CHAPTER 28

Medical Approaches to Ovarian Stimulation for Infertility

Nicholas S. Macklon and Bart C. J. M. Fauser

Introduction

CONCEPTS OF OVARIAN STIMULATION

Ovarian stimulation is a central component of many infertility therapies. At the outset of this chapter, it is important to emphasize that two different concepts of ovarian stimulation exist. These approaches differ in both the starting point (i.e., the type of patients) and end points (i.e., the aim of the medical intervention).

Ovulation Induction

In the strict sense of the term, ovulation induction refers to the triggering of ovulation, that is, the rupture of the preovulatory follicle and release of the oocyte. In the clinical context however, this term refers to the type of ovarian stimulation for anovulatory women aimed at restoring normal fertility by generating normo-ovulatory cycles (i.e., to mimic physiology and induce single dominant follicle selection and ovulation). Ovulation induction represents one of the most common interventions for the treatment of infertility.[1] Anovulation represents one of the few states of absolute infertility, but excellent cumulative pregnancy rates can be achieved if normal menstrual cyclicity is restored.

After the exclusion of intrinsic ovarian abnormalities (such as premature ovarian failure) follicle development can be stimulated by various pharmacologic compounds and normo-ovulatory cycles can usually be obtained. This can be achieved with appropriate monitoring of ovarian response and in the hands of skillful clinicians. Because of various more subtle inherent ovarian abnormalities in most of these women, especially in patients suffering from polycystic ovary syndrome (PCOS), the risks of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS) are considerable. However, the occurrence of these complications can be reduced to an acceptable level, especially with low-dose gonadotropin protocols.[2] The therapeutic window for an acceptable ovarian response is small, with a major individual (and to some extent cycle-to-cycle) variability in response. Approaches for gonadotropin ovulation induction include slowly and prudently surpassing the individual follicle-stimulating hormone (FSH) threshold for ongoing follicle development, as will be discussed later in this chapter.

Many other approaches for ovulation induction are available. These approaches include interfering with negative estrogen feedback, the use of insulin-sensitizing agents, and laparoscopic surgical methods.

Ovarian Hyperstimulation

This treatment modality has become an integral part of assisted reproductive technologies (ART). The aim is to bring more male and female gametes closer together and thereby increase the chances of pregnancy. The goal of ovarian hyperstimulation is to induce ongoing development of multiple dominant follicles and to mature many oocytes in order to improve chances for conception either in vivo (empirical ovarian hyperstimulation with or without intrauterine insemination, IUI) or in vitro with in vitro fertilization (IVF). This approach of interfering with physiologic mechanisms underlying single dominant follicle selection is usually applied in normo-ovulatory women. Although ovarian hyperstimulation can also be performed in anovulatory women, this approach should be clearly differentiated from ovulation induction. The physiologic concepts which underlie current approaches to ovulation induction and ovarian hyperstimulation are described later in this chapter.

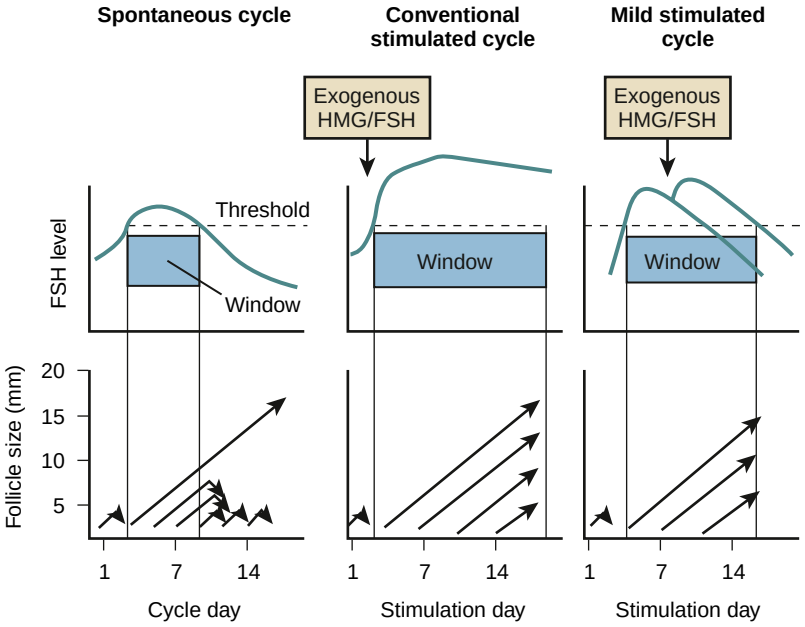


Figure 28-1. *The follicle-stimulating hormone (FSH) threshold and window concept for monofollicular selection* (left panel)*, as conventionally applied to achieve multifollicular development* (middle panel)*. Each* arrow *represents a developing follicle. The* right panel *represents the concept of extending the FSH window by administering exogenous FSH in the midfollicular phase to maintain FSH levels above the threshold allowing multifollicular development. HMG, human menopausal gonadotropin. (From Macklon NS, Stouffer RL, Giudice LC, Fauser BC. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocr Rev 27[2]:170-207, 2006.)*

CONCEPTS OF FOLLICLE DEVELOPMENT REGULATION RELEVANT TO OVARIAN STIMULATION

Initiation of growth of primordial follicles, also referred to as primary recruitment, occurs continuously and in a random fashion and development from the primordial up to the preovulatory stage takes several months.[3,4] The great majority of primordial follicles which enter this development phase undergo atresia prior to reaching the antral follicle stage. The regulation of early follicle development and atresia and the degree to which early stages of follicle development are influenced by FSH remain unclear, but evidence suggests that the transforming growth factor β superfamily and factors regulating apoptosis (i.e., programmed cell death) are involved.[5] Only at more advanced stages of development do follicles become responsive to FSH and obtain the capacity to convert the theca cell–derived substrate androstenedione (AD) to estradiol (E_2) by the induction of the aromatase enzyme.

Owing to demise of the corpus luteum during the late luteal phase of the menstrual cycle, E_2, inhibin A, and progesterone levels fall. This results in an increased frequency of pulsatile gonadotropin-releasing hormone (GnRH) secretion inducing rising FSH levels at the end of the luteal phase.[6,7] Although each growing follicle may initially have an equal potential to reach full maturation, only those follicles that happen to be at a more advanced stage of maturation during this intercycle rise in FSH (levels surpassing the so-called *threshold* for ovarian stimulation) gain gonadotropin dependence and continue to grow[2] (Fig. 28-1). This process is referred to as cyclic, gonadotropin-dependent recruitment as opposed to the above-mentioned initial, gonadotropin-independent recruitment of primordial follicles.[4] Based on indirect observations it is believed that the cohort size of healthy early antral follicles recruited during the luteofollicular transition is around 10 per ovary.[3,8,9] During the subsequent follicular phase, FSH levels plateau during initial days[10,11] and are gradually suppressed thereafter by ovarian inhibin B[12] and E_2[13] negative feedback. A rise in inhibin B occurs just after the intercycle rise in FSH. It may therefore be proposed that inhibin B limits the duration of the FSH rise. Decremental follicular phase FSH levels (effectively restricting the time when FSH levels remain above the threshold, referred to as the FSH *window*) (see Fig. 28-1) appear to be crucial for selection of a single dominant follicle from the recruited cohort.[10] Only one follicle escapes from atresia by increased sensitivity for stimulation by FSH and luteinizing hormone (LH).[2] The important concept of increased sensitivity of the dominant follicle for FSH has been confirmed by human studies showing developing follicles to exhibit a variable tolerance for GnRH antagonists-induced gonadotropin withdrawal.[14,15] On the other hand, early stages of follicle development being independent from gonadotropins is confirmed in hypophysectomized women presenting with preovulatory graafian follicles within 2 weeks after the initiation of ovarian stimulation with exogenous gonadotropins.[16]

A central role has also been demonstrated for LH in monofollicular selection and dominance in the normal ovulatory cycle.[17,18] Although granulosa cells from early antral follicles respond only to FSH, those from mature follicles also contain LH receptors and therefore become responsive to both FSH and LH. The maturing dominant follicle may become less dependent on FSH because of the ability to respond to LH. It is suggested that the leading follicle continues its development owing to LH responsiveness, whereas smaller follicles enter atresia because of insufficient support by decreasing FSH concentrations during the late follicle phase. The dominant follicle can be distinguished by ultrasound from other cohort follicles by a size greater than 10 mm diameter.[9] The concept of both endocrine and autocrine up-regulation is supported by several other observations that characterize the dominant follicle, including the in vitro induction of aromatase

enzyme activity,[19] ovarian morphology,[20] and endocrine changes in follicle fluid[21] and serum. These observations all show that enhanced E_2 biosynthesis is closely linked to preovulatory follicle development.

These concepts of follicular development and selection have come to underlie contemporary approaches to therapeutic ovulation induction in women suffering from anovulatory infertility. Moreover, our increasing understanding of the processes underlying monofollicular selection has enabled the development of new approaches to ovarian hyperstimulation for assisted reproduction treatments.

PREPARATIONS USED FOR OVARIAN STIMULATION

Evidence of the endocrine pituitary-gonadal axis arose early in the 20th century, when it was observed that lesions of the anterior pituitary resulted in atrophy of the genitals. The first convincing evidence supporting the existence of two separate gonadotropins (initially referred to as Prolan A and Prolan B) was provided by Fevold and Hisaw in 1931, and both LH and FSH were subsequently isolated and purified. In 1928 Aschheim and Zondek described the capacity of urine from pregnant women to stimulate gonadal function. The concept of stimulating ovarian function by the exogenous administration of gonadotropin preparations has intrigued investigators for many decades. As early as 1938, Davis and Koff had already described the ability of purified pregnant mare serum to induce ovulation in humans by intravenous administration. However, these initial attempts had to be stopped due to species differences and resulting antibody formation impacting on efficacy and safety. Not until 1958 did Gemzell describe the first successful use for ovulation induction of gonadotropin preparations derived from human pituitaries. Shortly thereafter, Lunenfeld reported the clinical use of gonadotropin extracts from urine of postmenopausal women (for an historical overview, see Gruhn and Kazer[22] and Lunenfeld[23]).

A second important development allowing for ovarian stimulation on a large scale was a fine example of medical serendipity. The first estrogen antagonist tested in cancer patients was found to induce ovulation.

Clomiphene Citrate

In the late 1950s the first nonsteroidal estrogen antagonist (MER-25) was tested in patients to assess the efficacy of the compound in women with cystic mastitis, breast cancer, endometrial hyperplasia, or endometriosis. Some of these women with endometrial hyperplasia were of reproductive age and suffering from long-standing amenorrhea due to the Stein-Leventhal syndrome. To the great surprise of the investigators, the initiation of the medication in these women was followed by the recommencement of menstrual cycles.[24] Shortly thereafter, the ovulation-inducing capacity of the next generation of closely related anti-estrogens (MRL/41; clomiphene citrate, CC) (Fig. 28-2) was recognized.[25] More than 30 years later, CC is still the most applied drug for infertility therapies worldwide, accounting for around two thirds of all prescriptions.

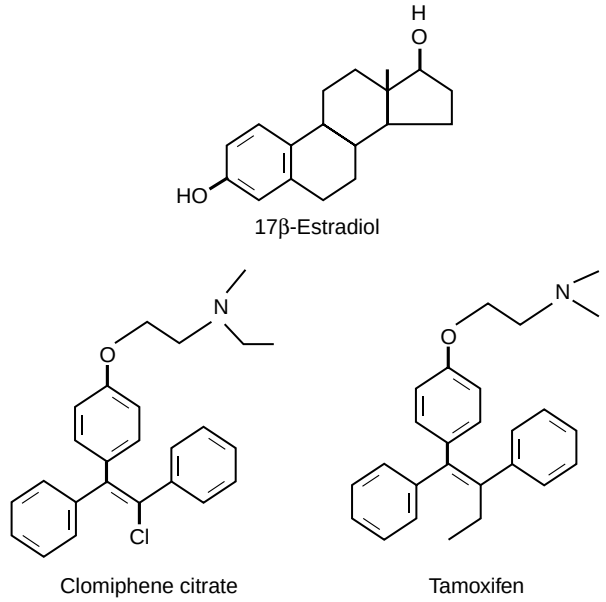


Figure 28-2. *Structure of 17β-estradiol and the anti-estrogenic triphenylethylene derivates clomiphene citrate and tamoxifen.*

CC is a racemic mixture of two stereoisomers. The enclomiphene isomer has a relatively short half-life, whereas the zuclomiphene isomer has an extended clearance. The two isomers demonstrate different patterns of agonistic and antagonistic activity in vitro.[26] Stimulation of ovarian function is elicited by raised pituitary FSH secretion due to blockage of E_2 steroid feedback by CC. Overall a 50% to 60% increase of serum FSH levels above baseline has been described.[27] The exact nature of the mechanism of action of CC is still uncertain.[28] Induced changes in other systems, like insulin-like growth factor (IGF) may partly explain the capacity of CC to stimulate the ovary.[27] However, anti-estrogenic effects at the uterine level (cervical mucus production and endometrial receptivity) are believed to underlie the observed discrepancy between achieved ovulation and pregnancy rates. The impact of a concomitant rise in LH on ovarian response to CC is also uncertain. CC for ovulation induction is considered to be relatively safe because steroid negative feedback remains intact. The oral route of administration and low costs represent additional advantages of this preparation. CC was originally developed for clinical use by the Merrel company in 1956, and it is still considered to represent the first-line treatment strategy in most anovulatory infertility. In addition, this compound was a central component in the early days of IVF[29,30] and is still often applied for the empirical treatment of unexplained infertility, alone or in combination with IVF.

Gonadotropin Preparations

Clinical experiments in the late 1950s demonstrated that extracts derived from the human pituitary could be used to stimulate gonadal function.[31] Subsequently, experiments involving the extraction of both the gonadotropic

hormones LH and FSH from urine of postmenopausal women led to the development of human menopausal gonadotropin (hMG) preparations. From the early 1960s these preparations were used for the stimulation of gonadal function in the human.[32] It soon became clear that hMG was a very potent compound. Its ability to directly stimulate the ovaries was accompanied by the inherent risks of ovarian hyperstimulation. Initial use in the treatment of anovulation was associated with high rates of multiple pregnancy and OHSS. The potential for dangerous complications induced the need for monitoring of ovarian response and dose adjustment. More recently introduced low-dose protocols applied in conjunction with intense ovarian response monitoring have substantially contributed to improved treatment outcomes.

Initial attempts in the 1970s by Edwards and Steptoe to enable the conception of a baby through IVF also involved hMG stimulation protocols. Because of a lack of pregnancies (presumed due to abnormal luteal function) it was decided to switch to natural cycle IVF. It was an unstimulated cycle that led to the conception of the first IVF baby Louise Brown, who was born on July 25, 1978.[33] Subsequent IVF pregnancies were reported from Australia to occur after ovarian stimulation with CC.[29] The more widespread use of hMG for successful IVF was developed thereafter in the United States.[34] For over two decades, gonadotropin preparations have also been extensively applied for ovarian stimulation in ovulatory women for empirical treatment of unexplained subfertility. Here the aim is to increase monthly fecundity rates by increasing the number of oocytes available for fertilization in vivo (with or without the additional use of IUI). These trends, and the rapid expansion in the use of IVF treatment, underlie the enormous increase in worldwide demand and sales for gonadotropin preparations.

The early extraction techniques were very crude, requiring around 30 L of urine to manufacture enough hMG needed for a single treatment cycle. The FSH to LH bioactivity ratio of these early preparations was 1:1. As purity improved, it was necessary to add human chorionic gonadotropin (hCG) in order to maintain this ratio of bioactivity.[35] These initial preparations were very impure with many contaminating proteins; only less than 5% of the proteins present were bioactive. Bioactivity of gonadotropin preparations continues to be assessed by the crude in vivo rat ovarian weight gain Steehlman and Pohley assay. This rather anachronistic technique has the disadvantage of allowing considerable batch-to-batch inconsistency in bioactivity. However, improved protein purification technology allowed for the production of hMG with reduced amounts of contaminating nonactive proteins and eventually the development of purified urinary FSH (uFSH) preparations by using monoclonal antibodies since the late 1980s. The currently available pure products allow for less hypersensitivity reactions, and less painful subcutaneous administration. Because of the worldwide increased need for gonadotropin preparations, demands for postmenopausal urine increased tremendously and adequate supplies could no longer be guaranteed. In addition, concern regarding the limited batch-to-batch consistency along with possibilities of urine contaminants emerged.[26]

Through recombinant DNA technology and the transfection of human genes encoding for the common α subunit and hormone-specific β subunit of the glycoprotein hormone (Fig. 28-3) into Chinese hamster ovary cell lines,[36] the large-scale in vitro production of human recombinant FSH (recFSH) has been realized.[37] The first pregnancies using this novel preparation in ovulation induction[38] and in IVF[39] were reported in 1992. Since then, numerous large-scale, multicenter studies have been undertaken, demonstrating their efficacy and safety. The recombinant products offer improved purity, consistency, and large-scale availability. Because of its purity, recFSH can now be administered by protein weight rather than

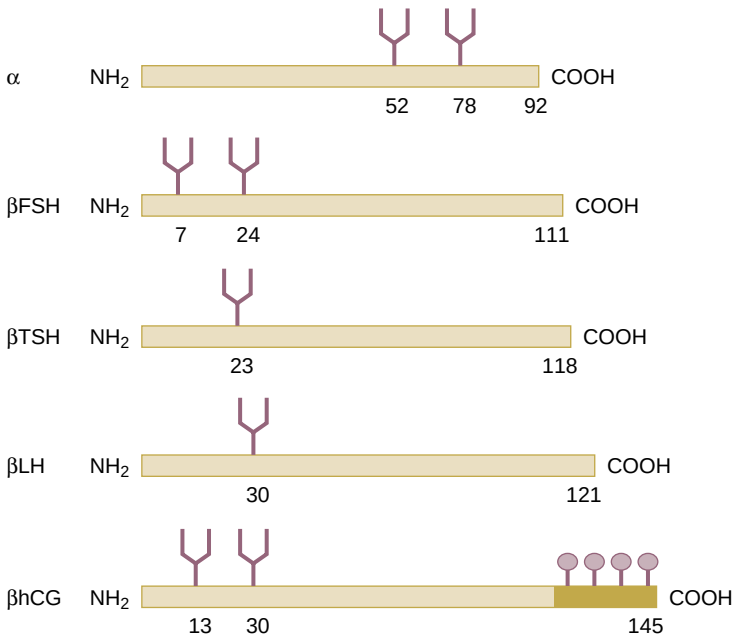


Figure 28-3. *Structure of the common α subunit and hormone-specific β subunit of the human glycoprotein hormones: follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG).*

bioactivity, and so-called "filled-by-mass" preparations[40] are now available for clinical use. During recent years, recLH and rechCG have also been introduced for clinical application.[26] Finally, a long-acting recFSH agonist (a chimeric hormone generated by the fusion of the carboxy-terminal peptide of hCG to the FSH β chain) is undergoing clinical IVF trials, and the birth of the first healthy child using this preparation was reported in 2003.[41]

GnRH Analogs

In 1971, the small decapeptide gonadotropin-releasing hormone (GnRH) was isolated and its structure elucidated by Schally and Guillemin (Fig. 28-4). Some years later, both investigators jointly received the Nobel prize for this discovery. Amino acid substitutions have revealed the significance of specific regions for its stability, receptor binding, and activation of the gonadotrope cells. This decapeptide is secreted by the hypothalamus into the portal circulation in an intermittent fashion, stimulating the pituitary gonadotropes to synthesize and secrete LH and FSH. Early studies demonstrated that pituitary down-regulation could be induced by the continued administration of GnRH.[42] Clinically safe GnRH agonists were developed relatively easily by replacing one or two amino acids. An increased potency could be achieved by replacing glycine for D-amino acids at position 6 and by replacing Gly-NH_2 at position 10 by ethylamide.[43] Such simple structural changes render these compounds more hydrophobic and more resistant to enzymatic degradation. The administration of GnRH agonists induces an initial stimulation of gonadotropin release for 2 to 3 weeks (the so-called "flare effect") followed by a down-regulation (or desensitization) due to the clustering and internalization of pituitary GnRH receptors.

GnRH agonists have been used clinically since 1981 to induce a "chemical castration" for steroid-dependent disease states such as fibroids and endometriosis in females and prostate cancer in males. The first paper concerning its use in IVF for the prevention of a premature LH rise also appeared in the early 1980s.[44] Shortly thereafter, the use of GnRH agonists such as buserelin, triptorelin, or leuprorelin to down-regulate the pituitary prior to administration of gonadotropins (a combination that became known as the "long protocol") became the standard of care. The recent clinical introduction of GnRH antagonists may ultimately lead to a new standard of care in IVF practice.

It has taken almost three decades to develop GnRH antagonists with acceptable safety and pharmacokinetic characteristics. The first-generation antagonists were developed by replacing amino acids histidine at position 2 and tryptophan at position 3, but these compounds suffered from low potency. In second-generation compounds, the activity was increased by incorporating a D-amino acid at position 6. However, the widespread clinical application of these compounds was hampered by frequent anaphylactic responses due to histamine release. By introducing further replacements at position 10, third-generation compounds were developed.[45,46] Subsequently both the compounds ganirelix and cetrotide were shown to be safe and efficacious in IVF. These third-generation GnRH antagonist were registered in 2001 for use in IVF. The immediate suppression and recovery of pituitary function renders these compounds appropriate for short-term use in IVF. Commencing administration in the late follicular phase of the stimulation cycle still prevents premature luteinization. Further extended use of GnRH antagonist in steroid-dependent disease states such as endometriosis or myomas will depend on the development of depot or slow release formulations.

