artificially prepared cycle. The NC can be a truly natural or a modified NC, while an artificial cycle involves sequential administration of estrogen and progesterone and is commonly named as a programmed cycle or a hormone treatment cycle. A mild ovarian stimulation can also be employed for FET.

True Natural Cycle Frozen Embryo Transfer

A true NC FET relies on ultrasound and hormonal monitoring to synchronize the endometrium with the embryo for transfer. After ruling out ovarian and endometrial abnormalities with an initial scan early in the cycle, monitoring typically resumes between cycle days 8 and 12, depending on the patient's cycle length—earlier for shorter cycles and later for longer ones. Ovulation is commonly determined by detecting the endogenous LH surge, which is usually identified through urine or serum LH measurements. While urinary LH tests can be employed outside the clinic, their reliability depends on patient adherence. A rise in serum LH levels by 180% from the previous measurement is often used to confirm the onset of the LH surge, although the precise definition an LH surge is still debated (**Figure 31.6**).[966]

Since decidualization is triggered by progesterone elevation, the LH surge is used as a surrogate marker for progesterone rise. However, interindividual and intercycle variations in the interval between LH and progesterone raise questions about how these variations affect embryo–endometrium synchronization. The first day that serum progesterone exceeds 1.0 to 1.5 ng/mL, marking the start of secretory transformation, is the least commonly used—but the most biologically plausible—tool for determining the opening of the implantation window. In an NC blastocyst transfer is typically scheduled for the sixth day after detecting the LH surge, the fifth day after observing follicle collapse, or the fifth day after a progesterone rise exceeding 1.0 or 1.5 ng/mL. However, whether live birth rates differ across these methods and the optimal approach remains unclear.

NC FET is only feasible for women with regular, predictable menstrual cycles. Its advantages include the absence of medical intervention and the presence of a corpus luteum, which is absent in programmed cycles (discussed further on). The absence of a corpus luteum in programmed cycles has been linked to compromised maternal cardiovascular adaptation to pregnancy, as well as increased risks of hypertensive disorders of pregnancy (~1.8 times higher), postpartum hemorrhage (~2.6 times higher), macrosomia, and postterm births (both ~1.6 times higher) compared to NC FET or stimulated-cycle FET.[967,968]

While luteal support with exogenous progesterone in NC FET might seem counterintuitive as the corpus luteum typically provides sufficient progesterone, current evidence suggests it increases live birth rates.[969–971] Monitoring midluteal serum progesterone may be helpful if routine luteal phase support is not provided; however, midluteal progesterone levels do not appear predictive of pregnancy outcomes when luteal support is routinely administered.

Drawbacks of NC FET include the unpredictability of the ET date, which may be inconvenient for patients and clinic staff, and the need for more frequent clinic visits—although this may involve only one additional visit compared to modified NC or stimulated-cycle FET.[972] Additionally, NC FET has a slightly higher cycle cancellation rate (~8%) due to failure to detect the LH surge.

Despite these limitations, NC FET is a favorable option for women with regular cycles due to its association with better obstetric outcomes. This is particularly relevant for women at higher risk of hypertensive disorders of pregnancy, postpartum hemorrhage, macrosomia, or postterm delivery. While pregnancy rates are generally comparable to other methods, the improved obstetric profile makes NC FET an appealing choice for many patients.

Modified Natural Cycle Frozen Embryo Transfer

The key distinction between a true NC and a modified natural cycle (mNC) lies in the use of an exogenous hCG injection in mNC to mimic the endogenous LH surge, similar to ovarian stimulation protocols. Monitoring in mNC follows the same approach as in NC: an initial baseline transvaginal ultrasonographic evaluation during the first three days of the

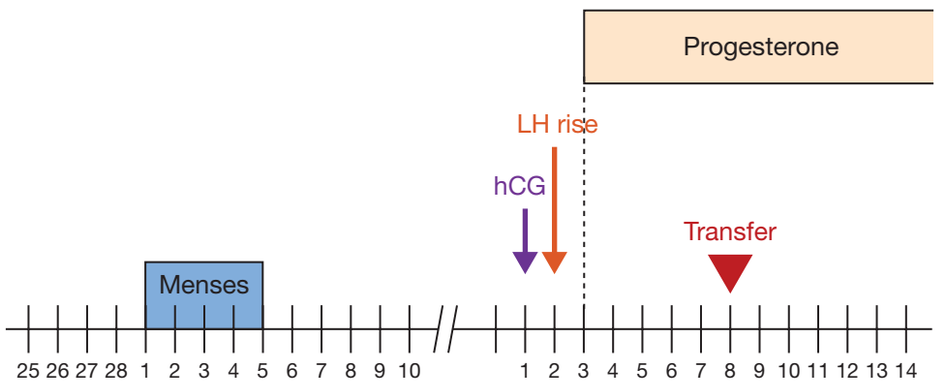

FIGURE 31.6 ET is on the seventh day after the hCG injection in a modified natural cycle or on the sixth day after the detection of the LH surge in a pure natural cycle. ET, embryo transfer; hCG, human chorionic gonadotropin; LH, luteinizing hormone.

menstrual cycle is followed by a second one between cycle days 8 and 12. If the leading follicle exceeds 16 mm in size and the endometrium appears favorable, an hCG injection is administered, and ET is scheduled—5 days later for a day-3 embryo or 7 days later for a blastocyst (**Figure 31.6**). If the follicle and endometrium are not ready at the second evaluation, additional monitoring occurs every 1 to 2 days at the physician's discretion.

A spontaneous LH surge prior to hCG administration can disrupt embryo–endometrium synchronization. Consequently, monitoring serum LH and progesterone levels is often performed alongside transvaginal ultrasonographic evaluation of follicle development. If an LH surge is detected, the hCG injection may be omitted, and ET can be scheduled to align with the surge, ensuring synchrony and preventing premature endometrial advancement.[973,974] However, if progesterone levels also rise as a result of the spontaneous LH surge, the cycle may need to be canceled due to the inability to appropriately time the ET to maintain embryo–endometrium synchronization.

Midluteal serum progesterone concentration does not seem to correlate with clinical pregnancy rates in mNC cycles and can therefore be omitted.[975] Routine luteal serum progesterone monitoring for individualized luteal phase support in mNC is generally unnecessary.

Studies comparing NC and mNC have used varying protocols, particularly in the timing of ET relative to hCG administration and the management of spontaneous LH surges before the hCG trigger. However, available evidence does not indicate clinically significant differences in pregnancy rates between NC and mNC FET.

The primary advantage of mNC is the precise timing of luteal phase initiation, which can reduce the need for frequent monitoring visits. A retrospective study suggests that triggering can occur when the follicle size is 13 to 22 mm and serum progesterone is less than 1.5 ng/mL on the day of the trigger. This may provide a 7-day window for scheduling FET, although further studies are needed to confirm this approach.[976] In addition, luteal phase support appears unnecessary in mNC due to the luteotropic effect of hCG.[975,977]

mNC combines the benefits of a corpus luteum with simpler monitoring compared to NC and requires only a single injection, making it a preferred protocol for women with regular menstrual cycles. The only notable drawback, compared to programmed FET cycles, is a slightly higher cycle cancellation rate (~2%) due to insufficient follicular growth, even in women with regular cycles.[978]

Mild Ovarian Stimulation Cycle Frozen Embryo Transfer

FET in an ovarian stimulation (OS) cycle is a viable option for both ovulatory and anovulatory women who can respond to stimulation with oral antiestrogens or gonadotropins. However, its benefits in regularly ovulating women remain controversial.[979–982]

While gonadotropins have historically been used for OS FET, letrozole has emerged as a preferred alternative due to its oral administration, cost-effectiveness, and favorable safety profile. Letrozole is typically administered at 2.5 to 5 mg/d for 5 days, starting on days 3 to 5 of a spontaneous or induced menstrual cycle. Monitoring begins 3 to 4 days after the last dose, with ovulation triggered when the dominant follicle reaches ≥17 mm in size and the endometrium is deemed suitable. ET is then scheduled relative to the trigger: 5 days later for day-3 embryos and 7 days later for blastocysts (**Figure 31.7**).

