PART 3

REPRODUCTIVE TECHNOLOGIES

CHAPTER 30

Medical Approaches to Ovarian Stimulation for Infertility

Bart C.J.M. Fauser

Concepts of Ovarian Stimulation

- *Ovarian stimulation can be applied for the medical treatment of anovulatory infertility (i.e., ovulation induction) or for infertility treatment in ovulatory women.*

Ovarian stimulation is a central component of many infertility therapies. At the outset, it is important to emphasize that two different concepts of ovarian stimulation exist: ovulation induction and ovarian stimulation. These approaches differ in both the starting point (i.e., the type of patients treated) and end points (i.e., the aim of the medical intervention). The reader is referred to Chapters 22 and 31 for additional coverage of this topic.

Ovulation Induction

In the strict sense of the term, ovulation induction refers to the triggering of ovulation, that is, the rupture of the preovulatory graafian 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 normoovulatory 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 (e.g., premature ovarian failure [POF] currently referred to as *primary ovarian insufficiency* [POI]), follicle development can be stimulated by various pharmacologic compounds, and normoovulatory 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) along with the major individual differences in ovarian response to stimulation, 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.[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 by using antiestrogens or aromatase inhibitors, the use of insulin-sensitizing agents, and laparoscopic surgical methods.

Ovarian Stimulation

This treatment modality has become an integral part of assisted reproductive technologies (ART). The aim of ART is to bring more male and female gametes closer together and thereby increase the chances of pregnancy. The goal of ovarian stimulation is to induce ongoing development of multiple dominant follicles and to mature many oocytes to

Abstract

Ovarian stimulation is an integral part of many different infertility treatments. Since the 1970s, ovarian stimulation has been applied in ovulatory women diagnosed with unexplained infertility aiming to increase the number of developing follicles and the number of oocytes for fertilization in vivo. Ovarian stimulation is often combined with intrauterine insemination of sperm. Since the early 1980s, ovarian stimulation has become an essential part of in vitro fertilization (IVF) aiming to improve pregnancy rates by providing the laboratory multiple oocytes for fertilization and early embryo development. Medical ovulation induction has now matured providing good cumulative live birth rates. In skillful hands and with proper ovarian response monitoring, chances for complications are low for ovulation induction. The aim of this intervention is to mimic physiologic circumstances in anovulatory women, hence, single dominant follicle development and ovulation. However, a tendency to hyperrespond to ovarian stimulation is a well-known feature of polycystic ovary syndrome (PCOS). Moreover, recent studies have shown the usefulness of novel drugs such as the aromatase inhibitor, letrozole, next to the antiestrogen clomiphene citrate or exogenous gonadotropins. In everyday practice, ovulation induction is often ignored in favor of IVF, although no direct comparative trials have been reported to date.

Any form of ovarian stimulation increases the chances of pregnancy per cycle, but it is at the expense of increased complication rates, most importantly multiple pregnancies and ovarian hyperstimulation syndrome (OHSS). This holds especially true for ovarian stimulation aiming at maturing multiple dominant follicles for fertilization either in vivo. Various strategies may significantly reduce chances for OHSS.

Regarding IVF, numerous new treatment modalities have been introduced over the years—often with insufficient evidence of safety and efficacy—using different compounds and dose regimens for ovarian stimulation, gonadotropin-releasing hormone analogue cotreatment, oocyte maturation trigger, interventions preceding stimulation, and luteal phase supplementation. The most important clinical challenge is to find the right balance between improving chances for success (birth of a healthy child) with reasonable cost, acceptable patient discomfort, and a minimal complication rate. New developments are rendering ovarian stimulation less intense and more individualized.

Keywords

Ovulation induction
ovarian stimulation
polycystic ovary syndrome (PCOS)
in vitro fertilization (IVF)
intracytoplasmic sperm injection (ICSI)
intrauterine insemination (IUI)
ovarian hyperstimulation syndrome (OHSS)
follicle-stimulating hormone (FSH)
luteinizing hormone (LH)
androgens
antiestrogens
clomiphene citrate
aromatase inhibitors
letrozole
insulin sensitizers
metformin
gonadotropin-releasing hormone (GnRH) agonist
GnRH antagonist

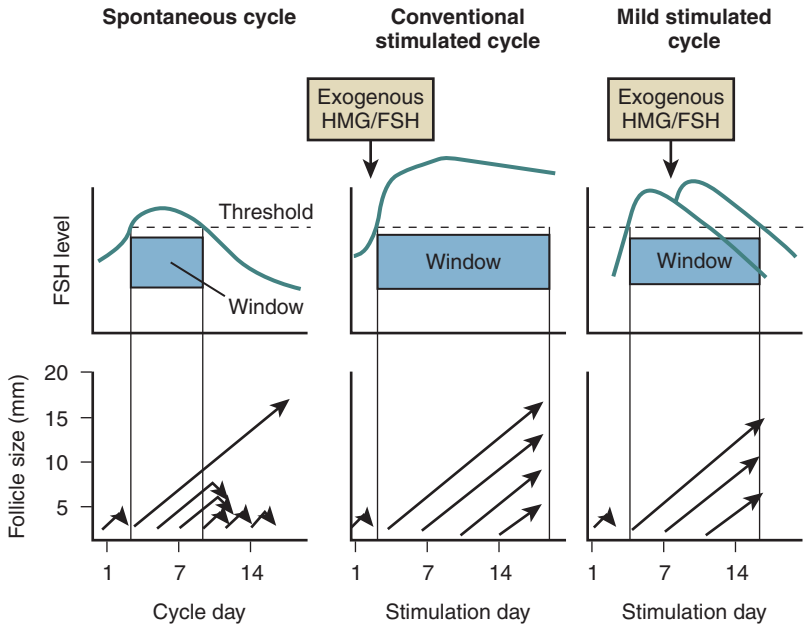


FIGURE 30.1 The follicle-stimulating hormone *(FSH)* threshold and window concept for monofollicular selection *(left panel)*, as conventionally applied to achieve multifollicular development *(middle panel)*. Each *arrow* represents a developing follicle. The *right panel* represents the concept of 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. *(Modified 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.)*

improve chances for conception either in vivo (empirical ovarian stimulation 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 normoovulatory women. Although ovarian hyperstimulation can also be performed in anovulatory women, this approach should be clearly differentiated from ovulation induction. The physiologic concepts that underlie current approaches to ovulation induction and ovarian hyperstimulation are described later in this chapter.

Concepts of Follicle Development Relevant to Ovarian Stimulation

- *Decreasing serum FSH concentrations during the follicular phase of the normal menstrual cycle are fundamental for single dominant follicle selection in the human.*

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 (see Chapter 8).[3,4] The great majority of primordial follicles that 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 (TGF)-β superfamily and factors regulating apoptosis (i.e., programmed cell death) are involved.[5,6] 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.[7,8] 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 (Fig. 30.1).[2] This process is referred to as *cyclic, gonadotropin-dependent recruitment* as opposed to the previously 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.[9,10] During the subsequent follicular phase, FSH levels plateau during initial days[11,12] and are gradually suppressed thereafter by ovarian inhibin B[13] and E_2[14] 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. 30.1) appear to be crucial for selection of a single dominant follicle from the recruited cohort.[11] Only one follicle escapes from atresia by increased sensitivity for stimulation by FSH and luteinizing hormone (LH).[2] This 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

antagonist-induced gonadotropin withdrawal.[15,16] 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.[17]

A central role has also been demonstrated for LH in monofollicular selection and dominance in the normal ovulatory cycle.[18-20] 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.[10] The concept of both endocrine and autocrine upregulation is supported by several other observations that characterize the dominant follicle, including the in vitro induction of aromatase enzyme activity,[21] ovarian morphology,[22] and endocrine changes in follicle fluid[23] 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

- *The history of ovarian stimulation with exogenous compounds in the human goes back almost a century. Many compounds and regimens have subsequently been developed.*
- *The history is of interest, and there are a few Nobel prizes in that historical recounting, but it does not guide current practice/patient care, so somewhat elective.*

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 resulting in antibody formation impacting 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 a historical overview, see Gruhn and Kazer[24] and Lunenfeld[25]).

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.[26] Shortly thereafter, the ovulation-inducing capacity of the next generation of closely related antiestrogens (MRL/41; clomiphene citrate [CC]) (Fig. 30.2) was recognized.[27] More than half a century later, CC is still the most applied drug for infertility therapies worldwide, accounting for around two-thirds of all prescriptions.

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.[28] 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

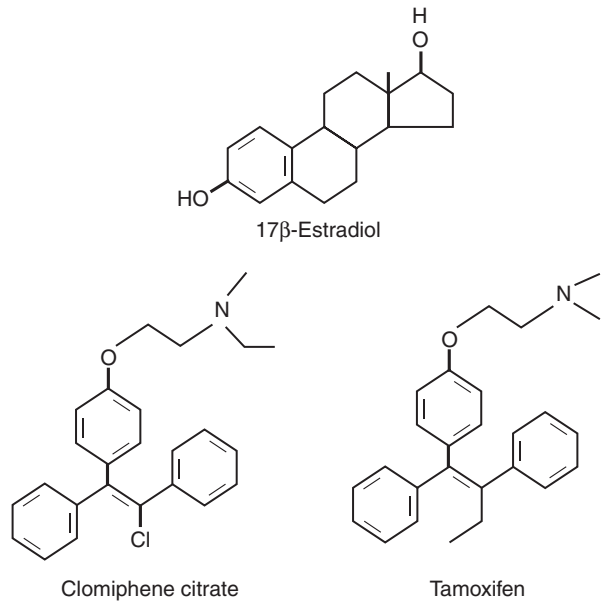


FIGURE 30.2 Structure of 17β-estradiol and the antiestrogenic triphenylethylene derivates clomiphene citrate and tamoxifen.

been described.[29] The exact nature of the mechanism of action of CC is still uncertain.[30,31] Induced changes in other systems, such as insulin-like growth factor (IGF), may partly explain the capacity of CC to stimulate the ovary.[30] However, antiestrogenic 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[32,33] and is still often applied for the empirical treatment of unexplained infertility.

Gonadotropin Preparations

Clinical experiments in the late 1950s demonstrated that extracts derived from the human pituitary could be used to stimulate gonadal function.[34] 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.[35] 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 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.[36] Subsequent IVF pregnancies were reported from Australia to occur after ovarian stimulation with CC.[32] The more widespread use of hMG for successful IVF was developed thereafter in the United States.[37] For over 2 decades, gonadotropin preparations have also been extensively applied for ovarian stimulation in ovulatory women for empirical treatment of unexplained subfertility. The aim here 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. These initial preparations were very impure with many contaminating proteins; only less than 5% of the proteins present were bioactive. As purity improved, it was necessary to add human chorionic gonadotropin (hCG) to maintain this ratio of bioactivity.[38] 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.

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.[39] 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.[39]

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. 30.3) into Chinese hamster ovary cell lines,[40] the large-scale in vitro production of human recombinant FSH (recFSH) has been realized (see Chapter 2).[41] The first pregnancies using this novel preparation in ovulation induction[42] and in IVF[43] 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 bioactivity, and so-called *filled-by-mass* preparations[44] are now available for clinical use. Subsequently, recombinant LH (recLH) and recombinant hCG (rechCG) have also been developed and introduced for clinical application.[29] Finally, a long-acting recFSH agonist (a man-made chimeric hormone generated by the fusion of the carboxy-terminal peptide [CTP] of hCG to the FSH-β chain) has recently been introduced into the clinic after efficacy and safety had been established in large sample size trials where IVF clinics from all over the world participated.[45-47] Moreover, the first recFSH produced by a human cell line has recently been tested,[48] and recFSH biosimilars have been introduced on the market.[49]

Gonadotropin-Releasing Hormone Analogues

In 1971, the small decapeptide GnRH was isolated and its structure was elucidated by Schally and Guillemin (Fig. 30.4). 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 downregulation

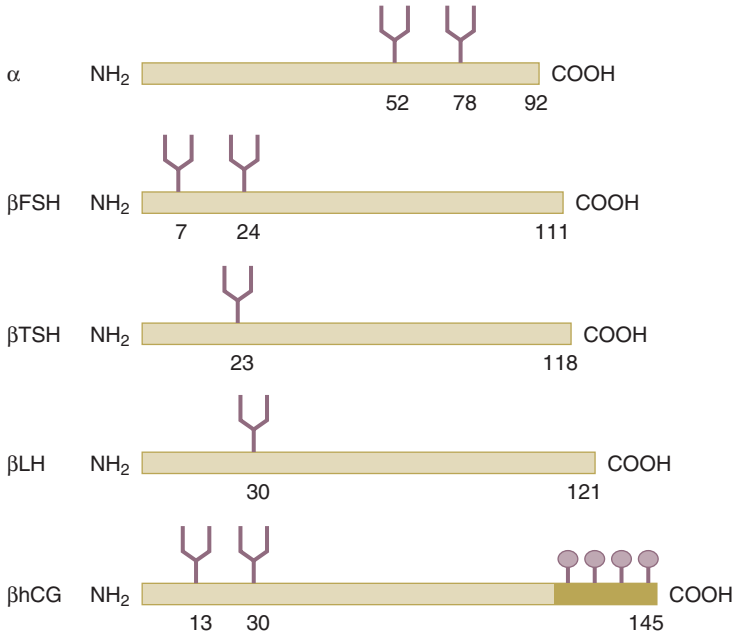


FIGURE 30.3 Structure of the common α subunit and hormone-specific β subunit of the human glycoprotein hormones: follicle-stimulating hormone *(FSH)*, thyroid-stimulating hormone *(TSH)*, luteinizing hormone *(LH)*, and human chorionic gonadotropin *(hCG)*.

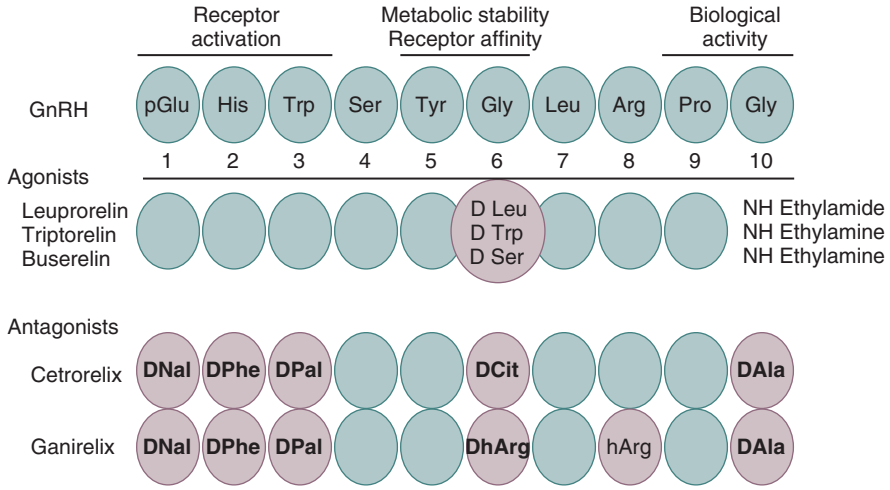


FIGURE 30.4 Structure of the native decapeptide gonadotropin-releasing hormone *(GnRH)*, as well as the modified, commercially available GnRH agonists and antagonists. *Arg*, Arginine; *D*, dextro; *DAla*, D-alanine; *DCit*, D-citroline; *DhArg*, D-homoarginine; *DNal*, D-naphthylalanine; *DPal*, D-pyridylalanine; *DPhe*, D-phenylalanine; *Gly*, glycine; *hArg*, homoarginine; *His*, histidine; *Leu*, leucine; *pGlu*, pyroglutamate; *Pro*, proline; *Ser*, serine; *Trp*, tryptophan; *Try*, tyrosine.

could be induced by the continued administration of GnRH.[50]

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.[51] 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.[50] Shortly thereafter, the use of GnRH agonists such as buserelin, triptorelin, or leuprorelin to downregulate the pituitary prior to administration of gonadotropins (a strategy that became known as the "long protocol") became the standard of care. The more recent clinical introduction of GnRH antagonists has slowly changed practice in IVF, and currently over 50% of IVF cycles apply GnRH antagonist cotreatment.

It has taken almost 3 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.[51,52] Subsequently, both the compounds ganirelix and cetrotide were shown to be safe and efficacious in IVF. These third-generation GnRH antagonists 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. Clinical uptake of GnRH antagonists in IVF has been rather slow. The most recent meta-analysis has confirmed the use of GnRH antagonist cotreatment to be effective and safer.[53-55]

Outcomes of Ovarian Stimulation

- *Ovarian stimulation is a common intervention in infertility.*
- *Ovarian stimulation may be applied in normoovulatory women for empirical reasons or in the context of IUI or IVF.*
- *Another form of ovarian stimulation involves the medical treatment of anovulatory infertility aiming to restore normal ovarian function.*

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 normoovulatory cycles cannot always be achieved, so the chances of complications such as multiple pregnancy or OHSS should be taken seriously, especially in patients diagnosed with PCOS. Oligomenorrheic women may or may not have incidental spontaneous ovulations; therefore spontaneous pregnancies may occur. For obvious reasons, fertility specialists see only oligo/amenorrheic 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, type of drugs used, gonadotropin preparations and dose regimens used, the intensity of monitoring ovarian response to stimulation, and willingness to cancel the cycle in case of hyperresponse. An alternative option under those circumstances would be to convert ovulation induction to IVF. Cumulative live birth rates of ovulation induction have been reported to be around 75% to 80%,[56,57] with a coinciding incidence of multiple pregnancies of around 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 coagulability, 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.[58] Moderate to critical OHSS is very rare with CC but constitutes an important complication of gonadotropin use.[59] 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.[29] In addition to PCOS, risk factors for the development of OHSS include young age and low body weight.[59] The risk is further increased when adjuvant GnRH agonist treatment is employed.[60]

The contribution of ovulation induction treatment to the number of triplet and higher-order pregnancies is considerable.[60,61] 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.[62]

Ovarian Stimulation

As previously outlined, the aim of ovarian stimulation alone or in combination with assisted reproductive techniques is to bring an increased number of gametes (oocytes and sperm) in close proximity to augment pregnancy chances. Ovarian stimulation alone 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 are 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%.[63]

Initial studies suggested that a threefold increase in monthly probability of pregnancy can be achieved with empirical ovarian stimulation in the treatment of unexplained infertility (Fig. 30.5).[64] 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 were 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.[65] 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.[66] A recent, well-designed randomized controlled trial (RCT) comparing ovarian stimulation in unexplained infertility comparing gonadotropins, the aromatase inhibitor letrozole, and CC concluded gonadotropins to be superior in terms of cumulative clinical pregnancy rates, but again at the expense of a high multiple pregnancy rate of 32% (Fig. 30.6).[67] 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. Based on a 2-year experience in a large US 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.[68] 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

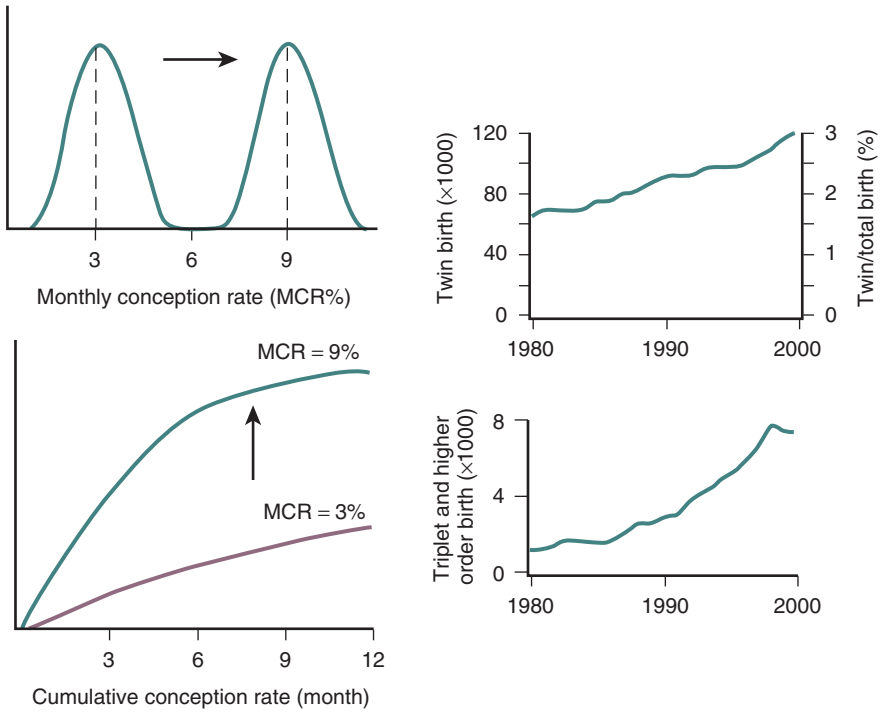


FIGURE 30.5 Monthly conception rate in unexplained infertility increasing from 3% to 9% per cycle due to ovarian stimulation and resulting increase in cumulative conception rates over a 12-month period *(left)*; increased occurrence of multiple pregnancies (twin, triplet, and higher order) between 1980 and 2000 associated with ovarian stimulation *(right)*. *(Left from Stovall DW, Guzick DS: Current management of unexplained infertility.* Curr Opin Obstet Gynecol *5:228–233,1993; right from Rowland Hogue CJ: Successful assisted reproduction technology: the beauty of one.* Obstet Gynecol *100:1017, 2002; and Jones HW: Multiple births: how are we doing?* Fertil Steril *79:17–21, 2003.)*

rate of multiple pregnancies arising from ovarian stimulation with or without IUI remains uncertain, however,[69] as few national registers record the outcome of ovarian stimulation. A recent summary of the European IVF monitoring consortium involving 34 countries, and a total of over 640,000 treatment cycles for the year 2012, reported a multiple delivery rate of 17.9%.[70]

A number of years ago, it was estimated that ovarian stimulation with or without IUI is responsible for around 30% of multiple births (Fig. 30.7). 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 stimulation for IVF is merely the factor allowing for the generation of multiples, but it is not the sole determining factor as in IUI. Unsurprisingly, the incidence of twin pregnancies following IVF without stimulation[71] or with ovarian stimulation combined with single embryo transfer (SET) is close to normal.[72,73] Over the years, the number of embryos transferred in IVF has decreased globally, but two to three embryos are still transferred in a significant proportion of IVF cycles in many countries around the world.