OUTCOMES OF OVARIAN STIMULATION

Ovulation Induction

Amenorrheic women with anovulation exhibit virtually no chance of spontaneous conception and ovulation induction may restore normal fertility. However, the aim of mimicking normo-ovulatory cycles cannot always be achieved, and therefore, the chances of complications such as multiple pregnancy or OHSS should be taken seriously, especially in PCOS patients. Oligomenorrheic women may

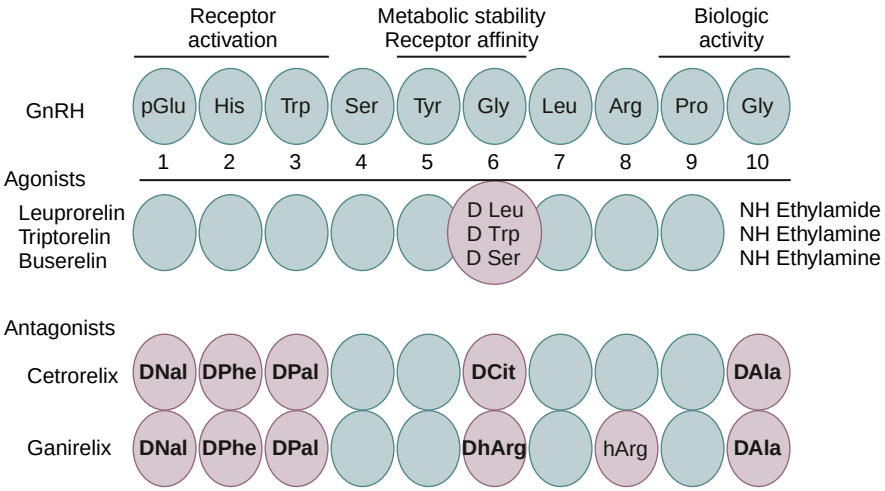


Figure 28-4. *Structure of the native decapeptide gonadotropin-releasing hormone (GnRH), as well as the modified, commercially available GnRH agonists and antagonists. Arg, arginine; D, dextro; DAla, D-alanine; DCit, D-citroline; DhArg, D-homoarginine; DNal, D-naphthylalanine; DPal, D-pyridylalanine; DPhe, D-phenylalanine; Gly, glycine; hArg, homoarginine; His, histidine; Leu, leucine; pGlu, pyroglutamate; Pro, proline; Ser, serine; Trp, tryptophan; Try, tyrosine.*

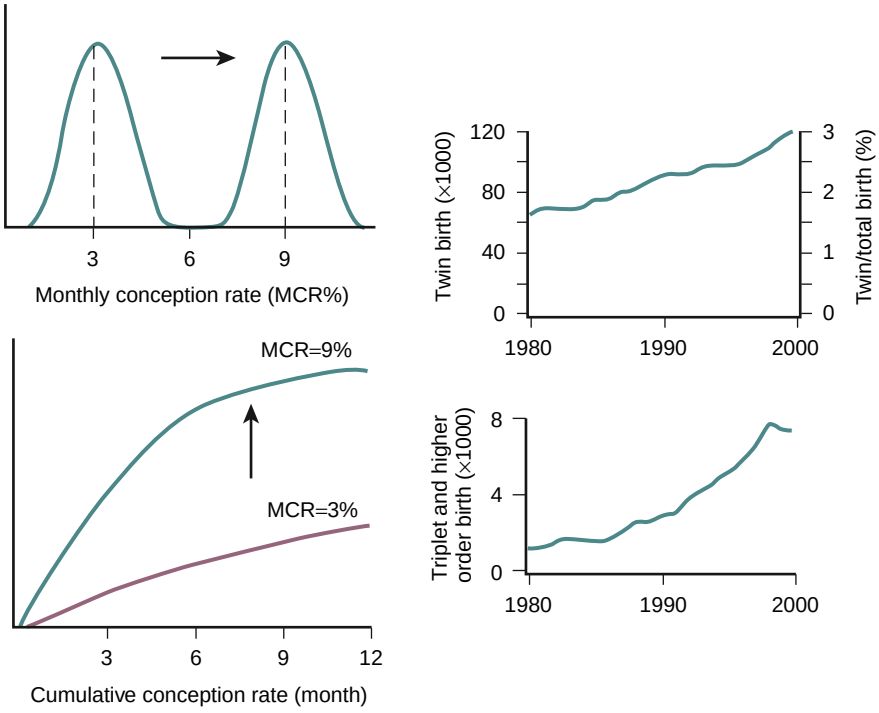


Figure 28-5. *Monthly conception rate in unexplained infertility increasing from 3% to 9% per cycle due to ovarian stimulation and resulting increase in cumulative conception rates over a 12-month period* (left)*; increased occurrence of multiple pregnancies (twin, triplet, and higher order) between 1980 and 2000 associated with ovarian stimulation* (right)*. (Left from Stovall DW, Guzick DS. Current management of unexplained infertility. Curr Opin Obstet Gynaecol 5:228-233,1993; right from Rowland Hogue CJ. Successful assisted reproduction technology: the beauty of one. Obstet Gynecol 100:1017, 2002; Jones HW. Multiple births: how are we doing? Fertil Steril 79:17-21, 2003.)*

or may not have incidental spontaneous ovulations, and therefore, spontaneous pregnancies may occur. For obvious reasons, fertility specialists see only oligomenorrheic women who have failed to conceive, and these patients will usually respond well to ovulation induction. The balance between success and complications resulting from ovulation induction is dependent on many factors, including patient characteristics, gonadotropin preparations and dose regimens used, the intensity of monitoring ovarian response to stimulation, and willingness to cancel the cycle in case of hyper-response. Cumulative success rates of ovulation induction are reported to be around 75%,[47] with a coinciding incidence of multiple pregnancies of more than 10% and of OHSS of less than 2%.

OHSS is a potentially life-threatening complication characterized by ovarian enlargement, high serum sex steroids, and extravascular fluid accumulation, primarily in the peritoneal cavity. In severe cases, hypotension, increased coagubility, reduced renal perfusion, and oliguria may occur. Deranged liver function tests, venous and arterial thrombosis, renal failure, and adult respiratory distress syndrome can ensue, and fatalities have been reported.[48] Moderate to critical OHSS is very rare with CC but constitutes an important complication of gonadotropin use.[49] The incidences of mild, moderate, and severe OHSS following gonadotropin ovulation induction have been reported to be 20%, 6% to 7%, and 1% to 2%, respectively.[26] In addition to PCOS, risk factors for the development of OHSS include young age and low body weight.[49] The risk is further increased when adjuvant GnRH agonist treatment is employed.[50]

The contribution of ovulation induction treatment to the number of triplet and higher order pregnancies is considerable.[51,52] It has been calculated that 40% of higher order multiple births in the United States could be attributed to the use of ovulation-inducing drugs without assisted reproduction.[51]

Ovarian Stimulation

As previously outlined, the aim of ovarian hyperstimulation alone or in combination with assisted reproductive techniques is to bring an increased number of gametes (oocytes and sperm) together in order to augment pregnancy chances. Hyperstimulation may give rise to a two- to fourfold increase in pregnancy rates. The associated risk of OHSS and the occurrence of twin and higher order multiple births is dependent on the magnitude of ovarian stimulation, the intensity of ovarian response monitoring, and the criteria applied for cycle cancellation should too many follicles develop. The overall incidence of severe ovarian OHSS associated with ovarian hyperstimulation is less than 5%.[53]

Initial studies suggested that a threefold increase in monthly probability of pregnancy can be achieved with empirical ovarian hyperstimulation in the treatment of unexplained infertility[54] (Fig. 28-5). Subsequently, a large multicenter study showed that ovarian hyperstimulation with gonadotropins and IUI both exhibit an independent additive effect on pregnancy chances. Moreover, overall cumulative pregnancy rates with this combined therapy was reported to be 33% within three cycles, but at the price of an unacceptably high multiple pregnancy rate of 20% for twins and 10% for higher-order multiple pregnancy.[55] It has been proposed that a similar cumulative pregnancy rate could be achieved by expectant management over a 6-month period, obviously with much lower chances of multiple pregnancy.[56] In an analysis from one

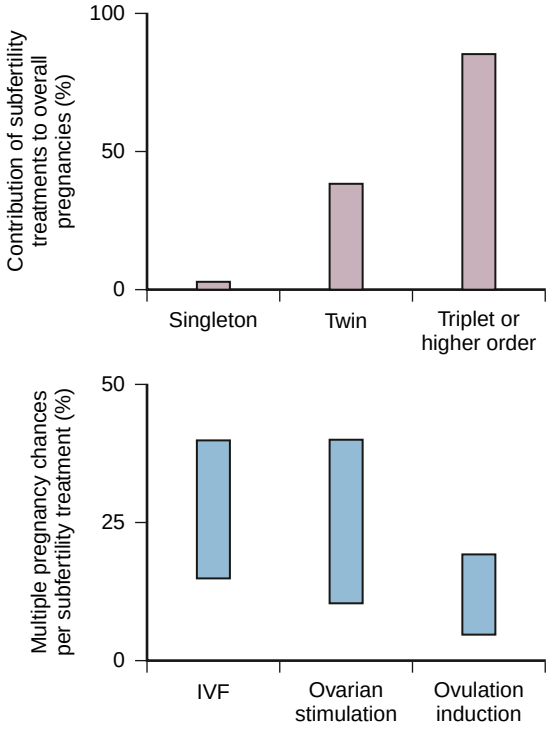
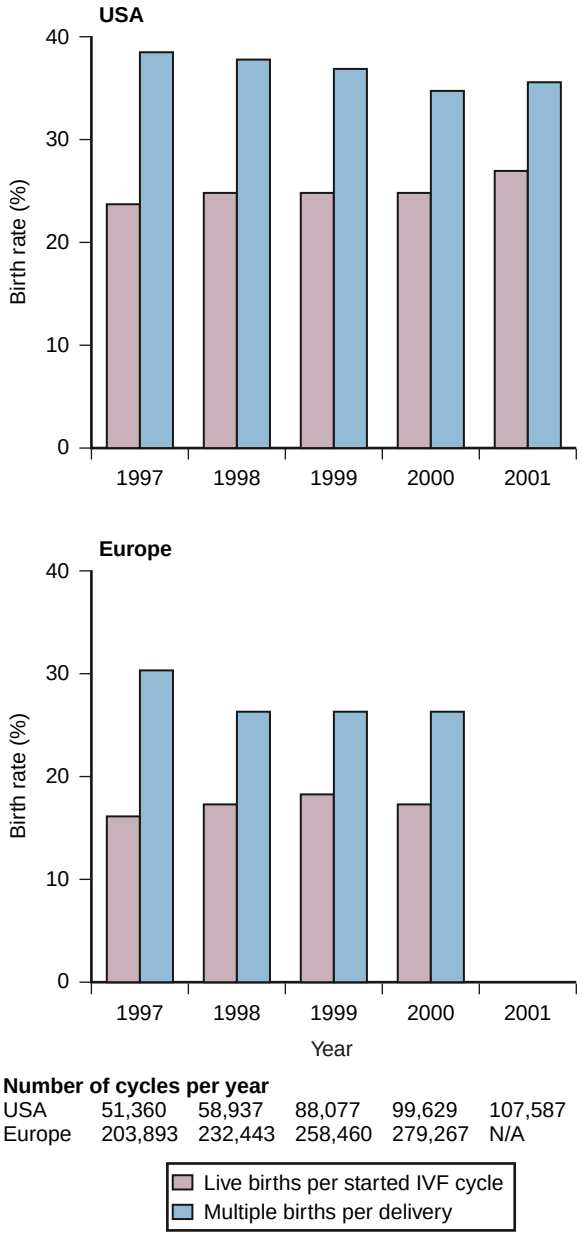


Figure 28-6. *Contribution of subfertility treatments to overall pregnancies* (upper) *and reported frequency of multiple pregnancy in relation to in vitro fertilization (IVF), ovarian stimulation, and ovulation induction* (lower). *(From Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet 365[9473]:1807-1816, 2005.)*

large European fertility center of 1878 pregnancies obtained from IUI cycles stimulated with gonadotropins, 16% were twins and 6% were triplets or higher order.[57] Less intense ovarian stimulation may reduce the incidence of higher order multiple pregnancies, but probably at the expense of a reduction in overall conception rate. On the basis of a 2-year experience in a large U.S. infertility clinic involving 3347 consecutive ovarian stimulation cycles (ovulation induction and ovarian hyperstimulation combined) in approximately 1500 women, a 30% pregnancy rate was described. Twenty percent of these pregnancies were twins, along with 5% triplets and 5% quadruplets or higher order.[58] The most worrying conclusion of this analysis was that the number of large antral follicles or serum E_2 levels during the late follicular phase had only limited value in predicting higher order multiple gestations. The true rate of multiple pregnancies arising from ovarian stimulation with or without IUI remains uncertain, however,[59] as few national registers record the outcome of ovarian stimulation. The European IVF Monitoring consortium reported twin rates among women under 40 years of age as 11.4% and triplet rates as 2.2% in 2003.[60] Although these data included natural cycle IUI treatments, the triplet rate was higher than that reported for IVF. It is estimated that ovarian stimulation with or without IUI is responsible for around 30% of multiple births (Fig. 28-6). It is easier to influence chances for multiple gestations after IVF, because the occurrence is primarily dependent on the number of embryos transferred. Therefore, ovarian hyperstimulation for IVF is merely the factor allowing for the generation of multiples, but not the sole determining factor like in IUI. Unsurprisingly, the incidence of twin pregnancies following IVF without stimulation[61] or with hyperstimulation combined with single embryo transfer is close to normal.[62,63] Over the years, the number of embryos transferred in IVF has decreased, but larger numbers continue to be transferred in the United States compared to Europe. On the basis of a large nationwide data set from the United Kingdom, it was reported that the number of embryos transferred could be reduced from three to two without a concomitant drop in overall pregnancy chances.[64] The policy of two embryo transfer was adopted by many major European IVF centers during the 1990s. Subsequently it was demonstrated that in young women in whom two high-quality embryos are transferred, the chances of a twin pregnancy are actually higher than for a singleton pregnancy.[65,66] An increasing number of leading centers in Europe are currently moving toward a policy of single embryo transfer in selected women. The number of embryos transferred in the United States is substantially higher, with current revised guidelines still recommending the number for transfer to be between two and five, depending on patient's age and prognosis.[67]

This trend to reduce the numbers of embryos transferred is beginning to be reflected in birth statistics. After many years of rising multiple births, a decline in the reported percentage of higher-order multiple births after IVF are beginning to appear. IVF registries for 1999 from the United States involving 88,000 initiated ART cycles indicate a continued slight overall improvement in pregnancy chances (currently 25% delivery rate per started cycle), with overall 32% twin pregnancies and 4.9% (n = 1024 deliveries) triplet and higher-order multiple birth.[68] In young women below 35 years of age, the overall multiple birth rate was 42%. Data from the European IVF-Monitoring Consortium show a continuing trend toward transferring fewer embryos. Whereas in 1999, in 49% of embryo transfer cycles three or more embryos were still being transferred,[69] in 2003, a similar analysis of over 280,000 cycles showed this figure to have fallen to 28%.[60] Single embryo transfer accounted for 16%.[60] Despite these trends, overall clinical pregnancy rate per retrieval of 24% rose in the same period from 24% to 26%. Twin deliveries accounted for 22% of pregnancies and triplet or higher order deliveries were 1.1%.[60] In general, overall IVF results in Europe are slightly lower compared to the United States, but with an overall reduced incidence of multiple and premature birth (Fig. 28-7).

Given the risks associated with ovarian stimulation, couples should be well counseled regarding their spontaneous chances for pregnancy prior to commencing therapy (Table 28-1). These chances are often underestimated[70] both by the doctor and by the patient and the price to be paid for interventions (the increased incidence of multiple pregnancies) may frequently be underemphasized. How should the increased chances for pregnancy on the one hand be balanced with the high complication rates inevitably associated with ovarian hyperstimulation? Until recently, fertility specialists tended to lean heavily in the direction of increasing pregnancy rates at all costs. In



Number of cycles per year

USA	51,360	58,937	88,077	99,629	107,587
Europe	203,893	232,443	258,460	279,267	N/A

Live births per started IVF cycle
Multiple births per delivery

Figure 28-7. *Rates of live births per started in vitro fertilization (IVF) cycle and multiple births. N/A, not available. (From Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet 365[9473]:1807-1816, 2005.)*

doing so, an iatrogenic epidemic of multigestation came about, with major health, psychosocial, and financial consequences.

Higher order multiple pregnancies have a major adverse impact on perinatal morbidity and mortality rates. Mortality rate is increased four- to sevenfold in twins and up to 20-fold in triplets.[52] Children born from multiple pregnancies have more chances for perinatal complications and subsequent health problems, chiefly associated with prematurity and low birth weight.[52] Chances for cerebral palsy are increased almost 50-fold in children from triplet pregnancies.[71] Even the second child from a twin pregnancy delivered at term presents with a significant increased risk for death due to complications of vaginal delivery.[72] Besides the medical and emotional burden, the financial costs associated with multiple pregnancies should be taken into account by policy makers. Obstetric and neonatal costs are increased five- to sevenfold in higher order multiples, and by the age of 8 costs for low-birth-weight children are increased eightfold.[52] Finally, possibilities of more subtle health risks which may be revealed only later in life should also be taken seriously.

Perhaps one strategy that may help improve the situation would be to agree to a new way of defining success from infertility therapy. The appropriate outcome measure should be shifted from pregnancy rate per treatment cycle toward live birth or preferably, healthy singleton child per started course of treatment.[63,73]

Ultimately, however, the risk/benefit ratio of ovulation induction and ovarian hyperstimulation is determined by the practice of the clinician. In the following sections we provide an overview of the medical approaches applied in contemporary practice.

Induction of Ovulation in Anovulatory Women

PRINCIPLES OF OVULATION INDUCTION

The aim of induction of ovulation in anovulatory women is to stimulate a single follicle to develop up to the preovulatory stage and subsequently ovulate. As stated before, this therapeutic goal should be clearly distinguished from two other forms of ovarian stimulation. First, ovulatory women with unexplained infertility may undergo a mild form of ovarian stimulation aimed at producing two or three follicles and an increased chance of fertilization in a given cycle. This treatment, which is frequently combined with IUI, is discussed later in the chapter. Second, ovarian hyperstimulation may be applied in ovulatory women undergoing IVF treatment, where multifollicular development is required to produce multiple oocytes. In contrast to these two therapeutic approaches, which produce a superphysiologic situation, ovulation induction aims to mimic the normal physiologic monofollicular ovulatory cycle. Ovulation induction is characterized therefore by tighter therapeutic margins and a need for careful monitoring and skilled management if success without complications is to be achieved. Ovarian surgical techniques such as laparoscopic drilling offer an alternative to medical therapies in this context. Again, the aim of this treatment paradigm is to institute monofollicular ovulatory cycles.

Anovulatory disorders account for around 25% of causes of infertility.[74] This proportion may increase with the rising prevalence of obesity. Anovulation is usually manifested as the absence (amenorrhea) or infrequent occurrence (oligomenorrhoea) of menstrual periods. Although oligomenorrhoea may be associated with occasional ovulation, the chance of a woman conceiving within a year of unprotected intercourse is clearly diminished unless therapeutic steps are taken. Many medical approaches have been developed to achieve the goal of inducing the monthly development of a single dominant follicle and

TABLE 28-1

Hypothetical Model of Cumulative Spontaneous Pregnancy Rates in Five Categories, According to Duration of Subfertility

		Cumulative Pregnancy Rate After (%)			
Category	*MFR (%)*	*6 mo*	*12 mo*	*24 mo*	*60 mo*
Superfertile	60	100	—	—	—
Normally fertile	20	74	93	100	—
Moderately subfertile	5	26	46	71	95
Severly subfertile	1	6	11	21	45
Infertile	0	0	0	0	0

MFR, monthly fecundity rate.
From Evers JLH. Female subfertility. Lancet 360:151-159, 2002.

subsequent ovulation. In recent years, increased understanding of the pathophysiology of ovarian dysfunction have enabled the development of clinical strategies which aim to mimic the endocrine control of normo-ovulatory cycles. Achieving this within the narrow therapeutic margins of stimulating single rather than multiple follicular developments remains a challenge to clinicians.

CLASSIFICATION OF ANOVULATION

Ovarian dysfunction can be readily classified in everyday clinical practice on the basis of the assessment of serum gonadotropin and estrogen levels in peripheral blood. This concise approach, currently known as the World Health Organization (WHO) classification of anovulation, was developed by Insler.[75] Amenorrhea may coincide with either low or normal E_2, whereas oligomenorrhea is associated with normal estrogens only. Low estrogens combined with low gonadotropin levels suggest a central origin of the disease at the hypothalamic-pituitary level.[76] This cause of anovulation occurs in less than 10% of infertile women and is termed WHO class 1. Low estrogens in combination with high gonadotropins suggest defective ovarian function per se, usually on the basis of premature ovarian failure (POF) or ovarian dysgenesis. This cause of anovulation, termed WHO class 3, occurs in around 5% of infertile women. The majority (80% to 90%) of anovulatory women present with estrogen and FSH levels within normal limits. LH levels may be increased in these women. Polycystic ovary syndrome (PCOS), exhibiting FSH and E_2 concentrations within the normal range, represents the great majority of these women. Recently, new criteria for the diagnosis of PCOS have been supported by the ASRM (American Society for Reproductive Medicine) and ESHRE (European Society of Human Reproduction and Embryology). The so-called Rotterdam consensus criteria are broader than the NIH (National Institutes of Health) criteria primarily because polycystic ovaries are now included. The incidence of PCOS as defined by the Rotterdam criteria is therefore higher.[77]

An additional cause of anovulation with an endocrine etiology is hyperprolactinemia which may present with normal or reduced gonadotropin and E_2 concentrations. This may be considered as a variant of WHO class 1 anovulation because high serum prolactin levels suppress GnRH release by the hypothalamus by altering opioid receptor stimulation. Hyperprolactinemia may also present with normal gonadotropin and E_2 concentrations, and may then be considered as a variant of WHO class 2. The pathophysiology and treatment of hyperprolactinemia are dealt with in Chapter 3.

PREPARATIONS FOR TREATING ANOVULATION

Anti-Estrogens

Background. The most widely used anti-estrogen for treating anovulation is CC, the development and pharmacology of which are addressed in the introduction to this chapter. In terms of its relative efficacy, safety, cost, and ease of use, it remains some 40 years after its introduction into clinical practice the most important therapeutic agent in use. The principal indication for CC is the treatment of anovulatory infertility in women with an intact hypophyseal-pituitary-ovarian axis. In this role it remains the first-line therapy. Given orally in the early to midfollicular phase, it causes a 50% rise in the endogenous serum FSH level,[78] thus stimulating follicle growth. This rise in FSH is accompanied by a similar rise in serum LH levels. Limitation of the duration of administration to 5 days is aimed at allowing FSH levels to fall in the late follicular phase and the mechanisms for monofollicular development and ovulation to operate. However, elevated gonadotropin levels may persist into the late follicular phase in some women.[79] The long half-life zuclomiphene isomer (which exhibits predominant estrogen agonist activity) has been shown to persist and accumulate across consecutive cycles of treatment.[80] However, the resulting concentrations are well below those demonstrated to have any effects in vitro and are unlikely to be of clinical significance.

Preparations and Regimens. The conventional starting dose of CC is 50 mg/day, starting from day 2 until day 5 of the menstrual cycle, for 5 consecutive days. In normogonadotropic amenorrheic women, treatment can be initiated following a progesterone-induced withdrawal bleeding. Whether CC is commenced on cycle day 1 or 5 does not appear to affect outcomes.[81] Should 50 mg/day fail to elicit follicle growth, the dose should be increased to 100 mg/day in the subsequent cycle, followed by

150 mg/day, which is usually considered to be the maximum dose beyond which alternative treatments are indicated. The LH surge occurs between 5 and 12 days following the last day of CC administration. Intercourse is therefore advised for a week from the fifth day after the last day of CC administration. Some advocate hCG administration as a surrogate for the LH surge to trigger ovulation and to time intercourse. However, recent studies showed no improvement in outcomes, despite the increased monitoring required to time hCG administration.[82,83]

Clinical Outcome. Between 60% and 85% of anovulatory women will become ovulatory with CC, and 30% to 40% will become pregnant.[84] In a meta-analysis based on four placebo-controlled studies in oligomenorrheic patients, the odds ratio with CC was 6.8 for ovulation and 4.2 for pregnancy.[85]

Why some women with WHO class 2 anovulation do not respond to CC is not fully understood. Altered individual requirements for FSH at the ovarian level, the local intraovarian effect of autocrine or paracrine factors, and variations in FSH receptor expression or FSH receptor polymorphisms may contribute. A number of studies have pointed to overweight as a factor.[84] In a multivariate analysis of factors found to predict outcome of CC ovulation indication, the free androgen index (FAI), body mass index (BMI), presence of amenorrhea (as opposed to oligomenorrhea), and ovarian volume were found to be independent predictors of ovulation.[86] The possibility of using clinical data to individualize treatment and optimize outcomes is discussed later in this chapter.