For ovulation triggering, either hCG or a GnRHa can be used; however, hCG is generally preferred. GnRHa trigger results in rapid luteolysis without hCG support, potentially negating the benefits of having a corpus luteum. Some protocols include monitoring endogenous LH levels, and if a spontaneous LH surge occurs, FET is scheduled accordingly (eg, 4 days post surge for day-3 embryos and 6 days for

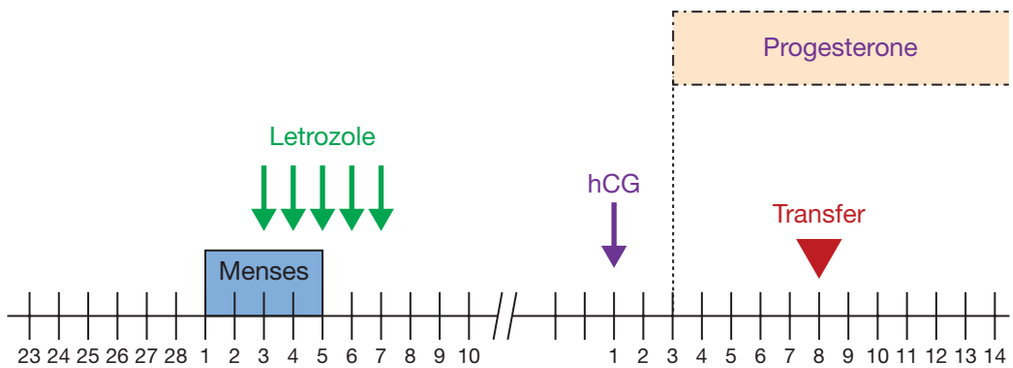

FIGURE 31.7

blastocysts). Luteal phase support (LPS) with progesterone is commonly applied in OS FET but may not be necessary after an hCG-triggered cycle.

Although most studies comparing letrozole OS FET are retrospective, two large-scale studies involving 850 and 2,409 cycles provide valuable insights.[983,984] These studies suggest that letrozole OS FET achieves approximately 1.5 times higher pregnancy and live birth rates than programmed FET or NC FET. Available data also indicate that anovulatory women with PCOS may achieve higher live birth rates with OS FET cycles compared to programmed cycles.[984,985] Despite the limited quality of evidence, letrozole OS FET appears to be an attractive option, especially for anovulatory women with PCOS.

Notably, gonadotropins were frequently added to letrozole when a dominant follicle failed to develop, raising questions about the relative cost-effectiveness and convenience of letrozole OS FET compared to gonadotropin OS FET. Further research is needed to better define the most efficient OS FET protocols and to optimize outcomes in different patient populations.

Programmed Cycle Frozen Embryo Transfer

Programmed FET cycles involve administering estrogen to stimulate endometrial proliferation and suppress ovarian follicular activity, thereby preventing spontaneous ovulation and untimely endometrial progesterone exposure. Progesterone is subsequently introduced to induce secretory transformation, opening the window of implantation. Both hormones are continued post FET to provide luteal phase support, as there is no corpus luteum in programmed cycles.

The primary advantage of programmed FET cycles is their ease of scheduling and monitoring, due to the flexibility of the estrogen phase. This makes them particularly suitable for women with anovulatory or irregular cycles, such as those with PCOS, ovarian insufficiency, or menopause. However, the absence of a corpus luteum in these cycles is a significant drawback. It is associated with increased risks of hypertensive disorders of pregnancy, postpartum hemorrhage, macrosomia, and postterm delivery.[986] These risks depend on the patient's baseline risk profile, making programmed cycle a more acceptable choice for women at low risk. Additional disadvantages include potential medication side effects, higher costs, and the need for strict adherence to the protocol. The choice between a programmed and an ovulatory cycle should be a shared decision with the patient, guided by a thorough discussion of the benefits and limitations.

Routine use of gonadotropin-releasing hormone agonists (GnRHa) for pituitary suppression in programmed cycles is not supported by evidence.[987,988] Due to hypoestrogenic side effects and additional complexity, GnRHa is reserved for rare cases where estrogen alone fails to suppress follicular growth, often due to patient noncompliance.

Monitoring begins with a baseline ultrasound at the start of a spontaneous or induced menstrual cycle to rule out ovarian and endometrial abnormalities. Estrogen can be administered via oral (eg, estradiol valerate, typically 6 mg/d), transdermal, or vaginal routes, with similar efficacy. However, data on the vaginal route are limited.[989,990] While endometrial proliferation usually occurs within 5 to 7 days, extending the estrogen phase to at least 10 days may reduce miscarriage risks. Prolonged estrogen exposure beyond 23 days, however, has been associated with decreased live birth rates and increased pregnancy loss, emphasizing the importance of timely progression to the next phase.[991–993]

A follow-up ultrasound confirms appropriate endometrial development and follicular suppression before progesterone initiation. Live birth occurs across a wide range of serum estradiol levels prior to progesterone start, and the added value of routine hormonal monitoring is controversial.[991,994–1000] If monitoring detects signs of follicular growth, ovulation, or a hyperechogenic endometrial stripe, serum progesterone should be measured to rule out ovulation. Elevated progesterone levels necessitate postponing FET to prevent embryo–endometrium asynchrony.

ET timing depends on the developmental stage of the cryopreserved embryo: day 3 embryos are transferred on the fourth day and blastocysts on the sixth day of progesterone administration.[1001–1004] For slow-growing blastocysts, transferring on the seventh day of progesterone may improve outcomes, although this is based on retrospective subgroup analyses and requires further investigation[1003] (**Figure 31.8**).

Progesterone can be delivered via intramuscular, vaginal, subcutaneous, or oral routes. Intramuscular injections remain a common choice in the United States despite being less patient-friendly. This preference is supported by a randomized controlled trial that showed lower pregnancy rates with vaginal progesterone (200 mg micronized progesterone twice daily) compared to intramuscular progesterone (50 mg daily) or a combination.[1005] It is noteworthy that this study has been criticized for the dose used in the vaginal progesterone arm, and other studies show no clear superiority among methods.[987,1006,1007] Consequently, vaginal preparations are commonly preferred for convenience worldwide.

Maintaining optimal serum progesterone levels is critical. When using vaginal progesterone alone, serum progesterone thresholds of more than 10 ng/mL on the day of FET are associated with better outcomes.[996,1008–1010] If levels fall below 10 ng/mL, supplementation with injectable, rectal, or oral progesterone may yield comparable outcomes to those with adequate levels.[1011] Notably, most available data on injectable progesterone pertain to subcutaneous formulations.