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.[74] 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.[75,76] The great majority of centers in Northern Europe have now adopted a SET policy in the great majority of IVF patients. Not surprisingly the multiple pregnancy rate dropped dramatically, but surprisingly the cumulative (fresh and frozen embryo transfer from the same oocyte harvest) live birth rate remained the same.[77-80] In general, overall IVF results in Europe remain slightly lower compared to the United States but with an overall reduced incidence of multiple and premature birth (Fig. 30.8).

Given the risks associated with ovarian stimulation, couples should be well counseled regarding their spontaneous chances for pregnancy prior to commencing empirical therapy for unexplained infertility (Table 30.1). These chances are often underestimated.[81]

Higher-order multiple pregnancies have a major adverse impact on perinatal morbidity and mortality rates. Mortality rate is increased 4- to 7-fold in twins and up to 20-fold in triplets.[61] Children born from multiple pregnancies have more chances for perinatal complications and subsequent health problems, chiefly associated with prematurity and low birth weight.[62] Chances for cerebral palsy are increased almost 50-fold in children from triplet pregnancies.[82] 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.[83] Besides the medical and emotional burden, the financial costs associated with multiple pregnancies should be considered by policy makers. Obstetric and neonatal costs are increased fivefold to sevenfold in higher-order multiples, and by the

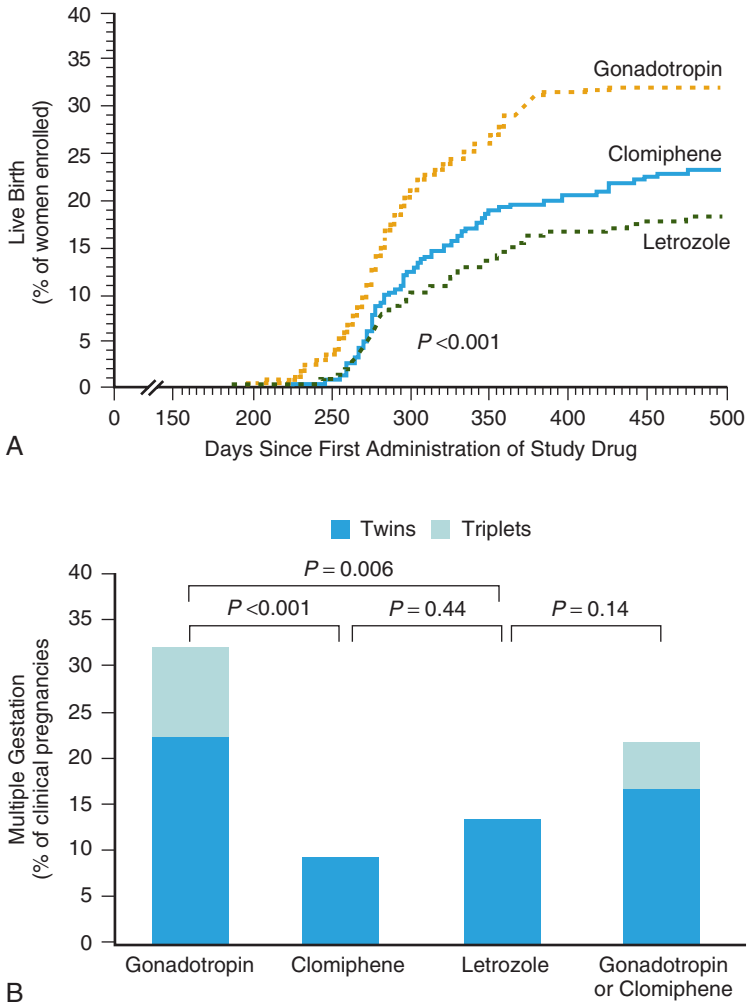


FIGURE 30.6 Cumulative live birth rates (A) and percent of multiple gestation (B) applying ovarian stimulation with gonadotropins, clomiphene, or letrozole for unexplained infertility. *(From Diamond MP, Legro RS, Coutifaris C, et al: Letrozole, gonadotropin, or clomiphene for unexplained infertility.* N Engl J Med *373[13]:1230-1240, 2015.)*

age of 8, costs for low-birth-weight children are increased eightfold.[62] Finally, possibilities of more subtle health risks that 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 may be shifted from pregnancy rate per treatment cycle toward health live birth started course of treatment (which may involve multiple treatment cycles) in the context of cost, burden of treatment, and complication rates.[84]

Induction of Ovulation in Anovulatory Women

- *The term ovulation induction should be reserved for the medical treatment of anovulatory infertility.*
- *Good results in terms of cumulative singleton live birth can be achieved by skilled hands and with proper ovarian response monitoring.*
- *Trials directly comparing outcomes of ovulation induction versus IVF are urgently needed.*

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 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 stimulation may be applied in ovulatory women undergoing IVF treatment where multifollicular development is required to produce multiple oocytes.

In contrast, 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.[85] 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 subsequent ovulation. In recent years, increased understanding of the pathophysiology of ovarian dysfunction has enabled the development of clinical strategies that aim to mimic the endocrine control of normoovulatory cycles.

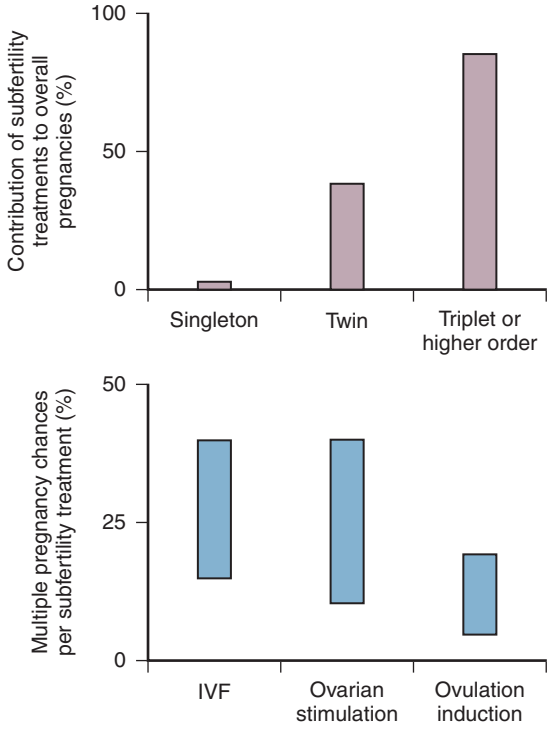


FIGURE 30.7 Contribution of subfertility treatments to overall pregnancies *(upper)* and reported frequency of multiple pregnancy in relation to in vitro fertilization *(IVF)*, ovarian stimulation, and ovulation induction *(lower)*. *(From Fauser BC, Devroey P, Macklon NS: Multiple birth resulting from ovarian stimulation for subfertility treatment.* Lancet *365[9473]:1807–1816, 2005.)*

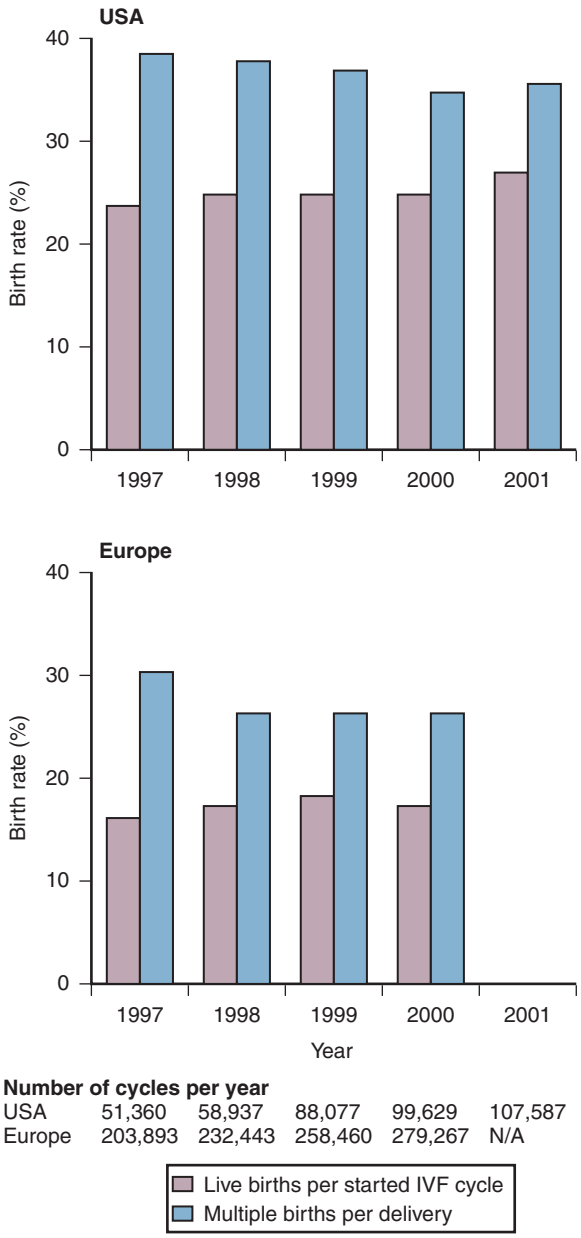


FIGURE 30.8 Rates of live births per started in vitro fertilization *(IVF)* cycle and multiple births. *N/A,* Not available. *(From Fauser BC, Devroey P, Macklon NS: Multiple birth resulting from ovarian stimulation for subfertility treatment.* Lancet *365[9473]:1807–1816, 2005.)*

Table 30.1 Hypothetical Model of Cumulative Spontaneous Pregnancy Rates in Five Categories, According to Duration of Subfertility

		Cumulative Pregnancy Rate After (%)			
Category	**MFR (%)**	**6 Months**	**12 Months**	**24 Months**	**60 Months**
Superfertile	60	100	—	—	—
Normally fertile	20	74	93	100	—
Moderately subfertile	5	26	46	71	95
Severely 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.

Achieving this within the narrow therapeutic margins of stimulating single rather than multiple follicular developments remains a challenge to clinicians.

The second European Society of Human Reproduction and Embryology (ESHRE) and Association of Reproductive Managers (ASRM) sponsored PCOS consensus workshop acknowledged that much attention should be paid to the condition of the woman (in terms of food intake, lifestyle, and smoking habits) before embarking on any form of ovulation induction.[86] Such a periconception strategy emphasizes the general observation that chances for pregnancy complications and compromised children outcomes are directly related to the health status of the woman before getting pregnant (Boxes 30.1 and 30.2).

Classification of Anovulation

Ovarian dysfunction can be readily classified in everyday clinical practice based on the assessment of serum gonadotropin and estrogen levels in peripheral blood. This concise approach, known as the World Health Organization (WHO) classification of anovulation, was originally developed by Insler and colleagues.[87] Amenorrhea may coincide with either low or normal E_2, whereas oligomenorrhea is associated only with normal estrogens. Low estrogens combined with low gonadotropin levels suggest a central origin of the disease at the hypothalamic-pituitary level.[88] 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 based on POF (currently referred to as POI) or ovarian dysgenesis. This cause of anovulation, termed WHO class 3, occurs in around 5% of infertile women and around 1% to 2% of the general female population. Anti-müllerian hormone (AMH) may help to identify women with POI with some residual ovarian activity.[89]

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. 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 and ESHRE. The so-called Rotterdam consensus criteria are broader than the National Institutes of Health (NIH) criteria primarily because polycystic ovaries are now included. The incidence of PCOS as defined by the Rotterdam criteria is therefore higher.[90]

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 discussed in detail in Chapter 3.

Box 30.1 Most Pertinent Current Issues Related to Ovulation Induction

- Clomiphene versus letrozole as first-line treatment
- Should adjunct drugs be tried next to clomiphene citrate before second-line treatment?
- Efficacy and safety of gonadotropins for ovulation induction in everyday practice
- Short- and long-term pros and cons of ovarian surgery compared to gonadotropins ovulation induction
- Ovulation induction outcomes compared to in vitro fertilization as first-line treatment in terms of success, cost, burden of treatment, and complications

Box 30.2 Most Pertinent Current Issues Related to Ovarian Stimulation for In Vitro Fertilization

- Does generating more oocytes improve overall outcomes of IVF?
- Most appropriate starting does for FSH
- Is there enough evidence to individualize the FSH starting dose?
- Usefulness of changing the FSH dose during stimulation
- Usefulness of additional compounds (such as clomiphene, letrozole, androgens, LH/hCG, growth hormone, and others)
- Intensity and way of ovarian response monitoring
- Cotreatment with GnRH agonist/antagonist
- Pretreatment with steroids
- Is ovarian stimulation associated with impaired endometrial receptivity?
- Most appropriate intervention in case of low ovarian response
- Mild ovarian stimulation
- Oocyte maturation triggering by GnRH agonist trigger
- Luteal phase supplementation

FSH, Follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *IVF,* in vitro fertilization; *LH,* luteinizing hormone.

Preparations for Treating Anovulation

- *The medical treatment of anovulation can be performed using different drugs.*
- *The preferred drug should be viewed in the context of ease of use, cost, efficacy, and risks.*

Antiestrogens

Background

The most widely used antiestrogen 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 50 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 for most clinicians. Given orally in the early to midfollicular phase, it causes a 50% rise in the endogenous serum FSH level,[91] 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.[92] The long half-life zuclomiphene isomer (which exhibits predominant estrogen agonist activity) has been shown to persist and accumulate across consecutive cycles of treatment.[93] 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.[94] 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.[95,96]

Clinical Outcome

Between 60% and 85% of anovulatory women will become ovulatory with CC, and 30% to 40% will become pregnant.[86] 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.[97] 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 (see Chapter 2). A number of studies have pointed to being overweight as a negative factor.[98] 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.[98]

The occurrence of ovulation can be identified using temperature charts and midluteal urinary pregnanediol or serum progesterone measurements.[86] Although results of large trials indicate that monitoring by ultrasound is not mandatory to ensure good outcomes,[86] 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%,[98] with conception rates per cycle around 22%.[86] 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 antiestrogenic effects of CC on the reproductive tract have been particularly implicated. Negative effects on tubal transport, quantity and quality of cervical mucus,[29] and the endometrium[99] 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[100] 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.[26] The multiple pregnancy rate is less than 10%, and OHSS is rare.[86] The putative increased risk of ovarian cancer reported to be associated with the use of CC for more than 12 months[101] has led CC to be licensed for just 6 months of use in some countries.

Similar to CC, tamoxifen is 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.[102] 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).[103] 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 initially 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 (Fig. 30.9).[104,105]

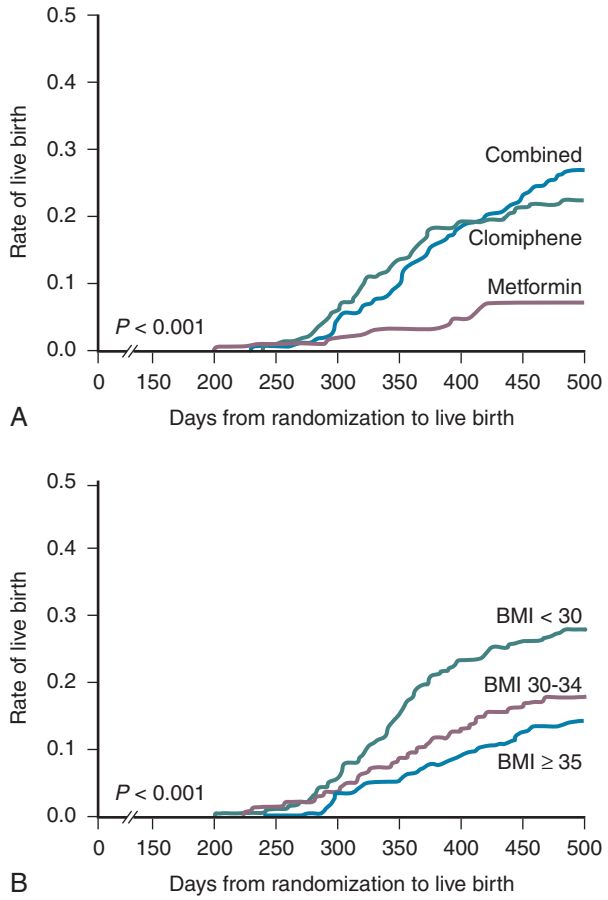


FIGURE 30.9 Kaplan-Meier curves for live births following ovulation induction with clomiphene, metformin, or both treatments combined (A) and body mass index *(BMI)* (B). *(Modified from Legro RS, Barnhart HX, Schlaff WD, et al: Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome.* N Engl J Med *356:551–566, 2007.)*

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.[106,107] 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.[108,109] It is recommended that metformin be commenced at 500 mg/day orally, rising to 500 mg 3 times a day over 7 to 10 days.[108] 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.[110,111]

Clinical Outcome

The majority of studies on the outcome following metformin therapy are small and uncontrolled or simply case series.[112] 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 to 6.69) for metformin versus placebo and 4.41 (CI, 2.37 to 8.22) for metformin and clomiphene versus clomiphene alone.[113] However, a recent large multicenter study has clarified the role of metformin as an alternative first-line ovulation induction agent in women with PCOS.[105] In this study, 626 women with PCOS were randomized to receive 50 to 150 mg CC plus placebo from cycle day 3 to 7500 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 Fig. 30.9. 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. A significant proportion of women using metformin discontinue treatment because of side effects. Pharmacogenomic studies on genes involved in metformin transport and action and effectiveness of in ovulation induction have been reported, but the findings are not yet translatable to clinical application.[114,115]

It has been suggested that metformin may reduce the rate of miscarriage compared with CC-derived pregnancies. However, in the study of Legro and colleagues, the rate of first trimester loss did not differ significantly between the treatment groups, although the study was not powered to detect this.[105]

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%.[110] 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. 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 (Box 30.3).[116] A number of studies have appeared suggesting a role for metformin to ameliorate these complications. However, conflicting results have been reported and most of these studies are not randomized or suffer from small numbers and surrogate outcomes.[117,118]

Aromatase Inhibitors

Background

In recent years the use of aromatase inhibitors to mimic the actions of CC has been proposed.[119] Rather than antagonizing estrogen feedback activity at the hypothalamic-pituitary axis,

this approach aims at reducing the amount of estrogens being synthesized. Aromatase inhibitors block the conversion of AD and T to estriol (E_3) and E_2, respectively.[120] This increases gonadotropin secretion, resulting in stimulation of ovarian follicles.[119] 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, letrozole, has been the focus of study as a potential therapeutic agent for inducing ovulation.