The occurrence of ovulation can be identified by the use of temperature charts and midluteal urinary pregnanediol or serum progesterone measurements.[84] Although results of large trials indicate that monitoring by ultrasound is not mandatory to ensure good outcomes,[87] the practice in many centers is to monitor the first cycle to allow adjustment of dose where necessary. The cumulative pregnancy rate in ovulatory women with CC in 6 to 12 months of treatment is around 70%,[86] with conception rates per cycle around 22%.[84]

Why do some women who become ovulatory with CC not conceive? Reasons include patient selection, the regimen used, and the presence of other causes of subfertility. The anti-estrogenic effects of CC on the reproductive tract have been particularly implicated. Negative effects on tubal transport, quantity and quality of cervical mucus,[26] and the endometrium[88] have all been reported.

Miscarriage rates of 13% to 25% are reported. Although these numbers appear high, they are similar to the spontaneous miscarriage rate[89] and those observed in infertile women undergoing IVF. In general, it does not appear that the miscarriage rate is significantly increased in anovulatory women treated with CC.

Side Effects and Complications. Apart from hot flushes, which may occur in up to 10% of women taking CC, side effects are rare. Nausea, vomiting, mild skin reactions, breast tenderness, dizziness, and reversible hair loss have been reported, but less than 2% of women are affected. The mydriatic action of CC may cause reversible blurred vision in a similar number.[24]

The multiple pregnancy rate is less than 10%, and OHSS is rare.[84] The putative increased risk of ovarian cancer reported to be associated with the use of CC for more than 12 months[90] has led CC to be licensed for just 6 months of use in some countries.

Tamoxifen is, like CC, a nonsteroidal selective estrogen receptor modulator (SERM). In contrast to CC, tamoxifen contains only the zu-isomer and appears to be less antiestrogenic at the uterine level. The possible advantages of tamoxifen over CC include an agonistic effect at the endometrium. Many uncontrolled studies in the area of ovulation induction have suggested that tamoxifen may be a safe and efficacious alternative to CC. A meta-analysis of four randomized controlled studies revealed tamoxifen to be as effective as CC in inducing ovulation. However, despite the theoretical benefits, no significant improvement in pregnancy rates was observed compared with CC.[91] Clinicians should therefore base their choice of treatment on familiarity with the given regimen.

Insulin-Sensitizing Agents

Background. The role of insulin-resistance in the pathogenesis of ovarian dysfunction in many PCOS patients has led to the introduction of insulin-sensitizing agents as adjuvant or sole treatment regimens for the induction of ovulation. The most extensively studied insulin-sensitizing drug in the treatment of anovulation is metformin. Metformin (dimethylbiguanide) is an orally administered drug used to lower blood glucose concentrations in patients with non-insulin-dependent diabetes mellitus (NIDDM).[92] It is antihyperglycemic in action, and increases sensitivity to insulin by inhibiting hepatic glucose production and by increasing glucose uptake and utilization in muscle. These actions result in reduced insulin resistance, lower insulin secretion, and reduced serum insulin levels.

Many papers have been published in recent years advocating the clinical usefulness of this compound for ovulation induction. The absence of well designed and properly powered studies did not dampen enthusiasm for metformin in this context, and it has been widely introduced into clinical practice. Recently, however, two large, placebo-controlled randomized studies comparing metformin to CC and metformin as adjunctive therapy to CC have shown no benefit of metformin.[87,93]

Preparations and Regimens. The first studies reporting the use of metformin as an ovulation induction agent suggested that metformin improved insulin sensitivity; lowered LH and total and free testosterone concentrations; and increased FSH and sex hormone–binding globulin levels.[94,95] This, and subsequent uncontrolled studies, indicated that correction of hyperinsulinemia has a beneficial effect in anovulatory women, by increasing menstrual cyclicity, improving spontaneous ovulation, and thus promoting fertility.[94,96,97] It is recommended that metformin be commenced at 500 mg/day orally, rising to 500 mg three times a day over 7 to 10 days.[96] Depending on response, this may be increased to 1000 mg twice a day. The optimal duration of treatment remains unclear. However, most studies reporting a beneficial effect from metformin

have shown this within 2 to 4 months.[98,99] Given the side effects of metformin (see later discussion), should the patient remain anovulatory, it is recommended by some that alternative therapy be instituted after 3 months.[98] In those who respond to treatment, metformin should be continued for 6 to 12 months once ovulatory cycles are established.

Clinical Outcome. The majority of studies on the outcome following metformin therapy are small and uncontrolled or simply case series.[100] Most of the available data on restoration of menses following metformin therapy are on predominantly obese hyperinsulinemic women with PCOS. Similarly, studies of the ability of metformin to induce ovulation have been primarily carried out in obese women. In a meta-analysis of 15 studies involving 543 participants with PCOS, metformin was found to be effective in achieving ovulation with odds ratios of 3.88 (CI 2.25-6.69) for metformin versus placebo and 4.41 (CI 2.37-8.22) for metformin and clomiphene versus clomiphene alone.[101] Metformin was also shown to have a significant effect on pregnancy rates in combination with clomiphene (OR 4.4, CI 1.96-9.85). This meta-analysis suggested that women with PCOS who failed to ovulate with CC should receive combination therapy with metformin ahead of moving to gonadotropin therapy, particularly obese women. However, a recent large multicenter study has clarified the role of metformin as an alternative first-line ovulation induction agent in women with PCOS.[87] In this study, 626 women with PCOS were randomized to receive 50 to 150 mg CC plus placebo from cycle day 3 to 7, 500 to 2000 mg daily doses of extended release metformin plus placebo, or a combination of metformin and CC. Treatment was continued for up to 6 months. Obesity was not an exclusion criterion. The results of this study are summarized in Figure 28-8. The primary end point of live birth rate was 22.5% after treatment with CC, compared with a significantly lower rate of just 7.2% following metformin treatment. Combination therapy with both metformin and CC yielded a live birth rate of 26.8%, which did not differ significantly from that achieved with CC treatment alone. The relatively poor performance of metformin in terms of live birth rates was partly explained by a low conception rate, which was just 21.7% following metformin, compared with 39.5% in the CC group. In terms of ovulation rates alone, the combination of metformin and CC was superior to either individual therapy. However, in a study of 228 women randomized to receive CC plus metformin or CC plus placebo, no significant difference in ovulation rates was observed (64% versus 72%, respectively).[93] A significantly larger proportion of women in the metformin group discontinued treatment because of side effects (16% versus 5%).

It has been suggested that metformin may reduce the rate of miscarriage compared with CC-derived pregnancies. However, in the study of Legro et al., the rate of first trimester loss did not differ significantly between the treatment groups, although the study was not powered to detect this.[87] Regarding side effects, gastrointestinal complaints were more common in those receiving metformin. However, no multiple pregnancies arose after metformin treatment, compared with a 6% twinning rate following CC. Metformin was also shown to have a positive effect on insulin sensitivity and BMI. However, these benefits were not translated into higher pregnancy rates.

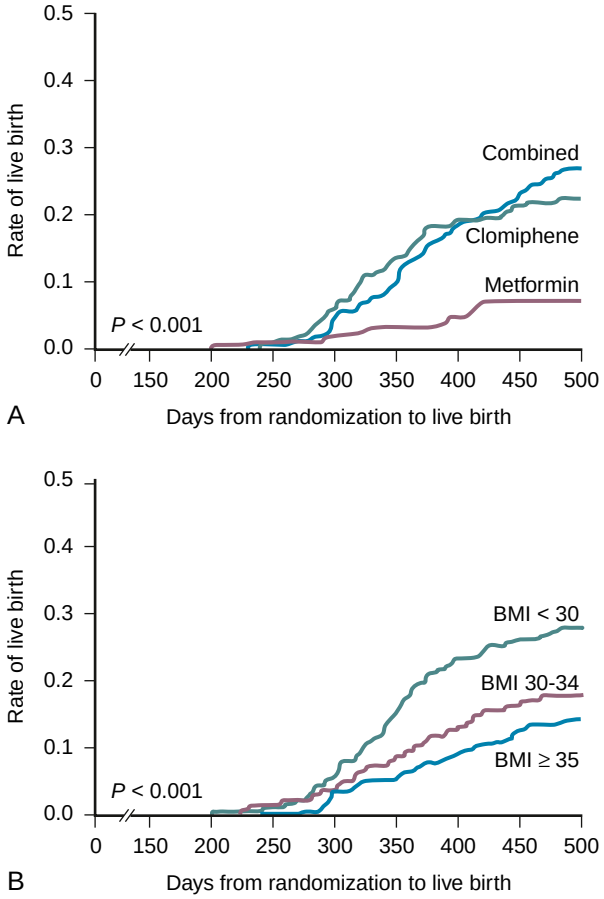


Figure 28-8. *Kaplan-Meier curves for live births following ovulation induction with clomiphene, metformin, or both treatments combined (**A**) and body mass index (BMI) (**B**). (From Legro RS, Barnhart HX, Schlaff WD, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med 356:551-566, 2007.)*

Since women with WHO type 2 anovulatory infertility frequently demonstrate a hyper-response to FSH, it has been proposed that metformin may also have an adjuvant role to gonadotropin ovulation induction by correcting hypersinsulinemia and reducing hyperandrogenism, and hence normalizing the response of the patient to gonadotropin stimulation.[96] In a study of CC-resistant women with PCOS who were randomized to pretreatment with metformin or no pretreatment prior to ovulation induction with FSH, the incidence of multiple follicular development was reduced in those receiving metformin beforehand.[102] In a randomized trial comparing metformin co-administration versus placebo during rFSH treatment in 32 CC-resistant women with PCOS no differences were observed in indices of insulin sensitivity or ovarian response during rFSH treatment.[103] In another similar randomized study, metformin co-administration was observed to normalize the endocrine profile and increase the rate of monofollicular cycles.[104] More adequately powered studies are now required to further elucidate the role of metformin as an adjunctive therapy to ovulation induction with gonadotropins.

Metformin therapy has also been proposed to aid weight loss in obese women with PCOS. Many studies have now examined the effect of metformin on BMI, and the evidence is conflicting. However, the majority of observational studies addressing weight loss with metformin have revealed a reduction in the BMI of 1% to 4.3%.[98] More recently, a double-blinded randomized trial compared metformin 850 mg twice daily treatment with placebo in 143 PCOS women with a BMI greater than 30. After 6 months' treatment no significant difference in weight loss or menstrual frequency was observed.[105] In contrast, lifestyle modification was to improve cycle regularity by improving weight loss.

Attention has turned in recent years to the possible benefits and safety of metformin administration during pregnancy. PCOS pregnancies demonstrate a greater incidence of perinatal and maternal complications such as gestational diabetes, preeclampsia, and premature delivery[106] (Box 28-1. A number of studies have appeared suggesting a role for metformin to ameliorate these complications. However, most of these studies are not randomized or suffer from small numbers and surrogate outcomes. Although metformin crosses the placenta, there is no clear evidence of toxicity when taken during pregnancy.[84] Larger well-designed studies are still required to address the possible therapeutic benefits of metformin during pregnancy in women with PCOS.

Side Effects and Complications. Metformin has been used for many years for the treatment of diabetic patients and appears to be safe for long-term use, with few side effects reported. Rarely, lactic acidosis may occur[96] if hepatic or renal disease is present, and these patients should be excluded before commencing therapy. The main side effects of metformin are nausea and diarrhea, which may occur in 10% to 25% of patients and contribute to the weight loss effects observed with metformin. If these symptoms persist despite lowering the dose, alternative therapy should be given. For this reason, metformin should be started at a low dose that gradually rises (see earlier discussion).

BOX 28-1

Maternal and Perinatal Risks Associated with Polycystic Ovary Syndrome

Maternal
- Gestational diabetes*
- Pregnancy-induced hypertension*
- Preeclampsia
- Delivery by cesarean section

Neonatal
- Admission to a neonatal intensive care unit
- Perinatal mortality
- Premature deliveries

*Outcome confirmed by subgroup analysis of higher validity studies.

From Boomsma CM, Eijkemans MJ, Hughes EG, et al. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod update 12:673-683, 2006.

Gonadotropins

Background. Women with WHO class 2 anovulation who fail to ovulate or conceive following ovulation induction with anti-estrogens can be successfully treated with exogenous gonadotropins. Exogenous gonadotropins have been widely used for the treatment of anovulatory infertile women since 1958.[2,23] Improvements in purification techniques led to increasing relative amounts of the active ingredients and the first urine-derived preparation containing only FSH (uFSH) became available in 1983. The development and application of production techniques based on immunoaffinity chromatography with monoclonal antibodies enabled the production of highly purified uFSH. In the 1980s, recombinant DNA technology led to the development and, later, the clinical introduction of human recombinant FSH (recFSH). This advance promised not only unlimited availability, but improved purity and batch-to-batch consistency compared to urinary derived products.

The development of recombinant gonadotropins also provided the opportunity to elucidate more clearly the physiology of ovarian E_2 synthesis. During further follicular development, LH has a synergistic action with FSH. Theca cells are stimulated by LH to convert cholesterol into androstenedione (AD) and testosterone (T) by cytochrome P450 side chain cleavage oxidases and 3β-hydroxysteroid dehydrogenase. Aromatase activity in the granulosa cells is induced by FSH and converts AD and T into estrone and E_2. The involvement of two cell types (granulosa and theca cells) and two hormones (LH and FSH) to produce estrogens from cholesterol has led to the concept of the "two cell, two gonadotropin" theory. In addition to stimulating aromatase activity, FSH also induces LH receptors and further increases FSH receptor formation while stimulating DNA and protein synthesis by the cell.[107] Clinical observations in the treatment of anovulatory women have supported this concept.

In the treatment of WHO class 1 (hypogonadotropic hypogonadal) anovulation, women with intact pituitary function can be treated with pulsatile GnRH therapy to restore the periodic release of FSH and LH. The treatment of hypogonadotropic women with FSH alone leads to follicular development but not pregnancy.[16] Exogenous LH is therefore required to treat this form of anovulatory infertility. Until recently, hMG was the only source of exogenous LH for this group of patients. Now recLH offers the possibility for a more sophisticated and individualized approach to treatment.

Recent studies have demonstrated the safety and appropriate dose required to effect follicle development and subsequent pregnancy. It has been established that resting levels of at least 0.5 to 1 IU should be sufficient to provide maximal stimulation to thecal cells.[108] In a study of hypogonadotropic women undergoing treatment with recFSH and recLH, a dose of 75 IU per day of recLH was observed to result in follicular development and pregnancy. However, further increases in LH levels above the threshold

level needed to gain a response did not appear to induce a greater degree of ovarian stimulation.[109]

Preparations and Regimens. In addition to urinary derived FSH products, recFSH has been clinically available since 1996 in the form of follitropin alpha and follitropin beta. More recently, a long-acting recFSH, an LH, and an hCG have been added to the clinical arsenal for ovarian stimulation.

In order to achieve development of a single dominant follicle with exogenous gonadotropins, specific treatment and monitoring protocols are needed. The two most frequently encountered in the literature and in clinical practice are the low-dose step-up and, more recently, step-down protocols (Fig. 28-9). The initially described standard step-up protocol had a starting dose of FSH 150 IU/day.[110] However, this regimen was associated with a high complication rate. Multiple pregnancy rates of up to 36% were reported, and ovarian hyperstimulation occurred in up to 14% of treatment cycles.[2] As a result, this protocol has been largely abandoned.

The concept of the FSH threshold proposed by Brown[111] postulated that FSH concentrations must exceed a certain level before follicular development will proceed (see Fig. 28-1). Once this level is reached, normal follicular growth requires only a minor further increase above this threshold. Exposure to excessive FSH serum concentrations may lead to excessive follicular development. This concept formed the theoretical basis for low-dose step-up regimens for ovulation induction. A low-dose, step-up protocol designed to allow the FSH threshold to be reached gradually has now become the most widely used regimen, reducing the risk of excessive stimulation and development of multiple preovulatory follicles. The initial starting dose of FSH is 37.5 to 50 IU/day.[84] The dose is increased by 50% if after 14 days, no response is observed on ultrasonography (and serum estradiol monitoring).[84] The detection of an ovarian response is an indication to continue the current dose until hCG can be given to trigger ovulation. If equal daily doses of FSH are given from the beginning of the follicular phase, steady-state serum FSH concentrations are reached after 5 to 7 days.[112] During step-up regimens elevated FSH serum concentrations may occur during the late follicular phase which may, in a similar manner, interfere with selection of a single dominant follicle. Previous suppositions that steroid negative feedback remained intact during low-dose step-up regimens have not been substantiated by scientific data.

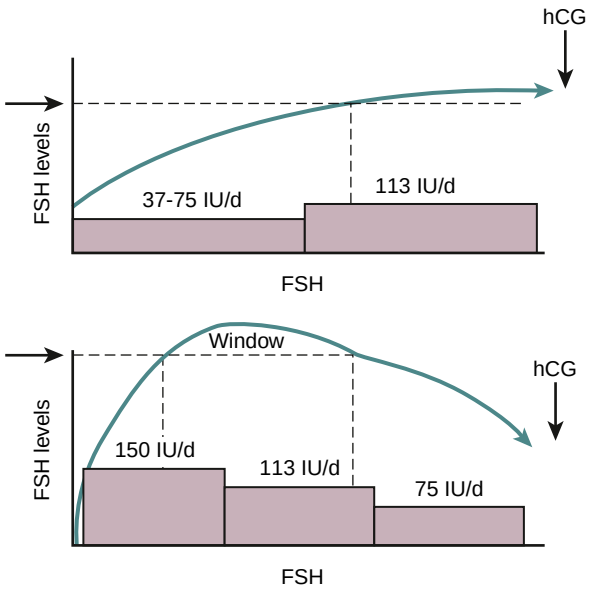


Figure 28-9. *Schematic representation of serum follicle-stimulating hormone (FSH) levels and daily dose of exogenous FSH during low-dose step-up or step-down regimens for ovulation induction.*

In contrast to the concept of the FSH threshold on which the low-dose step-up protocol is based, the concept of the FSH "window" stresses the significance of the duration of FSH elevation above the threshold level, rather than the magnitude of elevation of FSH for single dominant selection.[13,113] This concept was substantiated by the demonstration that elevating FSH levels high above the threshold level for a short period of time in the early follicular phase does not increase the number of dominant follicles[114] (Fig. 28-10). Conversely, when the physiologic decrease of FSH in a normal cycle is prevented by administration of FSH in the late follicular phase, the augmented sensitivity for FSH allows several follicles to gain dominance[115] (Fig. 28-11). As demonstrated previously in the monkey model, when the negative feedback effect of estradiol on gonadotropin production is suppressed by administration of anti-estrogens, selection of the preovulatory follicle is overridden.[116] Further studies regarding the process of selection of the dominant follicle in the normal cycle have indicated that throughout the cycle up to 10 nondominant follicles (measuring between 2 and 10 mm in diameter) can be visualized by transvaginal ultrasound. The dominant follicle itself can be identified once it has reached a diameter beyond 9 mm.[9] Endocrine studies have confirmed that E_2 levels in the serum[10] and follicle fluid[21] begin to rise only after a dominant follicle is present. The above-mentioned initial research findings provided the theoretical basis for developing and monitoring a step-down regimen of ovulation induction.

It was subsequently demonstrated that the late follicular phase FSH profile during a step-down regimen closely resembled serum FSH levels in the spontaneous cycle.[117] Moreover, a median daily fall of 5% to 10% in serum FSH levels was observed in women treated with the step-down regimen, and in those treated with a low-dose step-up regimen a reduction was observed in just 39% of treated women.[118] In the majority of women, the FSH levels remained stable in the late follicular phase.

With the aim of rapidly achieving the FSH threshold for stimulating follicle development, step-down regimens normally begin therapy with 150 IU/day, started shortly after a spontaneous or progesterone-induced bleed. This dose is continued until a dominant follicle (≥10 mm) is observed. The dose is then decreased to 112.5 IU/day followed by a further decrease to 75 IU/day 3 days later, which is continued until hCG is administered to induce ovulation.[119] Should no ovarian response be observed after 3 to 5 days, the FSH starting dose should be continued.

For some patients an initial dose of 150 IU/day is too high, reflecting major individual differences in the FSH

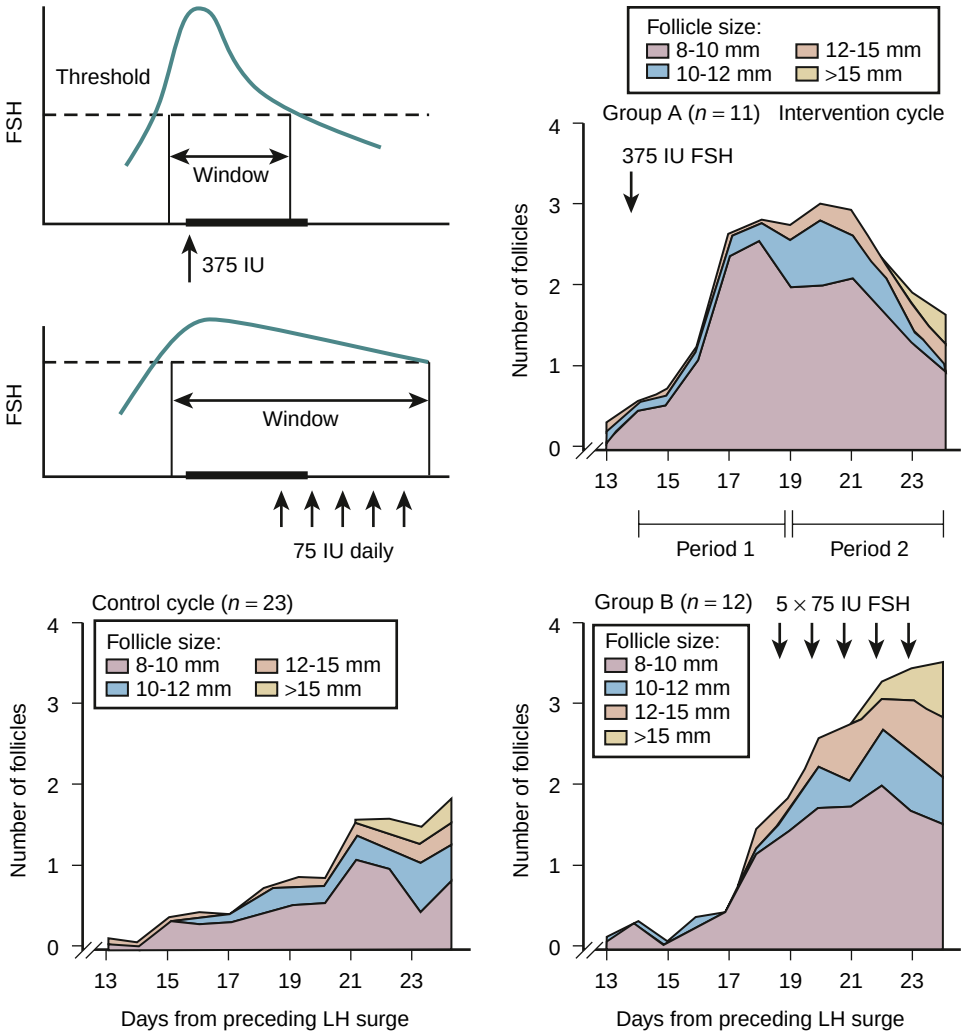


Figure 28-10. *Observations substantiating the follicle-stimulating hormone (FSH) threshold/window concept in the human. Differences in follicle growth response in normo-ovulatory women can be observed to be dependent on whether exogenous FSH is administered either as a single bolus during the early follicular phase* (top left) *or as daily low doses during the mid-late follicular phase. LH, luteinizing hormone. (From Schipper I, Hop WC, Fauser BC. The follicle-stimulating hormone [FSH] threshold/window concept examined by different interventions with exogenous FSH during the follicular phase of the normal menstrual cycle: duration, rather than magnitude, of FSH increase affects follicle development. J Clin Endocrinol Metab 83:1292-1298, 1998.)*

threshold. The appropriate starting dose may be determined by using the low-dose step-up regimen for the first treatment cycle in order to assess the individual FSH response dose.[120] Patients who demonstrate good follicular growth with a fixed regimen of 75 IU/day, (the "good responders" who might have been at risk of OHSS with the normal starting dose of the step-down regimen) can thus be identified. Conversely, those who do not respond with ongoing follicle growth to the initial dose should have the daily dosage increased. The second cycle may then be initiated as a step-down regimen with a starting dose 37.5 IU above the effective dose in the preceding low-dose step-up cycle.[119]

Experience to date has indicated that the major drawback of the step-down regimen is the risk that the initial starting dose is too high for some patients. In an effort to overcome this problem, sequential low-dose step-up and step-down regimens have been proposed.[121] Starting with a step-up regimen, the FSH dose is reduced when the leading follicle has reached 14 mm diameter. Comparisons with a group treated with a low-dose step-up regimen showed the incidence of monofollicular cycles to be similar. This approach requires further evaluation and the problem of the large individual variability in the dose of exogenous FSH required for monofollicular development remains to be properly addressed.