Estrogen and progesterone are continued after a positive pregnancy test to compensate for the absence of a corpus luteum. Although the luteoplacental shift typically occurs in the seventh gestational week of spontaneous pregnancies, extending luteal phase support until the 10th week provides a

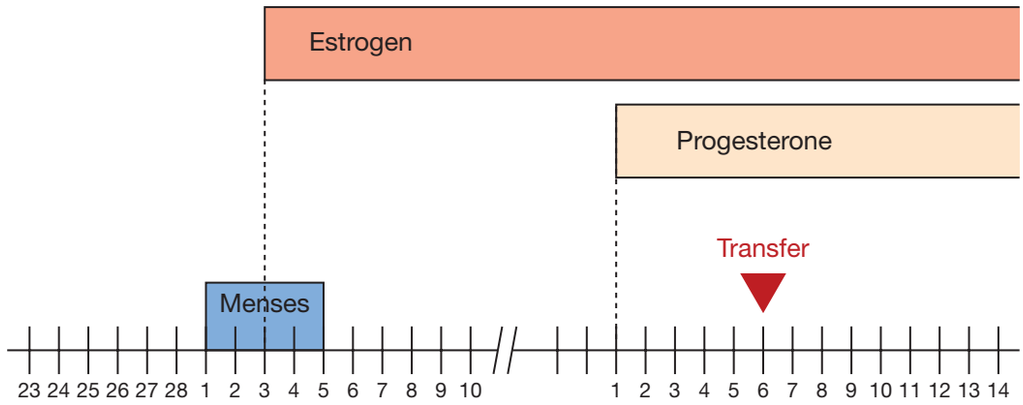

FIGURE 31.8

safety margin for FET pregnancies. Practices vary, with some clinicians stopping treatment as early as the seventh week and others continuing until the 12th week.

OUTCOMES OF IVF

IVF outcomes have improved steadily throughout the years since its introduction into clinical practice. Early on, IVF offered only a modest chance for success and was reserved appropriately for couples who had no option or had failed all other available forms of treatment. As technology and outcomes improved, IVF became a realistic and attractive option for an increasing number of couples. The advent of ICSI revolutionized the treatment of severe male factor infertility and greatly contributed to the growth of ART. Now, ART is often the first and the best option for a large proportion of infertile couples.

IVF success rates may be expressed in several ways, using different numerators and denominators. The two most common numerators are pregnancy and live birth, the latter being the most relevant measure. **Approximately 15% of pregnancies result in miscarriage, and about 3% in induced abortion, stillbirth, or an ectopic pregnancy.**[4] Pregnancy or live birth rates may be calculated as a percentage of cycle starts, retrievals, or transfers.

For the year 2022, the US registry recorded a total of 435,426 cycles of ART nationwide. Among these, 40,039 were fertility preservation cycles. The overall live birth rate was 37.5% per cycle. Results vary considerably by age.

Multiple Gestation

Following the introduction of ART into medical practice, there has been a substantial increase in multiple gestations. In 1998, 2 per 1,000 live births were triplet and higher-order multiple births, representing a more than 6.7-fold increase since 1971. Fertility treatments were responsible for more than 80% of these triplet and higher-order births, and half of these were the result of IVF treatment.[1012] The proportion of high-order multiple or twin deliveries has significantly declined since 1998 due to a trend toward reduction in ET order. In 2022, 4.5% of all births in the United States resulting from ART were multiples, a rate close to the 3% multiple-infant birth rate in the general population.[4] The higher maternal and neonatal risks associated with multiple pregnancies, their greater financial and social costs, and the host of factors that contribute to the high incidence of multiple births are reviewed in detail in Chapter 30. Consequently, discussion here is limited to issues specifically relating to multiple gestations that result from ART.

Success rates increase with the number of embryos transferred to a point beyond which only the multiple pregnancy rate further increases.[916,917] Increasing recognition of the problems associated with multiple pregnancies, and adoption of blastocyst transfers and euploid blastocyst transfers, led to a continuous increase in single ETs, and in 2022, 85.9% of all ETs were single ETs in the United States. Resultantly, 95% of ART live births were singletons.[4]

Offspring of IVF

Studies of the offspring resulting from IVF have raised concerns that the children may be at increased risk for prematurity, LBW, birth defects, genetic and epigenetic abnormalities, vascular and metabolic abnormalities, delayed neurologic development, and cancer.

Preterm Birth and Low Birth Weight

As previously discussed, ART is associated with increased incidence of multiple pregnancy, which, in turn, is associated with increased incidence of preterm birth and LBW. Therefore, to better assess the impact of ART itself on these parameters, one should compare the outcomes of singleton pregnancies. In a 2012 systematic review including 28,000 ART compared with non-ART singletons, the relative risks

for preterm delivery less than 37 weeks and less than 32 weeks were 1.54 (95% CI = 1.47–1.62) and 1.68 (95% CI = 1.48–1.91), respectively. The findings for LBW (<2,500 g) and very low birth weight (VLBW, <1,500 g) were also increased with odds ratios of 1.65 (95% CI = 1.56–1.75) and 1.93 (95% CI = 1.72–2.17), respectively. The risk for small for gestational age (SGA) was also increased by 40% in ART babies (OR = 1.39, 95% CI = 1.27–1.52).[1013] The risks of preterm delivery and LBW are declining over time.[1013–1018] An analysis of data from more than 92,000 ART infants born in Denmark, Finland, Norway, and Sweden confirmed both the increased risk and the improvement in the perinatal outcomes of singleton ART children.[1019] The adjusted odds ratios in singletons for preterm birth (PB) less than 37 weeks, PB less than 32 weeks, LBW less than 2,500 g, VLBW less than 1,500 g, and, finally, SGA were found to be 2.47, 3.80, 2.94, 4.77, and 1.83, respectively, in the analysis for 1988 to 1992, whereas the odds ratios for the 2003 to 2007 period were considerably lower, at 1.50, 2.06, 1.49, 2.11, and 1.13, respectively. The PB and LBW risks were similar in ART and spontaneously conceived twins, and the risks did not change significantly over time.[1019]

ART singleton pregnancies were also associated with a higher risk of antepartum hemorrhage (RR = 2.11, 95% CI = 1.86–2.38), hypertensive disorders of pregnancy (RR = 1.30, 95% CI = 1.04–1.62), gestational diabetes (RR = 1.31, 95% CI = 1.13–1.53), and caesarean section (RR = 1.58, 95% CI = 1.48–1.70), compared to spontaneously conceived singletons.[1020] Increased rate of pregnancy complications such as antepartum hemorrhage, placenta previa, and hypertensive disorders of pregnancy, along with more precautious antenatal care of ART pregnancies, may be at least partly responsible for the higher rate of PB.