Box 30.3 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

Clinical Outcomes

When given in the early follicular phase, letrozole reduces estrogen feedback at the pituitary-hypothalamic axis, causing a subsequent increase in gonadotropin secretion. This was shown in monkeys to stimulate follicle development.[121] 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.[119] 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.[122]

Although the concept of applying aromatase inhibitors as an alternative to CC or as adjuvant therapy to CC or gonadotropins seems attractive and preliminary data on pregnancy outcome were encouraging,[123] a more recent systematic review and meta-analysis, involving 13 RCTs, concluded that aromatase inhibitors should not be recommended as first-line ovulation induction treatment in the absence of good quality evidence of efficacy.[124]

A large sample size RCT published in 2014, performed by the NICHD Reproductive Medicine network, comparing letrozole and CC as first-line treatment in 750 women diagnosed with PCOS, surprisingly reported a cumulative live birth rate of 28 versus 19%, respectively (Fig. 30.10).[125] Altogether, based on current knowledge, letrozole can be

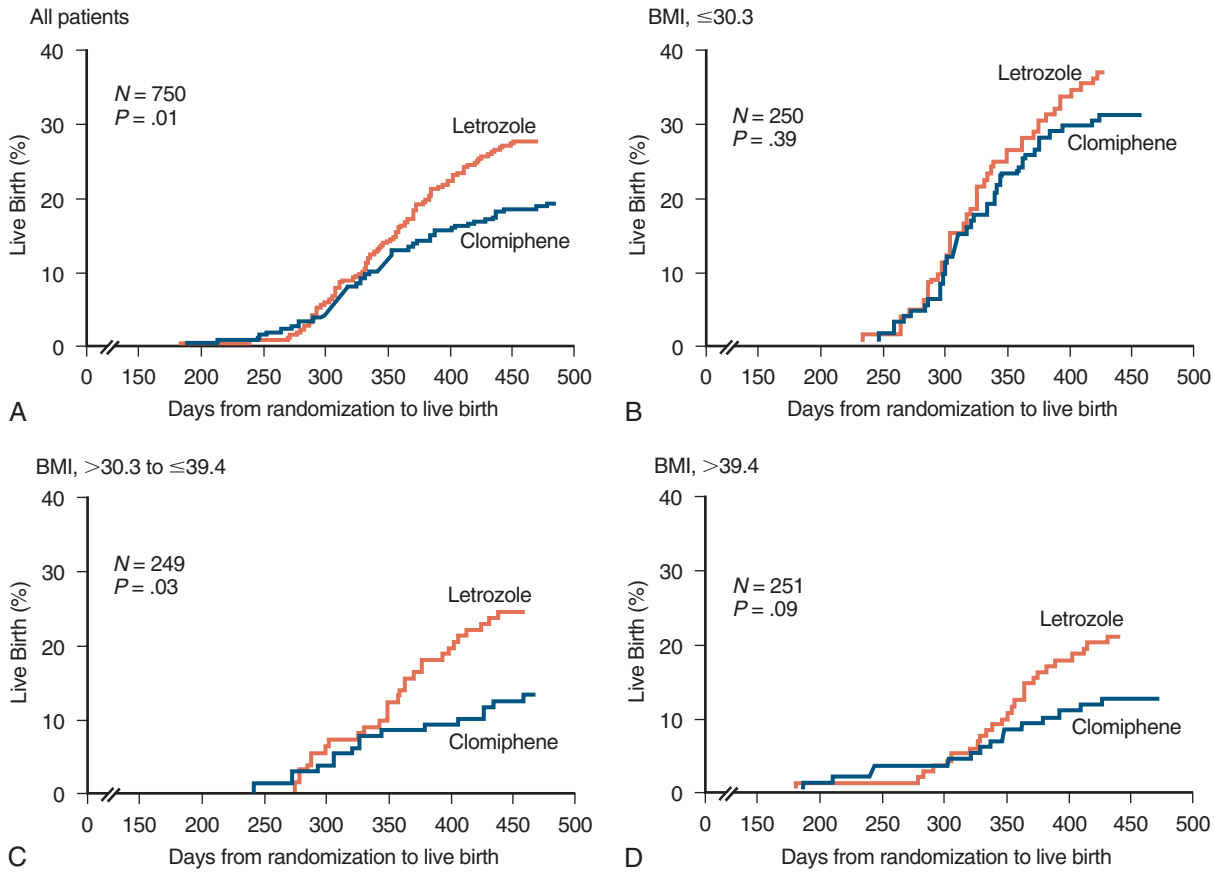


FIGURE 30.10 Live birth rates for all women (and separate for different body weight categories) using either letrzole or clomiphene for ovulation induction in polycystic ovary syndrome. *(From Legro RS, Brzyski RG, Diamond MP, et al: Letrozole versus clomiphene for infertility in the polycystic ovary syndrome.* N Engl J Med *371[2]:119–129, 2014. Erratum in:* N Engl J Med *317[15]:1465, 2014.)*

considered a realistic alternative for first line ovulation induction in PCOS. However, the generalizability in these findings remains a matter of debate since the study was performed in a severely obese population, and resulting CC success rates are much lower than reported elsewhere.

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 enthusiasm for undertaking additional clinical studies has been inhibited because an association was reported between letrozole and fetal toxicity. However, a more recent analysis of outcomes in 911 newborns conceived following CC or letrozole treatment showed no difference in the overall rates of major and minor congenital malformations.[126]

Gonadotropins

Background

Women with WHO class 2 anovulation who fail to ovulate or conceive following ovulation induction with antiestrogens can be successfully treated with exogenous gonadotropins. Exogenous gonadotropins have been widely used for the treatment of anovulatory infertile women since 1958.[2,25] 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 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 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 "two-cell, two-gonadotropin" concept. In addition to stimulating aromatase activity, FSH also induces LH receptors and further increases FSH receptor formation on granulosa cells while stimulating DNA and protein synthesis by the cell.[127] 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.[17] 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 or rechCG offers the possibility for a more sophisticated approach to treatment.

Recent studies have demonstrated the safety and appropriate dose required to affect follicle development and subsequent pregnancy. It has been established that baseline levels of at least 0.5 to 1 IU LH should be sufficient to provide maximal stimulation to thecal cells.[128] 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.[129]

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 (corifollitropin alpha), a recLH, and a rechCG have been added to the clinical arsenal for ovarian stimulation.

To achieve development of a single dominant follicle with exogenous gonadotropins, specific treatment and monitoring protocols are needed. The most frequently encountered dose regimen in the literature and in clinical practice is the low-dose step-up protocol. The initially described standard step-up protocol used a starting dose of FSH 150 IU/day.[130] However, this regimen was associated with a high complication rate. Multiple pregnancy rates of up to 36% were reported, and OHSS occurred in up to 14% of treatment cycles.[2] As a result, this protocol has been abandoned.

The concept of the FSH threshold proposed by Brown[131] postulated that FSH concentrations must exceed a certain level before follicular development will proceed (see Fig. 30.1). 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 forms 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 recommended initial starting dose of FSH is 37.5 to 50 IU/day.[86] The dose is increased by 50% if no response is observed on ultrasonography after 14 days (and serum E_2 monitoring). 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.[132] 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.

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.[133] 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.[134] 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 (Fig. 30.11).[135] As demonstrated previously in the monkey model, when the negative feedback effect of E_2 on gonadotropin production is suppressed by administration of antiestrogens, selection of the preovulatory follicle is overridden.[136] 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.[10] Endocrine studies have confirmed that E_2 levels in the serum[11] and follicular fluid[23] begin to rise only after a dominant follicle is present. The abovementioned initial research findings provided the theoretical basis for developing and monitoring a step-down regimen of ovulation induction.

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.[137] Studies focusing on further reducing the starting dose have reported the feasibility of commencing with 50 IU or 37.5 IU.

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 treated with the step-up regimen, presumably reducing the risk of multiple pregnancy and hyperstimulation.[138]

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

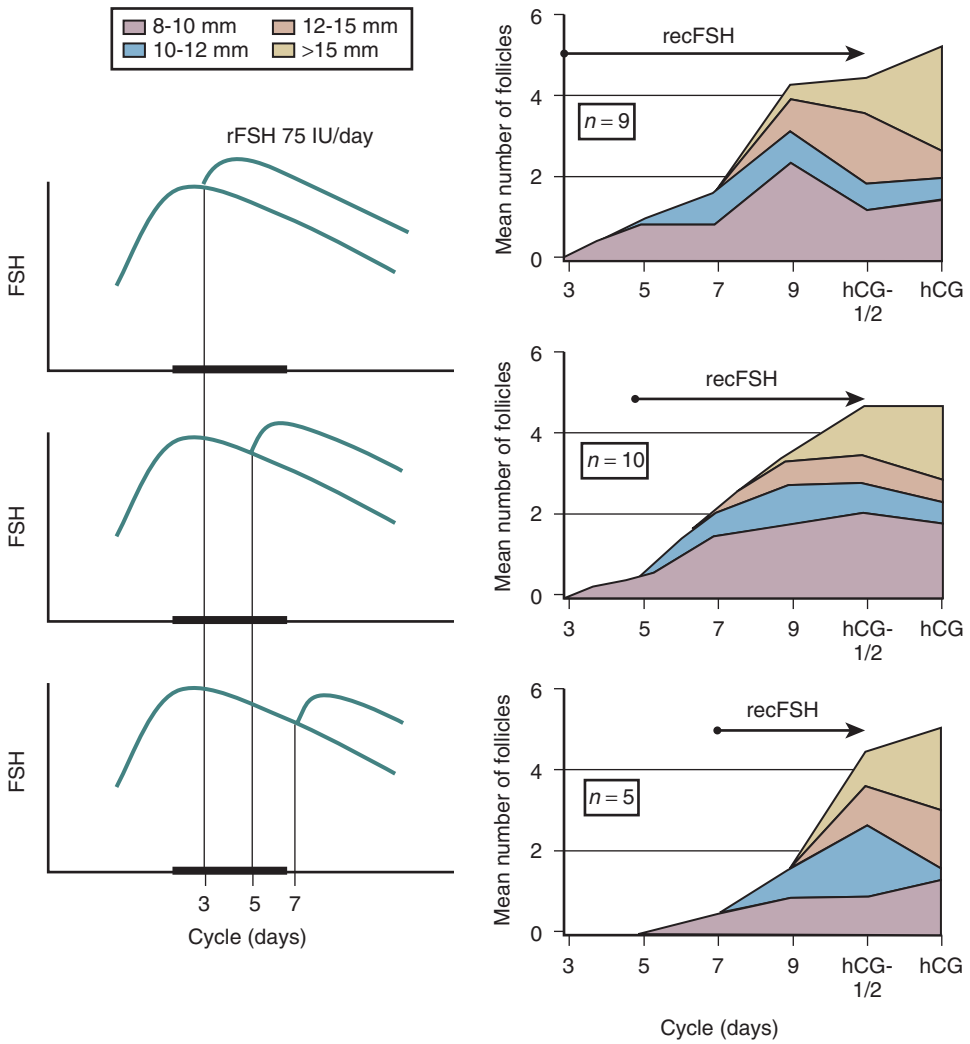


FIGURE 30.11 Observations substantiating the follicle-stimulating hormone *(FSH)* window concept in humans. Multiple dominant follicle growth can be observed in normoovulatory women receiving daily low doses of exogenous FSH, starting on cycle day 3, 5, or 7. *hCG,* Human chorionic gonadotropin; *recFSH,* recombinant FSH. *(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.)*

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 OHSS.[60] The total dose of recFSH needed and duration of treatment was less, and the complication rates were similar. In a later meta-analysis of RCTs 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 to 1.8). Furthermore, the odds ratios for pregnancy rate (OR 0.95; 95% CI, 0.64 to 1.41), miscarriage rate (OR 1.26; 95% CI, 0.59 to 2.7), multiple pregnancy rate (OR 0.44; 95% CI, 0.16 to 1.21), and OHSS (OR 1.55; 95% CI, 0.5 to 4.84) showed no significant difference between recFSH and uFSH.[139] In terms of 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.[140]

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.[141] 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.[19] Supplementation of LH activity may offer advantages in some patients by hastening large follicle development and therefore shortening the duration of treatment.[142] Moreover, the work of Zeleznik and coworkers[18] 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 hyperresponse to recFSH were randomized to continue treatment with the addition of either placebo or recLH.[143] 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. 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 to 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 to prevent multiple pregnancies and OHSS. Measurements of serum E_2 may also be useful.[144] 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.[145]

As confirmed recently, high cumulative singleton live birth rates of almost 80% can be achieved using CC as first line and low-dose FSH as second-line ovulation induction treatment in PCOS, with 14% multiple pregnancy rates (Fig. 30.12).[56,57] PCOS women with a poor prognosis for ovulation induction, in whom alternative treatment strategies such as IVF may be considered, can best be identified by age, duration of infertility, and body weight.

Pulsatile Gonadotropin-Releasing Hormone

Background

In the normoovulatory woman, the pattern of GnRH pulse stimulation alters with the phase of the menstrual cycle, effecting differential gonadotropin synthesis and secretion.[146] 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 midfollicular or late follicular phase, GnRH frequency increases, favoring LH secretion.[147] 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.[148] 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.[149] 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. The lower dose should be used initially to minimize the likelihood of multiple pregnancies.[150] 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.[151]

Clinical Outcomes

Pulsatile GnRH administration is primarily indicated for women with hypogonadotropic hypogonadal anovulation

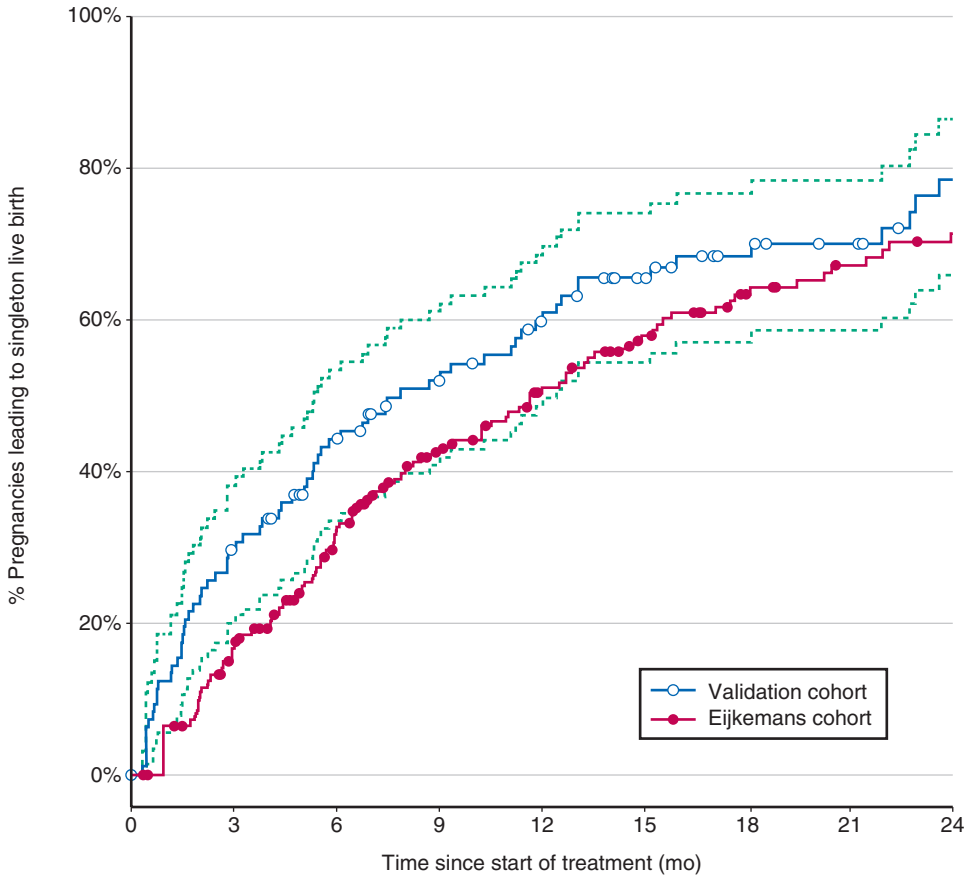


FIGURE 30.12 Cumulative pregnancy rates leading to single live birth following ovulation induction in polycystic ovary syndrome. *(Modified from Veltman-Verhulst SM, Fauser BC, Eijkemans MJ: High singelton live birth rate confirmed after ovulation induction in women with anovulatory polycystic ovary syndrome: validation of a prediction model for clinical practice.* Fertil Steril *98:761–768, 2012.)*

(WHO type 1) who have normal pituitary function.[152] 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.[150,153] Lower ovulation and pregnancy rates have been observed in women with WHO type 2 anovulation, including PCOS.[154] This may be because anovulation in PCOS in part reflects the effects of a persistent, rapid frequency of GnRH stimulation of the pituitary, causing increased LH and T levels.[147] 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.[155]

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, pharmacokinetic studies comparing the two routes have shown that the plasma GnRH profiles are damped after subcutaneous administration and that bioavailability is reduced.[156] However, the increased convenience offered by the subcutaneous route has led to this approach being favored by many. Smaller devices and more patient friendly delivery systems continue to be developed.

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.[157] 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.[158]

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.[159] However, others have observed restoration of the menstrual cycle.[160] 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.[161] 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.[162] However, conclusions as to the efficacy, optimal regimen, and safety of opiate antagonists for inducing ovulation cannot yet be made. No randomized controlled studies demonstrating their value for this condition have yet been published, and opiate antagonists remain at best a 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).

Kisspeptin

Drugs intervening with the pre-GnRH kisspeptin system are currently being tested for their potential use for both ovarian stimulation and ovarian suppression.

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.[163] Although the source of high androgen secretion in anovulatory women with PCOS is primarily ovarian, 50% to 70% also demonstrate excessive adrenal androgen levels.

To normalize (without suppressing) adrenal steroid production, daily oral doses of dexamethasone (0.25 to 0.5 mg) or prednisone (5 to 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 questionable. In a study of women with PCOS, the chance of ovulation after glucocorticoid suppression of adrenal androgens was not predicted by either basal dehydroepiandrosterone sulfate (DHEAS) levels or suppressed levels, and limited effects on ovulation were observed.[164] 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.[163]

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.[165] Further studies indicated that premature luteinization could be prevented by employing GnRH agonists, but no clear difference in pregnancy rates was demonstrated.[166] Although a meta-analysis of five prospective studies[167] 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 and hMG does not improve pregnancy and OHSS rates and should therefore not be recommended as a standard treatment for this patient group.[168]

Conflicting data on the effects on ovulation and pregnancy rates, combined with reports of severe OHSS with adjuvant 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 that 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.[169] 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 metabolical indices including insulin resistance.[170] 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.[171-173] A relatively modest reduction in weight has been shown to increase the frequency of ovulation in obese anovulatory women to more than 70%.[174] Energy restriction acting to temporarily improve insulin sensitivity may be important,[173] because improvements in endocrine and clinical parameters occurred maximally during the period of energy restriction. During subsequent weight maintenance, many benefits were reversed.[173]

The evidence for the benefits of weight loss combined with recent data confirming BMI to be a major factor influencing outcome of ovulation induction[86] make the treatment of obesity an important adjuvant treatment that should precede ovulation induction.[86] 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.[175-177]

Tobacco Smoking

Epidemiologic data provide strong evidence for a causal association between cigarette smoking and other lifestyle

factors and decreased fertility. For a recent review of the impact of smoking and other lifestyle factors on fertility treatment outcomes, see Homan and colleagues.[178] Dose-dependent effects of smoking have been reported in relation to the duration to conception.[179] Moreover, there is evidence of increased risk of early pregnancy loss in smokers[180] and a reduced mean age at menopause.[181] 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.[182] 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.

Ovarian Stimulation in the Empirical Treatment of Unexplained Subfertility

- *Despite numerous studies, the added value of ovarian stimulation for the empirical treatment of unexplained infertility remains uncertain, especially in the context of multiple pregnancy related cost and complications.*
- *Empirical ovarian stimulation is often combined with intrauterine insemination, although both interventions can be used separately.*

Principles of Ovarian Stimulation

The aim of ovarian stimulation is to intervene in the mechanisms regulating single dominant follicle selection to mature multiple follicles and obtain multiple oocytes for fertilization in vivo (either after timed intercourse or IUI) or IVF. Ovarian stimulation is usually performed in normoovulatory infertile women 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 incidences of multiple pregnancies can be controlled by limiting the number of embryos transferred.

Obviously, oligovulatory and anovulatory women may also qualify for either IUI or IVF after failed ovulation induction. Ovarian stimulation 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 hyperrespond to stimulation protocols.[183]

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 hyperresponse 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").[184] An excessive number of follicles for ovulation induction or hyperstimulation for IUI may be reduced by follicle puncture[185] 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.[186,187] This is not surprising if the pathophysiologic background of low response is taken into consideration. Low response to ovarian stimulation may be the first sign of advanced ovarian aging.[188] Women with a previous low response to stimulation have been shown to enter menopause at an earlier age.[189]

During the normal menstrual cycle, the midcycle 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 stimulation. 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[190] 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 regarding 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 midluteal phase serum progesterone assay, tubal patency is established by hysterosalpingogram, and male factor infertility is 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.[63] 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.[66]

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 implantation, and finally endometrial receptivity—cannot be studied accurately as yet.

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.[191,192] Similar cumulative pregnancy rates may be achieved with expectant management for 6 to 12 months.[66] Expectant management may represent the most favorable approach in many young women with a short duration of infertility.

Results are frequently reported from combined interventions such as ovarian stimulation 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.[193] Treatment with CC was associated with significantly reduced odds ratios of pregnancy per woman compared to gonadotropins (OR 0.41; 95% CI, 0.17 to 0.8). As far as complications are concerned, no significant differences could be found for miscarriage (OR 0.61; 95% CI, 0.09 to 4) or multiple birth (OR 1.1; 95% CI, 0.2 to 7). The incidence of OHSS or cycle cancellation rates could not be assessed.

For unexplained infertility, the combination of IUI with ovarian stimulation 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[194] 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.[195] Other studies have indicated that ovarian hyperstimulation with both CC and gonadotropins improve the fecundity rate compared to IUI alone.[69] However, a study comparing intracervical insemination alone with FSH in combination with IUI showed a statistically higher pregnancy rate with the latter treatment combination.[65] 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 and IUI than with intracervical insemination alone.[196] The number needed to treat with FSH in combination with IUI to obtain an extra pregnancy above that obtained with IUI alone is even greater.[196]

When the costs of multiple pregnancies arising from multiple follicle development are considered, the cost effectiveness of FSH and IUI combined therapy for this indication may be limited. Cost-effectiveness analyses have led to the conclusion that IUI with or without stimulation should precede IVF.[197] In clinical practice, the benefits of ovarian stimulation in combination with IUI need to be weighed against the additional discomfort and costs of monitoring applied, often unsuccessfully,[68] to avoid multiple pregnancy.[198] Clearly, more studies are needed to elucidate the optimal approach to treating unexplained infertility and the role ovarian hyperstimulation should play.