Clinical Outcomes. In what remains one of the largest series describing outcomes using the low-dose step-up regimen, 225 women with PCOS, with ovulation and pregnancy rates of 72% and 45%, respectively, were reported.[122] Studies focusing on further reducing the starting dose have reported the feasibility of commencing with 50 IU or 37.5 IU.[119]

In a randomized trial comparing outcomes following the low-dose step-up versus step-down protocol, the clinical benefits of a more physiologic means of stimulating follicle development were reflected in an incidence of monofollicular cycles of 88% compared to 56% observed in women

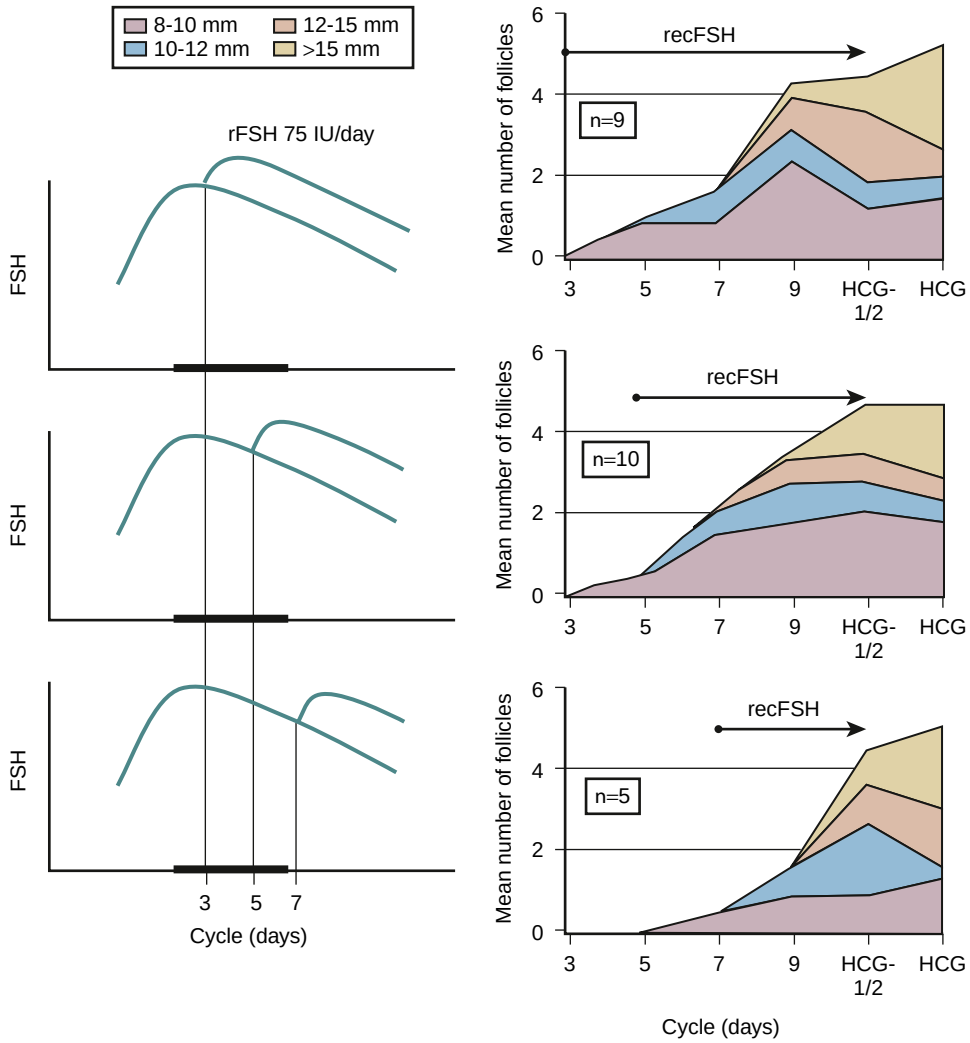


Figure 28-11. *Observations substantiating the follicle-stimulating hormone (FSH) window concept in the human. Multiple dominant follicle growth can be observed in normo-ovulatory women receiving daily low doses of exogenous FSH, starting on cycle day 3, 5, or 7. (From Hohmann FP, Laven JS, de Jong FH, et al. Low dose exogenous FSH initiated during the early, mid or late follicular phase can induce multiple dominant follicle development. Hum Reprod 16:846-854, 2001.)*

treated with the step-up regimen, presumably reducing the risk of multiple pregnancy and hyperstimulation[118] (Table 28-2). Potential health and economic benefits were also apparent because those treated with the step-down regimen required a mean duration of treatment of just 9 days, as opposed to 18 days in women treated with the low-dose step-up regimen.

A multicenter randomized study comparing the step-up versus step-down protocol using recFSH reported a shorter duration of stimulation when the step-down protocol was used.[123] The cumulative rate of clinical gestations did not differ between the two groups, but in contrast to the findings of an earlier single-center study,[118] the step-up protocol was associated with a higher rate of monofollicular development and a lower rate of ovarian hyperstimulation (see Table 28-2). These differences may reflect the necessity for increased skill and care in monitoring step-down stimulation cycles, which is easier to ensure in a single-center setting. Largely for this reason, low-dose step-up protocols remain the most widely used approach.

The degree to which the type of FSH compound employed may influence outcomes in ovulation induction continues to be subject of some controversy. Two meta-analyses comparing the effectiveness of daily uFSH to daily hMG for inducing ovulation in women with PCOS who had not responded to CC demonstrated no difference in pregnancy rate per treatment cycle. However, the women given FSH were less likely to have moderately severe or severe ovarian hyperstimulation syndrome.[50] The total dose of recFSH needed and duration of treatment was less, and the complication rates were similar. In a later meta-analysis of randomized controlled trials comparing recFSH with uFSH for ovulation induction in women with CC-resistant PCOS, no significant differences were demonstrated for the ovulation rate (OR 1.19, 95% CI 0.78-1.8). Furthermore, the odds ratios for pregnancy rate (0.95, 95% CI 0.64-1.41), miscarriage rate (1.26, 95% CI 0.59-2.7), multiple pregnancy rate (0.44, 95% CI 0.16-1.21), and ovarian hyperstimulation syndrome (1.55, 95% CI 0.5-4.84) showed no significant difference between recFSH and uFSH.[124] In terms of

TABLE 28-2

Comparison of Ovarian Response and Clinical Outcome Following the Low-Dose Step-Up and the Step-Down Regimens for Gonadotropin Induction of Ovulation

	Low-Dose Step-Up			Step-Down	
Response and Outcome	*Hamilton (1991)*	*Hull (1991)*	*Christin-Maitre (2003)*	*Christin-Maitre (2003)*	*van Santbrink (1995)*
Number of patients	100	144	44	39	82
Number of cycles	401	459	85	72	234
Duration treatment (days)	14	NR	15	10	11
Ampules per cycle	19	NR	13	13	14
Ovulation rate (%)	72	74	70	61	91
Monofollicular cycles					
% of ovulatory cycles	73	NR	77	35	62
% of all started cycles	55	NR	68	32	56
Pregnancy rate (%)					
Per started cycle	11	11	19	16	16
Per ovulatory cycle	16	15	NR	NR	17
Cumulative pregnancy rate (%)	55	NR	37	31	47
Multiple pregnancy rate (%)	4	11	12	25	8
Ongoing singleton pregnancy rate (%)	7	10	NR	NR	12
Ovarian hyperstimulation syndrome rate (%)	1	NR	2	11	2

NR, not recorded.

cost-effectiveness, a recent randomized study showed that a lower total dose of recFSH than highly purified urinary FSH was required to achieve the same outcomes. This translated into a 9.4% cost reduction in favor of recFSH.[125]

The success in early clinical studies of pure FSH preparations, increasingly devoid of LH, has served to enhance the impression that excess LH is detrimental to oocyte development and chances of pregnancy following therapeutic intervention. However, a number of recent clinical studies, together with an increasing understanding of the function played by LH in oocyte maturation, have begun to redefine the role of LH as a therapeutic agent in anovulatory fertility. In normogonadotropic anovulation, endogenous LH does not normally require supplementation. Indeed, the focus on LH in this group of patients has been primarily directed at reducing the potential detrimental effects associated with excessive LH.[126] More recently, however, the demonstration of the importance of late follicular LH in optimizing dominant follicle development oocyte quality has reopened the debate as to the role of LH in ovulation induction.[18] Supplementation of LH activity may offer advantages in some patients by hastening large follicle development and therefore shortening the duration of treatment.[127] Moreover, the work of Zeleznik and co-workers[17] referred to a potential therapeutic role for LH in effecting monofollicular stimulation as part of a sequential ovarian stimulation protocol following initiation with recFSH. This concept has been supported in a study in which anovulatory women with a hyper-reponse to recFSH were randomized to continue treatment with the addition of either placebo or recLH.[128] In those in whom LH was administered, a trend toward fewer preovulatory follicles was observed. As the availability of recombinant gonadotropins leads to increasing knowledge of the processes of follicular development and selection, further refinements in the efficacy and safety of ovulation induction are likely.

Adverse Effects and Complications. The complications of ovulation induction with gonadotropins are primarily related to excessive ovarian stimulation. Although the aim of therapy is monofollicular growth, multiple follicular developments may occur, causing symptoms of OHSS. Moreover, the development of multiple follicles raises the real risk of multiple pregnancies. In order to increase the chance of therapeutic success and reduce the risks of complications, careful monitoring of treatment is required. Ovarian response to gonadotropin therapy is monitored using ultrasound to measure follicular diameter. The scans, usually performed every 2 or 4 days, should be focused on identifying follicles of intermediate size; hCG (5000-10,000 IU subcutaneously or intramuscularly) is given on the day that at least one follicle measures more than 18 mm. If more than three follicles larger than 15 mm are present, stimulation should be stopped, hCG withheld, and use of a barrier contraceptive advised in order to prevent multiple pregnancies and OHSS. Measurements of serum E_2 may also be useful.[119] Ovarian stimulation with gonadotropins has not been shown to be associated with long-term risks. Urinary derived FSH is associated with a theoretical risk of transmission of prion proteins. However, the risk of infection is considered to be minimal and not in itself a reason to prescribe recFSH over uFSH.[129]

Pulsatile GnRH

In the normo-ovulatory woman, the pattern of GnRH pulse stimulation alters with the phase of the menstrual cycle, effecting differential gonadotropin synthesis and secretion.[130]

During the luteal-follicular transition, pulses occur every 90 to 120 minutes. This slow pulse frequency, in the presence of low E_2 and inhibin A levels, favors FSH production. In the mid- or late follicular phase GnRH frequency increases, favoring LH secretion.[131] In the luteal phase, the production of progesterone increases hypothalamic opioid activity, thus slowing GnRH pulse secretion. This again favors FSH secretion in the luteofollicular transition.

The application of pulsatile GnRH therapy has been demonstrated to be an effective reliable and safe alternative to gonadotropin therapy for treating this form of anovulation.[132] Due to the intact ovarian-pituitary feedback system during pulsatile GnRH treatment, the resulting serum FSH and LH concentrations remain within the normal range and the chances of multifollicular development and ovarian hyperstimulation are therefore low. Little ovarian response monitoring is therefore needed during treatment.

The intravenous route appears superior to the subcutaneous route.[133] In order to mimic the normal pulsatile release of GnRH, a pulse interval of 60 to 90 minutes is used with a dose of 2.5 to 10 μg per pulse.[133] The lower dose should be used initially in order to minimize the likelihood of multiple pregnancies.[134] The dose should then be increased to the minimum dose required to induce ovulation. Pulsatile GnRH administration may be continued throughout the luteal phase until menses or a positive pregnancy test. Alternatively, it may be discontinued after ovulation, and the corpus luteum supported by hCG.[43]

Clinical Outcomes. Pulsatile GnRH administration is primarily indicated for women with hypogonadotropic hypogonadal anovulation (WHO class 1) who have normal pituitary function.[135] In these patients cumulative pregnancy rates of 83% to 95% after six cycles have been reported, with multiple pregnancies accounting for 3% to 8% of pregnancies.[134,136] Lower ovulation and pregnancy rates have been observed in women with WHO type 2 anovulation, including PCOS.[137] This may be because anovulation in PCOS in part reflects the effects of a persistant, rapid frequency of GnRH stimulation of the pituitary, causing increased LH and T levels.[131] In a recent meta-analysis of four trials comparing pulsatile GnRH with gonadotropins for ovulation induction in women with PCOS, the small size and short follow-up of the studies meant that the authors were unable to draw conclusions on their relative effectiveness.[138]

Regular menstruation occurring approximately every 4 weeks indicates that the woman is having ovulatory cycles. Ultrasonography and measurements of serum progesterone are not usually needed for monitoring therapy. Local complications such as phlebitis may occasionally be encountered when intravenous administration is used. To avoid this, pulsatile GnRH can be administered subcutaneously. This route is certainly simpler than the intravenous approach. However, pharmakinetic studies comparing the two routes have shown that the plasma GnRH profiles are damped after subcutaneous administration, and that bioavailability is reduced.[139] However, the increased convenience offered by the subcutaneous route has led to this approach being in favored by many.

Aromatase Inhibitors

In recent years the concept of using aromatase inhibitors to mimic the action of CC has been proposed.[140] Rather than antagonizing estrogen feedback activity at the hypothalamic-pituitary axis, this approach aims at reducing the amount of estogens being synthesized. Aromatase inhibitors block the conversion of AD and T to estriol (E_3) and estrodiol (E_2), respectively.[141] This increases gonadotropin secretion, resulting in stimulation of ovarian follicles.[140] Aromatase inhibitors have been in clinical use for more than 20 years, primarily in the treatment of postmenopausal patients with advanced breast cancer. The more recently developed third generation of aromatase inhibitors are characterized by their potency in inhibiting the aromatase enzyme without significantly inhibiting inhibition of other steroidogenesis enzymes. One of the third generation compounds, letrazole, has been the focus of study as a potential therapeutic agent for inducing ovulation.

Clinical Outcomes. When given in the early follicular phase, letrozole reduces estrogenic feedback at the pituitary-hypothalamic axis, causing a subsequent increase in gonadotropin secretion. This was shown in monkeys to stimulate follicle development.[142] Subsequent small clinical studies employing a dose of 2.5 mg/day from day 3 to day 7 of the menstrual cycle have suggested that it may be an effective ovulatory agent in CC-resistant women.[140] A local effect at the ovary to increase sensitivity to FSH by blocking the conversions of androgens to estrogens has also been proposed, because accumulating intraovarian androgens may increase FSH receptor gene expression.[143] On the other hand, significantly increased intraovarian androgen/estrogen ratios may also induce follicle atresia.

Although the concept of applying aromatase inhibitors as an alternative to CC or as adjuvant therapy to CC or gonadotropins is attractive, and preliminary data on pregnancy outcome were encouraging,[140,144] adequately powered comparative controlled randomized studies are still awaited.

Adverse Effects and Complications. Although letrozole has a half-life that allows rapid disappearance following cessation of treatment in the midfollicular phase, the possible effects of this drug on ensuing pregnancy remain to be clarified. The performance of further clinical studies has been inhibited because an association was reported between letrozole and fetal toxicity. However, a recent analysis of outcomes in 911 newborns following conceived by CC or letrozole showed no difference in the overall rates of major and minor congenital malformations.[145] In the absence of sufficiently powered, randomized controlled trials establishing efficacy and safety, the routine clinical use of aromatase inhibitors is not advocated.[84]

Opioid Antagonists

Background. Endogenous opioid peptides have been shown to play an important role in regulating the pulsatile secretion of gonadotropins by inhibiting the hypothalamic

pulse generator that directs GnRH secretion.[146] Infusion of the opiate receptor antagonist naloxone was shown to increase serum LH levels when administered during the late follicular and luteal phase of the cycle.[147]

Clinical Outcomes. Several groups have used naltrexone, an orally active opioid receptor antagonist, to treat ovulatory disorders, with varying degrees of success. The earlier observation that gonadal steroids enhance opioid modulation of gonadotropin secretion was postulated to explain the inability of two groups to demonstrate an increase in gonadotropin secretion or resumption of ovulation in women with WHO class 1 anovulation.[148] However, others have observed restoration of the menstrual cycle.[149] In an uncontrolled study, 19 of 22 women with CC-resistant anovulation were observed to become ovulatory under naltrexone treatment (sometimes in combination with CC), with 12 conceiving.[150] Treatment with 25 mg twice daily was commenced on the first day of a spontaneous or progesterone-induced cycle and continued until a positive pregnancy test occurred or, if no response was observed, for 21 days of treatment. Others have employed doses of up to 100 mg/day.[151] Conclusions as to the efficacy, optimal regimen, and safety of opiate antagonists for inducing ovulation cannot yet be made, however. No randomized controlled studies demonstrating their value for this condition have yet been published, and opiate anatagonists remain at best second-line, alternative therapy.

Dopamine Agonists

These agents are primarily used in the treatment of anovulation secondary to hyperprolactinemia. The treatment of hyperprolactinemia and the agents available for treatment are covered in detail elsewhere (see Chapter 3).

ADJUNCTIVE THERAPIES

Dexamethasone

Glucocorticoids have been proposed as a useful adjuvant to both CC and gonadotropin ovulation induction in women with PCOS with a therapeutic rationale based on reducing ovarian androgen levels, improving ovulatory function, and reducing resistance to ovulation induction agents.[152] Although the source of high androgen secretion in anovulatory women with PCOS is primarily ovarian, 50% to 70% also demonstrate excessive adrenal androgen levels.[152]

In order to normalize (without suppressing) adrenal steroid production, daily oral doses of dexamethasone (0.25-0.5 mg) or prednisone (5-10 mg) have been employed in a continuous regimen. Although widely used, the value of adjuvant corticosteroid administration with CC or gonadotropins for ovulation induction remains uncertain. In a study of women with PCOS, the chance of ovulation after glucocorticoid suppression of adrenal androgens was not predicted by either basal DHEAS (dehydroepiandrosterone sulfate) levels or suppressed levels, and limited effects on ovulation were observed.[153] A randomized controlled study in 80 women with CC resistance and normal serum DHEAS levels showed significantly higher ovulation and pregnancy rates when 2 mg/day dexamethasone was added from cycle day 2 to 12 to CC 100 mg.[154]

While major complications from the adjuvant use of low-dose glucocorticoids are rare, weight gain is a common problem. Other reported side effects include glucose intolerance and osteoporosis. Given possible side effects, their use should remain as a second-line therapy subject to further research.

Gonadotropin-Releasing Hormone Agonists

Adjuvant GnRH agonist treatment has also been proposed to improve outcomes and reduce complications of ovulation induction. Early uncontrolled studies indicated that the concomitant use of GnRH agonist with ovarian stimulation regimens in women with PCOS was safe and improved treatment outcome.[155] Further studies indicated that premature luteinization could be prevented by employing GnRH agonists, but no clear difference in pregnancy rates was demonstrated.[156] Although, a meta-analysis of five prospective studies[157] suggested that improved pregnancy rates could be achieved at similar ovulation rates when GnRH agonists were also employed, a later systematic review concluded that GnRH agonist as an adjunct to FSH/hMG does not improve pregnancy and OHSS rates, and should therefore not be recommended as a standard treatment for this patient group.[158]

Conflicting data on the effects on ovulation and pregnancy rates, combined with reports of severe OHSS with adjuvent GnRH agonist therapy and the additional burden for the patient of prolonged treatment cycles, mean that adjuvant GnRH agonists remain a second-line therapy in conjunction with FSH stimulation.

The availability of GnRH antagonists provides new opportunities to modify ovulation induction regimens. Particular attributes of GnRH antagonists which might be of value in this context include their competitive binding properties, immediate suppression of the pituitary without a flare-up effect, and rapid resumption of gonadal function on discontinuation. However, few studies have appeared which further explore its role in this clinical context.