The risk of stillbirth between the 22nd and the 28th gestational weeks is increased 2-fold (95% CI = 1.55–2.78) for ART singletons compared to those spontaneously conceived.[1021] However, the risk of stillbirth seems similar after the 28th gestational week. The ART twins have a lower risk of stillbirth compared to spontaneously conceived twins; however, when only dizygotic twins are considered, the risk seems similar in the two groups. The risk of early neonatal and infant deaths for ART singletons and twins seems similar with spontaneously conceived singletons and twins.[1021]

Parental characteristics in couples with infertility may contribute to the poorer perinatal outcome. In a 2013 meta-analysis of 14 studies comparing the perinatal outcomes of pregnancies conceived without treatment after a long time to pregnancy interval with pregnancies conceived within 12 months of attempting pregnancy, they found that the risks of preterm delivery (adjusted OR = 1.31, 95% CI = 1.21–1.42), LBW (adjusted OR = 1.34, 95% CI = 1.21–1.48), and SGA (adjusted OR = 1.17, 95% CI = 1.03–1.33) were increased in women with a long time to pregnancy interval.[1022] A study comparing the perinatal outcomes of women treated with ART, women with subfertility but spontaneous pregnancies, and fertile women in a total of 334,628 births and fetal deaths in Massachusetts revealed that the risks for both PB and LBW were significantly higher for the ART group (adjusted OR = 1.23, 95% CI = 1.08–1.41, and OR = 1.26, 95% CI = 1.08–1.47, respectively) compared with the subfertile group and that risks in both the ART and the subfertile groups were higher than those among the fertile group (adjusted OR = 1.53, 95% CI = 1.40–1.67 and OR = 1.51, 95% CI = 1.37–1.67 for the ART group, and OR = 1.24, 95% CI = 1.12–1.38 and OR = 1.20, 95% CI = 1.06–1.36 for the subfertile group, respectively).[1023] There were no significant differences between the three groups for SGA in this study. These results indicate that subfertility itself may be partly responsible for the poorer perinatal outcome.

Some of the increased risk may be attributable to the endometrial environment. The stimulation associated with ART may impair implantation. The risk of PB was found to be similar between singletons from FET and spontaneous conceptions, whereas the risk was lower compared to ART singletons from fresh ETs (adjusted OR = 0.85, 95% CI = 0.76–0.94; frozen vs fresh ET).[1024] Furthermore, the risk of PB in singletons with a "vanishing co-twin" was significantly increased compared to a single gestation (adjusted OR = 1.73, 95% CI = 1.54–1.94).[1016] Concerning number of embryos transferred, a meta-analysis of three studies revealed that the risk of PB was similar for singletons from single ET and those from double ET (adjusted OR = 0.83, 95% CI = 0.64–1.06, $P = 0.21$),[1024] while another study utilizing publicly available data from five states found that singletons born following single ET had equivalent obstetric outcomes to non-ART singletons; however, the singletons following double ET were more likely to be preterm less than 37 weeks and less than 32 weeks and have LBW or VLBW.[1025]

In a population-based retrospective registry study comparing neonatal and maternal outcome after 4,819 blastocyst transfers with 25,747 cleavage-stage transfers and 1,196,394 spontaneous conceptions, perinatal mortality and the risk of placental complications were significantly higher in the blastocyst transfer compared to the cleavage-stage transfer group (adjusted OR = 1.61, 95% CI = 1.14–2.29, for perinatal mortality, and adjusted OR = 2.08, 95% CI = 1.70–2.55 and adjusted OR = 1.62, 95% CI = 1.15–2.29 for placenta previa and placental abruption, respectively).[1026] The risks for placenta previa and placental abruption were also significantly increased in the blastocyst transfer group compared to spontaneous conceptions (adjusted OR = 6.38, 95% CI = 5.31–7.66 and adjusted OR = 2.31, 95% CI = 1.70–3.13, respectively).

Large for Gestational–Age Babies

Singletons conceived after FET have an increased risk of being LGA and of having macrosomia, defined as a birth weight greater than 4,500 g.[1025,1027,1028] A Nordic study that compared the perinatal outcomes of 6,647 singletons conceived

after FET with 42,242 singletons conceived after fresh ET and 288,542 spontaneously conceived singletons found significantly increased risks of both LGA and macrosomia in the FET group. The adjusted ORs for LGA and macrosomia were 1.45 (95% CI = 1.27–1.64) and 1.58 (95% CI = 1.39–1.80), respectively, compared to the fresh ET group and 1.29 (95% CI = 1.15–1.45) and 1.29 (95% CI = 1.15–1.45), respectively, compared to spontaneously conceived children.[1028] Similarly, a meta-analysis of three national registry-based cohort studies revealed significantly higher risk of LGA and macrosomia in both FET versus fresh singletons and FET versus natural conceptions.[1025] In another analysis that aimed to distinguish the intrinsic maternal factors from those related to the freezing and thawing processes, the prevalence of LGA was compared in consecutive sibling pairs, where one sibling was born after FET and the other after fresh ET. The first sibling group consisted of 550 singletons, with the first sibling born after fresh ET and the second one after FET, whereas the second sibling group consisted of 116 singletons, with the first sibling born after FET and the second sibling after fresh ET. In the cohort with the first child born after fresh ET, the risk of LGA in the FET sibling was significantly higher, with an adjusted OR of 3.45 (95% CI = 1.33–8.33). In the latter cohort, with the first sibling born after FET, the risk of LGA was also increased, albeit lower than in the first cohort. The risk remained increased after adjustment for parity and birth order in the complete sibling cohort, indicating the contribution of the freezing and thawing procedures per se.[1025] With increasing reliance on cryopreserved ETs and improving laboratory conditions, large prospective studies are awaited to determine the impact of embryo cryopreservation on birth weight.

Congenital Anomalies

The risk of birth defects in ART babies has been the subject of many studies, albeit with several shortcomings. These limitations include different definitions of major malformations, time, and extent of evaluation for birth defects and the duration of follow-up, inclusion of pregnancies terminated for fetal malformations and stillbirths, use of inappropriate control groups (eg, spontaneous conceptions in normal vs subfertile couples), and diversity of ART techniques (IVF vs ICSI, fresh vs FET, cleavage vs blastocyst transfer, hatching, etc) used.[1029,1030]

Several meta-analyses revealed a 30% to 40% increase in relative risk of birth defects in singleton and multiple ART pregnancies combined, compared to spontaneous concepti ons.[1013,1017,1029,1031–1034] A 2012 systematic review including 46 studies with 124,468 children conceived by ART compared to spontaneously conceived children showed a significantly increased risk of birth defects (RR = 1.37, 95% CI = 1.26–1.48) in the former. When IVF and ICSI pregnancies were compared separately to spontaneous conceptions, the RR of birth defects was higher for ICSI children than IVF children (1.58 vs 1.30, respectively); however, the difference between the two groups was not statistically significant (P = 0.11). Furthermore, analysis of 24 studies that compared 46,890 IVF babies with 27,754 ICSI babies revealed no significant difference in risk of birth defects (RR = 1.05, 95% CI = 0.91–1.20).[1034] A 2013 meta-analysis confirmed the former and the risk ratio for birth defects was found to be 1.32 (95% CI = 1.24–1.42) in 92,671 ART children. The risk was further increased (RR = 1.42, 95% CI = 1.29–1.56) when studies evaluating only major malformations were analyzed. Additionally, the RR for singleton ART pregnancies was 1.36 (95% CI = 1.30–1.43), whereas analysis of studies in ART twin pregnancies adjusted for distribution of zygosity revealed a pooled risk of 1.26 (95% CI = 0.99–1.60) compared to non-ART twins.[1032] Similarly to the results of the 2012 study, there was no significant difference in the pooled risk estimates in ICSI versus non-ART children (RR = 1.37) compared to IVF versus non-ART children (RR = 1.36).[1032]