Preparations for Ovarian Stimulation

- *Both IVF and IUI are rarely performed in the unstimulated natural cycle.*
- *IVF is usually applied using extremely costly and complex medication regimens, requiring intense ovarian response monitoring. Burden of treatment and chances for complications are significant.*
- *Mild IVF aims to reduce cost, complexity, and risks associated with ovarian stimulation with comparable overall efficacy.*
- *The added value of ovarian stimulation in the context of IUI is still uncertain.*

Clomiphene Citrate

Preparations and Regimens

Daily doses of 50 to 100 mg are given usually from days 5 until 9,[28] 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%.[198] A meta-analysis based on six randomized trials[199] concluded that CC administration was superior to no treatment, with an odds ratio for clinical pregnancies of 2.4 (95% CI 1.2 to 4.6) per patient and 2.5 (1.4 to 4.6) per cycle. As stated before, an earlier meta-analysis[194] indicated an independent significant improvement in pregnancy rates for clomiphene, exogenous FSH, and IUI. Most recent analysis suggests no benefit of CC in unexplained infertility.[199]

Adverse Effects and Complications

Adverse effects include hot flushes, mood swings, headaches, 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 (more than 12 months) may be associated with a slight increase in the risk of ovarian epithelial cancer.[101]

Letrozole

The standard of ovarian stimulation for unexplained infertility by either CC or FSH was recently challenged by yet another well-designed RCT involving a total of 900 women.[200] Observed clinical pregnancy rates were 36%, 28%, and 22% for gonadotropin, CC, or the aromatase inhibitor letrozole, respectively (see Fig. 30.6). Pregnancy chances with letrozole were significantly reduced. However, the study was powered for multiple pregnancies, which was 22% (including 10 triplet pregnancies) in the gonadotropin arm, which is still unacceptably high.[201]

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 based on 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 hyperresponse (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 cotreatment during gonadotropin hyperstimulation have demonstrated a reduced incidence of a premature LH rise but no significant improvement in pregnancy rates.[202] However, this approach renders ovarian stimulation protocols even 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 to 2.9).[194] 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%).[198] A subsequent large multicenter study[65] 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.[65] 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,[68] 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 addressing these issues, and that there is a need to individualize treatment to ensure optimal outcomes.

Ovarian Stimulation for In Vitro Fertilization

- *Ovarian stimulation protocols for IVF have become extremely complex, costly, and time consuming.*
- *The preferred intensity of ovarian stimulation for IVF remains a matter of debate, focusing on the optimal number of oocytes retrieved in the context of cumulative chances for success, burden of treatment, cost, and complications).*

Therapeutic Approaches

The general aim of ovarian stimulation in this clinical context is to induce the development of multiple dominant follicles 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 (see Fig. 30.11).[29] 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.[28] The paradigm of so-called "controlled" ovarian stimulation by high doses of exogenous gonadotropins and GnRH agonist long protocol cotreatment 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 questioned by some.[73,202]

The ovarian stimulation protocols required to produce a large number of follicles have become extremely complex and costly over the years,[28,52] creating considerable burden of treatment, side effects, risks of complications, and the need for intense monitoring of ovarian response.[203] The total number of retrieved oocytes required to achieve a single live birth is somewhere around 20, suggesting a very substantial oocyte wastage.[204] 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.[205] 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 chronologic age and endocrine and ultrasound aging parameters assessed before the initiation of treatment, as will be discussed later.[189,206]

However, the widely applied approach to increase gonadotropin doses administered in case of insufficient ovarian response has little scientific foundation. The occurrence of a severe hyperresponse comes as a surprise in most cases.[59,194] However, recent research indicates that the initial AMH level may represent a useful tool to identify women and risk for an exaggerated response to ovarian stimulation.[207] Severe OHSS is induced by hCG and is therefore often 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. Current approaches also include the triggering of an endogenous LH surge inducing final oocyte maturation by a bolus dose of GnRH agonist.[208] For obvious reasons, such an approach can only be used in stimulation protocols employing GnRH antagonist cotreatment.

Slowly, ovarian stimulation protocols have shifted from the use of hMG to uFSH to recFSH.[209] 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,[19] 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.[71] Cumulative pregnancy and live birth rates over four cycles of 42% and 32%, respectively, have been reported.[210] 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.[211]

More recently a modified natural cycle[212] has been proposed in which GnRH antagonists are instituted to prevent premature ovulation, and low-dose exogenous gonadotropin cotreatment is given as add-back to prevent a GnRH antagonist induced involution of follicle development. Using this approach, which (similar to natural cycle IVF) aims to achieve monofollicular development, cumulative pregnancy rates of 44% have been reported over nine cycles of treatment.[212]

First significant steps have been undertaken in recent years towards individualized dosing based on initial screening characteristics, such as female age, baseline AMH concentrations, and body weight.[48] This large multicenter, multinational study involving 265 women not only demonstrated that the number of oocytes retrieved is clearly dependent on baseline AMH, next to the dose of exogenous FSH (Fig. 30.13). More importantly the study convincingly elucidated that more oocytes does not give rise to more good quality blastocysts, emphasizing the concept that oocyte quality and quantity may not necessarily go hand in hand.

Background

After the first baby born following IVF in a natural cycle,[39] four normal IVF pregnancies were reported following ovarian stimulation with CC.[35] In subsequent years, many groups reported IVF results following CC, with or without gonadotropin cotreatment.[213] Combined CC and hMG regimens were considered the standard of care before GnRH agonist cotreatment to induce pituitary downregulation came into use. (For a comprehensive historical overview, see reference 195.) The advantages of these combined regimens included reduced requirements for hMG and higher luteal phase progesterone levels, alleviating the need for luteal phase supplementation.[32] Recent studies have reported clinical outcomes of combined regimens applying CC, gonadotropins, and GnRH antagonist.[32]

CC usually induces the development of at least two follicles, which may sometimes elicit a premature LH rise. Because CC is therapeutically active through interference with estrogen feedback, this compound cannot be combined with GnRH agonist cotreatment 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 and 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%.[214]

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.[213] 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. By intuition, most clinicians start with a low dose of gonadotropins in case of expected hyperresponse, whereas higher daily dose are employed in patients with expected or previously observed poor response. Scientific data to back up these approaches are limited. Based on seven RCTs involving a total of 2563 cycles, although

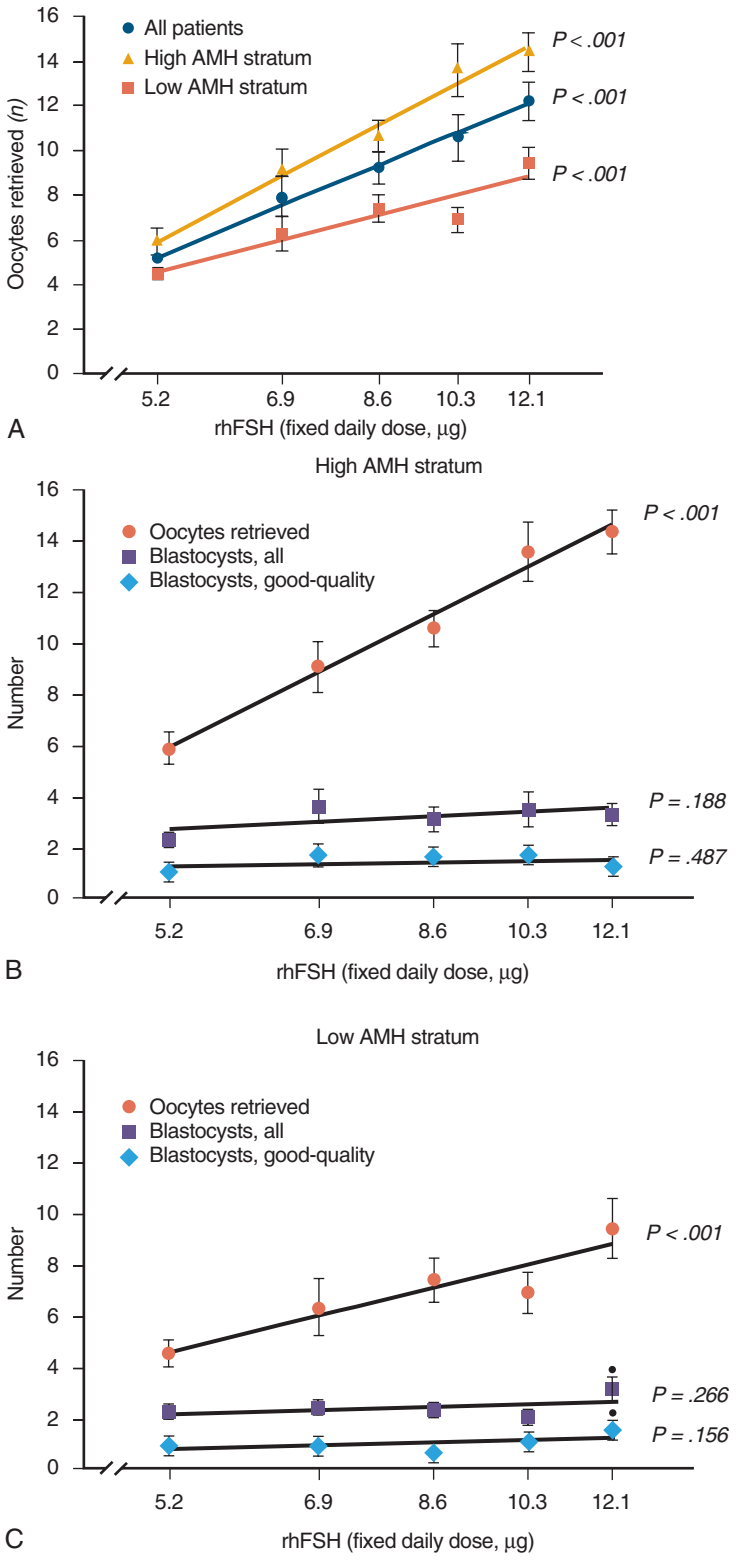


FIGURE 30.13 Number of oocytes retrieved in relation to the recombinant human follicle-stimulating hormone *(rhFSH)* dose for all patients undergoing in vitro fertilization and separate for the high and low anti-müllerian hormone *(AMH)* strata (A). Number of blastocysts generated in relation to the number of oocytes retrieved, separate for the low (B), and high (C) AMH strata. *(From Arce JC, et al: Ovarian response to recombinant human follicle-stimulating hormone: a randomised antimüllerian hormone-stratified, dose-response trial in women undergoing in vitro fertilization/intracytoplasmic sperm injection.* Fertil Steril *102:1633–1640, 2014.)*

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 in women with an expected normal ovarian response to stimulation.[215]

A novel recFSH produced by a human cell line has been tested clinically,[48] and recFSH biosimilars have been introduced to the market in various European countries. A chimeric FSH agonist (so-called recFSH-CTP) generated by the fusion of the CTP of hCG (responsible for its prolonged metabolic clearance compared to LH) with the FSH-β chain has recently been introduced in the IVF clinic.[45] Corifollitropin alpha can be administered with a 7-day injection free interval and has been shown to be safe with high efficacy.[216]

The type, duration, and dosing of GnRH analogue cotreatment to suppress endogenous pituitary gonadotropin release (as will be discussed later in the chapter) may also affect the preferred gonadotropin preparation, dose, and starting day. 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.[29] A number of studies have indicated that excessively suppressed late follicular phase LH concentrations may be detrimental for clinical IVF outcome.[217,218] 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.[29] 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,[219] or when cycles with or without the addition of exogenous LH are compared.[220]

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 to stimulate a more homogeneous cohort of follicles for IVF.[18,19,20]

Preparations and Regimens

To allow for the clinical introduction of recFSH, large-scale, multicenter, comparative trials in IVF were published from 1995 onward.[222] It was arbitrarily chosen for all initial studies that recFSH would only be compared with uFSH and not hMG. 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[223] as well as health economics studies[224,225] indicate a slightly improved outcome for recFSH compared to uFSH. However, recently published multicenter, company-sponsored trials reported similar clinical outcomes comparing uFSH versus recFSH or hMG versus recFSH,[226,227] which was confirmed in the most recent meta-analysis.[228]

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.[184] Studies of the efficacy of this approach thus far undertaken have been limited and inconclusive. Adequate doses for gonadotropin preparations may also vary, depending on whether GnRH agonist or antagonist cotreatment is used.[229] Major individual differences in body weight may also determine response.[230] 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 that has been poorly characterized so far and may also vary depending on GnRH agonist or antagonist cotreatment. It is surprising to conclude that very few of the previously mentioned questions regarding applied dose regimens can be answered based on 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 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.[231] Moreover, an RCT in which higher versus standard dose of FSH was administered to expected poor responders showed no difference in pregnancy rates.[232] Altogether, a meta-analysis[215] encompassing all RCTs comparing different FSH doses failed to show a difference in favor of high-dose regimens where pregnancy chances are concerned (Fig. 30.14), 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.[233]

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.[234] Although there is evidence that OC pretreatment may aid in the scheduling of IVF cycles when GnRH anatagonists are used, there is no evidence at present that they improve live birth rates, and some data suggest that pregnancy rates may even be reduced.

In addition, scheduling in GnRH antagonist cotreated cycles could be performed by E_2 alone rather than OCs[235] or by altering the timing of the hCG trigger.[236]

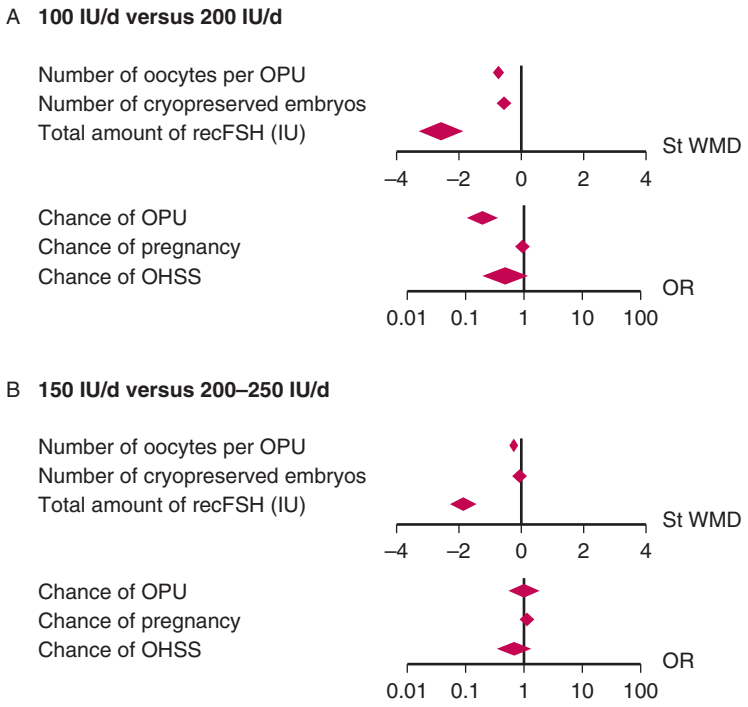


FIGURE 30.14 Summary of clinical outcomes from a meta-analysis comparing different follicle-stimulating hormone doses for ovarian stimulation for in vitro fertilization. (A) Comparison A: 100 versus 200 IU/day. (B) Comparison B: 150 versus 200 to 250 IU/day. *OHSS,* Ovarian hyperstimulation syndrome; *OPU,* oocyte pick up; *OR,* odds ratio; *recFSH,* recombinant follicle-stimulating hormone; WMD, weighted mean difference. *(Graph from Sterrenburg MD, Veltman-Verhulst SM, Eijkemans MJ, et al: Clinical outcomes in relation to the daily dose of recombinant follicle-stimulating hormone for ovarian stimulation in in vitro fertilization in presumed normal responders younger than 39 years: a meta-analysis.* Human Reprod Update *17:184–196, 2011.)*

Another intriguing and clinically relevant question remains whether pretreatment or cotreatment with any compound could improve ovarian responsiveness to stimulation. Many compounds and interventions have been applied clinically, but most attention in recent years has been towards the use of androgens in low response patients. The biological rationale for such interventions is far from solid, but it is speculated that early preantral (gonadotropin independent) growth of follicles may be improved. Although the most recent meta-analysis involving eight studies using DHEA (880 subjects total) and four applying testosterone (350 subjects) suggested improved overall pregnancy/live birth rates (OR 1.81 and 2.6, respectively),[237] a recent small sample size RCT showed no difference with regarding oocytes numbers retrieved.[238]

Gonadotropin-Releasing Hormone Agonist Cotreatment

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.[32] 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[61] allowed for the complete suppression of pituitary gonadotropin release during ovarian stimulation protocols for IVF.[32] Induced pituitary downregulation indeed resulted in significantly reduced cancellation rates and improved overall IVF outcome.[239,240] Moreover, the approach of GnRH agonist cotreatment 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 downregulation 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 quiescence has been achieved. Moreover, it is uncertain whether ovarian response to exogenous stimulation is affected by GnRH agonist cotreatment.[241] 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[242,243] 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.[32]

Gonadotropin-Releasing Hormone Antagonist Cotreatment

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 cotreatment with GnRH antagonist can be restricted to the time in the cycle at risk for a premature rise in LH (i.e., the midfollicular to late follicular phase of the cycle).[32]

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 cotreatment regimen, it was initiated on cycle day 6. However, in principle, GnRH antagonists need only be given when there is follicular development and rising E_2 levels that 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 to 1.05).[244]

The most recent meta-analysis comparing IVF/intracytoplasmic sperm injection (ICSI) with either GnRH agonist or antagonist cotreatment involving a total of 11 RCTs and 2300 women demonstrated no difference with respect to live birth rates (OR 1.02; 95% CI, 0.85-1.23) (Figs. 30.15, 30.16, and 30.17).[55] Moreover, chances for OHSS were significantly reduced with an OR of 0.61. Despite slow acceptance and uptake, the most recent estimates suggest that the majority of IVF cycles worldwide are now with GnRH antagonist.

Despite improving outcomes, the debate regarding the advantages and disadvantages of GnRH antagonist compared with GnRH agonists continues (Box 30.4).[53]

Box 30.4 Advantages and Disadvantages for the Use of Gonadotropin-Releasing Hormone Antagonists in In Vitro Fertilization

ADVANTAGES

- Prevention of premature LH increase is easier and takes less time.
- GnRH antagonists are not associated with an initial acute stimulation of gonadotropins and steroid hormones (so-called flare effect). The initial stimulation by GnRH agonists can induce ovarian cyst formation.
- No hot flushes are observed with GnRH antagonists.
- Inadvertent administration of the GnRH analogue in early pregnancy can be avoided, as GnRH antagonist is administered in the follicular phase of the menstrual cycle.
- Requirements for exogenous gonadotropins are reduced, rendering ovarian stimulation less costly.
- Duration of ovarian stimulation protocols is shortened, improving patient discomfort.
- Reduced rate of OHSS with similar efficacy.

DISADVANTAGES

- Still more experience with GnRH agonist cotreatment.
- 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.

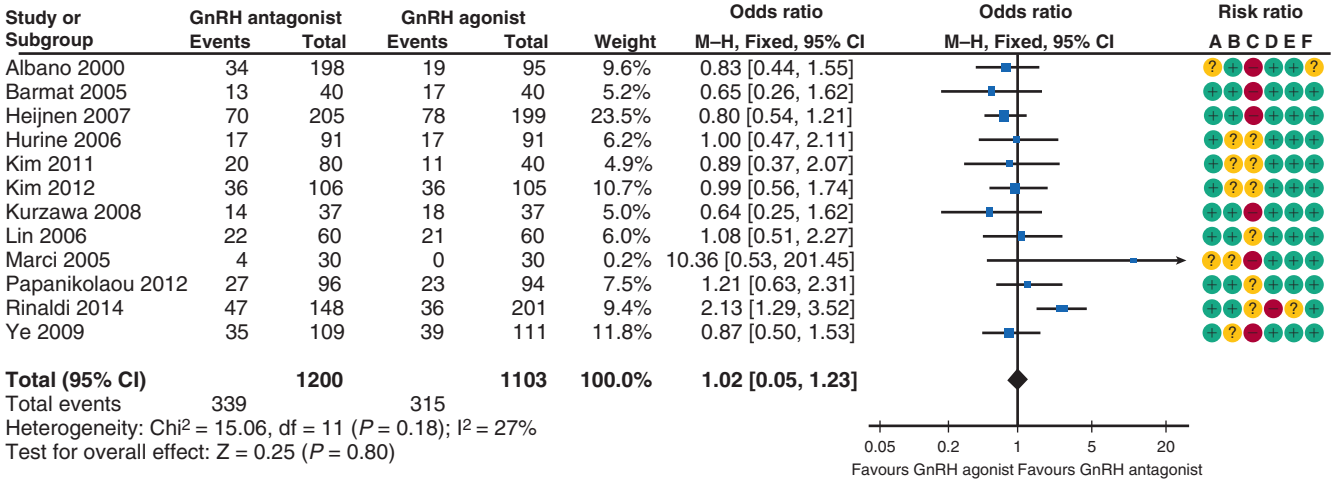
GnRH, Gonadotropin-releasing hormone; *LH*, luteinizing hormone; *OHSS*, ovarian hyperstimulation syndrome.