ADDITIONAL TREATABLE FACTORS INFLUENCING THE BALANCE OF EFFICACY AND RISKS

Obesity

Among women with WHO class 2 anovulation, obesity may be present in up to 50%. In addition to enhancing the features of insulin resistance mentioned earlier, overweight (BMI > 32) is also associated with reproductive dysfunction, despite regular menstrual cycles.[159] In recent years, considerable attention has been given to the role of lifestyle factors and management in improving outcomes in obese anovulatory women. Even a small (2% to 5%) reduction in weight has been shown to improve metabolic indices including insulin resistance.[160] In addition, weight loss can lead to a rise in sex hormone–binding globulin (SHBG) concentrations, a decrease in FAI and T levels, and improvement in

cyclicity.[161-163] A relatively modest reduction in weight has been shown to increase the frequency of ovulation in obese anovulatory women to more than 70%.[164] Energy restriction acting to temporarily improve insulin senstivity may be important,[163] because improvements in endocrine and clinical parameters occurred maximally during the period of energy restriction. During subsequent weight maintenance, many benefits were reversed.[163]

The evidence for the benefits of weight loss, combined with recent data confirming BMI to be a major factor influencing outcome of ovulation induction,[165] make the treatment of obesity an important adjuvant treatment that should precede ovulation induction.[84] Given the baseline risks of ovulation induction, and the possible risks of obesity for subsequent pregnancy and general health, weight loss in cases of obesity should be considered as a prerequisite to medical ovulation induction treatment.[166-168]

Tobacco Smoking

Epidemiologic data provide strong evidence for a causal association between cigarette smoking and decreased fertility. For a recent review of the impact of smoking and other lifestyle factors on fertility treatment outcomes, see Homan et al.[168] Dose-dependent effects of smoking have been reported in relation to the duration to conception.[169] Moreover, there is evidence of increased risk of early pregnancy loss in smokers[170] and a reduced mean age at menopause.[171] Although properly designed studies of the effect of smoking on outcomes of ovulation induction are scarce, data from studies in assisted conception point to detrimental effects on ovarian function and oocyte quality, which are likely to be applicable to the situation concerning ovulation induction.[172] In any discussion of infertility therapy, the clinician should emphasize the risks of smoking for outcome of treatment. Indeed, preconceptional care and lifestyle advice should be an integral part of the modern fertility clinic.[168]

Ovarian Stimulation in the Empirical Treatment of Unexplained Infertility

PRINCIPLES OF OVARIAN STIMULATION

The aim of ovarian stimulation is to intervene in the mechanisms regulating single dominant follicle selection in order to mature multiple follicles and obtain multiple oocytes for fertilization in vivo (either after timed intercourse or IUI) or in vitro (IVF). Ovarian stimulation is usually performed in normo-ovulatory infertile women in order to increase chances for pregnancy. However, the development of multiple follicles inherently also increases the undesired risk of (higher order) multiple pregnancies and OHSS. In IVF OHSS risks are reduced because of the puncture of all visible large follicles to retrieve the oocytes, and the incidence of multiple pregnancies can be controlled by limiting the number of embryos transferred.

Obviously, oligo/anovulatory women may also qualify for either IUI or IVF after failed ovulation induction. Hyperstimulation may also be performed in these women, aiming at multiple follicle development. It should again be emphasized that this condition of hyperstimulation in these patients is distinctly different from ovulation induction in which the aim is to mimic physiology and stimulate ongoing growth and ovulation of a single dominant follicle. However, these patients are usually difficult to manage because of an unpredictable major individual variability in response and a tendency to hyper-respond to stimulation protocols.[173]

Although daily administration of ovary stimulating agents allows for dose adjustments based on individual ovarian response monitoring, the clinical evidence for the efficacy of such an approach is scant. A hyper-response may be counteracted by a dose decrease or the complete cessation of exogenous gonadotropins for some days (the latter strategy is referred to as "coasting").[174] An excessive number of follicles for ovulation induction or hyperstimulation for IUI may be reduced by follicle puncture[175] or cycle cancellation. When, in contrast, low ovarian response to standard stimulation is observed, recent evidence indicates that a gonadotropin dose increase does not result in improved outcome.[176] This is not surprising if the pathophysiologic background of low response is taken into consideration. Low response to ovarian hyperstimulation may be the first sign of advanced ovarian aging.[177] Women with a previous low response to hyperstimulation have been shown to enter menopause at an earlier age.[178]

During the normal menstrual cycle the mid-cycle LH surge represents the trigger for inducing final oocyte maturation, the rupture of the follicle and release of the oocyte, and finally luteinization of granulosa and theca cells allowing for the formation of the corpus luteum. As mentioned before, the synchrony of endocrine events inducing the LH surge is disrupted in ovarian hyperstimulation. Therefore, the endogenous LH surge is replaced by an exogenous hCG bolus injection, timed by the visualization of large graafian follicles upon ultrasound. Finally, these follicular phase interventions result in luteal phase abnormalities[179] requiring luteal phase supplementation by either hCG or exogenous progestins.

THERAPEUTIC APPROACHES

Unexplained infertility is usually diagnosed by exclusion, when standard infertility investigation shows no abnormalities. However, no agreement exists with regard to the preferred extent of standard investigation as well as the interpretation and prognostic value of many of these tests. Usually, ovulation is assessed by a mid-luteal phase serum progesterone assay, tubal patency is established by hysterosalpingogram, and male factor infertility excluded by semen analysis. Again, the interpretation of any of these tests is not without difficulty and many clinicians perform additional tests to further explore possible causes of infertility.[53] Hence, the term *unexplained infertility* is notoriously ambiguous and may mean anything in between undiagnosed infertility and normal fertility in which a pregnancy did not occur merely by chance. This may especially be the case in young women who have been attempting to conceive for a relatively short time.[56]

It should be realized that many biologically relevant processes important for obtaining a pregnancy—such as oocyte chromosomal constitution, subtle sperm abnormalities, in vivo conception, embryo transport and nidation, and finally endometrial receptivity—cannot be studied accurately as yet. It is to be expected that with the advancement of our understanding of these processes, the percentage of couples diagnosed with unexplained infertility, and therefore potential need for empirical ovarian hyperstimulation, will decrease.

When a couple presents with unexplained infertility, it is extremely important to assess chances of spontaneous pregnancy before commencing on any kind of empirical therapy. As mentioned before, ovarian hyperstimulation (with or without additional interventions such as IUI) may enhance pregnancy chances per cycle, but at the cost of patient stress and discomfort, chances for side effects such as multiple gestation and OHSS, and high costs[180,181] (see also Fig. 28-5). Similar cumulative pregnancy rates may be achieved with expectant management for 6 to 12 months.[56] Expectant management may represent the most favorable approach in young women with a short duration of infertility.

Results are frequently reported from combined interventions such as ovarian hyperstimulation and IUI. These studies are often uncontrolled, and few are sufficiently powered to differentiate between the independent effects of hyperstimulation and IUI and the potential additive effects of combining both interventions. In recent years, the picture has become clearer. Although the absolute treatment effect appears relatively limited, given the low cost and ease of administration, CC can be recommended as first choice medication for the treatment of unexplained infertility. In terms of pure efficacy, however, a meta-analysis of five trials indicated that gonadotropins may be superior to CC as ovarian stimulation agents for the treatment of unexplained infertility.[182] Treatment with CC was associated with significantly reduced odds ratios of pregnancy per woman compared to gonadotropins (OR 0.41; 95% CI 0.17-0.8). As far as complications are concerned, no significant differences could be found for miscarriage (OR 0.61; 95% CI 0.09-4) or multiple birth (OR 1.1; 95% CI 0.2-7). The incidence of OHSS or cycle cancellation rates could not be assessed.

For unexplained infertility, the combination of IUI with ovarian hyperstimulation potentially bypasses several possible barriers to fertility, including minor sperm abnormalities, sperm-cervical mucus interactions, timing of sperm delivery problems, and a possible beneficial effect of ovarian stimulation on endometrial receptivity. The most important benefit is likely to be the stimulation of multiple follicles. Although a meta-analysis by Hughes[183] has addressed questions relating to the benefits of FSH and IUI alone compared with combined therapy, less than a third of the studies included in the analysis make use of treated control subjects. Moreover, the conclusions that both FSH and IUI improve fecundity are derived from regression analysis and are open to discussion.[59,184] Other studies have indicated that ovarian hyperstimulation with both CC and gonadotropins improve the fecundity rate compared to IUI alone.[59] However, a study comparing intracervical insemination alone with FSH in combination with IUI showed a statistically higher pregnancy rate with the latter treatment combination.[55] The number needed to treat was 31 cycles. This implies that it would take 31 cycles of treatment before there would be one more singleton live birth with FSH/IUI than with intracervical insemination alone.[185] The number needed to treat of FSH in combination with IUI in order to obtain an extra pregnancy above that obtained with IUI alone is even greater.[185] When the costs of multiple pregnancies arising from multiple follicle development are taken into account, the cost effectiveness of FSH/IUI combined therapy for this indication may be limited. Cost-effectiveness analyses have led to the conclusion that IUI with or without hyperstimulation should precede IVF.[186] In clinical practice the benefits of ovarian hypertimulation in combination with IUI need to be weighed against the additional discomfort and costs of monitoring applied, often unsuccessfully,[58] to avoid multiple pregnancy. Clearly, more studies are needed to elucidate the optimal approach to treating unexplained infertility, and the role ovarian hyperstimulation should play. Although it is increasingly recognized that treatment success should be defined in terms of cumulative multiple cycles, at present cumulative live birth rates remain poorly reported, and comparisons with expectant management after multiple cycles have not been made.

PREPARATIONS FOR OVARIAN STIMULATION

Clomiphene Citrate

Preparations and Regimens. Daily doses of 50 to 100 mg are applied usually from days 5 until 9[26] and ovulation is triggered by exogenous hCG. Little ovarian response monitoring is required, and luteal support is probably not necessary.

Clinical Outcome. A retrospective analysis of 45 published reports conclude that the adjusted pregnancy rate per initiated cycle is 5.6% for CC alone, versus 8.3% for CC plus IUI compared to an estimated pregnancy rate from expectant management of 1.3%.[187] A meta-analysis on the basis of six randomized trials[188] concluded that CC administration was superior to no treatment, with an odds ratio for clinical pregnancies of 2.4 (95% CI 1.2-4.6) per patient and 2.5 (1.4-4.6) per cycle. As stated before, an earlier meta-analysis[183] indicated an independent significant improvement in pregnancy rates for clomiphene, exogenous FSH, and IUI.

Adverse Effects and Complications. Adverse effects include hot flushes, mood swings, headache, and visual disturbances. The principal complication remains multiple pregnancy, which occurs in around 10% of pregnancies, and a slightly increased chance for OHSS. Long-term use of CC (>12 months) may be associated with a slight increase in the risk of ovarian epithelial cancer.[90]

Gonadotropins

Preparations and Regimens. Usually exogenous gonadotropin administration is started around cycle day 3 to 5 at daily doses of 75 to 225 IU for several days in fixed

dose regimens. Thereafter, doses may be adjusted on the basis of ovarian response monitoring by ultrasound and or E_2 assays. The therapeutic window for gonadotropins achieving the desired goal (two to three preovulatory follicles) is rather small, and a considerable proportion of treatment cycles are canceled because of hyper-response (and the related increased chance of higher order multiple pregnancy) or because they fail to achieve multiple dominant follicle development. The need for cancellation is highly dependent on the stimulation protocol applied and the rigidity of cancellation criteria applied. This in turn depends on whether higher order multiple pregnancies are considered an acceptable side effect of treatment, or whether this should be seen as a failure of treatment to be prevented at any price. Moreover, premature luteinization during ovarian hyperstimulation for IUI may occur more frequently than generally assumed. This may have a detrimental impact on treatment outcome. Recent studies of GnRH antagonist co-treatment during gonadotropin hyperstimulation have demonstrated a reduced incidence of a premature LH rise but no significant improvement in pregnancy rates.[189] However, this approach renders ovarian stimulation protocols more complicated and expensive, increasing the frequency of hospital visits required for monitoring.

Clinical Outcome. A meta-analysis based on 5214 cycles reported in 22 trials concluded an odds ratio for pregnancies associated with FSH compared to expectant management of 2.35 (95% CI 1.9-2.9).[183] A retrospective analysis based on 45 previous papers concluded a significantly increased pregnancy rate occurred after either hMG alone (7.7%) or hMG plus IUI (17.1%).[187] A subsequent large multicenter study[55] confirmed that ovarian hyperstimulation with gonadotropins and IUI both exhibit an independent additive effect on pregnancy chances. The applied treatment regimen for ovarian hyperstimulation (150 IU/day FSH from cycle day 3 to 7) resulted in high frequency of conception. Overall cumulative pregnancy rates when this was combined with IUI therapy were reported to be 33% within three cycles, but at the price of an unacceptable high multiple pregnancy rate of 20% twins and 10% higher-order multiple pregnancy.[55] Women undergoing combined hyperstimulation and IUI were 1.7 times more likely to achieve a pregnancy in a given cycle compared to those receiving IUI alone. However, only 53% of these pregnancies resulted in a live birth with a substantial number of triplet and quadruplet births, despite the fact that fetal reduction has been applied in some of these women. Indeed, 30% of occurring pregnancies were multiples, including 9% triplets and quadruplets. No information was provided regarding perinatal mortality and morbidity rates.

Adverse Effects and Complications. Those effects relating to gonadotropins in general are discussed earlier. In the context of ovarian stimulation for the treatment of unexplained infertility, we again stress the risk of multiple pregnancy associated with the use of these drugs. The ability of careful monitoring to allow prevention of this complication is limited even in highly skilled hands,[58] and the decision to employ gonadotropins in the context of treating ovulatory women for unexplained infertility should be preceded by an open and informed discussion with the couple over the risks of treatment and the limitations of monitoring. It is clear that an individual approach is required when addessing these issues, and that there is a need to individualize treatment in order to ensure optimal outcomes.

Ovarian Stimulation for in Vitro Fertilization

THERAPEUTIC APPROACHES

The general aim of ovarian stimulation in this clinical context is to induce the development of multiple dominant follicles in order to be able to retrieve many oocytes to allow for inefficiencies in subsequent fertilization in vitro, embryo culture, and embryo selection for transfer and implantation (Fig. 28-12).[26] Hence, multiple embryos can be transferred in the great majority of patients and often spare embryos can be cryopreserved to allow for subsequent chances of pregnancy without the need for repeated ovarian stimulation and oocyte retrieval.[26] The paradigm of so-called "controlled" ovarian stimulation by high doses of exogenous gonadotropins and GnRH agonist long protocol co-treatment for IVF has constituted the gold standard for clinicians throughout the world since the early 1990s. It appears that large numbers of developing follicles is still considered a useful surrogate marker of successful IVF, whereas its significance in relation to the chance of achieving a pregnancy resulting in a healthy baby born is in doubt.[63,73,190] The ovarian stimulation protocols required to produce a large number of follicles have become extremely complex and costly over the years,[26,46] creating considerable side effects, risks of complications, and the need for intense monitoring of ovarian response.[191]

Physicians appear to be in control of ovarian stimulation owing to their ability to adjust the gonadotropin doses or the type of preparation on the basis of ovarian response monitoring. However, the major individual variability in response is out of the doctor's control and is an extremely important determining factor for both success and complications of IVF treatment.[192] A good ovarian response to standard stimulation indicates normal ovarian function and a good prognosis for successful IVF. A low ovarian response suggests ovarian aging and is therefore associated with poor IVF outcome. A low response can to some extent be predicted by chronological age and endocrine and ultrasound aging parameters assessed before the initiation of treatment, as will be discussed later.[178,193] However, the widely applied approach to increase gonadotropin doses administered in case of insufficient ovarian response has very little scientific foundation.[176] The occurrence of a severe hyper-response comes as a surprise in most cases and therefore cannot be predicted.[49,174] Severe OHSS is induced by hCG and is therefore associated with pregnancy. This can be prevented from happening by refraining from embryo transfer in the cycle at risk and cryopreserving all available embryos for transfer in another cycle.

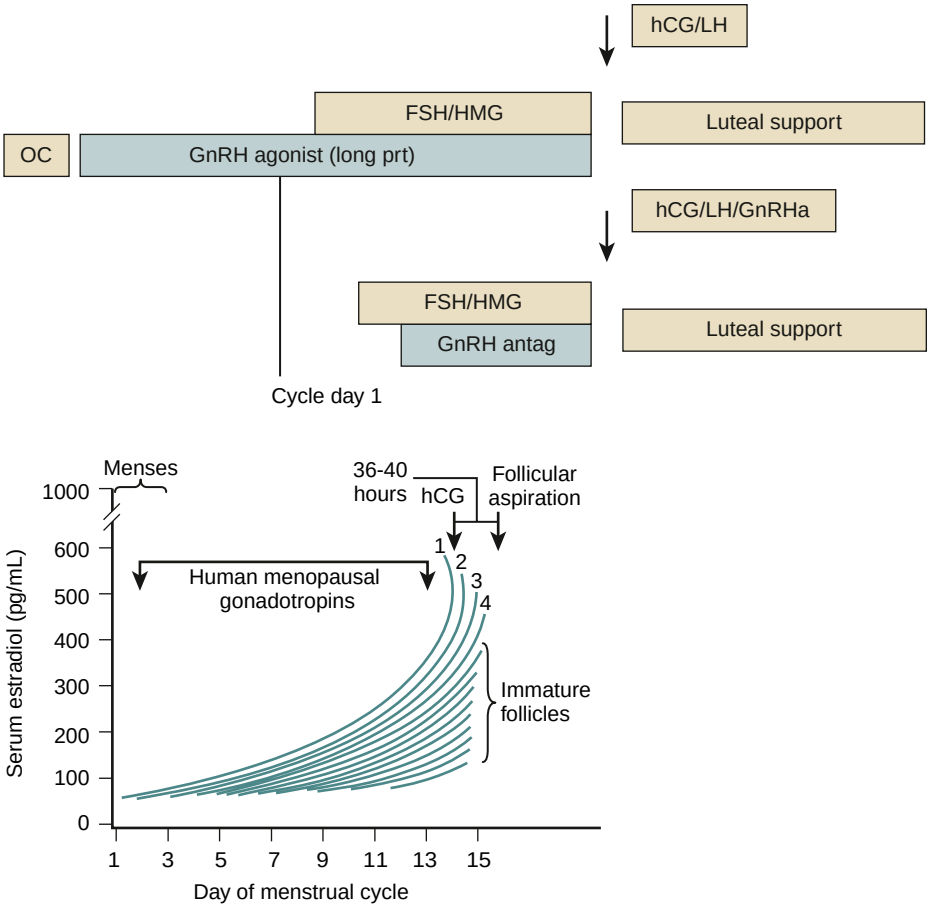


Figure 28-12. *Schematic representation of complex medication regimens involved in ovarian hyperstimulation for in vitro fertilization* (top)*, and the heterogeneous cohort of recruited and selected follicles* (bottom)*. antag, antagonist; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HMG, human menopausal gonadotropin; LH, luteinizing hormone; OC, oral contraceptives; prt, protocol. (Graph from Oehninger S, Hodgen GD. Introduction of ovulation for assisted reproduction programmes. Baillieres Clin Obstet Gynecol 4:451-573, 1990.)*

Slowly, ovarian stimulation protocols have shifted from the use of hMG to uFSH to recFSH.[194] In recent years several groups have focused on the potential significance of late follicular phase LH levels for clinical IVF outcome. Indeed, it has been shown that dominant follicle development can be stimulated exclusively by LH rather than FSH, opening new possibilities for therapeutic interventions,[18] as discussed in more detail later.

Despite the fact that the first child born after IVF was conceived in a spontaneous menstrual cycle, natural cycle IVF received little attention. The major focus has been the improvement of complex ovarian stimulation regimens. Natural cycle IVF offers major advantages such as negligible complications (arising from multiple pregnancy or OHSS), reduced patient discomfort, and a low cost. The efficacy of natural cycle IVF is hampered, however, by high cancellation rates due to premature ovulation or luteinization. A systematic review of 20 selected studies involving a total of 1800 cycles showed a 7.2% overall pregnancy rate per started cycle, and 16% per embryo transfer.[61] Cumulative pregnancy and live birth rates over four cycles of 42% and 32%, respectively, have been reported.[195] Despite the relatively high failure rate, the approach of natural cycle may still be cost effective. In one study, it was calculated that natural cycle IVF could be offered at 23% of the cost of a stimulated cycle.[195]

More recently a modified natural cycle[196] has been proposed, in which GnRH antagonists are instituted to prevent premature ovulation, and low-dose exogenous gonadotropin co-treatment is given as add-back to prevent a GnRH antagonist induced involution of follicle development. Using this approach, which (like natural cycle IVF) aims to achieve monofollicular development, cumulative pregnancy rates of 44% have been reported over 9 cycles of treatment.[197]

PREPARATIONS

Clomiphene Citrate

Background. After the first baby born following IVF in a natural cycle[33] four normal IVF pregnancies were reported following ovarian stimulation with CC.[29] In subsequent years, many groups reported IVF results following CC, with or without gonadotropin co-treatment.[198] Combined CC/hMG regimens were considered the standard of care before GnRH agonist co-treatment to induce pituitary down-regulation came into use. (For a comprehensive historical overview see reference 198.) The advantages of these combined regimens included reduced requirements for hMG and higher luteal phase progesterone levels alleviating the need for luteal phase supplementation.[26] Recent studies have reported clinical outcomes of combined regimens applying CC, gonadotropins, and GnRH antagonist.[26]

CC usually induces the development of at least two follicles, which may sometimes elicit a premature LH rise. By virtue of the fact that CC is therapeutically active through interference with estrogen feedback, this compound cannot

be combined with GnRH agonist co-treatment for prevention of a premature LH surge. Moreover, undesired antiestrogenic effects of CC at the level of the endometrium have been implicated by some in the observed discrepancy between relatively low embryo implantation rates coinciding with successful ovarian hyperstimulation.

Preparations and Regimens. CC administration is usually initiated on cycle day 2, 3, or 5, and given daily for 5 subsequent days with doses varying between 100 and 150 mg/day. In most applied regimens exogenous gonadotropin medication (150 IU/day) is initiated after cessation of CC. It seems that CC alone induces a limited but dose-dependent increase in the number of developing follicles. However, the addition of gonadotropins elicits a more intense ovarian response. Sufficiently powered randomized comparative trials to support one approach over the other are lacking.

Clinical Outcome. Reported outcome is variable in the literature, but in general pregnancy rates appear higher compared to natural cycle IVF, but lower compared to conventional gonadotropin/GnRH agonist protocols. Again, most studies are uncontrolled but an extensive summary of almost 40,000 cycles reported in the literature suggests an overall pregnancy rate per embryo transfer of 20.5%.[199]

Adverse Effects and Complications. Because of the relatively mild stimulation, the incidence of side effects or complications of CC treatment for IVF is low, as discussed earlier. Overall side effects are CC dose related and are completely reversible once medication is stopped.

Gonadotropins

Background. Gonadotropin preparations have been used for ovarian stimulation since the early days of IVF and were originally developed in the United States.[198] The daily administration of these preparations is usually efficacious in the induction and maintenance of growth of multiple dominant follicles, allowing for the retrieval of many oocytes for IVF. Preparations initially used were hMG (containing both LH and FSH bioactivity), followed by purified uFSH and more recently recFSH. No general consensus exists with regard to starting day and doses of gonadotropins. An overview of published randomized studies is given in Table 28-3. In conclusion, based on seven randomized controlled trials (RCTs) involving a total of 2563 cycles, although higher gonadotropin doses may result in the retrieval of 1 or 2 more oocytes, improved clinical outcomes in terms of pregnancy rates could not be demonstrated.

A chimeric FSH agonist (so-called recFSH-CTP), generated by the fusion of the carboxy-terminal peptide (CTP) of hCG (responsible for its prolonged metabolic clearance compared to LH) with the FSH-β chain has recently been undergoing phase 3 studies in IVF. The birth of a first healthy baby was reported in 2003 following the single injection of this novel compound in the early follicular phase of the cycle and a 7-day medication-free period (see Fig. 28-5).[41] Phase 2[200] and phase 3 studies are establishing the optimal dose and the clinical efficacy of this preparation in comparision to recFSH. It is anticipated that this latter development is going to represent a step forward in rendering stimulation regimens more patient friendly, but it is not to be expected that clinical outcome will improve.