In a recent and large registry-based study comparing the prevalence of birth defects among 64,861 live born infants conceived using ART, with 4,553,215 non-ART infants, including singleton and multiple pregnancies, the adjusted RR for nonchromosomal birth defects was 1.28 (95% CI = 1.15–1.42).[1035] Furthermore, tracheoesophageal fistula/esophageal atresia (aRR = 1.93, 95% CI = 1.40–2.67), rectal and large intestinal atresia/stenosis (aRR = 2.03, 95% CI = 1.51–2.74), and reduction deformity of the lower limbs (aRR = 2.18, 95% CI = 1.39–3.43) were significantly increased with ART use. Similarly, regarding singleton live born infants only, the adjusted RR for nonchromosomal birth defects was found to be 1.39 (95% CI = 1.21–1.59), and the risks for tracheoesophageal fistula/esophageal atresia (aRR = 1.90, 95% CI = 1.23–2.94) and rectal and large intestinal atresia/stenosis (aRR = 1.88, 95% CI = 1.26–282) were significantly higher for ART versus non-ART infants. Similar findings have been reported in a 2024 systematic review and meta-analysis, with comparable effect estimates.[1036] **Both earlier and more recent meta-analyses suggest a 30% to 40% relative increased risk of birth defects in ART children, from a baseline of 2% to 3% to 2.7% to 4%.**[1032,1037,1038]

However, similar to the data on obstetric risk, it is unclear if it is the actual exposure to ovarian hyperstimulation/embryo culture or the underlying infertility that is the primary driver of this effect. A large registry-based observational Australian study of approximately 310,000 deliveries compared the risk of birth defects in spontaneous conceptions, ART infants, spontaneous pregnancies in women who had a previous birth with ART, and spontaneous pregnancies in women with infertility. Mothers in the assisted conception group were older and more likely to be nulliparous, White, and of higher socioeconomic status. After multivariate adjustment, the increased risk for birth defects in the IVF/ICSI combined group (adjusted OR = 1.24, 95% CI = 1.09–1.41) and the ICSI group (adjusted OR = 1.57, 95% CI = 1.30–1.90) remained significant, whereas the

increased risk for the IVF group (adjusted OR = 1.07, 95% CI = 0.90–1.26) was no longer significant. Regarding risks of other types of assisted conception compared to spontaneous conceptions, gamete intrafallopian transfer, intrauterine insemination, and the use of clomiphene citrate at home were associated with a significantly increased risk of birth defects, whereas clinically supervised ovulation induction, low-dose hormonal stimulation, timed intercourse, and donor insemination were not. Furthermore, adjusted risk of birth defects showed a 25% relative increase in spontaneously conceived children of women who have had a previous ART pregnancy (adjusted OR = 1.25, 95% CI = 1.01–1.56). Borderline increased risk of birth defects was also observed in spontaneously conceived children of women with a history of infertility (OR = 1.29, 95% CI = 0.99–1.68).[1039] Another study comparing the prevalence of birth defects in the Danish national birth registry between fertile couples (time to pregnancy ≤12 months) and those who conceived spontaneously after 12 months addressed the effect of subfertility per se.[1040] The risk for malformations was increased in the latter group (OR = 1.20, 95% CI = 1.07–1.35), and the overall prevalence of congenital malformations increased with increasing time to pregnancy. A meta-analysis calculated that subfertility was responsible for about 40% of the increased risk of birth defects associated with ART and reported an adjusted overall odds ratio of 1.01 (95% CI = 0.82–1.23), indicating no significant increased risk associated with ART.[1041]

Chromosomal, Genetic, and Epigenetic Abnormalities

Limited evidence suggests that the prevalence of chromosomal abnormalities in children conceived with ART is not different from that in children conceived naturally. Nonetheless, concerns persist that the use of sperm from infertile men, and ICSI itself, might increase the risk of conceiving a child with a chromosomal or genetic defect, because infertile men (and women) are more likely than fertile men (and women) to have a genetic abnormality that may contribute to their infertility and that their children may inherit.

Genomic imprinting describes the process that limits expression of the imprinted gene from one parental allele (maternal or paternal). For a number of genes involved in early embryonic growth and placental and neurologic development, transcription is normally restricted to one allele. Usually, the maternal allele is active in imprinted genes involved in fetal development, and the paternal allele is active in genes involved in placental growth. Imprinting disorders can arise via several mechanisms, including mutations in an imprinted gene, uniparental disomy (both copies of a gene coming from one parent), and changes in DNA methylation. There is reason for concern that elements of ART might predispose to imprinting disorders; imprints are established during meiosis and at the time of each meiotic division in the oocyte (the first occurring at ovulation and the second at fertilization), and they are exposed to treatments and manipulations during ART. Any excess risk for imprinting disorders that might relate to ART is difficult to detect because the disorders are quite rare (1 in 12,000 births). Nonetheless, three of the nine known disorders have been linked to ART, including Beckwith–Wiedemann syndrome,[750–752,1042–1044] Angelman syndrome,[749,1045,1046] and maternal hypomethylation syndrome.[757] Although earlier studies suggested an increased incidence of Beckwith–Wiedemann syndrome in ART children, later cohort studies did not confirm an increased risk of imprinting disorders.[754,1047,1048] In addition, the relative risks of these syndromes were the same in subfertile women with or without ART, and thus, the increased prevalence of imprinting disorders, if any, has been attributed to subfertility rather than ART procedures themselves.[1049] It is very challenging to estimate the impact of ART on these rare imprinting disorders, and large prospective cohort studies are needed.

Vascular and Metabolic Abnormalities

There are a number of cohort studies suggesting that cardiovascular and general metabolic health may be mildly compromised in children conceived via ART. A comparison of 233 ART singletons with 233 spontaneously conceived children from subfertile parents, after matching for age and gender, showed that ART children had significantly more peripheral adipose tissue.[1050] Height, weight, BMI, bone mineral content, bone mineral density, central fat measures, and pubertal stage were similar in both groups. Another study on the same cohort showed that ART children had significantly higher systolic and diastolic blood pressures than controls (systolic 109 ± 11 vs 105 ± 10 mm Hg, $P < 0.001$; diastolic 61 ± 7 vs 59 ± 7 mm Hg, $P < 0.001$, respectively).[1051] While fasting insulin concentrations and BMI were comparable, fasting glucose levels were significantly higher in the IVF group (5.0 ± 0.4 vs 4.8 ± 0.4 mmol/L $P = 0.005$).

Another study compared 106 ART with 68 spontaneously conceived children and found that the former had significantly higher blood pressures.[1052] BMI, fasting glucose-to-insulin ratio, high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid, apolipoprotein-A1, apolipoprotein-B, lipoprotein(a), leptin, adiponectin, high-sensitivity C-reactive protein (hsCRP), and high-sensitivity interleukin-6 (hs IL-6) were similar in both groups; only triglyceride levels were significantly increased in the IVF group compared to controls (59.5 ± 25.1 vs 52.4 ± 23.3 mg/dL, respectively $P = 0.031$). When SGA and LGA born children were analyzed separately, IVF children still had significantly higher blood pressures; however, the difference in triglyceride levels was no longer significant. A previous study of 69 ART and 71 spontaneously conceived children reported an improved lipid profile, including higher HDL levels (1.67 ± 0.04 mmol/L vs 1.53 ± 0.04 mmol/L, $P = 0.02$) and lower triglyceride levels (0.65 ± 0.04 mmol/L vs 0.78 ± 0.04 mmol/L, $P = 0.02$) in IVF children.[1053]

Systemic and pulmonary vascular functions of 65 ART and 57 spontaneously conceived children were compared, and BMI, systolic and diastolic blood pressures, glucose and insulin levels, HOMA, LDL, HDL, and triglyceride levels were similar.[1054] However, concerns were raised over endothelial cell function and risk for atherosclerosis; flow-mediated dilation of the brachial artery was approximately 25% less ($P < 0.0001$), pulse wave velocity was significantly faster ($P < 0.001$), and carotid intima-media thickness was significantly greater in ART children ($P < 0.0001$). Similarly, when the subjects were taken to a higher altitude and pulmonary artery pressure was measured by Doppler echocardiography, ART children had 30% higher systolic pulmonary artery pressure ($P < 0.0001$).