Approaches for Induction of Final Oocyte Maturation

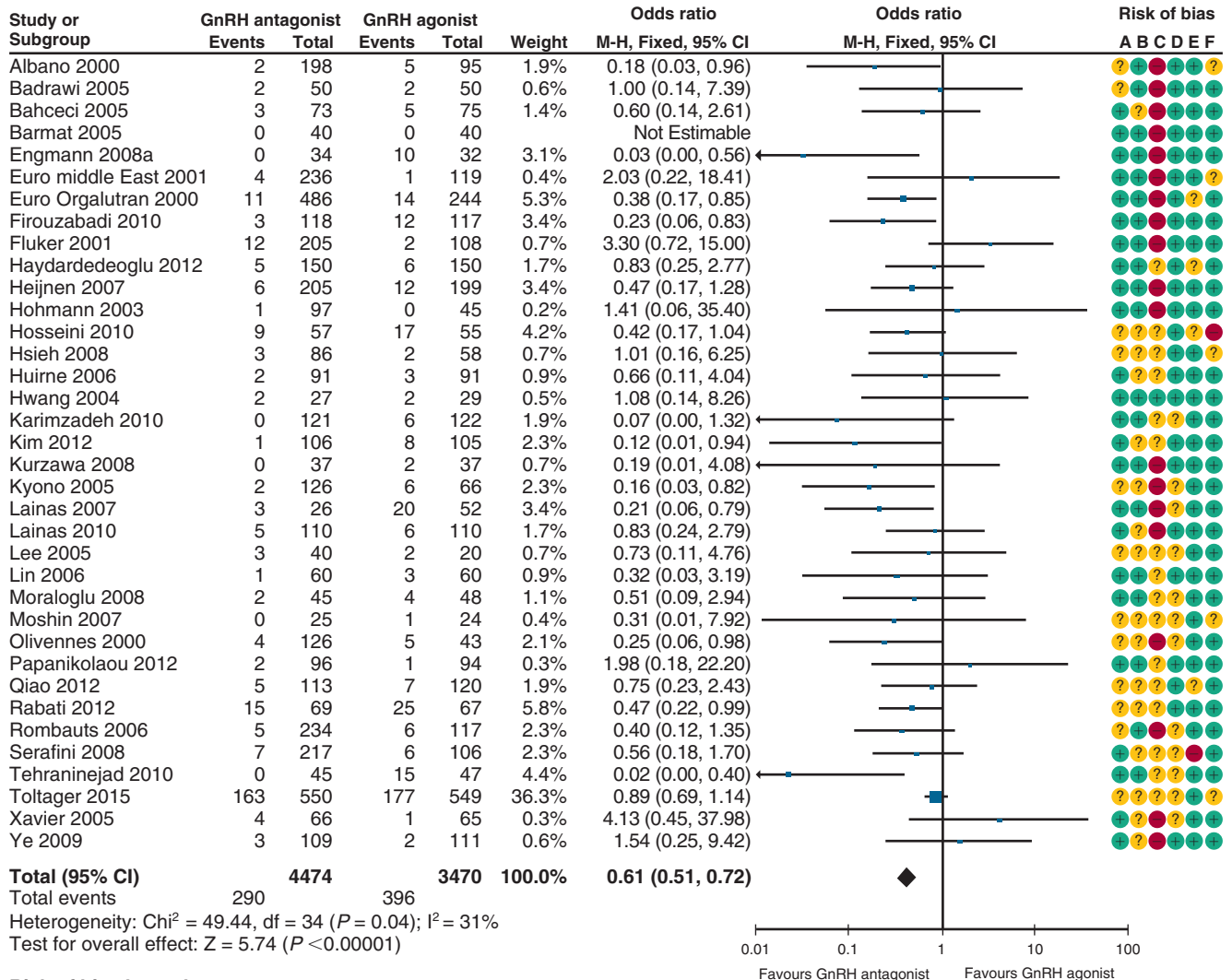
In the natural normoovulatory cycle, rupture of the dominant follicle and release of the oocyte are triggered by the midcycle surge of LH. This sudden enhancement of pituitary synthesis and release of LH (and FSH) is elicited by high late-follicular phase E_2 levels in combination with slightly elevated progesterone levels.[246] In stimulated cycles for IVF, estrogen levels are prematurely elevated, which may induce unpredictable but advanced LH rises. As mentioned before, GnRH agonist cotreatment is required 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 stages of oocyte maturation before oocyte retrieval along with corpus luteum formation in IVF.[32] Exogenous hCG is also implicated in sustained luteotropic activity[242] due to its prolonged circulating half-life.[246] Unfortunately, hCG is therefore also believed to contribute to chances of developing OHSS.[184]

Initial studies during ovarian hyperstimulation for IVF (before the widespread use of GnRH agonist cotreatment) showed that an endogenous LH surge could be reliably induced by the administration of GnRH or a bolus injection of GnRH agonist.[247] The induction of an endogenous LH (and FSH) surge is more physiologic compared to exogenous hCG because of the much shorter half-life.[248,249] Moreover, luteal phase steroid concentrations seem closer to the physiologic range,[250] which may improve endometrial receptivity.[251] As the follicular phase cotreatment with GnRH agonist has been the standard of care for over a decade, alternative approaches for the induction of oocyte maturation has received little attention. However, the suppressive effect of follicular phase GnRH antagonist administration can be reversed immediately by administering native GnRH or GnRH agonist.[252] 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 cotreatment with a GnRH antagonist. This was demonstrated by the observed gonadotropin surge and quality and fertilization rate of recovered oocytes. The concept of GnRH agonist triggering as a valid alternative to hCG is appealing due to the virtual elimination of OHSS.[252] However, the preferred luteal phase supplementation under those circumstances remains uncertain, and cases of OHSS following GnRH agonist trigger have been reported (for recent review, see reference 252a).

Recombinant LH and recombinant hCG have recently become available for clinical use. An early large randomized



A



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Incomplete outcome data (attrition bias)
(E) Selective reporting (reporting bias)
(F) Other bias

B

FIGURE 30.15 Meta-analysis comparing gonadotropin-releasing hormone *(GnRH)* antagonist versus agonist cotreatment during ovarian stimulation for in vitro fertilization. Showing no difference in live birth rates per woman (A) and decreased rates of ovarian hyperstimulation syndrome (B). *(Data from reference Al-Inany HG, Youssef MA, Ayeleke RO, et al: Gonadotropin-releasing hormone antagonists for assisted reproductive technology.* Cochrane Database Syst Rev *4, 2016.)*

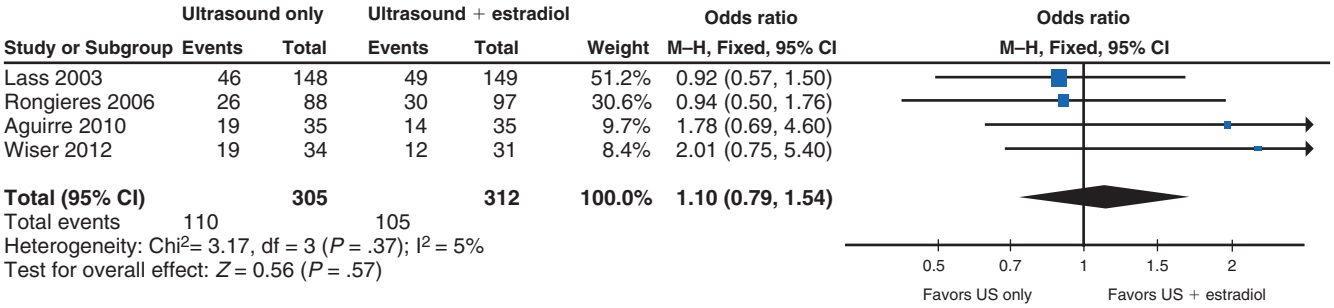


FIGURE 30.16 Meta-analysis comparing the monitoring of ovarian stimulation for in vitro fertilization with ultrasound only or in combination with estradiol serum assessment showing no difference regarding clinical pregnancy rates. *(Data from Kwan I, Bhattacharya S, Kang A, Woolner A: Monitoring of stimulated cycles in assisted reproduction [IVF and ICSI].* Cochrane Database Syst Rev *8:CD005289, 2014.)*

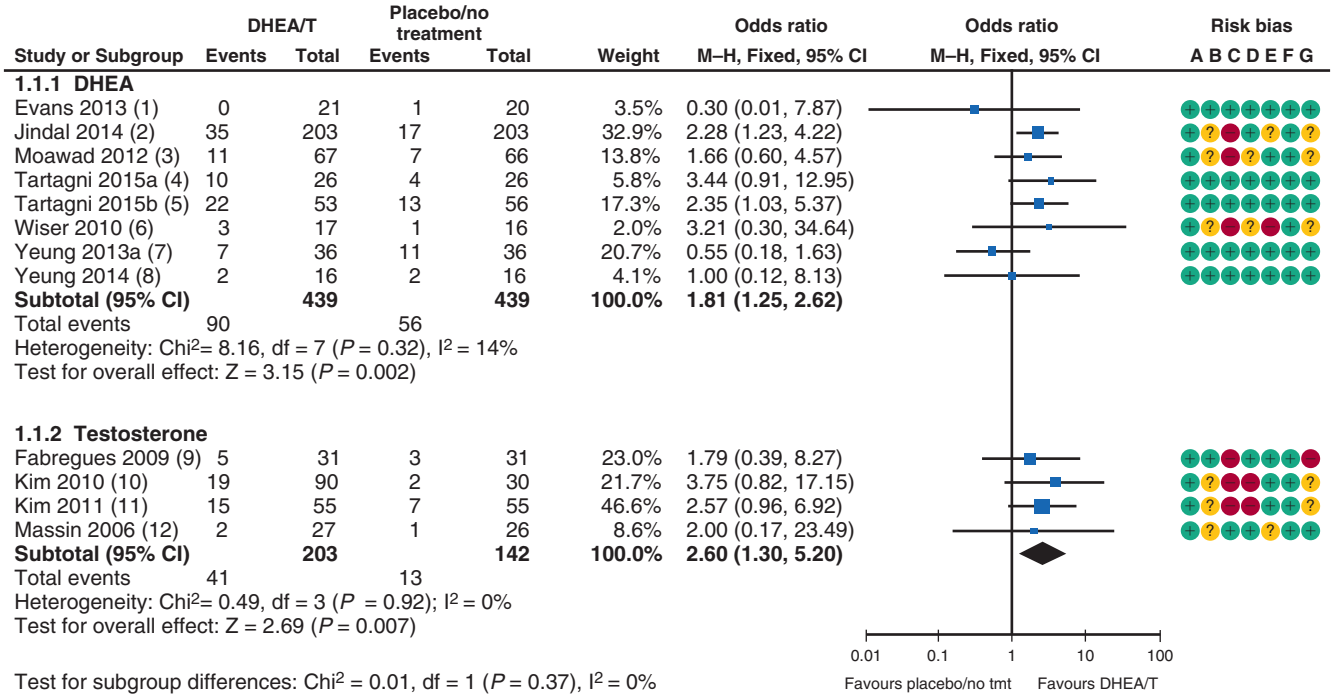


FIGURE 30.17 Meta-analysis concerning the addition of DHEA/T during ovarian stimulation for in vitro fertilization showing increased live birth/ongoing pregnancy rates. *(Data from Nagels HE, Rishworth JR, Siristatidis CS, Kroon B: Androgens [dehydroepiandrosterone or testosterone] for women undergoing assisted reproduction.* Cochrane Database Syst Rev *[11]:CD009749, 2015.)*

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.[253] 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,[250] randomized studies comparing this approach to hCG administration showed lower implantation and ongoing pregnancy rates.[254] Recent data indicate that standard luteal support regimens may be insufficient in this setting, and improved results may be achieved when this is addressed.[255,256]

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[39] 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 (Fig. 30.18).[257]

As mentioned earlier, GnRH agonist cotreatment became the standard of care for the prevention of a premature rise

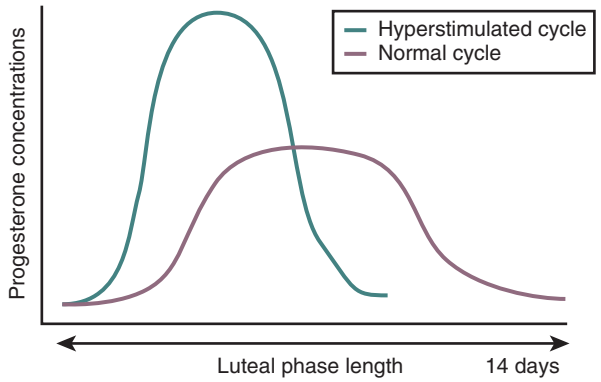


FIGURE 30.18 Schematic representation of changes in luteal phase length and endocrine profile induced by ovarian hyperstimulation for in vitro fertilization. *(From Jones HWJ: What happened? Where are we?* Hum Reprod *11[Suppl 1]:7–21, 1996.)*

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 prolonged pituitary recovery from downregulation during the luteal phase[258] resulted in lack of support of the corpus luteum by endogenous LH and advanced luteolysis.[259] It was observed shortly thereafter that the corpus luteum can be rescued by the administration of hCG,[32] 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,[184] luteal phase hCG support has been largely replaced over the years by luteal phase progesterone supplementation.[260] A recent meta-analysis of luteal support in stimulated IVF cycles involving a total of 69 studies and 16,327 women concluded supplementation by synthetic progesterone represents the current method of choice, with no improved outcomes using either estrogen or hCG supplementation.[261,262]

Attempts to secure pituitary recovery during the luteal phase by the early follicular phase cessation of GnRH agonist cotreatment all failed because it takes at least 2 to 3 weeks for LH secretion to recover.[32] 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.[263] Preliminary observations related to ovarian stimulation and GnRH antagonist cotreatment for IUI seem to favor this contention.[264] However, various studies in IVF applying GnRH antagonist cotreatment 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.[265-267] More detailed studies could confirm that early luteal and midluteal phase LH levels remained suppressed following the follicular phase administration of GnRH antagonist.[267,268]

Moreover, luteolysis is advanced in the nonsupplemented luteal phase after either hCG or GnRH agonist triggering of oocyte maturation.[267] 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.[202] Mild stimulation regimens resulting in lower serum steroid levels have therefore been advocated as a means of benefiting the luteal phase.[261]

Clinical Outcome of In Vitro Fertilization

- *IVF outcomes should be reported as cumulative live birth rate per stimulation cycle (hence from fresh and frozen embryo transfer) or per started treatment strategy (involving multiple cycles if needed).*
- *The added value of ovarian stimulation for IVF should be viewed not only in terms of live birth rates, but it should also include patient discomfort, side effects, chances for complication, and cost.*

The most recent report concerning IVF/ICSI in Europe relates to 2012 involving 18 countries and 369,000 cycles[70] and to 2013 for the United States (CDC report) concerning a total of 94,000 cycles (fresh embryo transfer and nondonor eggs) and close to 20,000 cycles using donor eggs. It is difficult to outline the overall success rates since this is greatly dependent on the age of women, embryo transfer policies, pregnancy versus healthy live birth rates, and calculations of rates per fresh transfer, fresh and frozen transfer from the same harvest, and even cumulative IVF cycles. Multiple (twin) pregnancy rates remain substantial, with different rates per continent.

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.[270] The drawbacks associated with profound ovarian stimulation for IVF include considerable patient discomfort such as weight gain, headaches, 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. The most common reason for treatment discontinuation is burden of treatment.[271]

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, and severe forms 0.1% to 0.2% of cycles.[67,224] 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 cotreatment, 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 twofold to fivefold increased incidence in case of multiple pregnancy. Overall OHSS chances are increased in case of high-dose gonadotropin stimulation aiming for a high oocyte yield, and chances have now convincingly shown to be reduced with GnRH antagonist cotreatment.

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. Finally, current efforts focus on individualized dosing of ovarian stimulation based on initial screening characteristics, such as female age, body weight, AMH concentrations, and antral follicle count.[272-274]

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. 30.5). (For recent reviews, see Fauser and colleagues[61] and Verberg and colleagues.[62]) 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.[61] Similar trends have been reported in European countries.[62] 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.[61] Although the available data indicate that the majority of twin births are still unrelated to infertility therapies,[61] 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. Overall, multiple births following IVF treatment are reduced but still significant.

Pregnancy complications include increased risk of miscarriage, preeclampsia, growth retardation, and preterm delivery. Perinatal mortality rates are at least 4-fold higher in twin births and at least 6-fold 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, respectively, and the risks for low-birth-weight infants are increased 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.[275]

New Approaches to Mild Ovarian Stimulation for In Vitro Fertilization

After the initial years of IVF, profound ovarian stimulation became the rule for more than 2 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 became very 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 slowly changing,[203,276] particularly given the growing tendency to transfer a reduced number of embryos. It has previously been demonstrated based on the UK national database that reducing the number of embryos transferred from three to two does not diminish chances of birth despite a reduction in risk of multiple birth.[86] In Europe, an increasing number of centers are performing SET in younger women. Emphasis may therefore now be directed toward the development of more simple and milder stimulation protocols[29,215,223,277] or the improvement of natural cycle IVF outcomes.[82,210,212] The increasing quality of embryo cryopreservation programs will serve to encourage the transfer of one embryo at a time.[278] Consequently, the current consensus now is that success rates of IVF should not only be provided for fresh embryo transfer, but cumulative for fresh and frozen from the same oocyte harvest. Some countries now almost exclusively transfer a single embryo only, generating good overall outcomes due to excellent cryopreservation results.

Previous studies in normoovulatory female volunteers[134,135] confirmed that the development of multiple dominant follicles can be induced by interfering with decremental FSH concentrations during the midfollicular to late follicular phase. As shown previously, this decrease is required for selecting a single dominant follicle,[11,12] in agreement with previous findings in the monkey model.[136,279] We were subsequently able to demonstrate that the initiation of exogenous FSH (fixed dose, 150 IU/day, GnRH antagonist cotreatment) 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 (Fig. 30.19).[280]

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 SET would lead to a similar outcome assessed over a 1-year period after initiation of treatment, while reducing patients' discomfort, multiple pregnancies, and costs compared with standard treatment.[73] The study included a total of 404 patients and observed in the mild approach a shorter duration of treatment per cycle, less medication needed, a reduction in twin pregnancies, and an equal chance of live birth after a year of treatment while reducing the total costs (Fig. 30.20).

Apart from clinical efficacy and costs (see later in the chapter), emotional stress should be considered an important side effect associated with IVF treatment. Following mild stimulation, patients reported fewer side effects and stress related to hormone treatment compared with conventional stimulation.[281] Consequently, dropout rates have been reported to be significantly reduced during mild stimulation (Fig. 30.21). Treatment-related stress has been found to be the most important reason patients drop out of IVF treatment.[282] The early dropout 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.[283]

Other novel protocols under investigation include the replacement of FSH by LH, an approach based on the

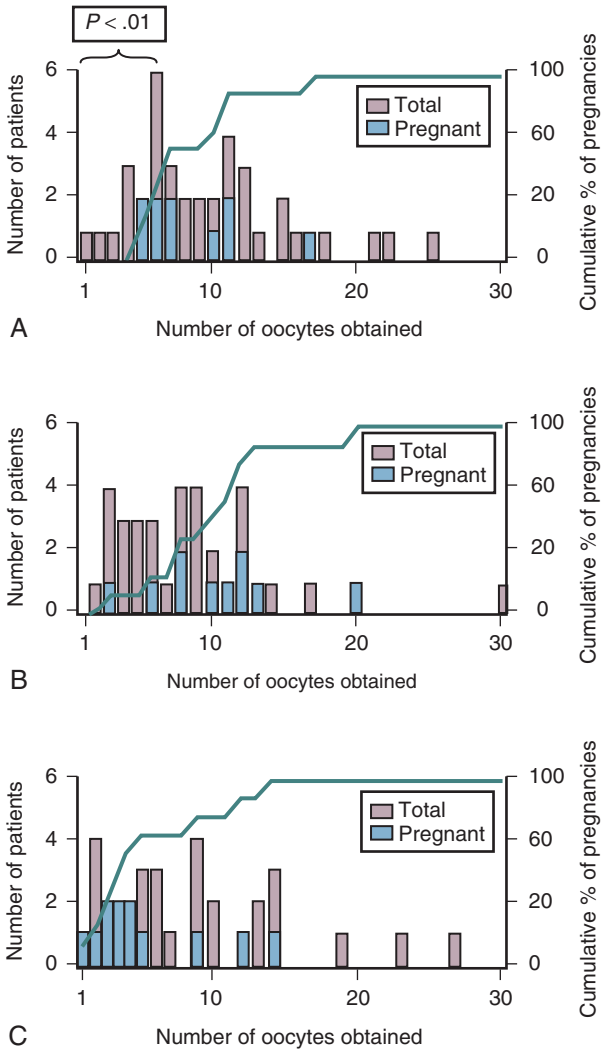


FIGURE 30.19 Number of women undergoing in vitro fertilization who did or did not achieve a pregnancy in relation to the number of oocytes retrieved, comparing conventional hyperstimulation with gonadotropin-releasing hormone *(GnRH)* agonist long protocol (A) with two mild stimulation protocols employing GnRH antagonist cotreatment (B and C). *(Modified 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.)*

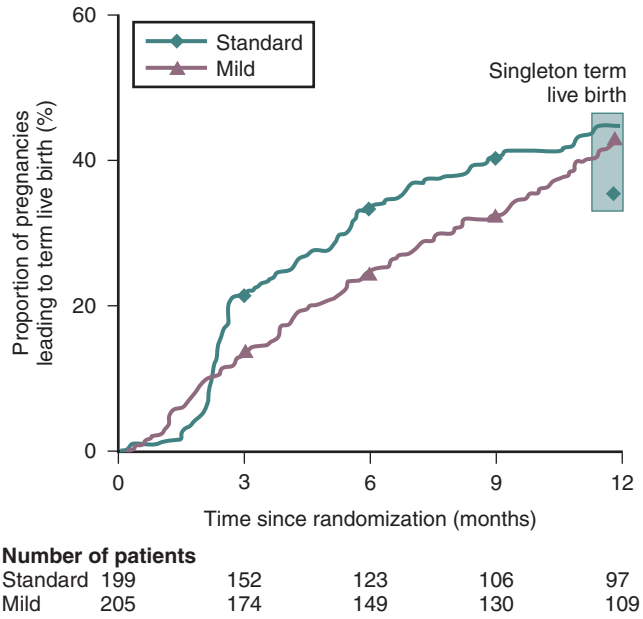


FIGURE 30.20 Proportions of pregnancies leading to cumulative term live birth within 12 months after starting in vitro fertilization. *Mild:* mild ovarian stimulation with gonadotropin-releasing hormone *(GnRH)* antagonist and single embryo transfer. *Standard:* standard ovarian stimulation with GnRH 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.)*

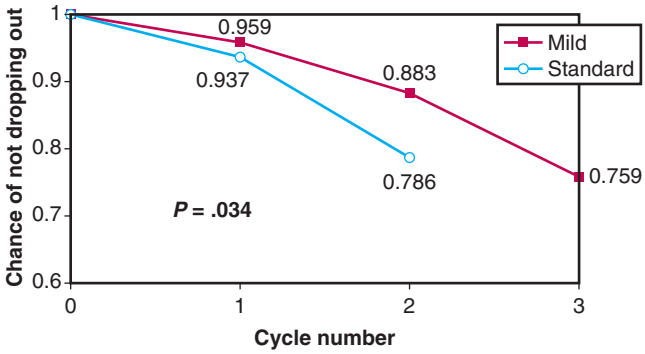


FIGURE 30.21 Chances of not dropping cut from successive in vitro fertilization *(IVF)* treatment cycles applying either mild IVF or standard IVF. *(Modified from Verberg et al: Why do couples drop-out from IVF treatment? A prospective cohort study.* Hum Reprod *23[9]:2050–2055, 2008.)*

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.[167] Several RCTs[20,284,285] 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 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.[286-289] 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.[290] This issue of oocyte quality versus quantity remains unsettled to date.