The type, duration, and dosing of GnRH analog co-treatment to suppress endogenous pituitary gonadotropin release (as will be discussed later) may also affect the preferred gonadotropin preparation. Classical principles teach us that both LH and FSH are required for adequate ovarian estrogen biosynthesis and follicle development. Theca cell–derived androgen production (which is under LH control) is mandatory as a substrate for the conversion to estrogens by FSH-induced aromatase activity of granulosa cells.[26] A number of studies have indicated that excessively suppressed late follicular phase LH concentrations may be detrimental for clinical IVF outcome.[201,202] Under these circumstances the use of urinary preparations containing both LH and FSH activity or the addition of recLH or rechCG next to exogenous FSH may be useful.[26] It is uncertain as yet, however, for which patients this approach may be beneficial. Recent meta-analyses failed to show clinically relevant differences in relation to late follicular phase LH concentrations,[203] or when cycles with or without the addition of exogenous LH are compared.[204]

Recently the concept that exogenous LH is capable of selectively stimulating the development of the more mature dominant follicles has been developed. A shift from FSH to LH preparations during stimulation may therefore be useful in order to stimulate a more homogeneous cohort of follicles for IVF.[17,18]

Preparations and Regimens. To allow for the clinical introduction of recombinant FSH, large-scale, multicenter, comparative trials in IVF were published from 1995 onward.[205] It should be noted, however, that these studies, including several hundreds of women, were sponsored by pharmaceutical companies. The results should therefore be interpreted with an appropriate degree of caution. For instance, it was arbitrarily chosen for all initial studies that recFSH would only be compared with uFSH and not hMG, although the latter preparation was still considered to be the gold standard by the majority of clinicians. Several independent comparative trials have been published since then, but sample size of these single-center studies was usually insufficient to allow for the detection of relatively small differences. An early meta-analysis[206] as well as health economics studies[207,208] indicate a slightly improved outcome for recFSH compared to uFSH. In addition, a meta-analysis involving a limited number of IVF studies comparing recFSH versus hMG suggested comparable outcomes.[209] However, recently published multicenter, company-sponsored trials reported similar clinical outcomes comparing uFSH versus recFSH, or hMG versus recFSH.[210]

Many different regimens are applied with little if any proof of their efficacy and safety. Different starting days and doses are applied worldwide along with incremental or decremental doses. In case of imminent OHSS resulting from the development of too many follicles, the possibility of complete cessation of gonadotropin administration (coasting) has been advocated by several investigators.[174] Studies of the efficacy of this approach thus far undertaken have been limited and inconclusive. Adequate doses

TABLE 28-3

Randomized Controlled Trials Comparing Different Gonadotropin Doses for Ovarian Stimulation for in Vitro Fertilization

Reference	*Patients*	n	*Study Design*	*Conclusion (High- vs Low-Dose Regimen)*
van Hooff, HR '93	Low response after 5 d 225 IU/d hMG	64	225 vs 450 IU/d from day 5 of stimulation GnRH ag, long prt	No difference E_2, follicle #
Hoomans, HR '99	<39 yr, nl cycle, nl indic, BMI < 29 kg/m^2	165	150 IU rFSH vs 225 IU/d uFSH, fixed GnRH ag, long prt	Same oocyte #, same ongoing PR
Out, HR '99	30-39 yr, nl cycle, nl indic, BMI < 29 kg/m^2	199	100 vs 200 IU/d rFSH, fixed GnRH ag, long prt	More oocytes, same clinical PR
Out, HR '00	30-39 yr, nl cycle, nl indic, BMI < 29 kg/m^2	205	150 vs 250 IU/d rFSH, fixed GnRH ag, long prt	Similar oocyte # (also in older age!)
De Jong, FS '00	<38 yr, nl cycle, nl indic	15	100 vs 150 IU/d rFSH, fixed, late start GnRH antagonist	Reduced cycle cancellations in high-dose group
Latin-Am, FS '01	30-39 yr, nl cycle, nl indic, BMI < 29 kg/m^2	201	150 vs 250 IU/d rFSH, fixed GnRH ag, long prt	same oocyte #, same vital PR, 2 cases OHSS in high-dose group
Out, HR '01	<38 yr, nl cycle, male factor	91	100 vs 200 IU/d rFSH, fixed GnRH ag, long prt	More oocytes, same vital PR, 4 cases OHSS in high-dose group
Wilkland, HR '01	<39 yr, nl cycle, nl indic, BMI < 30 kg/m^2	60	150 vs 225 IU/d rFSH, fixed GnRH antagonist	More oocytes (9 vs 11), same ongoing PR in high-dose group
Yong, FS '03	<40 yr, nl cycle, nl FSH, BMI < 34 kg/m^2	120	150 vs 225 IU/d rFSH, fixed GnRHa, long prt	Same oocyte #, same embryo, same PR, 4 cases OHSS in high-dose group
Popovic, HR '03	<39 yr, nl cycle, nl indic, first cycle, nl FSH	267	individual (100-250)* vs fixed 150 IU/d rFSH GnRHa, long prt	More nl response (5-14 oocytes), higher ongoing PR in individual dose group
Hohmann, JCEM '03	20-38 yr, nl cycle, nl indic, BMI 19-29 kg/m^2	142	cycle day 2 vs day 5 start 150 IU/d rFSH GnRH antagonist, flexible start	Shorter stimulation, higher cancellations, similar ongoing PR in late start group
Out, HR '04	<39 yr, nl cycle, nl FSH, BMI < 29 kg/m^2	257	150 vs 200 IU/d rFSH GnRH antagonist	Same oocyte #, same embryo #, same vital PR
Aboulghar, HR '04	< 40 yr, nl cycle, nl indic	150	150-300 IU/d hMG: same dose vs 75 IU/d increase on day start GnRH antagonist	Same oocyte #, same embryo #, same clinical PR
Klinkert, HR '05	Expected low response (low AFC), nl cycle	52	150 vs 300 IU/d rFSH, fixed GnRHa, long prt	Same oocyte #
Propst, FS '06	< 38 yr, nl indic, nl FSH, BMI < 33 kg/m^2	60	150-300 IU/d rFSH, same dose vs 75 IU/d increase on day start GnRH antagonist	Same E_2 levels, same implantation #, same PR, same live birth #
Baart, HR '07	< 38 yr, nl cycle, nl indic, BMI 19-29 kg/m^2	111	150 IU/d rFSH (late start), GnRH antag vs 225 IU/d rFSH, GnRHa long prt	Fewer oocytes, fewer embryos, fewer aneuploid embryos in late-start GnRH antagonist group
Heijnen, Lancet '07	<38 yr, nl cycle, nl indic, BMI < 29 kg/m^2	404	150 IU/d rFSH (late start), GnRH antag vs 150 IU/d rFSH, GnRHa long prt	Fewer oocytes, fewer embryos in late-start GnRH antagonist group†

*Based on prediction model (including follicle number, ovarian volume, age, smoking, and Doppler).
†Pregnancy rates are not given because ovarian stimulation protocols were combined, with differences in embryo transfer policies.
AFC, antral follicle count; ag, agonist; antag, antagonist; BMI, body mass index; E_2, estrodiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; GNRHa, GNRH analog; hMG, human menopausal gonadotropin; indic, indications; nl, normal; OHSS, ovarian hyperstimulation syndrome; PR, pregnancy rate; prt, protocol; rFSH, recombinant FSH; uFSH, urinary FSH.

for gonadotropin preparations may also vary, depending on whether GnRH agonist or antagonist co-treatment is used.[211] Major individual differences in body weight may also determine response.[212] Because endogenous gonadotropins are suppressed by GnRH antagonists for a limited period of time (as will be discussed later), less exogenous FSH is required. The ideal day of initiation of gonadotropin therapy is another variable which has been poorly characterized so far, and may also vary dependent on GnRH agonist or antagonist co-treatment. It is surprising to conclude that very few of the above-mentioned questions with regard to applied dose regimens can be answered on the basis of scientific evidence by properly designed studies.

Usually starting doses vary between 100 and 300 IU/day and doses are often altered depending on the observed individual ovarian response. A typical daily starting dose would currently be 150 to 225 IU in Europe and 225 to 300 IU in the United States. Only few randomized studies regarding dose regimens can be found in the literature. A single-center RCT from Rotterdam showed that a doubling of the hMG dose in low responders after a 225 IU/day dose for 5 days is not efficacious compared to continued similar doses.[213] Moreover, an RCT in which higher versus standard dose of FSH was administered to expected poor responders showed no difference in pregnancy rates.[214] Table 28-3 summarizes further comparative studies, which taken together fail to show a difference in favor of high-dose regimens, indicating that the widely applied practice of a gonadotropin dose increase in case of low response is not efficacious.

The approach of starting exogenous FSH early during the luteal phase of the preceding cycle recognizes the physiologic principle of early recruitment of a cohort of follicles for the next cycle.[2] However, this protocol did not result in improved ovarian response in women with a low oocyte yield during previous IVF attempts.[215]

The perceived need to allow programming of oocyte retrieval led to a number of studies addressing the role of oral contraceptives (OCs) for this indication. Fixed schedule protocols were developed by a number of groups in which OCs were administered in advance of ovarian stimulation and planned follicle aspiration. Despite their apparent efficacy, ease of administration, and fewer side effects, subsequent randomized studies comparing OCs to GnRH agonists as a means of preventing premature luteinization showed the superiority of the latter and because of this, OCs are no longer widely used for this indication. To facilitate the planning of the initiation of exogenous gonadotropins in a GnRH antagonist cycle, independent of the menstrual period, OC pretreatment has been evaluated in a number of small studies and a recent meta-analysis.[216] Although there is evidence that OC pretreatment may aid in the scheduling of IVF cycles when GnRH anatagonists are used, at present there is no evidence that they improve live birth rates.[216]

Gonadotropin-Releasing Hormone Agonist Co-treatment

During initial studies with hMG stimulation of multiple follicle development for IVF it became apparent that a premature LH peak occurred in around 20% to 25% of cycles, due to positive feedback activity by high serum E_2 levels during the midfollicular phase of the stimulation cycle.[26] This advanced exposure to high LH resulted in premature luteinization of follicles and either cycle cancellation due to follicle maturation arrest or severely compromised IVF outcome. The clinical development of GnRH agonists in the early 1980s[45] allowed for the complete suppression of pituitary gonadotropin release during ovarian stimulation protocols for IVF.[26] Induced pituitary down-regulation indeed resulted in significantly reduced cancellation rates and improved overall IVF outcome.[217,218] Moreover, the approach of GnRH agonist co-treatment did facilitate scheduling of IVF and timing of oocyte retrieval. Frequently used preparations include buserelin, triptorelin, nafarelin, and leuprorelin. To some degree, the extent and duration of pituitary suppression are dose related, but surprisingly few dose finding studies have been performed. In addition, randomized studies comparing different GnRH agonists are scarce.

Due to the intrinsic agonist activity of the compound, pituitary down-regulation is preceded by an initial stimulatory phase (referred to as the "flare" effect) which lasts for around 2 weeks. In this long protocol, GnRH agonist treatment therefore usually commences in the luteal phase in the preceding cycle and is continued until hCG administration. Stimulation with gonadotropins is started when pituitary and ovarian quiesence has been achieved. Moreover, it is uncertain whether ovarian response to exogenous stimulation is affected by GnRH agonist co-treatment.[219] Some women suffer from serious hypoestrogenic side effects, such as mood changes, sweating, and flushes. Alternative approaches include the short (and sometimes ultrashort) protocols in which the initial flare effect of GnRH agonist treatment is used to stimulate the ovaries. Attempts to discontinue GnRH agonist administration during the ovarian stimulation phase[220,221] have not shown beneficial effects. Reported clinical results of these alternative clinical protocols remain variable, and the GnRH agonist long protocol has remained the standard of care for over a decade.[26]

Gonadotropin-Releasing Hormone Antagonist Co-treatment

Two third-generation GnRH antagonists (cetrorelix and ganirelix) became available for large-scale clinical studies around 1995. Previous generations of the antagonist suffered from problems with pharmaceutical formulation and related bioavailability along with the local or systemic induction of histamine release. The potential advantage of a GnRH antagonist is that pituitary gonadotropin secretion is suppressed immediately after initiation of therapy. Therefore the co-treatment with GnRH antagonist can be restricted to the time in the cycle at risk for a premature rise in LH (i.e., the mid- to late follicular phase of the cycle).[26]

Both single, high-dose and multiple, low-dose GnRH antagonist regimens have been described. Multiple, daily dose regimens are most widely used at present. Initial dose finding studies suggested that a daily injection of 0.25 mg represents the minimal effective dose to suppress a premature LH rise in most patients. In all phase 3 comparative trials of the daily GnRH antagonist co-treatment regimen, it was initiated on cycle day 6. However,

Citation	Year	Rate 1 Antagonists		Rate 2 Agonists		Effect	Lower	Upper	*P* Value
Albano	2000	34 / 198	.17	19 / 95	.20	.83	.44	1.55	.56
European	2000	97 / 486	.20	61 / 244	.25	.75	.52	1.08	.12
Olivennes	2000	22 / 126	.17	9 / 43	.21	.80	.34	1.90	.61
North American	2001	60 / 208	.29	36 / 105	.34	.78	.47	1.28	.32
Middle East	2001	72 / 236	.31	37 / 119	.31	.97	.60	1.57	.91
Akman	2001	4 / 24	.17	5 / 24	.21	.76	.18	3.26	.71
Hohmann	2003	18 / 111	.16	10 / 58	.17	.93	.40	2.17	.86
Martinez	2003	4 / 21	.19	3 / 23	.13	1.57	.31	8.01	.59
Franco	2003	3 / 14	.21	2 / 6	.33	.55	.07	4.56	.57
Hwang	2004	8 / 27	.30	8 / 29	.28	1.11	.35	3.53	.87
Sauer	2004	9 / 24	.38	9 / 25	.36	1.07	.33	3.41	.91
Xavier	2005	7 / 66	.11	8 / 65	.12	.85	.29	2.48	.76
Loutradis	2005	9 / 58	.16	12 / 58	.21	.70	.27	1.83	.47
Malmusi	2005	5 / 30	.17	5 / 30	.17	1.00	.26	3.89	1.00
Marci	2005	4 / 30	.13	0 / 30	.00	10.36	.53	201.45	.06
Cheung	2005	3 / 33	.09	2 / 33	.06	1.55	.24	9.94	.64
Check	2005	8 / 30	.27	5 / 30	.17	1.82	.52	6.38	.35
Barmat	2005	13 / 40	.33	17 / 40	.43	.65	.26	1.62	.36
Bahceci	2005	29 / 73	.40	33 / 75	.44	.84	.44	1.61	.60
Badrawi	2005	11 / 50	.22	13 / 50	.26	.80	.32	2.02	.64
Schmidt	2005	3 / 24	.13	3 / 24	.13	1.00	.18	5.53	1.00
Lee	2005	13 / 41	.32	8 / 20	.40	.70	.23	2.11	.52
Fixed combined (22)		**436/1950**		**305/1226**		**.86**	**.72**	**1.02**	**.08**

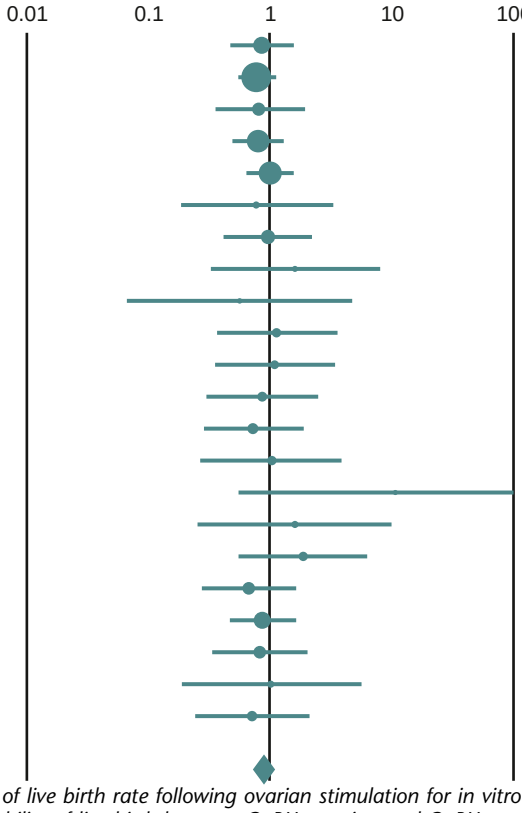


Figure 28-13. *Meta-analysis showing odds ratio (OR) of live birth rate following ovarian stimulation for in vitro fertilization in combination with either a GnRH agonist or a GnRH antagonist. The probability of live birth between GnRH agonists and GnRH antagonists was not significantly different (OR, 0.86; 95% CI 0.72-1.02; P = 0.085; heterogeneity, P = 0.99; fixed effect model). (From Kolibianakis EM, Collins J, Tarlatzis BC, et al. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. Hum Reprod Update 12:651-671, 2006.)*

in principle, GnRH antagonists need only be given when there is folliclular development and rising E_2 levels which might give rise to a premature elevation in pituitary LH release due to positive feedback mechanisms. However, a meta-analysis of four studies comparing fixed with flexible regimens showed a trend toward lower pregnancy rates following the flexible protocol (OR 0.7, 95% CI 0.47-1.05).[222] The first meta-analysis published comparing outcomes following co-treatment with GnRH antagonist versus GnRH agonist[211] based on five multicenter RCTs concluded that the GnRH antagonist is as efficient as GnRH agonist in preventing a premature LH surge in IVF (OR 1.76, 95% CI 0.75-4.16). However, a small but significant reduction in pregnancies was observed per started cycle (OR 0.79, 95% CI 0.63-0.99). Since then, protocols have been refined, and a recent meta-analysis of later studies has shown no difference in live birth rates[223] (Fig. 28-13).

Concerns have been raised regarding the possibility of direct effects of GnRH antagonists on the embryo. However, no adverse effects were observed on the freeze–thaw embryos of GnRH antagonist cycles.[224] Possible detrimental effects of GnRH antagonists at the endometrial level and on follicle development have not been confirmed.[26] Moreover, recent studies have indicated that gonadotropin regimens do not need to be adjusted when GnRH antagonists are commenced.[225,226] Furthermore, exogenous LH is probably not required next to FSH.[203,204,227]

Despite improving outcomes the debate regarding the advantages and disadvantages compared with GnRH agonists continues.[228] A summary of the advantages and disadvantages for the use of GnRH antagonists in IVF is given in Box 28-2.

APPROACHES FOR INDUCTION OF FINAL OOCYTE MATURATION

In the natural normo-ovulatory cycle, rupture of the dominant follicle and release of the oocyte are triggered by the mid-cycle surge of LH. This sudden enhancement of pituitary synthesis and release of LH (and FSH) is elicited by high late-follicular phase E_2 levels in combination with slightly elevated progesterone levels.[229] In stimulated cycles for IVF, estrogen levels are prematurely elevated, which may induce unpredictable but advanced LH rises. As mentioned before, GnRH agonist co-treatment is required in order to prevent this from happening. Consequently, exogenous hCG should be used during the late follicular phase under these circumstances to replace the endogenous LH surge. This approach has been considered the standard of care for the induction of final

BOX 28-2

Advantages and Disadvantages for the Use of GnRH Antagonists in IVF

Advantages

Prevention of premature LH increase is easier and takes less time.

GnRH antagonists are not associated with an acute stimulation of gonadotropins and steroid hormones.

The initial stimulation by GnRH agonists can induce cyst formation, which is avoided with GnRH antagonists.

No hot flushes are observed with GnRH antagonists.

Inadvertent administration of the GnRH analog in early pregnancy can be avoided as GnRH antagonist is administered in the midfollicular phase.

Requirements for exogenous gonadotropins are reduced, rendering ovarian stimulation less costly.

Duration of ovarian stimulation protocols is shortened, improving patient discomfort.

Disadvantages

GnRH antagonist co-treatment represents a novel approach and more knowledge is necessary for its optimization.

GnRH antagonists offer less flexibility regarding cycle programming as compared with the long GnRH agonist protocol.

Reduced ability to gain an orderly daily volume of oocyte retrievals compared with GnRH agonist, although this can be improved by using the oral contraceptive pill.

GnRH, gonadotropin-releasing hormone; IVF, in vitro fertilization; LH, luteinizing hormone.