While metabolic effects of ART are not well established, some studies indicate that ART may have an unfavorable effect on the vascular system. However, the differences observed are inconsistent between studies, quite small for each parameter, and of unknown biologic significance. The long-term consequences, if there are any, of these initial findings are to be determined in subsequent studies.

Behavioral and Cognitive Aspects

Multiple studies have examined whether children conceived via IVF were at a greater risk of psychomotor delay, altered socialization, or emotional problems. A 2009 study compared behavioral and socioemotional functions of 139 children born after IVF with 143 children conceived spontaneously by subfertile couples.[1055,1056] The results were corrected for parity, birth weight, prematurity, gestational age, and mother's educational level. ART children had significantly fewer externalization problems, thought and attention problems, and aggressive behavior. A 2011 study using the same cohort showed that ART children scored comparably in total problem scale, internalizing, anxious/depressed behavior, somatic complaints, social problems, thought problems, attention problems, aggressive behavior, and rule-breaking behavior with spontaneously conceived children.[1056] Moreover, there were no differences in externalization and withdrawn/depressed behavior between the two groups. These findings suggest that ART does not seem to cause any behavioral effects in adolescents. A 2001 study compared socioemotional functions of 38 spontaneously conceived children, 49 adopted children, and 34 ART children with a mean age of 11, using interviews and a number of standardized questionnaires that were filled by children, their parents, and teachers.[1057] Likewise, ART children were found to have similar social and emotional functions when compared with the other groups. **Overall, ART children seem to have normal social and emotional functions, at least in early life**.

A study comparing information processing, attention, and visual-motor function in children conceived spontaneously and by ART reported similar general cognitive ability, information processing, and attention (speed, accuracy and sustainability of attention, capacity of mental shifting, memory) in both groups.[1058] While the ART group scored significantly lower in motor speed and motor coordination, the results were still within the normal range. Similar intelligence quotient (IQ), visual-motor coordination, visual memory, and verbal comprehension abilities are reported in 51 IVF children and 51 control children.[1059] Cognitive development of 86 ICSI, 83 IVF, and 85 natural conceptions reported a crude difference in IQ of 3.9 (95% CI = −0.7 and 8.4) between ICSI and IVF children and 6.8 (95% CI = 2.0 and 11.6) between ICSI and natural conception children. Nonetheless, mean IQ was within the normal range in all groups.[1060]

Assessing neurocognitive outcomes for ART has methodologic challenges such as lack of blinding, being underpowered, using nonstandardized assessment tools, low participation rates, and selection bias.[1061] Still, available evidence suggests that cognitive and motor functions are not affected by method of conception.[1062,1063]

Cancer

Another concern about ART is its possible association with childhood cancers. Many studies have been conducted to determine whether offspring born following ART treatment have an increased risk of developing various cancers.

A 2013 meta-analysis of 25 studies showed an increased risk (RR = 1.42, 95% CI = 0.96–1.8).[1064] Only 2 of the 25 studies in the meta-analysis included untreated subfertile controls; neither of these studies identified a link between ART and cancer risk. In the same year, a large British study included 106,103 ART children born from 83,967 pregnancies between 1992 and 2008 and identified 108 children diagnosed with cancer. Expected number of cancer cases was 109.7 for the same cohort, resulting in a standardized incidence ratio of 0.98 (95% CI = 0.81 and 1.19) for the study group.[1065] A more recent large population study in 2014 included ART babies born between 1982 and 2007 in Sweden, Denmark, Finland, and Norway.[1066] A total of 91,796 ART children were compared with 358,419 spontaneously conceived children. After adjusting for country, maternal age, parity, gender, gestational age, birth defects, and chromosomal defects, hazard ratio for cancer in ART children was not significant: 1.08 (95% CI = 0.91–1.27). While 2 in 1,000 children were diagnosed with cancer in the ART group, this rate was 1.8 in 1,000 spontaneously conceived children. Risks for two cancer types were significantly increased in ART children, central nervous system tumors (adjusted HR = 1.44, 95% CI = 1.01–2.05), and malignant epithelial neoplasms (adjusted HR = 2.03, 95% CI = 1.06–3.89). However, the authors underline that this contributes very little to the absolute risk for these cancers, and overall patients can be reassured about safety of ART procedures. A French National registry study involving 8,526,306 children, including 133,965 born after fresh ET and 66,165 after FET,

reported similar overall cancer risk for children born after fresh or FETs and naturally conceived children. However, an increased risk of leukemia was reported with frozen ETs (HR = 1.61, 95% CI = 1.04–2.50) or fresh ETs (HR = 1.42, 95% CI= 1.06–1.92). It should be noted that there were only 45 and 20 cases in frozen and fresh transfer analyses.[1067] **In conclusion, although some studies inconsistently show increased risk for certain cancer types, ART children do not seem to be at significant risk for childhood cancers.**

Key Points

- Concerns about the health and welfare of children born after ART are reasonable and understandable.
- The available data indicate that ART is associated with an increased risk of multiple gestation, preterm delivery, LBW, congenital anomalies, and the complications associated with these outcomes.
- The concerns, although justified, are not cause for undue alarm. Children born from ART are generally healthy.

OOCYTE DONATION

Until approximately 25 years ago, women with ovarian failure were understandably considered irreversibly sterile, but advances in ART have changed that view. Oocyte donation now offers women with premature ovarian insufficiency, premature reproductive aging, and surgical removal of the ovaries and even women beyond their normal reproductive years a very realistic chance of pregnancy.

A successful pregnancy established in one woman (the recipient) using oocytes from another (the donor) was first reported in 1983. The original technique involved intracervical artificial insemination of a normal volunteer with sperm from the male partner of an infertile woman, uterine lavage during the preimplantation interval, and transfer of the recovered embryo to the uterus of the infertile female partner who received a programmed regimen of hormone replacement designed to synchronize endometrial and embryo development.[1068] Numerous ethical and technical problems prevented wide application. That same year saw the first report of a pregnancy established by ovum donation, IVF, and transfer to a cycling recipient.[1069] Within another year, the first successful pregnancy resulting from oocyte donation and IVF in a woman with ovarian failure was reported.[1070]

Oocyte donation is now commonly achieved by IVF using oocytes retrieved from healthy young donors after controlled ovarian hyperstimulation and the sperm of the recipient's partner, with the resulting embryos then transferred to the uterus of the recipient.[1071] Although straightforward in concept, the requirements for successful ovum donation IVF are many and complicated. The unique and key features of a donor oocyte IVF cycle relate to the need for embryo/endometrial synchronization and exogenous hormonal support of early pregnancy until the luteal-placental shift. Other important issues relate to donor recruitment, selection, and screening.

Indications

There are a number of accepted indications for ovum donation IVF—ovarian failure, genetically transmitted disease, declining or absent ovarian function, advanced reproductive age, and persistent poor oocyte quality in IVF cycles.[1072] Women with ovarian failure from any cause (X chromosome abnormalities; idiopathic gonadal dysgenesis or premature oocyte depletion; previous surgery, irradiation, or chemotherapy; autoimmune disease) are candidates. Also included are women who carry specific heritable disorders not amenable to PGT or who reject PGT and women with a DOR due to age or other causes who have a poor prognosis for successful IVF using their own oocytes. Oocyte donation is also an option for male same-sex couples who choose parenthood through assisted reproduction.