Toward Individualized Treatment Algorithms

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.[291] 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. 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.[131] Following multivariate analysis, the FAI, BMI, presence of amenorrhea (as opposed to oligomenorrhea), and ovarian volume were found to be independent predictors of ovulation.[292] 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.[293]

In a subsequent study, those factors that 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[175] (Fig. 30.22) that may assist in the selection of patients for clomiphene therapy and selection of those for whom this first-line treatment will be of little value. In this latter group, early recourse to gonadotropin therapy is indicated.[294]

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

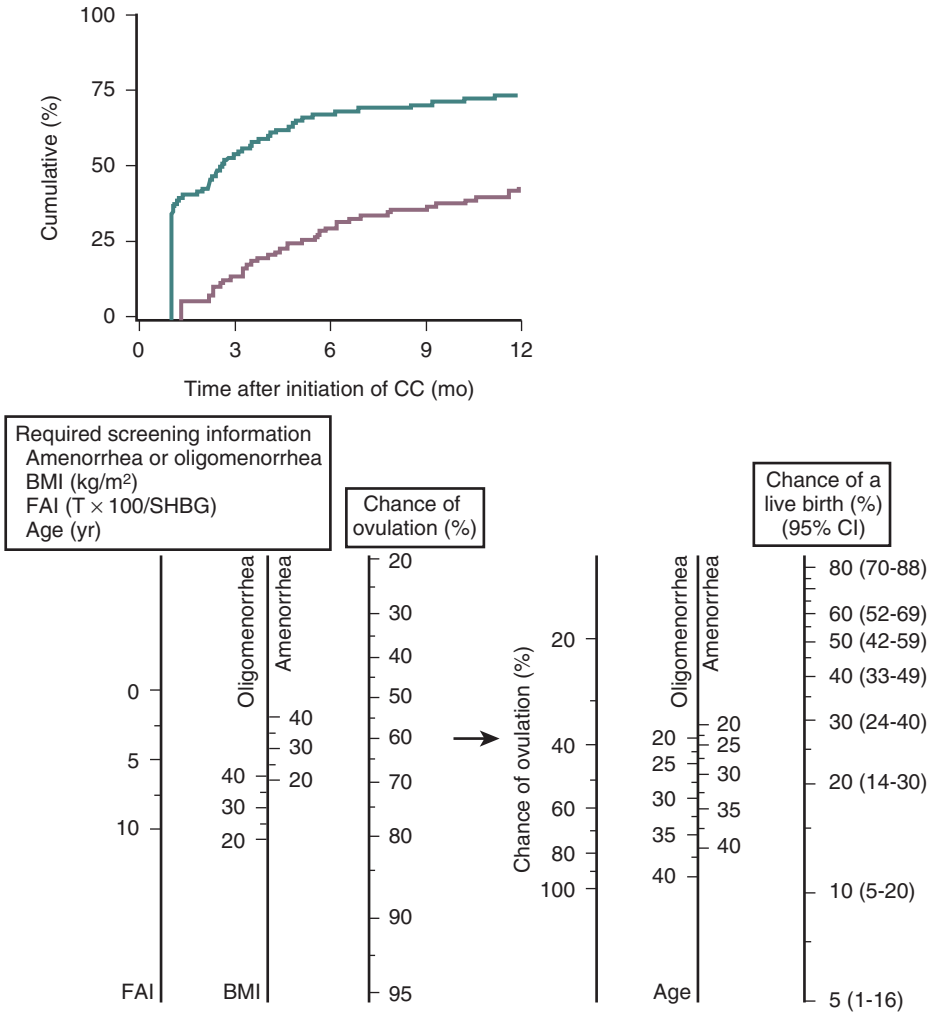


FIGURE 30.22 Cumulative percentage of patients who ovulate or conceive following the initiation of clomiphene citrate *(CC; top)*, and a two-step nomogram predicting chances of live birth following clomiphene citrate based on initial screening characteristics *(bottom)*. *BMI*, Body mass index; *FAI*, free androgen index; *SHBG*, sex hormone–binding globulin. *(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.)*

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, that is, their individual FSH threshold for stimulation. A prediction model has recently been developed that may be used to determine the individual FSH response dose (which is presumably closely related to the FSH threshold).[295] 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.[295] 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.[294] 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,[183] simple patient characteristics combined with endocrine factors were again shown to enable (limited) prediction of outcome following FSH ovulation induction.[296] 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. 30.23).[56] This observation has been confirmed 10 years later.[294]

Regarding IVF treatment, it appears that the most prominent factor determining outcome is the individual variability in ovarian response to stimulation. Rather than exhibiting the desired response, women can present with either a hyporesponse or a hyperresponse to ovarian 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.[186,231]

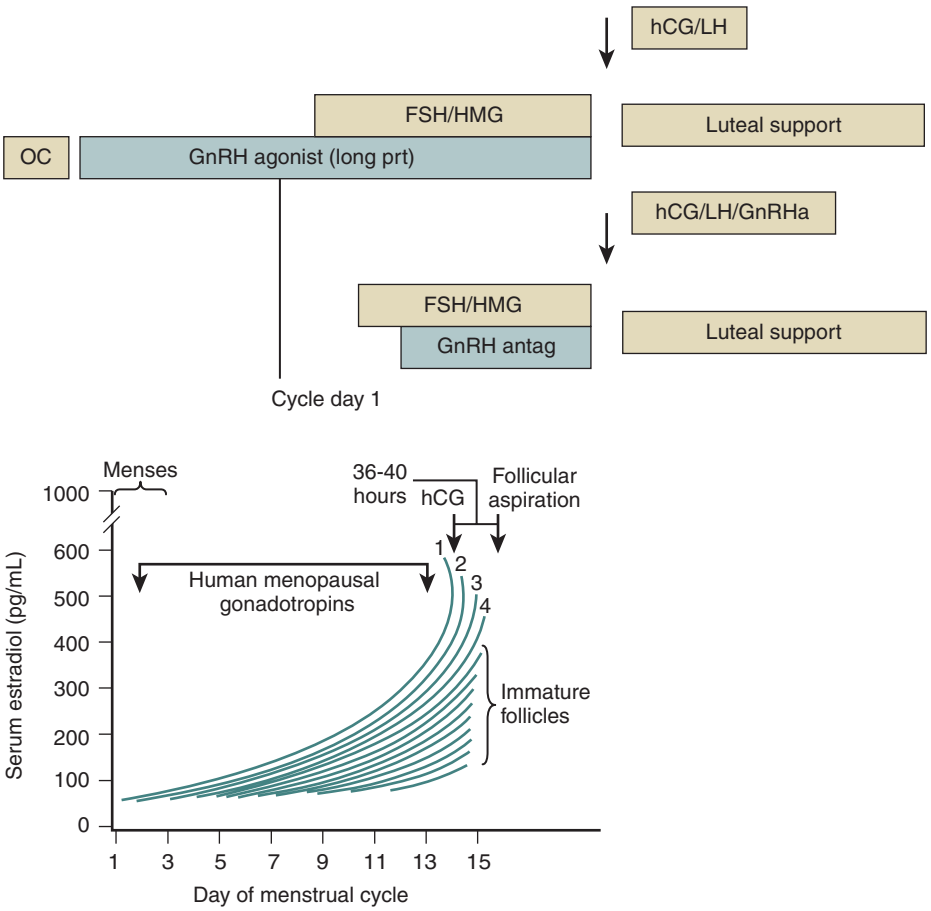


FIGURE 30.23 Schematic representation of complex medication regimens involved in ovarian hyperstimulation for in vitro fertilization *(top)* and the heterogeneous cohort of recruited and selected follicles *(bottom)*. *Antag,* Antagonist; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotropin-releasing 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.)*

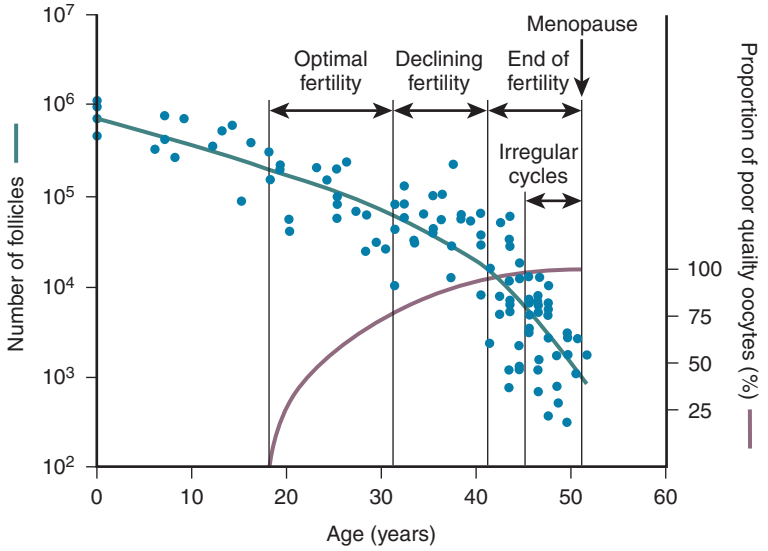


FIGURE 30.24 The decline in follicle number and the increase in poor-quality oocytes in relation to reproductive events with increasing female age. *(From Broekmans FJ, Knauff EA, te Velde ER, et al: Female reproductive ageing: current knowledge and future trends.* Trends Endocrinol Metab *18[2]:58–65, 2007.)*

This may help in counseling the patient because the chances of successful IVF in these women is extremely low.

Poor ovarian response appears to be related to ovarian aging[188] (and early menopause) (Fig. 30.24).[189] In IVF, the association between poor ovarian response due to diminished ovarian reserve with cycle cancellation and poor success rates is well established.[57] Age is an important predictor of IVF outcome.[298] However, chronologic 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 of age. The quantity and quality of the primordial follicle pool diminishes with age, reducing ovarian reserve.[299] This results in a decline in both therapy-induced and spontaneous pregnancies.[300] However, some women older than 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 that may enable the prediction of poor or good response. This would open the way to improved counseling and patient selection for IVF.

The first endocrine marker for ovarian reserve is the early follicular phase FSH level,[301] which has been shown to be an independent predictor to age of IVF outcome.[302] 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.[302] 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.[302] Older women (older than 40 years old) 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.[302] 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 non-pregnancy was observed.[303]

Other markers may therefore have an adjunctive value when diagnosing diminished ovarian reserve. The ultrasound measurement of the number of antral follicles present on cycle day 3 has been shown in a number of studies to predict poor ovarian response. 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.[304] At present, no single reliable marker for ovarian reserve has been identified.[302]

AMH, a member of the TGF-β superfamily, has been proposed as another 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,[305] and recent studies have shown that poor response to IVF can be predicted by reduced baseline serum AMH concentrations.[302] Additional research firmly established AMH as the most useful marker for ovarian response prediction and individualized gonadotropin dosing.[305,306]

Until recently, hyperresponse (and the threat of OHSS) came as a surprise in the great majority of cases.[205] Early recognition of women at risk may give rise to effective, altered stimulation protocols and improved safety.[184] It is now clearly established that AMH is the best marker to identify women at risk for developing OHSS, and dosing strategies can be modified accordingly.

The use of nomograms for individualizing FSH dose for ovarian stimulation in IVF may optimize the risk to benefit

dose of FSH in IVF. In recent years, several models have been developed based on multiple regression analysis.[307,308] Factors consistently observed to be predictive of the number of oocytes obtained were age, the total number of antral follicles, and smoking status.[309] Others have suggested that ovarian volume and blood flow as measured by Doppler ultrasound are also predictive factors.[304] A model combining all these factors has been developed to prescribe the optimal dose of rFSH that will yield 5 to 14 oocytes.[307] 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.[308] A recent RCT failed to demonstrate a benefit of individualized dosing (based on a number of screening parameters but not including AMH) over standard treatment.[310] As mentioned earlier, recent studies confirmed that the number of oocyte retrieved clearly depends on baseline AMH concentrations next to the dose of FSH.[48] Currently, individualized dosing based on initial AMH, age and body weight are being tested in multiple prospective comparative trials.

Health Economics of Ovarian Stimulation

- *Ovarian stimulation may improve success chances of IVF. However, added costs are considerable.*
- *Infertility interventions cannot be judged based on success rates only. Burden of treatment, cost, and risks should also be taken into consideration.*

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. A recent meta-analysis involving six RCTs concluded that the effectiveness of IVF for unexplained infertility remains questionable.[311] Moreover, the economic implications of a more widespread use of assisted reproduction should be considered seriously when making decisions regarding treatment.[312,313]

The diagnosis by exclusion of unexplained infertility and 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 with an advanced female age beyond 35.[314] Again, chances of spontaneous conception are usually underestimated both by the doctor and the patient.[98] On the other hand, 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.[286] Moreover, IVF is available in only 25% of the countries worldwide.[313] In contrast, 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.[314]

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 midterm 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 healthcare 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 considered. For a low-birth-weight child, the average cost of health care and education up to the age of 8 years is 17-fold higher than the costs for a normal birth weight child.[315] It has also been shown that multiple births contribute disproportionately to hospital inpatient costs, especially during the child's first year of life.[316]

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.[313]

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 SET.[317]

Conclusions and Future Perspective

- *It seems that major steps to improve success rates of IVF treatment are over.*
- *Fine tuning of IVF treatment should now become the focus of attention using access to care (affordability), reduced cost and treatment burden, and healthy babies born as the end points.*

Special care should also be taken to perform a proper infertility work-up 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 stimulation.

Milder forms of ovarian stimulation (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, cumulative pregnancy rates following the start of treatment over a given period of time (which may involve multiple cycles) may be similar.

A trend can be observed towards ovarian stimulation 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.[65] Newly introduced compounds to the field of ovulation induction such as insulin sensitizers and aromatase inhibitors may further improve outcomes. On the other hand, the incidence of multiple pregnancies can be reduced to none by employing SET policies in all PCOS patients undergoing IVF. In addition, chances for OHSS can be significantly reduced by cryopreserving all available embryos and transferring them one by one in subsequent unstimulated cycles.[318]

Large numbers of preovulatory follicles and oocytes subsequently retrieved have been applied as useful surrogate outcome parameters for successful IVF.[276] The debate regarding the optimal number of oocytes retrieved is still ongoing, with some advocating numbers such as 20 or even higher (in the context of completed families). 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 reconsidered 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. Cheaper oral compounds are increasingly offered to patients as alternative milder stimulation strategies. 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.

Based on a continued trend worldwide to reduce the number of embryos transferred along with distinct improvements in cryopreservation of supernumerary embryos, novel approaches of mild ovarian stimulation or even natural cycle IVF deserve reevaluation. The possible relationship between quantity of oocytes stimulated and quality (i.e., genetic competence) of embryos obtained[290] should be studied in greater detail. Once a general agreement is reached on the optimal number of oocytes retrieved for IVF, further studies should be undertaken to develop robust individualized dosing based on initial screening parameters such as female age, AMH, antral follicle count, and body weight. Individualizing ovarian stimulation to optimize outcomes between risks and desired outcomes may further improve with the development of pharmacogenetics. Preliminary clinical studies have shown that FSH receptor gene polymorphisms influence the ovarian response to stimulation in women undergoing IVF,[319] raising the possibility that genotyping could further aid in tailoring FSH dosing based on individual ovarian sensitivity.[274]

Top References

Broekmans FJ, Soules MR, Fauser BC: Ovarian aging: mechanisms and clinical consequences. *Endocr Rev* 30(5):465–493, 2009.

Broer SL, Broekmans FJ, Laven JS, et al: Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Hum Reprod Update* 20(5):688–701, 2014.

Diamond MP, Legro RS, Coutifaris C, et al: Letrozole, gonadotropin, or clomiphene for unexplained infertility. *N Engl J Med* 373(13):1230–1240, 2015.

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.

Gleicher N, Oleske DM, Tur-Kaspa I, et al: Reducing the risk of high-order multiple pregnancy after ovarian stimulation with gonadotropins. *N Engl J Med* 343(1):2–7, 2000.

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.

Legro RS, Barnhart HX, Schlaff WD, et al: Ovulatory response to treatment of polycystic ovary syndrome is associated with a polymorphism in the STK11 gene. *J Clin Endocrinol Metab* 93(3):792–800, 2008.

Legro RS, Brzyski RG, Diamond MP, et al: Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 371(2):119–129, 2014. Erratum in: *N Engl J Med*. 2014;317(15):1465.

Lunenfeld B: Historical perspectives in gonadotrophin therapy. *Hum Reprod Update* 10(6):453–467, 2004.

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

Pau CT, Cheang KI, Modi BP, et al: The role of variants regulating metformin transport and action in women with polycystic ovary syndrome. *Pharmacogenomics* in press, 2016.

Steures P, van der Steeg JW, Hompes PG, et al: Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. *Lancet* 368(9531):216–221, 2006.

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

Veltman-Verhulst SM, Fauser BC, Eijkemans MJ: High singelton live birth rate confirmed after ovulation induction in women with anovulatory polycystic ovary syndrome: validation of a prediction model for clinical practice. *Fertil Steril* 98:761–768, 2012.