*Adapted from Tarlatzis B, Fauser BCJM, Kolibianakis EM, et al. GnRH antagonists in ovarian stimulation for IVF. Hum Reprod Update 12:333-340, 2006.

stages of oocyte maturation before oocyte retrieval along with corpus luteum formation in IVF.[26] Exogenous hCG is also implicated in sustained luteotropic activity[220] due its prolonged circulating half-life.[230] Unfortunately, hCG is therefore also believed to contribute to chances of developing OHSS.[174]

Initial studies during ovarian hyperstimulation for IVF (before the widespread use of GnRH agonist co-treatment) showed that an endogenous LH surge could be induced reliably by the administration of GnRH or a bolus injection of GnRH agonist.[231] The induction of an endogenous LH (and FSH) surge is more physiologic compared to exogenous hCG because of the much shorter half-life.[232] Moreover, luteal phase steroid concentrations seem closer to the physiologic range[233] (Fig. 28-14), which may improve endometrial receptivity.[234] As the follicular phase co-treatment with GnRH agonist has been the standard of care for over a decade, alternative approaches for the

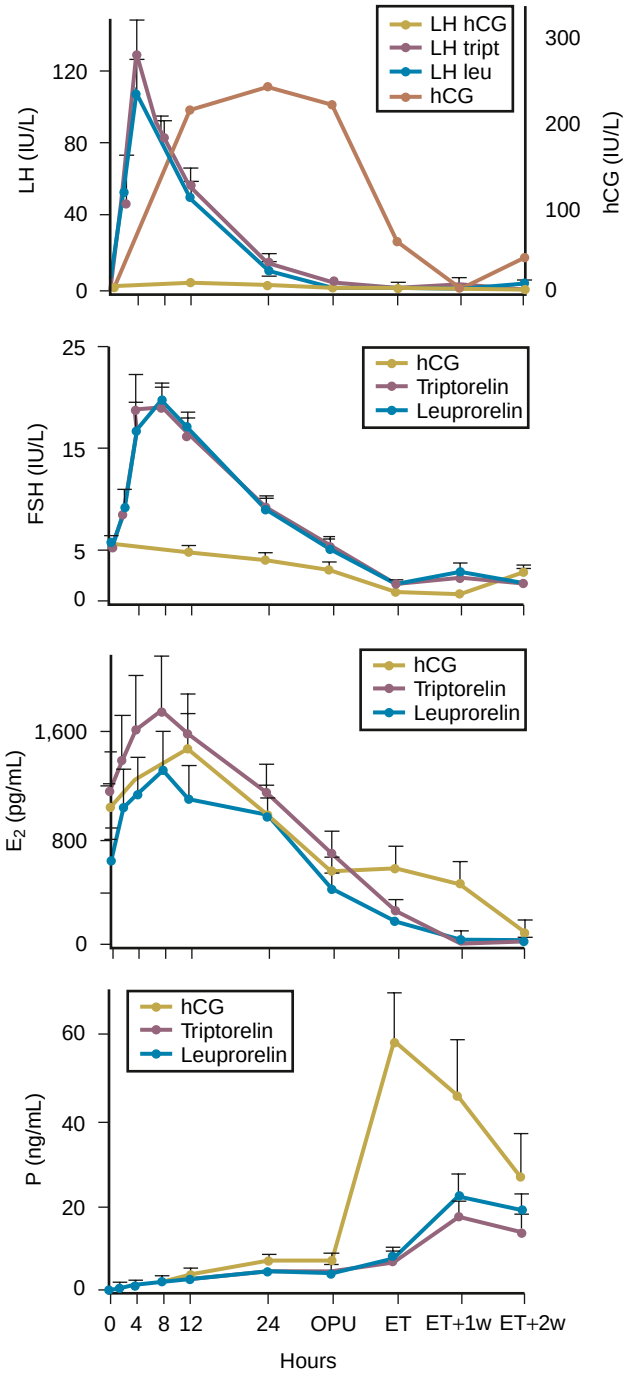


Figure 28-14. *Endocrine characteristics of the supplemented luteal phase following ovarian hyperstimulation for in vitro fertilization using exogenous follicle-stimulating hormone and gonadotropin-releasing hormone (GnRH) antagonist co-treatment, where oocyte maturation is induced by either human chorionic gonadotropin or the GnRH agonists triptorelin or leuprorelin. E_2, estradiol; ET, embryo transfer; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; OPU, ovum pickup; P, progesterone; w, week. (From Fauser BC, de Jong D, Olivennes F, et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian stimulation for in vitro fertilization. J Clin Endocrinol Metab 87:709-715, 2002.)*

induction of oocyte maturation has received little attention in recent years. However, the suppressive effect of follicular phase GnRH antagonist administration can be reversed immediately by administering native GnRH or GnRH agonist.[14,234] Indeed, a randomized trial confirmed that the triggering of final stages of oocyte maturation can be induced effectively by a single bolus injection of GnRH agonist even after the follicular phase co-treatment with a GnRH antagonist. This was demonstrated by the observed gonadotropin surge and quality and fertilization rate of recovered oocytes.[233]

Recombinant LH and recombinant hCG have recently become available for clinical use. An early large randomized trial comparing 250 µg rechCG versus 5000 IU uhCG for the induction of oocyte maturation in a total of 190 women undergoing IVF showed that the number of mature oocytes retrieved and luteal phase serum concentrations of progesterone and hCG concentrations were significantly higher.[235] Considering the short half-life of recLH two injections with a 1- to 3-day interval may be considered.

The introduction of GnRH antagonists into clinical practice now makes it possible to employ a bolus injection of GnRH agonist to induce an endogenous LH surge. Although previously shown to be effective in achieving this,[233] randomized studies comparing this approach to hCG administration showed lower implantation and ongoing pregnancy rates.[236] Recent data indicate that standard luteal support regimens may be insufficient in this setting, and improved results may be achieved when this is addressed. In a meta-analysis of 23 randomized studies, the use of GnRH agonist to trigger final oocyte maturation in IVF yielded a number of oocytes capable to undergo fertilization and subsequent embryonic cleavage comparable to that achieved with hCG.[237] However, the likelihood of an ongoing clinical pregnancy after GnRH agonist triggering was significantly lower as compared to standard hCG treatment. For women at risk of developing OHSS who have been co-treated with GnRH antagonists, replacing hCG with a GnRH agonist bolus has been shown in a randomized study to reduce the risk.[238]

LUTEAL PHASE SUPPLEMENTATION

Since the early days of IVF it has been described that the luteal phase of stimulated IVF cycles is abnormal. In fact, it was already stated in the first extended report on IVF by Edwards and Steptoe[33] that "the luteal phase of virtually all patients was shortened considerably after treatment with gonadotropins" and it was suggested that high follicular phase estrogen levels due to ovarian hyperstimulation might be involved. Initial studies in the United States in 1983 concerning hMG-stimulated IVF cycles also confirmed the occurrence of an abnormal luteal phase in IVF cycles with characteristic features of elevated progesterone levels along with a significantly reduced luteal phase length[239] (Fig. 28-15).

As mentioned earlier, GnRH agonist co-treatment became the standard of care for the prevention of a premature rise in LH. Typically, GnRH agonist treatment is initiated in the luteal phase of the preceding cycle and continued until the late follicular phase. It became apparent, however, that

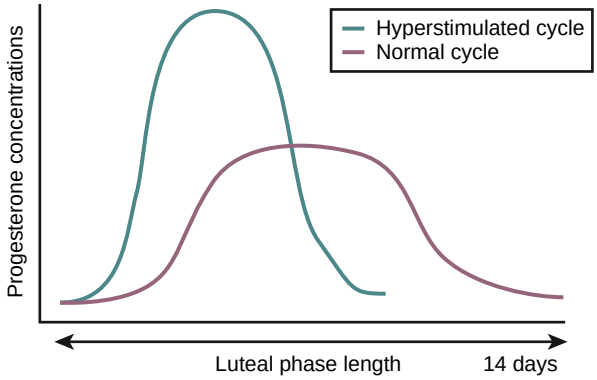


Figure 28-15. *Schematic representation of changes in luteal phase length and endocrine profile induced by ovarian hyperstimulation for in vitro fertilization (From Jones HW]. What happened? Where are we? Hum Reprod 11[Suppl 1]:7-21, 1996.)*

prolonged pituitary recovery from down-regulation during the luteal phase[240] resulted in lack of support of the corpus luteum by endogenous LH and advanced luteolysis.[241] It was observed shortly thereafter that the corpus luteum can be rescued by the administration of hCG,[26] and this treatment modality became the standard of care for luteal support during the late 1980s. Outcome was better compared to progesterone supplementation, but 5% of hCG-supplemented patients developed OHSS. Because of this association between hCG and OHSS,[174] luteal phase hCG support has been largely replaced over the years by luteal phase progesterone supplementation.[242] In a recent meta-analysis of luteal support in stimulated IVF cycles, both hCG (OR 2.72; 95% CI 1.56-4.9, $P < 0.05$) and progesterone (OR 1.57; CI 1.13-2.17, $P < 0.05$) were confirmed to result in an increased pregnancy rate compared with placebo.[243] However, hCG was clearly associated with an increased risk of OHSS. Natural micronized progesterone was not efficient if taken orally, but both the vaginal and intramuscular routes were effective and demonstrated comparable outcomes. With respect to the addition of estradiol, while it showed some benefit in long GnRH agonist protocols, there was no evidence to support its use in short GnRH agonist or GnRH antagonist protocols.

Attempts to secure pituitary recovery during the luteal phase by the early follicular phase cessation of GnRH agonist co-treatment all failed, because it takes at least 2 to 3 weeks for LH secretion to recover.[26] Because of the rapid recovery of pituitary gonadotropin release after discontinuation of GnRH antagonist it has been speculated that luteal phase supplementation may not be required following the late follicular phase administration of antagonist.[244] Preliminary observations related to ovarian stimulation and GnRH antagonist co-treatment for IUI seem to favor this contention.[245] However, various studies in IVF applying GnRH antagonist co-treatment have now clearly shown that luteolysis is also initiated prematurely resulting in a significant reduction in the length of the luteal phase along with greatly compromised chances for pregnancy.[246-248] More detailed studies could confirm that early and mid-luteal phase LH levels remained suppressed following the follicular phase administration of GnRH antagonist.[248,249]

Moreover, luteolysis is advanced in the nonsupplemented luteal phase after either hCG or GnRH agonist triggering of oocyte maturation.[248] Collectively, this indicates that high early luteal phase steroid production is primarily responsible for advanced luteolysis, due to massive negative feedback resulting in greatly suppressed LH secretion.[179] Mild stimulation regimens resulting in lower serum steroid levels have therefore been advocated as a means of benefiting the luteal phase.[243]

CLINICAL OUTCOME OF IVF

ESHRE IVF data for the year 2003 from 28 European countries involving a total of 725 clinics and over 360,000 IVF and ICSI cycles report major differences between countries, with an overall clinical pregnancy rate of 26.1% per retrieval and 29.6% per transfer for IVF (26.5% and 28.7%, respectively, for ICSI). Singleton deliveries involved 76.7% of pregnancies.[60] The most recent report of U.S. centers involving a total of 92,389 cycles performed in 2005 (www.cdc.gov/ART/index.htm) continue to indicate higher clinical pregnancy rates per retrieval, reaching 41% in women under 35 years. Respective live birth rates per cycle are also highly age dependent, ranging from 37% in women under 35 years, to 11% in women of 41 to 42 years of age. Respective twinning rates are 33% and 13%, while triplets or more constitute 4% to 5% of pregnancies. The percentage of miscarriages, fetal reduction procedures, or immature births following IVF are largely unknown. Next to differences in quality of fertility laboratories, this discrepancy in success rates may depend on how success is defined. Currently, live birth is defined as delivery of a fetus with a heartbeat from 20 weeks onward, and may also be associated with differences in indications for IVF, smoking habits, and the age of patients treated, along with the number of embryos transferred. In the United States, up to five embryos can be transferred, and in 2005 a mean of 2.5 embryos were transferred per cycle (www.cdc.gov/ART/index.htm).

ADVERSE EFFECTS AND COMPLICATIONS

Complications related to invasive IVF procedures such as oocyte retrieval and embryo transfer, predominantly involve infection and bleeding along with anesthesia problems.[250] The drawbacks associated with profound ovarian stimulation for IVF include considerable patient discomfort such as weight gain, headache, mood swings, breast tenderness, abdominal pain, and sometimes diarrhea and nausea. In this respect it is important to comprehend that after a first unsuccessful IVF attempt around 25% of patients refrain from a second cycle, even in countries where costs are covered by health insurance companies.[251]

OHSS is a potentially life-threatening complication characterized by ovarian enlargement, high serum sex steroids, and extravascular fluid accumulation, primarily in the peritoneal cavity. Mild forms of OHSS constitute around 20% to 35% of IVF cycles, moderate forms 3% to 6% of cycles, along with 0.1% to 0.2% severe forms.[48,174] To some extent, patients at risk of developing OHSS may be recognized by the following features: young age, PCOS, profound hyperstimulation protocols with GnRH agonist long protocol co-treatment, large numbers of preovulatory graafian follicles, high serum E_2 levels, high (>5000 IU) bolus doses of hCG to induce final oocyte maturation, the use of hCG for luteal phase supplementation, and finally the occurrence of pregnancy. In fact, the incidence of OHSS is directly related to hCG concentrations with a two- to fivefold increased incidence in case of multiple pregnancy.

Preventive strategies in case of imminent OHSS include cessation of exogenous gonadotropins for several days (coasting), follicular aspiration, prevention of pregnancy during the stimulation cycle by cryopreserving all embryos, or the prophylactic infusion of glucocorticoids or albumin. The risk of OHSS may also be lowered by using alternative strategies to induce oocyte maturation, such as inducing an endogenous LH surge by administration of a single bolus dose of GnRH agonist or the short half-life preparation of recLH instead of hCG.

The most important complication related to IVF treatment is multiple pregnancy. The magnitude of the problem has been discussed previously in this chapter (see Fig. 28-5). (For recent reviews see Fauser et al.[51] and Verberg et al.[52]) Between the years of 1980 and 2000, twin birth rates in the United States increased by 75%, and currently represent around 3% of total births.[51] Similar trends have been reported in European countries.[52] Although an association between increased female age and multiple gestation is clearly established, the delay in childbearing accounts for no more than 30% of the observed overall increase in multiple pregnancies.[51] Although the available data indicate that the majority of twin births are still unrelated to infertility therapies[51] up to 80% of higher order multiple births are considered to be due to ovarian stimulation and ART. Births resulting from infertility therapies account for around 1% to 3% of all singleton live births, 30% to 50% of twin births, and more than 75% of higher order multiples.

Pregnancy complications include increased risk of miscarriage, preeclampsia, growth retardation, and preterm delivery. Perinatal mortality rates are at least fourfold higher in twin, and at least sixfold higher in triplet, births compared with singleton births. Moreover, the risks of prematurity in twin and higher order multiple birth are increased 7- to 40-fold, and for low-birth-weight infants 10- to 75-fold, respectively. Adverse outcomes among children conceived through IVF are largely associated with multiple gestation.

Recent data are reassuring with respect to possible long-term health consequences such as ovarian cancer, breast cancer, and advanced menopausal age.[252]

NEW APPROACHES TO MILD OVARIAN STIMULATION FOR IVF

After the initial years of IVF, profound ovarian stimulation became the rule for more than two decades. The stimulation of growth of large numbers of follicles and the retrieval of many oocytes has been viewed as an acceptable marker of successful IVF treatment. Medication regimens to achieve profound ovarian stimulation are extremely

complex and expensive, take many weeks of frequent injections, and require intense monitoring. Moreover, patient discomfort and chances for serious side effects and complications are considerable. In addition, this profound stimulation gives rise to greatly abnormal luteal phase endocrinology, and its impact on endometrial receptivity and therefore IVF success is mostly unknown.

Attitudes toward profound ovarian stimulation are changing,[191,253] particularly given the growing tendency to transfer a reduced number of embryos. It has previously been demonstrated on the basis of the U.K. national database that reducing the number of embryos tranferred from three to two does not diminish chances of birth despite a reduction in risk of multiple birth.[64] In Europe, an increasing number of centers are carrying out single transfer in younger women. Emphasis may therefore now be directed toward the development of more simple mild stimulation protocols[26,191,196,254] or the improvement of natural cycle IVF outcomes.[61,195,197] The increasing quality of embryo cryopreservation programs will serve to encourage the transfer of one embryo at a time.[255]

Previous studies in normo-ovulatory female volunteers[114,115] confirmed that the development of multiple dominant follicles can be induced by interfering with decremental FSH concentrations during the mid- to late follicular phase. As shown previously, this decrease is required for the selection of a single dominant follicle.[9,10] These observations are in agreement with previous findings in the monkey model.[116,256] We were subsequently able to demonstrate that the initiation of exogenous FSH (fixed dose, 150 IU/day, GnRH antagonist co-treatment) as late as cycle day 5 results in a comparable clinical IVF outcome, despite a reduced duration of stimulation (number of ampules used) and increased cancellation rates[257] (Fig. 28-16).

To test the efficacy of this mild stimulation protocol in standard practice, a large randomized effectiveness study has been performed to analyze whether a strategy including the mild stimulation protocol in combination with single embryo transfer (SET) would lead to a similar outcome assessed over a 1-year interval after initiation of treatment, while reducing patients' discomfort, multiple pregnancies, and costs compared with standard treatment.[63] The study included a total of 404 patients and observed that because of the shorter duration of treatment per cycle, less medication needed, and a reduction in twin pregnancies, the mild approach led to an equal chance of live birth after a year of treatment while reducing the total costs (Fig. 28-17).

Apart from clinical efficiacy and costs (see later), emotional stress should be considered an important negative side effect associated with IVF treatment. Following mild stimulation, patients reported fewer side effects and stress related to hormone treatment and cycle cancellation compared with conventional stimulation.[258] Treatment-related stress has been found to be the most important reason patients drop out of IVF treatment.[259] The early drop-out of treatment deprives the couple of an optimal cumulative chance of achieving pregnancy, and therefore also impacts on the success of the respective IVF program. Mild stimulation might therefore have a positive impact on cumulative treatment success rates as it positively affects the chance that patients are willing to continue treatment following a failed attempt.

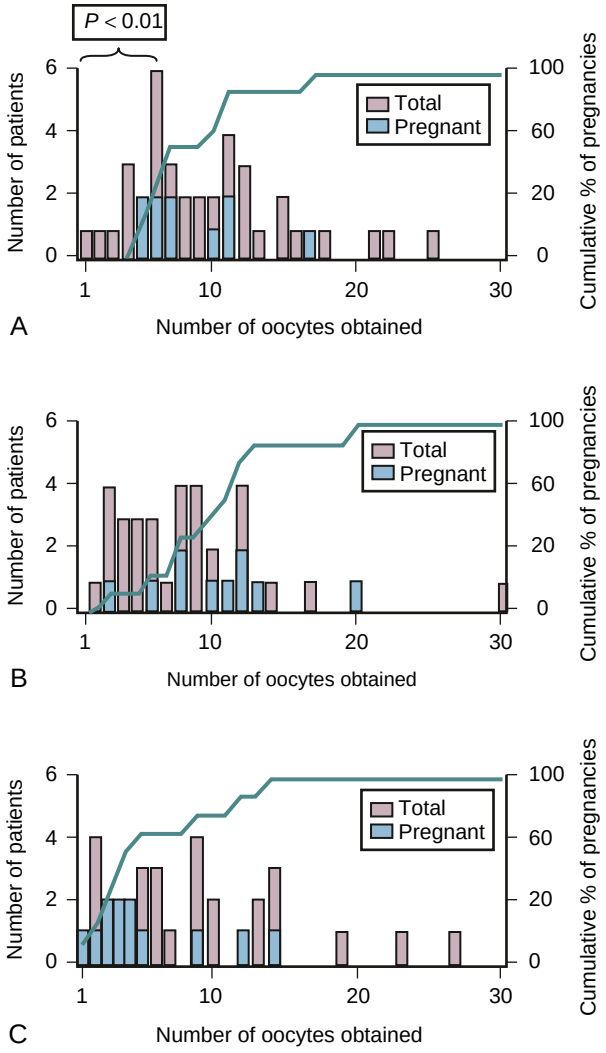
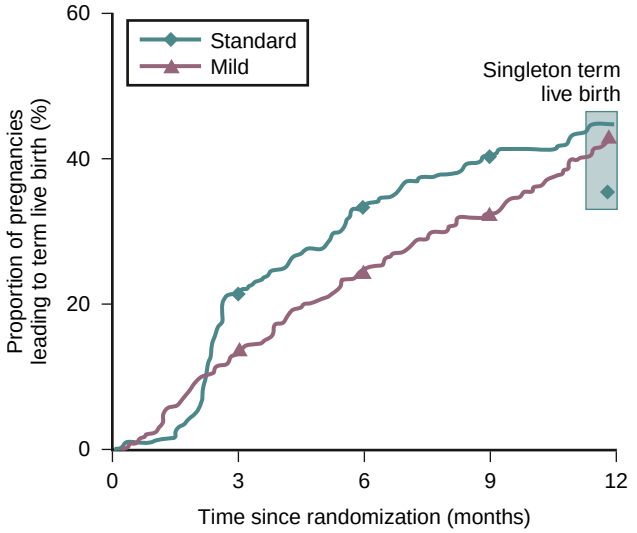


Figure 28-16. *Number of women undergoing in vitro fertilization who did or did not achieve a pregnancy in relation to the amount of oocytes retrieved, comparing conventional hyperstimulation with gonadotropin-releasing hormone (GnRH) agonist long protocol (**A**) with two mild stimulation protocols employing GnRH antagonist co-treatment (**B** and **C**). (From Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone [GnRH] antagonist cotreatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab 88:166-117, 2003.)*

Other novel protocols under investigation include the replacement of FSH by LH, an approach based on the acquired LH responsiveness of granulosa cells of dominant follicles. Besides the expected reduction of gonadotropin usage, this ovarian stimulation approach might also reduce the number of small, less mature follicles, possibly reducing the chance of OHSS, because smaller ovarian follicles are unlikely to be responsive to LH.[127] Three randomized controlled trials[260-262] have shown that this approach can result in a significant reduction in FSH needed and in the number of small follicles at final oocyte maturation. Pregnancy rates do not appear to be compromised. More



Number of patients

Standard	199	152	123	106	97
Mild	205	174	149	130	109

Figure 28-17. *Proportions of pregnancies leading to cumulative term live birth within 12 months after starting in vitro fertilization. Mild: mild ovarian stimulation with GnRH antagonist and single embryo transfer. Standard: standard ovarian stimulation with GnRH antagonist and dual embryo transfer. The shaded area represents the singleton live birth rate after 12 months. (From Heijnen EM, Eijkemans MJ, De Klerk C, et al. A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. Lancet 369[9563]:743-749, 2007.)*

extensive studies are required to determine the critical threshold for FSH replacement by LH stimulation and the most appropriate dosage of LH or hCG.