Donor Oocyte Recipients

With a few exceptions, the pretreatment evaluation and screening of couples seeking oocyte donation are virtually identical to that recommended before conventional IVF. Psychological counseling is an important element of the evaluation and helps to identify couples with unresolved concerns or fears and to ensure that both partners are fully committed to the effort.

Women with Turner syndrome may be considered candidates for ovum donation and deserve specific mention. **Evidence indicates that pregnancy may pose unique and serious risks for women with Turner syndrome, who often have cardiovascular malformations involving the aortic root.** Like women with Marfan syndrome, women with Turner syndrome are at increased risk for aortic dissection during pregnancy, presumably relating to the increased cardiovascular demands. **The maternal risk of death from rupture or dissection of the aorta in pregnancy may be 2% or higher.**[1073] Women with Turner syndrome expressing interest in oocyte donation should be carefully evaluated, to include echocardiography or magnetic resonance imaging, with any significant abnormality best regarded as a contraindication to oocyte donation. In general, even women with normal evaluations should be discouraged because aortic dissection may still occur. Those who choose to proceed require careful observation and frequent reevaluation during pregnancy.[1074]

Controlled Endometrial Development

In spontaneous cycles, endometrial proliferation and secretory maturation are driven by sex steroid production

associated with follicular growth, ovulation, and luteal function; development of the endometrium and the embryo is naturally synchronized. In ovum donation cycles, that same careful synchronization must be orchestrated. The "window of endometrial receptivity," the interval during which implantation normally occurs, is relatively narrow and spans approximately 3 days, perhaps as much as 5 days.[1075,1076] The opening and duration of the implantation window are controlled primarily by the duration of progesterone exposure. The length of the preceding proliferative phase is extremely flexible and can vary widely,[1075] as occurs naturally in oligo-ovulatory women.

To synchronize endometrial development with the embryos to be transferred, recipients with functioning ovaries may be first down-regulated with a long-acting GnRHa, treatment that women with ovarian failure obviously do not require. In either case, a programmed regimen of sequential estrogen and progesterone replacement is used to simulate the NC and to promote normal endometrial growth and maturation, the same way as in FET cycles (discussed previously). A wide variety of treatment regimens have been used successfully to achieve controlled endometrial development and maturation.

Estrogen can be administered orally (micronized estradiol 4–6 mg daily) or transdermally (estradiol 0.2–0.4 mg daily). Both routes of administration are effective, and neither has proven superior, despite the widely varying serum estradiol levels that can result.[127,1077–1080] Oral and transdermal estrogen treatment regimens are designed to achieve serum levels that approximate those observed in the late follicular phase in NCs (200–400 pg/mL); equivalent doses of vaginal estrogen achieve markedly higher serum and tissue concentrations.[1081] The duration of estrogen therapy is quite flexible and can range from as short as 7 days to as long as 3 weeks or more.[1078] Progesterone can be administered intramuscularly, in doses designed to achieve serum concentrations approximating 20 ng/mL (50–100 mg daily),[1082,1083] and/or vaginally, in the form of suppositories, tablets, or gel (180–600 mg daily). Intramuscular administration yields substantially higher serum concentrations, but endometrial tissue levels are highest after vaginal treatment.[1077]

The methods and extent of monitoring vary widely among programs. Many use transvaginal ultrasonographic measurements of endometrial thickness, aiming to achieve a thickness greater than a minimum of 6 to 7 mm.[468,493,1084,1085]

To maximize the probability for successful implantation, ET must be carefully timed. In cycles where a fresh ET is planned, in order to achieve the same coordination of embryo and endometrial development that occurs in natural conception cycles, progesterone treatment in the recipient should begin on the day the donor undergoes retrieval.[938] Day 3 embryos (3 days after retrieval and fertilization) are transferred on the fourth day of progesterone therapy, and day 5 embryos on the sixth day.[127] Although the effective "transfer window" is wider than a single day, synchronous transfer provides a margin of safety and compensates for any minor variations in the speed of endometrial maturation. Flexibility in the duration of preliminary estrogen treatment in the recipient facilitates convenient scheduling. In general, estrogen therapy begins at 2 days before the time that stimulation begins in the donor, allowing ample time to achieve the desired degree of endometrial proliferation before the donor's retrieval (**Figure 31.9**). However, most oocyte donation programs currently use frozen oocytes, and synchronization of the donor and the recipient is not an issue. In cycles using frozen donor oocytes, the day of oocyte thaw is considered the day of retrieval for planning purposes.

Luteal Phase and Early Pregnancy Support

In naturally conceived early pregnancies, the rapidly rising levels of hCG first "rescue" and then stimulate the corpus luteum to maintain the high levels of estrogen and progesterone secretion necessary to ensure endometrial stability in support of early embryonic growth and development until the emerging placenta achieves the capacity to assume that responsibility. The donor oocyte recipient has no corpus luteum. Consequently, exogenous luteal support must be provided for the requisite interval. Normally, the luteal-placental transition is completed between 7 and 9 weeks of gestation (menstrual dates) or between 4 and 6 weeks after a blastocyst transfer.[1086] **Exogenous estrogen and progesterone treatment must therefore continue until at least 7 weeks, and many recommend treatment until approximately 10 weeks of gestation, for added safety.** Some prefer to monitor serum estradiol and progesterone concentrations during the early weeks of pregnancy, decreasing the dose of exogenous hormone treatment by half after observing a sharp rise in levels and discontinuing treatment after an additional week if concentrations continue to rise normally.

Oocyte Donors

The limited availability of suitable oocyte donors is the greatest obstacle in maintaining an active donor oocyte program. Donors may be a known relative or acquaintance of the recipient,[1087] but most are anonymous, young healthy volunteers recruited from the local population. In most metropolitan areas in the United States, oocyte donors are compensated for their time, inconvenience, and assumption of risk. Outside of the United States, such compensation is discouraged and, in some countries, is illegal.

The ASRM has provided detailed guidelines for the appropriate screening of candidate oocyte donors.[1072] In summary, donors should be preferably between 21 and 34 years of age. Donors aged younger than 21 years should undergo a psychological evaluation, and decision to proceed must be on an individual basis. For donors aged older than 34 years, the age of the donor should be disclosed to the recipient with information about the effect of donor age on pregnancy rates and cytogenetic risks. All donors should be healthy, without