References

See a full reference list on ExpertConsult.com

References

1. Collins JA, Hughes EG: Pharmacological interventions for the induction of ovulation. *Drugs* 50:480–494, 1995.
2. Fauser BC, Van Heusden AM: Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev* 18:71–106, 1997.
3. Gougeon A: Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* 17:121–155, 1996.
4. McGee EA, Hsueh AJ: Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 21:200–214, 1996.
5. Adhikari D, Liu K: Molecular mechanisms underlying the activation of mammalian primordial follicles. *Endocr Rev* 30:438–464, 2009.
6. Edson MA, Nagaraja AK, Matzuk MM: The mammalian ovary from genesis to revelation. *Endocr Rev* 30:624–712, 2009.
7. Hall JE, Schoenfeld DA, Martin KA, et al: Hypothalamic gonadotropin-releasing hormone secretion and follicle-stimulating hormone dynamics during the luteal-follicular transition. *J Clin Endocrinol Metab* 74:600–607, 1992.
8. le Nestour E, Marraoui J, Lahlou N, et al: Role of estradiol in the rise in follicle-stimulating hormone levels during the luteal-follicular transition. *J Clin Endocrinol Metab* 77:439–442, 1993.
9. Hodgen GD: The dominant ovarian follicle. *Fertil Steril* 38:281–300, 1982.
10. Pache TD, Wladimiroff JW, de Jong FH, et al: Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. *Fertil Steril* 54:638–642, 1990.
11. van Santbrink EJ, Hop WC, van Dessel TJ, et al: Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. *Fertil Steril* 64:37–43, 1995.
12. Schipper I, de Jong FH, Fauser BC: Lack of correlation between maximum early follicular phase serum follicle stimulating hormone concentrations and menstrual cycle characteristics in women under the age of 35 years. *Hum Reprod* 13:1442–1448, 1998.
13. Groome NP, Illingworth PJ, O'Brien M, et al: Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 81:1401–1405, 1996.
14. Baird DT: A model for follicular selection and ovulation: lessons from superovulation. *J Steroid Biochem* 27:15–23, 1987.
15. Fluker MR, Marshall LA, Monroe SE, et al: Variable ovarian response to gonadotropin-releasing hormone antagonist-induced gonadotropin deprivation during different phases of the menstrual cycle. *J Clin Endocrinol Metab* 72:912–919, 1991.
16. Hall JE: Gonadotropin-releasing hormone antagonists: effects on the ovarian follicle and corpus luteum. *Clin Obstet Gynecol* 36:744–752, 1993.
17. Schoot DC, Coelingh Bennink HJ, Mannaerts BM, et al: Human recombinant follicle-stimulating hormone induces growth of preovulatory follicles without concomitant increase in androgen and estrogen biosynthesis in a woman with isolated gonadotropin deficiency. *J Clin Endocrinol Metab* 74:1471–1473, 1992.
18. Sullivan MW, Stewart-Akers A, Krasnow JS, et al: Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. *J Clin Endocrinol Metab* 84:228–232, 1999.
19. Filicori M, Cognigni GE, Samara A, et al: The use of LH activity to drive folliculogenesis: exploring uncharted territories in ovulation induction. *Hum Reprod Update* 8:543–557, 2002.
20. Blockeel C, De Vos M, Verpoest W, et al: Can 200 IU of hCG replace recombinant FSH in the late follicular phase in a GnRH-antagonist cycle? A pilot study. *Hum Reprod* 24:2910–2916, 2009.
21. Erickson GF, Yen SCC: New data on follicle cells in polycystic ovaries: a proposed mechanism for the genesis of cystic follicles. *Semin Reprod Endocrinol* 2:231–243, 1984.
22. Bomsel-Helmreich O: Ultrasound and the preovulatory human follicle. *Oxf Rev Reprod Biol* 7:1–72, 1985.
23. van Dessel HJ, Schipper I, Pache TD, et al: Normal human follicle development: an evaluation of correlations with oestradiol, androstenedione and progesterone levels in individual follicles. *Clin Endocrinol (Oxf)* 44:191–198, 1996.
24. Gruhn JG, Kazer RR: *Hormonal regulation of the menstrual cycle: the evolution of concepts*, New York, 1989, Plenum Publishing.
25. Lunenfeld B: Historical perspectives in gonadotrophin therapy. *Hum Reprod Update* 10:453–467, 2004.
26. Kistner RW, Smith OW: Observations on the use of a nonsteroidal estrogen antagonist: MER-25. II. Effects in endometrial hyperplasia and Stein-Leventhal syndrome. *Fertil Steril* 12:121–141, 1961.
27. Greenblatt RB, Barfield WE, Jungck EC, et al: Induction of ovulation with MRL/41. Preliminary report. *JAMA* 178:101–104, 1961.
28. Glasier AF, Irvine DS, Wickings EJ, et al: A comparison of the effects on follicular development between clomiphene citrate, its two separate isomers and spontaneous cycles. *Hum Reprod* 4:252–256, 1989.
29. Macklon NS, Stouffer RL, Giudice LC, et al: The science behind 25 years of ovarian stimulation for in vitro fertilization. *Endocr Rev* 27:170–207, 2006.
30. Butzow TL, Kettel LM, Yen SS: Clomiphene citrate reduces serum insulin-like growth factor I and increases sex hormone-binding globulin levels in women with polycystic ovary syndrome. *Fertil Steril* 63:1200–1203, 1995.
31. Tarlatzis BC, Grimbizis G: Future use of clomiphene in ovarian stimulation. Will clomiphene persist in the 21st century? *Hum Reprod* 13:2356–2358, 1998.
32. Trounson AO, Leeton JF, Wood C, et al: Pregnancies in humans by fertilization in vitro and embryo transfer in the controlled ovulatory cycle. *Science* 212:681–682, 1981.
33. Cohen J, Fehilly CB, Fishel SB, et al: Male infertility successfully treated by in-vitro fertilisation. *Lancet* 1:1239–1240, 1984.
34. Gemzell C: Induction of ovulation with human gonadotropic hormones. *Recent Prog Horm Res* 21:179–204, 1965.
35. Lunenfeld B, Rabau E, Sulomivici S: [Treatment of amenorrhea by gonadotropic substances from women's urine]. *Harefuah* 64:289–292, 1963.
36. Edwards RG, Steptoe PC, Purdy JM: Establishing full-term human pregnancies using cleaving embryos grown in vitro. *Br J Obstet Gynaecol* 87:737–756, 1980.
37. Laufer N, DeCherney AH, Haseltine FP, et al: The use of high-dose human menopausal gonadotropin in an in vitro fertilization program. *Fertil Steril* 40:734–741, 1983.
38. Stokman PG, de Leeuw R, van den Wijngaard HA, et al: Human chorionic gonadotropin in commercial human menopausal gonadotropin preparations. *Fertil Steril* 60:175–178, 1993.
39. Balen AH, Lumholtz IB: Consensus statement on the bio-safety of urinary-derived gonadotrophins with respect to Creutzfeldt-Jakob disease. *Hum Reprod* 20:2994–2999, 2005.
40. Keene JL, Matzuk MM, Otani T, et al: Expression of biologically active human follitropin in Chinese hamster ovary cells. *J Biol Chem* 264:4769–4775, 1989.
41. Fauser BC: Developments in human recombinant follicle stimulating hormone technology: are we going in the right direction? *Hum Reprod* 13:36–46, 1998.
42. Donderwinkel PF, Schoot DC, Coelingh Bennink HJ, et al: Pregnancy after induction of ovulation with recombinant human FSH in polycystic ovary syndrome. *Lancet* 340:983, 1992.
43. Devroey P, Van SA, Mannaerts B, et al: First singleton term birth after ovarian superovulation with rhFSH. *Lancet* 340:1108–1109, 1992.
44. Hugues JN, Barlow DH, Rosenwaks Z, et al: Improvement in consistency of response to ovarian stimulation with recombinant human follicle stimulating hormone resulting from a new method for calibrating the therapeutic preparation. *Reprod Biomed Online* 6:185–190, 2003.
45. Beckers NG, Macklon NS, Devroey P, et al: First live birth after ovarian stimulation using a chimeric long-acting human recombinant follicle-stimulating hormone (FSH) agonist (recFSH-CTP) for in vitro fertilization. *Fertil Steril* 79:621–623, 2003.
46. Devroey P, Boostanfar R, Koper NP: A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. *Hum Reprod* 24:3063–3072, 2009.
47. Fauser BC, Mannaerts BM, Devroey P: Advances in recombinant DNA technology: corifollitropin alfa, a hybrid molecule with sustained follicle-stimulating activity and reduced injection frequency. *Hum Reprod Update* 15:309–321, 2009.
48. Arce JC, Nyboe Andersen A, Fernando-Sanchez M, et al: Ovarian response to recombinant human FSH, a randomised dose-response trial in women undergoing IVF/ICSI. *Fertil Steril* 102:1633–1640, 2014.
49. Rettenbacher M, Andersen AN, Garcia-Velasco JA, et al: A multi-centre phase 3 study comparing efficacy and safety of Bemfola(®) versus Gonal-f(®) in women undergoing ovarian stimulation for IVF. *Reprod Biomed Online* 30(5):504–513, 2015.

50. Porter RN, Smith W, Craft IL, et al: Induction of ovulation for in-vitro fertilisation using buserelin and gonadotropins. *Lancet* 2:1284–1285, 1984.
51. Huirne JA, Lambalk CB: Gonadotropin-releasing-hormone-receptor antagonists. *Lancet* 24:1793–1803, 2001.
52. Huirne JA, Lambalk CB, van Loenen AC, et al: Contemporary pharmacological manipulation in assisted reproduction. *Drugs* 64: 297–322, 2004.
53. Tarlatzis BC, Fauser BC, Kolibianakis EM, et al: GnRH antagonist in ovarian stimulation for IVF. *Hum Reprod Update* 12:333–340, 2006.
54. 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.
55. Al-Inany HG, Youssef MA, Ayeleke RO, et al: Gonadotropin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* (4):2016.
56. Eijkemans MJ, Imani B, Mulders AG, et al: High singleton live birth rate following classical ovulation induction in normogonadotrophic anovulatory infertility (WHO2). *Hum Reprod* 18:2357–2362, 2003.
57. Veltman-Verhulst SM, Fauser BC, Eijkemans MJ: High singelton live birth rate confirmed after ovulation induction in women with anovulatory polycystic ovary syndrome: validation of a prediction model for clinical practice. *Fertil Steril* 98:761–768, 2012.
58. Aboulghar MA, Mansour RT: Ovarian hyperstimulation syndrome: classifications and critical analysis of preventive measures. *Hum Reprod Update* 9:275–289, 2003.
59. ASRM Practice Committee: Ovarian hyperstimulation syndrome. *Fertil Steril* 86:S178–S183, 2006.
60. Nugent D, Vandekerckhove P, Hughes E, et al: Gonadotrophin therapy for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Syst Rev* (4):CD000410, 2000.
61. Fauser BC, Devroey P, Macklon NS: Multiple birth resulting from ovarian stimulation for subfertility treatment. *Lancet* 365:1807–1816, 2005.
62. Verberg MF, Macklon NS, Heijnen EM, et al: ART: iatrogenic multiple pregnancy? *Best Pract Res Clin Obstet Gynaecol* 21:129–143, 2007.
63. Aboulghar MA, Mansour RT, Serour GI, et al: Diagnosis and management of unexplained infertility: an update. *Arch Gynecol Obstet* 267:177–188, 2003.
64. Stovall DW, Guzick DS: Current management of unexplained infertility. *Curr Opin Obstet Gynecol* 5:228–233, 1993.
65. Guzick DS, Carson SA, Coutifaris C, et al: Efficacy of superovulation and intrauterine insemination in the treatment of infertility. National Cooperative Reproductive Medicine Network. *N Engl J Med* 340: 177–183, 1999.
66. te Velde ER, Cohlen BJ: The management of infertility. *N Engl J Med* 340:224–226, 1999.
67. Tur R, Barri PN, Coroleu B, et al: Risk factors for high-order multiple implantation after ovarian stimulation with gonadotrophins: evidence from a large series of 1878 consecutive pregnancies in a single centre. *Hum Reprod* 16:2124–2129, 2001.
68. Gleicher N, Oleske DM, Tur-Kaspa I, et al: Reducing the risk of high-order multiple pregnancy after ovarian stimulation with gonadotropins. *N Engl J Med* 343:2–7, 2000.
69. Verhulst SM, Cohlen BJ, Hughes E, et al: Intra-uterine insemination for unexplained subfertility. *Cochrane Database Syst Rev* (4):CD001838, 2006.
70. European IVF-Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE), Calhaz-Jorge C, de Geyter C, et al: Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. *Hum Reprod* 31(8):1638–1652, 2016.
71. Pelinck MJ, Hoek A, Simons AH, et al: Efficacy of natural cycle IVF: a review of the literature. *Hum Reprod Update* 8:129–139, 2002.
72. Gerris J, De Neubourg D, Mangelschots K, et al: Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. *Hum Reprod* 17:2626–2631, 2002.
73. Heijnen EM, Eijkemans MJ, de Klerk C, et al: A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. *Lancet* 369:743–749, 2007.
74. Templeton A, Morris JK: Reducing the risk of multiple births by transfer of two embryos after in vitro fertilization. *N Engl J Med* 339:573–577, 1998.
75. Hunault CC, Eijkemans MJ, Pieters MH, et al: A prediction model for selecting patients undergoing in vitro fertilization for elective single embryo transfer. *Fertil Steril* 77:725–732, 2002.
76. Hunault CC, te Velde ER, Weima SM, et al: A case study of the applicability of a prediction model for the selection of patients undergoing in vitro fertilization for single embryo transfer in another center. *Fertil Steril* 87:1314–1321, 2007.
77. Thurin A, Hausken J, Hillensjö T, et al: Elective single-embryo transfer versus double-embryo transfer in in vitero fertilization. *N Engl J Med* 351:2392–2402, 2004.
78. Veleva Z, Karinen P, Tomas C, et al: Elective single embryo transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. *Hum Reprod* 24:1632–1639, 2009.
79. McLernon DJ, Harrild K, Bergh C, et al: Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials. *BMJ* 21:c6945, 2010.
80. Kresowik JD, Stegmann BJ, Sparks AE, et al: Five-years of mandatory single-embryo transfer (mSET) policy dramatically reduces twinning rate without lowering pregnancy rates. *Fertil Steril* 96:1367–1369, 2011.
81. Evers JL: Female subfertility. *Lancet* 360:151–159, 2002.
82. Stromberg B, Dahlquist G, Ericson A, et al: Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet* 359:461–465, 2002.
83. Smith GC, Pell JP, Dobbie R: Birth order, gestational age, and risk of delivery related perinatal death in twins: retrospective cohort study. *BMJ* 325:1004, 2002.
84. Heijnen EM, Macklon NS, Fauser BC: What is the most relevant standard of success in assisted reproduction? The next step to improving outcomes of IVF: consider the whole treatment. *Hum Reprod* 19:1936–1938, 2004.
85. Hull MG, Glazener CM, Kelly NJ, et al: Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* 291:1693–1697, 1985.
86. The Thessaloniki ESHRE/ASRM-sponsored PCOS consensus workshop group: consensus on infertility treatment related to polycystic ovary syndrome. *Hum Reprod* 23:462–477, 2008.
87. Insler V, Melmed H, Mashiah S, et al: Functional classification of patients selected for gonadotropic therapy. *Obstet Gynecol* 32:620–626, 1968.
88. The ESHRE Capri Workshop: Anovulatory infertility. The ESHRE Capri Workshop Group. *Hum Reprod* 10:1549–1553, 1995.
89. Knauff EA, Eijkemans MJ, Lambalk CB, et al: Anti-mullerian hormone, inhibine B, and antral follicle count in young women with ovarian failure. *J Clin Endocrinol Metab* 94:786–792, 2009.
90. Broekmans FJ, Knauff EA, Valkenburg O, et al: PCOS according to the Rotterdam consensus criteria: change in prevalence among WHO-II anovulation and association with metabolic factors. *BJOG* 113:1210–1217, 2006.
91. Adashi EY: Clomiphene citrate: mechanism(s) and site(s) of action—a hypothesis revisited. *Fertil Steril* 42:331–344, 1984.
92. Polson DW, Kiddy DS, Mason HD, et al: Induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome: the difference between responders and nonresponders. *Fertil Steril* 51:30–34, 1989.
93. Young SL, Opsahl MS, Fritz MA: Serum concentrations of enclomiphene and zuclomiphene across consecutive cycles of clomiphene citrate therapy in anovulatory infertile women. *Fertil Steril* 71:639–644, 1999.
94. Dehbashi S, Vafaei H, Parsanezhad MD, et al: Time of initiation of clomiphene citrate and pregnancy rate in polycystic ovarian syndrome. *Int J Gynaecol Obstet* 93:44–48, 2006.
95. George K, George S, Chandy A, et al: hCG administration offers no outcome benefit over spontaneous ovulation in anovulatory women treated with clomiphene citrate. *Fertil Steril* 87(4):985–987, 2007.
96. Kosmas IP, Tatsioni A, Fatemi HM, et al: Human chorionic gonadotropin administration vs. luteinizing monitoring for intrauterine insemination timing, after administration of clomiphene citrate: a meta-analysis. *Fertil Steril* 87:607–612, 2007.
97. Hughes E, Collins J, Vandekerckhove P: Clomiphene citrate for ovulation induction in women with oligo-amenorrhoea. *Cochrane Database Syst Rev* (2):CD000056, 2000.
98. Imani B, Eijkemans MJ, te Velde ER, et al: Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab* 83:2361–2365, 1998.

99. Palomba S, Russo T, Orio F, et al: Uterine effects of clomiphene citrate in women with polycystic ovary syndrome: a prospective controlled study. *Hum Reprod* 21:2823–2829, 2006.
100. Macklon NS, Geraedts JP, Fauser BC: Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update* 8:333–343, 2002.
101. Rossing MA, Daling JR, Weiss NS, et al: Ovarian tumors in a cohort of infertile women. *N Engl J Med* 331:771–776, 1994.
102. Steiner AZ, Terplan M, Paulson RJ: Comparison of tamoxifen and clomiphene citrate for ovulation induction: a meta-analysis. *Hum Reprod* 20:1511–1515, 2005.
103. Bailey CJ, Turner RC: Metformin. *N Engl J Med* 29:574–579, 1996.
104. Moll E, Bossuyt PM, Korevaar JC, et al: Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial. *BMJ* 332:1485, 2006.
105. 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.
106. Velazquez EM, Mendoza S, Hamer T, et al: Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism* 43:647–654, 1994.
107. Nestler JE, Jakubowicz DJ, Evans WS, et al: Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. *N Engl J Med* 338:1876–1880, 1998.
108. Nestler JE, Stovall D, Akhter N, et al: Strategies for the use of insulin-sensitizing drugs to treat infertility in women with polycystic ovary syndrome. *Fertil Steril* 77:209–215, 2002.
109. Glueck CJ, Wang P, Fontaine R, et al: Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. *Metabolism* 48:511–519, 1999.
110. Haas DA, Carr BR, Attia GR: Effects of metformin on body mass index, menstrual cyclicity, and ovulation induction in women with polycystic ovary syndrome. *Fertil Steril* 79:469–481, 2003.
111. George SS, George K, Irwin C, et al: Sequential treatment of metformin and clomiphene citrate in clomiphene-resistant women with polycystic ovary syndrome: a randomized, controlled trial. *Hum Reprod* 18:299–304, 2003.
112. Seli E, Duleba AJ: Optimizing ovulation induction in women with polycystic ovary syndrome. *Curr Opin Obstet Gynecol* 14:245–254, 2002.
113. Lord JM, Flight IH, Norman RJ: Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *BMJ* 327:951–953, 2003.
114. Legro RS, Barnhart HX, Schlaff WD, et al: Ovulatory response to treatment of polycystic ovary syndrome is associated with a polymorphism in the STK11 gene. *J Clin Endocrinol Metab* 93(3):792–800, 2008. [Epub 2007 Nov 13].
115. Pau CT, Cheang KI, Modi BP, et al: The role of variants regulating metformin transport and action in women with polycystic ovary syndrome. *Pharmacogenomics* in press, 2016.
116. 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.
117. Morin-Papunen L, Rantala AS, Unkila-Kallio L, et al: Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. *J Clin Endocrinol Metab* 97:1492–1500, 2012.
118. Tang T, Lord JM, Norman RJ, et al: Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) fror women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* (16):CD003053, 2012.
119. Mitwally MF, Casper RF: Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. *Fertil Steril* 75:305–309, 2001.
120. Cole PA, Robinson CH: Mechanism and inhibition of cytochrome P-450 aromatase. *J Med Chem* 33:2933–2942, 1990.
121. Shetty G, Krishnamurthy H, Krishnamurthy HN, et al: Effect of estrogen deprivation on the reproductive physiology of male and female primates. *J Steroid Biochem Mol Biol* 61:157–166, 1997.
122. Weil S, Vendola K, Zhou J, et al: Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab* 84:2951–2956, 1999.
123. Casper RF, Mitwally MF: Use of aromatase inhibitor Letrozole for ovulation induction in women with polycystic ovarian syndrome. *Clin Obstet Gynecol* 54:685–695, 2011.
124. Misso ML, Wong JL, Teede HJ, et al: Aromatase inhibitors for PCOS: a systematic review and meta-analysis. *Hum Reprod Update* 18:301–312, 2012.
125. Legro RS, Brzyski RG, Diamond MP, et al: Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 371(2):119–129, 2014. Erratum in: *N Engl J Med*. 2014;317(15):1465.
126. Tulandi T, Martin J, Al-Fadhli R, et al: Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil Steril* 85:1761–1765, 2006.
127. Hsueh AJ, Bicsak TA, Jia XC, et al: Granulosa cells as hormone targets: the role of biologically active follicle-stimulating hormone in reproduction. *Recent Prog Horm Res* 45:209–273, 1989.
128. Chappel SC, Howles C: Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum Reprod* 6:1206–1212, 1991.
129. Burgues S: The effectiveness and safety of recombinant human LH to support follicular development induced by recombinant human FSH in WHO group I anovulation: evidence from a multicentre study in Spain. *Hum Reprod* 16:2525–2532, 2001.
130. Franks S, Mason HD, Polson DW, et al: Mechanism and management of ovulatory failure in women with polycystic ovary syndrome. *Hum Reprod* 3:531–534, 1988.
131. Brown JB: Pituitary control of ovarian function: concepts derived from gonadotrophin therapy. *Aust N Z J Obstet Gynaecol* 18:46–54, 1978.
132. Mizunuma H, Takagi T, Yamada K, et al: Ovulation induction by step-down administration of purified urinary follicle-stimulating hormone in patients with polycystic ovarian syndrome. *Fertil Steril* 55:1195–1196, 1991.
133. Fauser BC, Donderwinkel P, Schoot DC: The step-down principle in gonadotrophin treatment and the role of GnRH analogues. *Baillieres Clin Obstet Gynaecol* 7:309–330, 1993.
134. 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.
135. 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.
136. Zeleznik AJ, Hutchison JS, Schuler HM: Interference with the gonadotropin-suppressing actions of estradiol in macaques overrides the selection of a single preovulatory follicle. *Endocrinology* 117:991–999, 1985.
137. White DM, Polson DW, Kiddy D, et al: Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. *J Clin Endocrinol Metab* 81:3821–3824, 1996.
138. van Santbrink EJ, Fauser BC: Urinary follicle-stimulating hormone for normogonadotropic clomiphene-resistant anovulatory infertility: prospective, randomized comparison between low dose step-up and step-down dose regimens. *J Clin Endocrinol Metab* 82:3597–3602, 1997.
139. Bayram N, van Wely M, van der Veen F: Recombinant FSH versus urinary gonadotrophins or recombinant FSH for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Syst Rev* (2):CD002121, 2001.
140. Revelli A, Poso F, Gennarelli G, et al: Recombinant versus highly-purified, urinary follicle-stimulating hormone (r-FSH vs. HP-uFSH) in ovulation induction: a prospective, randomized study with cost-minimization analysis. *Reprod Biol Endocrinol* 4:38, 2006.
141. Shoham Z, Jacobs HS, Insler V: Luteinizing hormone: its role, mechanism of action, and detrimental effects when hypersecreted during the follicular phase. *Fertil Steril* 59:1153–1161, 1993.
142. Filicori M, Cognigni GE, Taraborrelli S, et al: Luteinizing hormone activity supplementation enhances follicle-stimulating hormone efficacy and improves ovulation induction outcome. *J Clin Endocrinol Metab* 84:2659–2663, 1999.
143. Loumaye E, Engrand P, Shoham Z, et al: Clinical evidence for an LH 'ceiling' effect induced by administration of recombinant human LH during the late follicular phase of stimulated cycles in World Health Organization type I and type II anovulation. *Hum Reprod* 18:314–322, 2003.
144. van Santbrink EJ, Fauser BC: Ovulation induction in normogonadotropic anovulation (PCOS). *Best Pract Res Clin Endocrinol Metab* 20:261–270, 2006.
145. Balen A: Is there a risk of prion disease after the administration of urinary-derived gonadotrophins? *Hum Reprod* 17:1676–1680, 2002.