There are indications that the degree of ovarian stimulation affects both the morphologic embryo quality and the chromosomal constitution of the developed embryos.[263,264] This phenomenon could be the result of interference with the natural selection of good quality oocytes or the exposure of growing follicles to the potentially negative effects of ovarian stimulation. A randomized trial concerning the chromosomal analysis of human embryos following mild ovarian stimulation for IVF showed a significantly higher proportion of euploid embryos compared to conventional ovarian stimulation, suggesting that through maximal stimulation the surplus of obtained oocytes results in chromosomally abnormal embryos.[265]

Toward Individualized Treatment Algorithms

As previously highlighted, the chance of achieving a spontaneous pregnancy is frequently underestimated by couples and their physicians.[70] An increasingly assertive patient population, who continue to delay childbearing for career, social, or other reasons is putting physicians under greater pressure to intervene in order to aid the couple in achieving their goal quickly and with minimal disruption to busy lives. Time is increasingly an issue for couples seeking to conceive, and the commercial pressures and competition between IVF centers can lead to couples being accepted into IVF programs without a sound indication. Yet the virtue of patience can pay dividends for many who are now subject to premature and unnecessary intervention. Most couples seeking help will present with subfertility rather than absolute infertility. On the basis of a modest range of investigations and certain individual characteristics, the chances of an individual couple conceiving spontaneously over a given period of time can be calculated. It is known for instance that the spontaneous monthly fecundity rate declines with increasing duration of subfertility. After 3 years the residual likelihood of spontaneous pregnancy in untreated couples with unexplained infertility falls to 40% and after 5 years to 20%.[70]

In recent years a number of prediction models for calculating individual chances of spontaneous conception in subfertile couples have been published.[266-268] The chance of conception over a given time frame can be calculated from the results of a number of fertility investigations and patient parameters such as age and duration of infertility. Caution is, however, required when applying a prediction model developed elsewhere to one's own patient population. Before a prediction model can be introduced into everyday clinical practice, prospective external validation is required. Furthermore, knowledge of the development cohort is important when selecting a model for application in one's own setting. The mean duration and degree of subfertility in a primary care population is less than in a tertiary population. As a result, the conclusions derived from model developed in academic centers may have limited relevance for primary subfertility management and vice versa.[70]

The majority of women undergoing ovulation induction have WHO class 2 anovulation. Although this is a highly heterogeneous group, the treatment for these women is the same.[269] The identification of patient characteristics predictive of ovulation induction outcome would allow the design of individual treatment regimens, and would provide useful information regarding the factors that determine the extent of ovarian dysfunction.[269] In recent years a number of studies addressing these issues have been published. In one study the criteria that could predict the response of women with WHO class 2 anovulation to treatment with CC were identified.[86] Following multivariate analysis, the free androgen index (FAI), body mass index (BMI), presence of amenorrhea (as opposed to oligomenorrhea), and ovarian volume were found to be independent predictors of ovulation. The area under the receiver operating curve in a prediction model using these factors was 0.82. By adding additional endocrine factors, the area under the curve increased to 0.86.[270] In a subsequent study, those factors which could predict conception following ovulation were studied. Multivariate analysis of a number of clinical, endocrine, and ultrasound characteristics revealed lower age and the presence of amenorrhea to be the only significant parameters for predicting conception. Initial LH levels were not found to be important. From these data, a nomogram was constructed[165] (Fig. 28-18) which may assist in the selection of patients for clomiphene therapy, and those for whom this first-line treatment will be of little value. In this latter group, early recourse to gonadotropin therapy is indicated.[271,272]

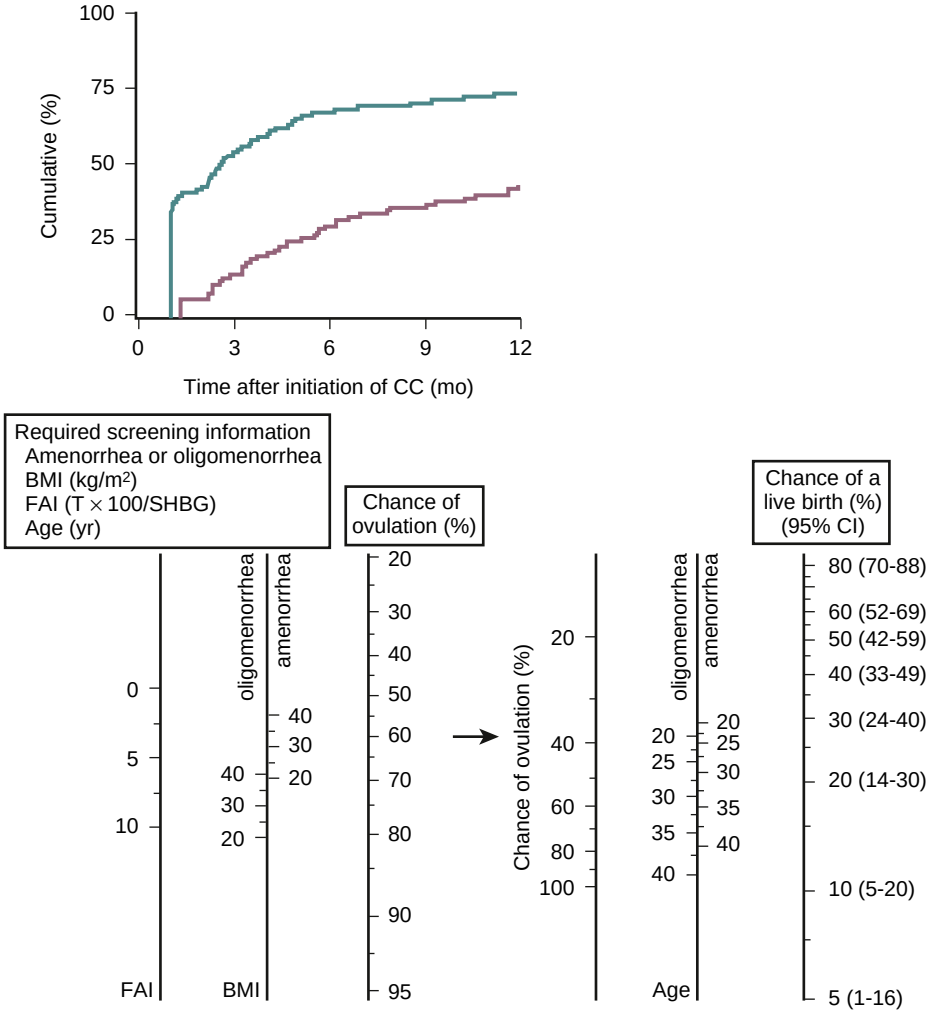


Figure 28-18. *Cumulative percentage of patients who ovulate or conceive following the initiation of clomiphene citrate, CC* (top)*, and a two-step nomogram predicting chances of live birth following clomiphene citrate on the basis of initial screening characteristics* (bottom)*. BMI, body mass index; FAI, free androgen index. (From Imani B, Eijkemans MJ, te Velde ER, et al. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertil Steril 77:91-97, 2002.)*

When gonadotropin therapy for ovulation induction is selected, the duration of treatment, the amount of gonadotropins administered, the associated risks of cycle-to-cycle variability, multifollicular development, OHSS, and multiple pregnancy might all be reduced if the starting dose were individualized. This would require the means to reliably predict the dose of FSH at which a given individual will respond by way of monofollicular selection to dominance—in other words, their individual FSH threshold for stimulation. A prediction model has recently been developed which may be used to determine the individual FSH response dose (which is presumably closely related to the FSH threshold).[120] Women about to undergo low-dose step-up ovulation induction with recFSH, were subject to a standard clinical, sonographic, and endocrine screening. The measured parameters were analyzed for predictors of the FSH dose on the day of ovarian response. In multivariate analysis, BMI, ovarian response to preceding CC medication (CC-resistant anovulation [CRA], or failure to conceive despite ovulatory cycles), initial free insulin-like growth factor-I (free IGF-I), and serum FSH levels were included in the final model.[120] In a subsequent analysis of women with PCOS who had undergone ovulation induction with the step-down regimen, a correlation was observed between the predicted individual FSH response dose and the number of treatment days before dominance was observed.[273] Application of this model may enable the administration of the lowest possible daily dose of exogenous gonadotropins to surpass the individual FSH threshold of a given patient and achieve follicular development and subsequent ovulation. Refining ovulation induction therapy in this way offers the prospect of improving safety, reducing the risk of multiple pregnancies, and improving the efficiency of gonadotropin ovulation induction.

The ability to predict clinical outcome from ovulation induction with gonadotropins would also be of value in the individualization of treatment regimens. In a prediction model for outcome after FSH ovulation induction[173] simple patient characteristics combined with endocrine factors were again shown to enable (limited) prediction of outcome following FSH ovulation induction. The most important end point for ovulation induction is overall singleton live birth. Data are now available to allow the prediction of a given couple achieving this from conventional ovulation induction strategies over an extended period of time (Fig. 28-19).[47]

Regarding IVF treatment, it appears that the most prominent factor determining outcome is the individual

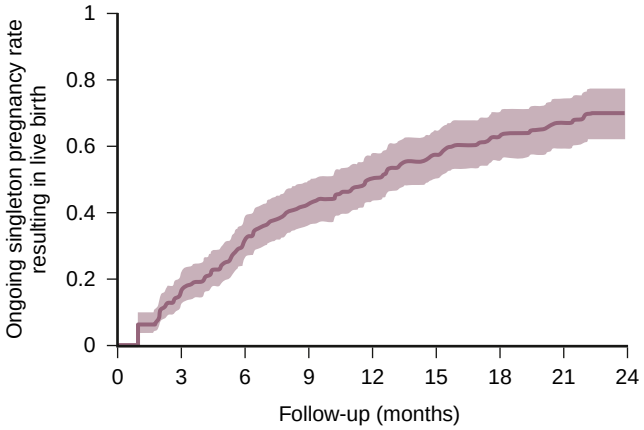


Figure 28-19. *Cumulative singleton live birth rate of 72% within 2 years after the initiation of a conventional ovulation induction algorithm (using clomiphene citrate as first-line and gonadotropins as second-line therapy) for the medical treatment of anovulatory infertility. (From Eijkemans MJ, Imani B, Mulders AG, et al. High ongoing singleton live birth rate following classical ovulation induction in anovulatory infertility. Hum Reprod 18:2357-2362, 2003.)*

variability in ovarian response to stimulation. Rather than exhibiting the desired response, women can present with either a hyporesponse or a hyper-response to stimulation. Studies undertaken so far have been unable to demonstrate a beneficial effect of gonadotropin dose increase in patients who exhibit a poor response to standard dose regimens.[176,213] This may help in counseling the patient, because the chances of successful IVF in these women will be extremely low.

Poor ovarian response appears to be related to ovarian aging[177] and early menopause[178] (Fig. 28-20). In IVF, the association between poor ovarian response due to diminished ovarian reserve with cycle cancellation and poor success rates is well established.[274] Age is an important predictor of IVF outcome.[275] However, chronological age is poorly correlated with ovarian aging. A major individual variability exists in follicle pool depletion within the normal range of menopausal age, as complete follicle pool exhaustion may occur between 40 and 60 years. The quantity and quality of the primordial follicle pool diminishes with age, reducing ovarian reserve.[276] This results in a decline in both therapy-induced and spontaneous pregnancies.[277] However, some women above 40 years of age will show a good response to ovarian stimulation and subsequently conceive with IVF, yet other women under 40 may fail to respond as a result of accelerated ovarian aging. In recent years attention has been given to the identification of sensitive and specific markers for ovarian aging which may enable prediction of poor or good response. This would open the way to improved counseling and patient selection for IVF.

The first and still most widely used endocrine marker for ovarian reserve is the early follicular phase FSH level,[278] which has been shown to be an independent predictor to age of IVF outcome.[279] More recent studies have indicated that while FSH level is a stronger predictor of cycle cancellation due to poor response and the number of oocytes collected at pick-up, age is more closely related to the chance of pregnancy.[279] In current practice, women with raised baseline FSH levels are usually advised against IVF treatment due to the anticipated poor outcome. However, although young women with high FSH levels demonstrate lower numbers of growing follicles and a high probability of cycle cancellation, normal ongoing pregnancy rates may be observed if oocytes and embryos are obtained.[279] Older women (>40 years) with normal baseline FSH levels may demonstrate lower cancellation rates, but the implantation rate per embryo and the ongoing pregnancy rates are lower than those observed in young women with elevated FSH.[279] FSH has been suggested to be of greater value in predicting ovarian reserve than other ovarian markers such as inhibin B. However, in a meta-analysis, baseline FSH levels showed only a moderate predictive performance for poor response and a low predictive performance for nonpregnancy was observed.[280] Other markers may therefore have an adjunctive value when diagnosing diminished ovarian reserve. The ultrasound measurement

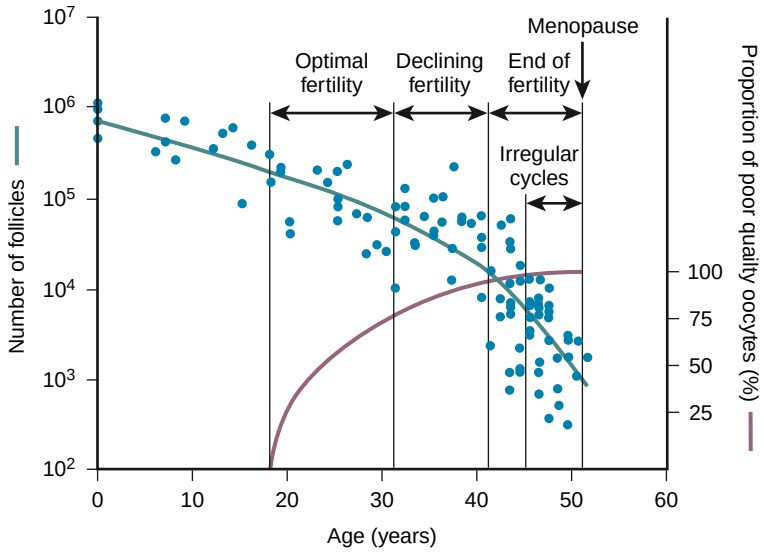


Figure 28-20. *The decline in follicle number and the increase in poor-quality oocytes in relation to reproductive events with increasing female age. (From Broekmans FJ, Knauff EA, te Velde ER, et al. Female reproductive ageing: current knowledge and future trends. Trends Endocrinol Metab 18[2]:58-65, 2007.)*

of the number of antral follicles present on cycle day 3 has been shown in a number of studies to predict poor ovarian reponse. Addition of basal FSH and inhibin B levels to a logistic model with the antral follicle count appears to further improve the performance of this marker.[281] At present no single reliable marker for ovarian reserve has been identified.[279] Anti-müllerian hormone (AMH), a member of the transforming growth factor-β superfamily, has been proposed as a novel candidate in this context. It is produced by granulosa cells of growing preantral and small antral follicles and is directly involved in primordial follicle pool depletion in the rat. Serum levels decline with age[282] and recent studies have shown that poor reponse to IVF can be predicted by reduced baseline serum AMH concentrations.[279] Although this hormone is a promising marker, additional prospective studies and multivariate analyses of potential factors are required in order to improve predictive potential.

To some extent, women likely to present with a reduced response to ovarian stimulation can be predicted, but hyper-response (and the threat of OHSS) comes as a surprise in the great majority of cases. It is uncertain, as yet, which patients are likely to present with hyper-response (at risk for OHSS).[192] Early recognition of women at risk may give rise to effective, altered stimulation protocols and improved safety.[174]

The use of normograms for individualising FSH dose for ovarian stimulation in IVF may optimize the risk/benefit dose of FSH in IVF. In recent years, several models have been developed based on multiple regression analysis.[283,284] Factors consistently observed to be predictive of the number of oocytes obtained were age, the total number of antral follicles, and smoking status.[285] Others have suggested that ovarian volume and and blood flow as measured by Doppler ultrasound are predictive factors. A model combining all these factors has been developed to prescribe the optimal dose of rFSH that will yield 5 to 14 oocytes.[283] In a prospective randomized study, the application of this model increased the proportion of "appropriate ovarian responses" and decreased the need for dose adjustments during ovarian stimulation.[284]

In those who do respond to ovarian hyperstimulation for IVF and for whom embryos are available for transfer, individualizing treatment in order to optimize outcomes should involve consideration of the number of embryos to be transferred. As stated earlier, although a trend toward the transfer of fewer embryos is now clear, fear of a lower chance of pregnancy may discourage couples and their physicians from transferring two embryos or less. This applies particularly when single embryo transfer is being considered. The ability to identify those treatment cycles in which single embryo transfer would avoid the risk of twin pregnancy without reducing the chance of achieving a singleton pregnancy would certainly encourage the adoption of single embryo transfer into clinical practice. In recent years, a number of authors have tried to identify those factors that may predict the chance of birth and of multiple birth on the basis of key characteristics of the patient, the cycle, and the embryos available for transfer.[57,286] Important determining factors thus far identified include the age of the woman, the duration of infertility, and the number of oocytes obtained following ovarian hyperstimulation. We have previously developed a prediction model to allow the chance of pregnancy and twin pregnancy to be assessed in a given transfer cycle should one or two embryos be transferred.[65] Application of this model allows a subgroup of young patients with good quality embryos to be identified for whom applying single embryo transfer could drastically diminish the twin rate without compromising singleton pregnancy rates.

As with models designed to predict spontaneous pregnancy, untested models for predicting IVF outcome can show a disappointing performance when used on patients from a different but plausibly similar population.[287] Therefore, before such a model can be applied in clinical practice, its reliability in predicting the selected outcomes should be validated on a different population to that on which it was developed. External validation of our model has demonstrated that it may be applied in different populations following a simple calibration process to adjust for local success rates.[66]

Health Economics of Ovarian Stimulation

Although a tendency to increased IVF consumption can be observed every year, IVF or IUI should not be routinely applied for all kinds of infertility problems. Assisted reproduction should not replace a proper infertility workup. Moreover, the economic implications of a more widespread use of assisted reproduction should be considered seriously when making decisions regarding treatment.[288,289]

The diagnosis by exclusion of unexplained infertility/subfertility is made in around 30% of couples in whom conventional diagnostic tests are normal. The prognosis for conception significantly decreases when the duration of infertility is at least 3 years along with an advanced female age beyond 35.[267] Again, chances of spontaneous conception are usually underestimated both by the doctor and the patient.[70] It appears that high costs prevent many couples with an indication for this treatment modality from undergoing IVF (i.e., undertreatment due to insufficient access to ART services). Data from the United States suggest that in states where IVF is not covered, only one third of couples with a valid indication for IVF actually undergo treatment.[290] Moreover, IVF is available in only 25% of the countries worldwide.[289] On the other hand, in a commercial environment couples may be exposed to risks associated with assisted reproduction too early (i.e., overtreatment under conditions in which expectant management might have been more appropriate). Indeed, in various European countries such as France, The Netherlands, and Sweden where IVF is covered by health insurance, a threefold higher use of IVF per capita compared to the United States can be observed.[289]

Cost-effective health care involves the achievement of a desired treatment goal at the lowest possible expenditure. IVF cost effectiveness should assess costs per live birth. So far, calculations of costs per live birth have only included direct costs related to neonatal care. The inclusion of indirect costs (i.e., including mid- and long-term health

sequelae such as mental retardation, cerebral palsy, and learning disabilities) would presumably double the overall costs.

The financial consequences of multiple pregnancies are substantial for both parents and health care providers. However, the economic impact of a multiple pregnancy is not limited to increased costs of maternal hospitalization and obstetric and neonatal (intensive) care. Lifetime costs for chronic medical care, rehabilitation, and special education related to extreme prematurity must also be taken into account. For a low-birth-weight child, the average cost of heath care and education up to the age of 8 years is 17-fold higher than the costs for a normal birth weight child.[291] It has also been shown that multiple births contribute disproportionately to hospital inpatient costs, especially during the child's first year of life.[292]

Because of the limited use of ovarian stimulating medication, the per cycle costs of mild stimulation IVF cycles will be lower than conventional stimulation approaches. However, to analyze the cost effectiveness of mild stimulation, the total cost per live birth should be analyzed. Besides the costs for medication, medical consultations and visits, laboratory charges (general, hormone and embryology), ultrasound procedures, IVF procedures (oocyte retrieval and embryo transfer), hospital charges, nurse coordinator costs, administrative charges, fees for anesthesia, costs for complications, travel expenses, and lost wages should be taken into account.[289]

Those who advocate milder strategies in IVF point to recent studies that show that the costs for IVF per year of treatment are comparable with conventional stimulation approaches, and the costs for the pregnancy and neonatal period are significantly lower following mild stimulation and single embryo transfer.[293]

Conclusions and Future Perspective

Any form of ovarian stimulation increases chances of pregnancy per cycle but at the expense of increased complication rates, most importantly multiple pregnancies (see Fig. 28-8) and OHSS. This holds especially true for ovarian hyperstimulation aiming at maturing multiple dominant follicles for fertilization either in vivo (following intercourse or IUI) or in vitro by IVF. With IVF, the incidence of occurring multiple pregnancies can be controlled by the number of embryos transferred. Moreover, various strategies may significantly reduce chances for OHSS. In skillful hands and with proper ovarian response monitoring, chances for complications are lowest for ovulation induction. The aim of this intervention is to mimic physiologic circumstances in anovulatory women, hence, single dominant follicle development and ovulation.

Special care should also be taken to carry out a proper infertility workup in order to diagnose other treatable infertility factors. This will also allow a proper assessment to be made of pregnancy chances for a given couple, either spontaneously or after infertility therapies. Along these lines, only patients with a proper indication will be exposed to the discomfort, risks, and costs associated with assisted reproduction and ovarian hyperstimulation.

Milder forms of ovarian hyperstimulation (or indeed none at all) may be considered for empirical treatment of unknown infertility (with or without IUI) due to the inherent risk of higher order multiple pregnancies. In general, however, the price to pay is a slightly lower pregnancy rate per cycle. Overall, assessment of cumulative pregnancy rates over a given period of time (which may involve multiple cycles) may be similar.

A trend can be observed toward hyperstimulation and assisted reproduction as first-line treatment in anovulatory infertility, especially PCOS. This shift in clinical practice is not based on sound scientific evidence. In fact, healthy live birth rates from conventional ovulation induction strategies are good, with acceptable rate of multiple pregnancies and OHSS.[47] Newly introduced compounds to the field of ovulation induction such as insulin sensitizers and aromatase inhibitors may further improve outcomes.

With regard to IVF, many new treatment modalities have been introduced over the years without proper preceding evaluation for efficacy and safety. The current most profound clinical challenge is to find the right balance between improving chances for success coinciding with an acceptable complication rate. The paradigm of so-called "controlled" ovarian hyperstimulation using maximum stimulation by exogenous gonadotropins together with the GnRH agonist long protocol has been taken for granted for more than a decade. Potential detrimental effects of this approach with regard to patient discomfort and safety, oocyte quality, corpus luteum function, and endometrial receptivity have been largely ignored. Large numbers of preovulatory follicles and oocytes subsequently retrieved have been applied as useful surrogate outcome parameters for successul IVF.[253] Maximum ovarian stimulation along with the transfer of large numbers of embryos in an attempt to maximize pregnancy rates per IVF cycle may by itself have a major impact on patient dropout rates, costs, and overall IVF outcome and should therefore be considered seriously. The introduction of GnRH antagonists allows for a careful reevaluation of current IVF strategies. We can now render stimulation protocols simpler, starting with a spontaneous menstrual cycle, allowing for more subtle interference with dominant follicle selection. Final stages of oocyte maturation can now also be stimulated, applying different drugs and strategies for the induction of an endogenous LH surge. Finally, effects of these altered follicular phase interventions on corpus luteum function and endometrial development (important for embryo implantation) should be assessed.

Especially in the light of a continued trend worldwide to reduce the number of embryos transferred, novel approaches of mild ovarian stimulation or even natural cycle IVF deserve reevaluation. It does not seem logical to stimulate the ovary profoundly in order to generate numerous embryos in case the aim is to transfer only one or two of them. Moreover, the possible relationship between quantity of oocytes stimulated and quality (i.e., genetic competence) of embryos obtained[265] should be studied in greater detail. Finally, in the light of a reduced number of fresh embryos being transferred, the continuing improvement of

techniques to cryopreserve supernumerary embryos such as vitrification (allowing couples additional pregnancy chances without having to go through ovarian stimulation and oocyte retrieval) seems of pivotal significance.

Individualizing ovarian stimulation in order to optimize outcomes between risks and desired outcomes is likely to improve in the future with the development of pharmacogenetics. Clinical studies have shown that FSH receptor gene polymorphisms can influence the ovarian response to stimulation in women undergoing IVF.[294] Genotyping of patients prior to treatment may therefore aid in tailoring FSH doses dependent on individual ovarian sensitivity.[272]

Choosing the "best" embryo for transfer is still problematic, because the assessment of embryo morphology is still crude and inaccurate. More information is urgently needed regarding randomized controlled trials replacing a single embryo with or without preimplantation genetic aneuploidy screening. The paradigm of measuring success in terms of positive pregnancy test per IVF retrieval or transfer treatment should shift in future studies to take into account the balance between the chance of a healthy live (singleton) birth per started IVF treatment, which may involve multiple cycles in relation to risks and complications, patient discomfort, and costs.[73] The health economics evaluation of IVF should be no different from other complex medical interventions. In this context, the impact of ovarian stimulation on embryo quality (applying aneuploidy blastomere screening through fluorescence in situ hybridization [FISH] procedures), corpus luteum function, and endometrial receptivity should be studied in greater detail.

The complete reference list can be found on the companion Expert Consult Web site at www.expertconsultbook.com.

Suggested Reading

Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet 365:1807-1816, 2005.

Fauser BC, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev 18(1):71-106, 1997.

Macklon NS, Stouffer RL, Giudice LC, Fauser BC. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocr Rev 27(2):170-207, 2006.

The Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Human Reprod 23(3):462-477, 2008.