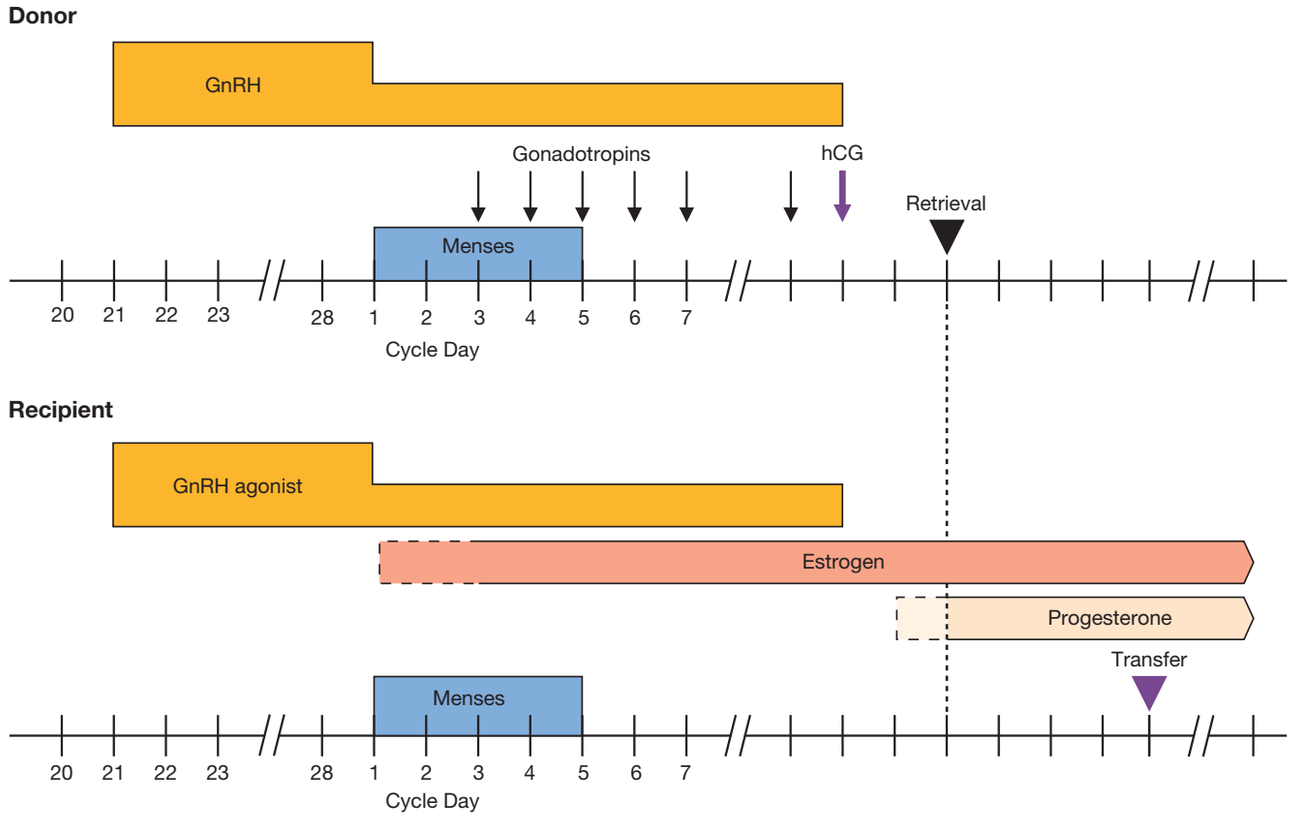

FIGURE 31.9

a history suggesting hereditary disease. While proven fertility is preferred, it is not mandatory. A thorough medical history and physical examination is performed to exclude those at high risk for sexually transmitted infections or genetically transmissible disease, and donors undergo standard preconception testing. In accordance with US federal law, candidate donors must also be thoroughly screened for sexually transmitted infections, including syphilis, hepatitis B (surface antigen and core antibody), hepatitis C (antibody), and HIV-1/HIV-2, using tests performed in a laboratory approved by the US Food and Drug Administration (FDA) for donor screening. Screening also includes tests for gonorrhea and chlamydia, and all tests must be performed within the 30 days immediately preceding oocyte retrieval or up to 7 days after acquisition. False-positive results exclude anonymous donors and repeated testing is not permitted. Other exclusion criteria can be found in the ASRM Practice Committee Recommendations for gamete and embryo donation.[1072] Written documentation of donor eligibility is also required. The donor should undergo appropriate genetic evaluation based on history, in accordance with ethnic background and current guidelines. Cystic fibrosis, spinal muscular atrophy, and thalassemia/hemoglobinopathy career screening should be performed on all donors. Consideration should be given to fragile X testing on donors. Screening for fragile X syndrome carrier status should be performed on all oocyte donors with a family history of fragile X–related disorders or an intellectual disability suggestive of fragile X syndrome. Pan-ethnic expanded carrier screening may be appropriate, and most donor egg banks perform expanded carrier screening for a large number of recessive mutations. Psychological evaluation by a qualified mental health professional is strongly recommended.[1072]

Vitrification has greatly improved the efficiency of oocyte cryopreservation, and since 2013, the use of vitrified/warmed oocytes for fertility preservation has no longer been considered experimental.[130] Fertilization and pregnancy rates are similar to IVF/ICSI with fresh oocytes when vitrified/warmed oocytes are used for young women. No increase in chromosomal abnormalities, birth defects, and developmental deficits has been reported in offspring born from cryopreserved oocytes when compared to pregnancies from conventional IVF/ICSI and the general population.[130] Egg banking simplified oocyte donation dramatically, by eliminating the need to synchronize donors and recipients, and has the added benefit of serving to decrease the number of unused frozen embryos resulting from conventional oocyte donation cycles.

Outcomes of Oocyte Donation

Experience with ovum donation has provided important insights into the mechanisms involved in the age-related decline in female fertility. The oocyte donation model effectively dissociates oocyte and uterine age. Success rates with conventional IVF decline steadily as age increases, most noticeably after age 35, and viable pregnancies are infrequent beyond age 42. **In contrast, the live birth rate in oocyte donation cycles varies little across all recipient age groups.** These data demonstrate that the declining developmental potential of aging oocytes is the limiting factor.

Data from the 2022 US national ART summary indicate that among 985 fresh and 2,817 frozen donor oocyte cycles performed across all age groups, 38.7% and 38.9% resulted in a live birth, respectively, with an average of 1.1 embryos transferred. Among 16,952 transfers of frozen embryos derived from donor oocytes, 45.8% resulted in a live birth, with an average of 1.1 embryos transferred.[4]

Because most recipients are over age 35, their pregnancies may be considered high-risk pregnancies. A 2017 systematic review and meta-analysis showed an increased risk of preterm delivery in oocyte donation pregnancies compared to nondonor oocyte pregnancies (OR = 1.45, 95% CI = 1.20–1.77). Similarly, the risk of LBW was higher after transfer of fresh embryos derived from donated oocytes compared to nondonor oocyte pregnancies (OR = 1.34, 95% CI = 1.12–1.60).[1088]

Two 2016 systematic reviews showed approximately 3-fold increased risk of preeclampsia in oocyte donation pregnancies compared with other methods of ART or natural conception. The risk of gestational hypertension was also increased significantly in oocyte donation pregnancies in comparison with other methods of ART or natural conception. The risks for preeclampsia and gestational hypertension were similarly increased in singleton and multiple gestations from donor oocytes.[1089,1090]

GESTATIONAL SURROGACY

Gestational surrogacy offers women without a functional uterus the opportunity to have genetic offspring. The techniques involved are no different than those applied in other forms of ART, but the ethical, legal, and psychosocial issues involved are complex.

Gestational surrogacy involves transfer of embryos to the uterus of a woman willing to carry a pregnancy on behalf of an infertile couple. **Surrogacy is an option for couples wherein the female partner has no uterus (congenital, hysterectomy), an irreparably damaged uterus (congenital malformation, severe intrauterine adhesions), or a medical condition for which pregnancy may pose a life-threatening risk. Surrogacy is also an option for male same-sex couples who choose parenthood through assisted reproduction.** The host carrier may be a relative, a friend, or someone with no attachment to the couple who may or may not be compensated for her service. Regardless of what the circumstances are, candidates for surrogacy should have previously given birth and undergo a thorough psychological evaluation. The legal status of gestational surrogacy varies widely among different states, and even where it has recognition, a formal legal contract is required to formalize agreements between the infertile couple and the surrogate.

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