146. Knobil E: The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res* 36:53–88, 1980.
147. Marshall JC, Eagleson CA, McCartney CR: Hypothalamic dysfunction. *Mol Cell Endocrinol* 183:29–32, 2001.
148. Filicori M, Flamigni C, Meriggiola MC, et al: Ovulation induction with pulsatile gonadotropin-releasing hormone: technical modalities and clinical perspectives. *Fertil Steril* 56:1–13, 1991.
149. Jansen RP: Pulsatile intravenous gonadotrophin releasing hormone for ovulation induction: determinants of follicular and luteal phase responses. *Hum Reprod* 8:193–196, 1993.
150. Braat DD, Berkhout G, ten Brug CS, et al: Multiple follicular development in women with normal menstrual cycles treated with pulsatile gonadotrophin-releasing hormone. *Hum Reprod* 6:1379–1383, 1991.
151. Filicori M: Gonadotrophin-releasing hormone agonists. A guide to use and selection. *Drugs* 48:41–58, 1994.
152. Martin KA, Hall JE, Adams JM, et al: Comparison of exogenous gonadotropins and pulsatile gonadotropin-releasing hormone for induction of ovulation in hypogonadotropic amenorrhea. *J Clin Endocrinol Metab* 77:125–129, 1993.
153. Homburg R, Eshel A, Armar NA, et al: One hundred pregnancies after treatment with pulsatile luteinising hormone releasing hormone to induce ovulation. *BMJ* 298:809–812, 1989.
154. Filicori M, Flamigni C, Dellai P, et al: Treatment of anovulation with pulsatile gonadotropin-releasing hormone: prognostic factors and clinical results in 600 cycles. *J Clin Endocrinol Metab* 79:1215–1220, 1994.
155. Bayram N, van Wely M, van der Veen F: Pulsatile gonadotrophin releasing hormone for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Syst Rev* (1):CD000412, 2004.
156. Handelsman DJ, Jansen RP, Boylan LM, et al: Pharmacokinetics of gonadotropin-releasing hormone: comparison of subcutaneous and intravenous routes. *J Clin Endocrinol Metab* 59:739–746, 1984.
157. Plosker SM, Marshall LA, Martin MC, et al: Opioid, catecholamine, and steroid interaction in prolactin and gonadotropin regulation. *Obstet Gynecol Surv* 45:441–453, 1990.
158. Yen SS, Quigley ME, Reid RL, et al: Cetel NS: Neuroendocrinology of opioid peptides and their role in the control of gonadotropin and prolactin secretion. *Am J Obstet Gynecol* 152:485–493, 1985.
159. Couzinet B, Young J, Brailly S, et al: Even after priming with ovarian steroids or pulsatile gonadotropin-releasing hormone administration, naltrexone is unable to induce ovulation in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab* 80:2102–2107, 1995.
160. Wildt L, Leyendecker G, Sir-Petermann T, et al: Treatment with naltrexone in hypothalamic ovarian failure: induction of ovulation and pregnancy. *Hum Reprod* 8:350–358, 1993.
161. Roozenburg BJ, van Dessel HJ, Evers JL, et al: Successful induction of ovulation in normogonadotrophic clomiphene resistant anovulatory women by combined naltrexone and clomiphene citrate treatment. *Hum Reprod* 12:1720–1722, 1997.
162. Rossmanith WG, Monn M, Benz R: Effects of chronic opioid antagonism on gonadotrophin and ovarian sex steroid secretion during the luteal phase. *Clin Endocrinol (Oxf)* 49:343–351, 1998.
163. Elnashar A, Abdelmageed E, Fayed M, et al: Clomiphene citrate and dexamethazone in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective placebo-controlled study. *Hum Reprod* 21:1805–1808, 2006.
164. Azziz R, Black VY, Knochenhauer ES, et al: Ovulation after glucocorticoid suppression of adrenal androgens in the polycystic ovary syndrome is not predicted by the basal dehydroepiandrosterone sulfate level. *J Clin Endocrinol Metab* 84:946–950, 1999.
165. Fleming R, Haxton MJ, Hamilton MP, et al: Successful treatment of infertile women with oligomenorrhoea using a combination of an LHRH agonist and exogenous gonadotrophins. *Br J Obstet Gynaecol* 92:369–373, 1985.
166. Charbonnel B, Krempf M, Blanchard P, et al: Induction of ovulation in polycystic ovary syndrome with a combination of a luteinizing hormone-releasing hormone analog and exogenous gonadotropins. *Fertil Steril* 47:920–924, 1987.
167. Schoot DC, Pijlman B, Stijnen T, et al: Effects of gonadotropin releasing hormone agonist addition to gonadotropin induction of ovulation in polycystic ovary syndrome patients. *Eur J Obstet Gynecol Reprod Biol* 45:53–58, 1992.
168. Hughes E, Collins J, Vandekerckhove P: Gonadotrophin-releasing hormone analogue as an adjunct to gonadotropin therapy for clomiphene-resistant polycystic ovarian syndrome. *Cochrane Database Syst Rev* (2):CD000097, 2000.
169. Zaadstra BM, Seidell JC, Van Noord PA, et al: Fat and female fecundity: prospective study of effect of body fat distribution on conception rates. *BMJ* 306:484–487, 1993.
170. Moran LJ, Brinkworth G, Noakes M, et al: Effects of lifestyle modification in polycystic ovarian syndrome. *Reprod Biomed Online* 12:569–578, 2006.
171. Pasquali R, Gambineri A, Biscotti D, et al: Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *J Clin Endocrinol Metab* 85:2767–2774, 2000.
172. Crosignani PG, Colombo M, Vegetti W, et al: Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. *Hum Reprod* 18:1928–1932, 2003.
173. Moran LJ, Noakes M, Clifton PM, et al: Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 88:812–819, 2003.
174. Huber-Buchholz MM, Carey DG, Norman RJ: Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. *J Clin Endocrinol Metab* 84:1470–1474, 1999.
175. 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.
176. Norman RJ, Davies MJ, Lord J, et al: The role of lifestyle modification in polycystic ovary syndrome. *Trends Endocrinol Metab* 13:251–257, 2002.
177. Balen AH, Dresner M, Scott EM, et al: Should obese women with polycystic ovary syndrome receive treatment for infertility? *BMJ* 332:434–435, 2006.
178. Homan GF, Davies M, Norman R: The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Hum Reprod Update* 13:209–223, 2007.
179. Baird DD, Wilcox AJ: Cigarette smoking associated with delayed conception. *JAMA* 253:2979–2983, 1985.
180. Klonoff-Cohen H: Female and male lifestyle habits and IVF: what is known and unknown. *Hum Reprod Update* 11:179–203, 2005.
181. Midgette AS, Baron JA: Cigarette smoking and the risk of natural menopause. *Epidemiology* 1:474–480, 1990.
182. Zenzes MT, Reed TE, Casper RF: Effects of cigarette smoking and age on the maturation of human oocytes. *Hum Reprod* 12:1736–1741, 1997.
183. Mulders AG, Eijkemans MJ, Imani B, et al: Prediction of chances for success or complications in gonadotrophin ovulation induction in normogonadotrophic anovulatory infertility. *Reprod Biomed Online* 7:170–178, 2003.
184. Delvigne A, Rozenberg S: Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. *Hum Reprod Update* 8:559–577, 2002.
185. Albano C, Platteau P, Nogueira D, et al: Avoidance of multiple pregnancies after ovulation induction by supernumerary preovulatory follicular reduction. *Fertil Steril* 76:820–822, 2001.
186. Venetis CA, Kolibianakis EM, Tarlatzis TB, et al: Evidence-based management of poor ovarian response. *Ann N Y Acad Sci* 1205:199–206, 2010.
187. Polyzos NP, Devroey P: A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? *Fertil Steril* 96:1058–1061, 2011.
188. Beckers NG, Macklon NS, Eijkemans MJ, et al: Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for in vitro fertilization exhibit follicular phase characteristics suggestive of ovarian aging. *Fertil Steril* 78:291–297, 2002.
189. Broekmans FJ, Knauff EA, te Velde ER, et al: Female reproductive ageing: current knowledge and future trends. *Trends Endocrinol Metab* 18:58–65, 2007.
190. Fauser BC, Devroey P: Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. *Trends Endocrinol Metab* 14:236–242, 2003.
191. Crosignani PG, Walters DE, Soliani A: The ESHRE multicentre trial on the treatment of unexplained infertility: a preliminary report. European Society of Human Reproduction and Embryology. *Hum Reprod* 6:953–958, 1991.
192. Melis GB, Paoletti AM, Ajossa S, et al: Ovulation induction with gonadotropins as sole treatment in infertile couples with open

tubes: a randomized prospective comparison between intrauterine insemination and timed vaginal intercourse. *Fertil Steril* 64:1088–1093, 1995.
193. Athaullah N, Proctor M, Johnson NP: Oral versus injectable ovulation induction agents for unexplained subfertility. *Cochrane Database Syst Rev* (3):CD003052, 2002.
194. Hughes EG: The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: a meta-analysis. *Hum Reprod* 12:1865–1872, 1997.
195. Stewart JA: Stimulated intra-uterine insemination is not a natural choice for the treatment of unexplained subfertility. Should the guidelines be changed? *Hum Reprod* 18:903–907, 2003.
196. Collins J: Stimulated intra-uterine insemination is not a natural choice for the treatment of unexplained subfertility. Current best evidence for the advanced treatment of unexplained subfertility. *Hum Reprod* 18:907–912, 2003.
197. Goverde AJ, McDonnell J, Vermeiden JP, et al: Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. *Lancet* 355:13–18, 2000.
198. ESHRE Capri Workshop Group: Intrauterine insemination. *Hum Reprod Update* 15:265–277, 2009.
199. Hughes E, Collins J, Vandekerckhove P: Clomiphene citrate for unexplained subfertility in women. *Cochrane Database Syst Rev* (3):CD000057, 2000.
200. Diamond MP, Legro RS, Coutifaris C, et al: Letrozole, gonadotropin, or clomiphene for unexplained infertility. *N Engl J Med* 373(13):1230–1240, 2015.
201. Fauser BC: Unexplained infertility-ongoing transatlantic debate. *Nat Rev Endocrinol* 12:2016.
202. van der Gaast MH, Eijkemans MJ, van der Net JB, et al: Optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online* 13:476–480, 2006.
203. Fauser BC, Devroey P, Yen SS, et al: Minimal ovarian stimulation for IVF: appraisal of potential benefits and drawbacks. *Hum Reprod* 14:2681–2686, 1999.
204. Stoop D, Ermini B, Polyzos NP, et al: Reproductive potential of a metaphase II oocyte retrieved after ovarian stimulation: an analysis of 23354 ICSI cycles. *Hum Reprod* 27:2030–2035, 2012.
205. Kligman I, Rosenwaks Z: Differentiating clinical profiles: predicting good responders, poor responders, and hyperresponders. *Fertil Steril* 76:1185–1190, 2001.
206. Van Rooij IA, Broekmans FJ, te Velde ER, et al: Serum anti-mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 17:3065–3071, 2002.
207. Broer SL, Dolleman M, Opmeer BC, et al: AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. *Hum Reprod Update* 17:46–54, 2011.
208. Humaiden P: Agonist trigger: what is the best approach? Agonist trigger and low dose hCG. *Fertil Steril* 97:529–530, 2012.
209. Zwart-van Rijkom JE, Broekmans FJ, Leufkens HG: From HMG through purified urinary FSH preparations to recombinant FSH: a substitution study. *Hum Reprod* 17:857–865, 2002.
210. Nargund G, Waterstone J, Bland J, et al: Cumulative conception and live birth rates in natural (unstimulated) IVF cycles. *Hum Reprod* 16:259–262, 2001.
211. Nargund G, Fauser BC, Macklon NS, et al: The ISMAAR proposal on terminology for ovarian stimulation for IVF. *Hum Reprod* 22:2801–2804, 2007.
212. Pelinck MJ, Vogel NE, Arts EG, et al: Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: a cohort study. *Hum Reprod* 22:2463–2470, 2007.
213. Cohen J, Trounson A, Dawson K, et al: The early days of IVF outside the UK. *Hum Reprod Update* 11:439–459, 2005.
214. Kenny DT: In vitro fertilisation and gamete intrafallopian transfer: an integrative analysis of research, 1987-1992. *Br J Obstet Gynaecol* 102:317–325, 1995.
215. Sterrenburg M, Veltman-Verhulst SM, Eijkemans MJ, et al: Clinical outcomes in relation to the daily dose of recombinant follicle-stimulating hormone for ovarian stimulation in in vitro fertilization in presumed normal responders younger than 39 years: a meta-analysis. *Hum Reprod Update* 17:184–196, 2011.
216. Devroey P, Fauser BC, Platteau P, et al: Induction of multiple follicular development by a single dose of long-acting recombinant follicle-stimulating hormone (FSH-CTP, corifollitropin alfa) for controlled ovarian stimulation before in vitro fertilization. *J Clin Endocrinol Metab* 89:2062–2070, 2004.
217. Westergaard LG, Laursen SB, Andersen CY: Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. *Hum Reprod* 15:1003–1008, 2000.
218. Fleming R, Rehka P, Deshpande N, et al: Suppression of LH during ovarian stimulation: effects differ in cycles stimulated with purified urinary FSH and recombinant FSH. *Hum Reprod* 15:1440–1445, 2000.
219. Kolibianakis EM, Kalogeropoulou L, Griesinger G, et al: Among patients treated with FSH and GnRH analogues for in vitro fertilization, is the addition of recombinant LH associated with the probability of live birth? A systematic review and meta-analysis. *Hum Reprod Update* 13:445–452, 2007.
220. Baruffi RL, Mauri AL, Petersen CG, et al: Recombinant LH supplementation to recombinant FSH during induced ovarian stimulation in the GnRH-antagonist protocol: a meta-analysis. *Reprod Biomed Online* 14:14–25, 2007.
221. Deleted in review.
222. Out HJ, Mannaerts BM, Driessen SG, et al: A prospective, randomized, assessor-blind, multicentre study comparing recombinant and urinary follicle stimulating hormone (Puregon versus Metrodin) in in-vitro fertilization. *Hum Reprod* 10:2534–2540, 1995.
223. Daya S: Updated meta-analysis of recombinant follicle-stimulating hormone (FSH) versus urinary FSH for ovarian stimulation in assisted reproduction. *Fertil Steril* 77:711–714, 2002.
224. Daya S, Ledger W, Auray JP, et al: Cost-effectiveness modelling of recombinant FSH versus urinary FSH in assisted reproduction techniques in the UK. *Hum Reprod* 16:2563–2569, 2001.
225. Sykes D, Out HJ, Palmer SJ, et al: The cost-effectiveness of IVF in the UK: a comparison of three gonadotrophin treatments. *Hum Reprod* 16:2557–2562, 2001.
226. Agrawal R, Holmes J, Jacobs HS: Follicle-stimulating hormone or human menopausal gonadotropin for ovarian stimulation in in vitro fertilization cycles: a meta-analysis. *Fertil Steril* 73:338–343, 2000.
227. Andersen AN, Devroey P, Arce JC: Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. *Hum Reprod* 21:3217–3227, 2006.
228. van Wely M, Kwan L, Burt AL, et al: Recombinant versus gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. *Cochrane Database Syst Rev* (16):CD005354, 2001.
229. Al-Inany H, Aboulghar M: GnRH antagonist in assisted reproduction: a Cochrane review. *Hum Reprod* 17:874–885, 2002.
230. Mannaerts B, Shoham Z, Schoot D, et al: Single-dose pharmacokinetics and pharmacodynamics of recombinant human follicle-stimulating hormone (Org 32489*) in gonadotropin-deficient volunteers. *Fertil Steril* 59:108–114, 1993.
231. van Hooff MH, Alberda AT, Huisman GJ, et al: Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: a randomized study. *Hum Reprod* 8:369–373, 1993.
232. Klinkert ER, Broekmans FJ, Looman CW, et al: Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. *Hum Reprod* 20:611–615, 2005.
233. Rombauts L, Suikkari AM, MacLachlan V, et al: Recruitment of follicles by recombinant human follicle-stimulating hormone commencing in the luteal phase of the ovarian cycle. *Fertil Steril* 69:665–669, 1998.
234. Griesinger G, Venetis CA, Marx T, et al: Oral contraceptive pill pretreatment in ovarian stimulation with GnRH antagonists for IVF: a systematic review and meta-analysis. *Fertil Steril* 90:1055–1063, 2008.
235. Hauzman EE, Zapata A, Bermejo A, et al: Cycle scheduling for in vitro fertilization with oral contraceptive pills versus oral estradiol valerate: a randomized, controlled trial. *Reprod Biol Endocrinol* 11:96, 2013.
236. Tremellen KP, Lane M: Avoidance of weekend oocyte retrievals during GnRH antagonist treatment by simple advancement or delay of hCG administration does not adversely affect IVF live birth outcomes. *Hum Reprod* 25(5):1219–1224, 2010.
237. Nagels HE, Rishworth JR, Siristatidis CS, et al: Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction. *Cochrane Database Syst Rev* CD009749, 2015.

238. Bosdou JK, Venetis CA, Dafopoulos K, et al: Transdermal testosterone pretreatment in poor responders undergoing ICSI: a randomized clinical trial. *Hum Reprod* 31(5):977–985, 2016.
239. Smitz J, Devroey P, Camus M, et al: Addition of Buserelin to human menopausal gonadotrophins in patients with failed stimulations for IVF or GIFT. *Hum Reprod* 3:35–38, 1988.
240. Hughes EG, Fedorkow DM, Daya S, et al: The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. *Fertil Steril* 58:888–896, 1992.
241. Hugues JN, Cedarin-Durnerin I: Revisiting gonadotrophin-releasing hormone agonist protocols and management of poor ovarian responses to gonadotrophins. *Hum Reprod Update* 4:83–101, 1998.
242. Beckers NG, Laven JS, Eijkemans MJ, et al: Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for in-vitro fertilization. *Hum Reprod* 15:43–49, 2000.
243. Damianaki T, Vaxevanoglou T, Kapetanakis E: Prospective study of a modified gonadotropin-releasing hormone agonist long protocol in an in vitro fertilization program. *Fertil Steril* 61:709–713, 1994.
244. Al-Inany H, Aboulghar MA, Mansour RT, et al: Optimizing GnRH antagonist administration: meta-analysis of fixed versus flexible protocol. *Reprod Biomed Online* 10:567–570, 2005.
245. Deleted in review.
246. Hoff JD, Quigley ME, Yen SS: Hormonal dynamics at midcycle: a reevaluation. *J Clin Endocrinol Metab* 57:792–796, 1983.
247. Gonen Y, Balakier H, Powell W, et al: Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for in vitro fertilization. *J Clin Endocrinol Metab* 71:918–922, 1990.
248. Damewood MD, Shen W, Zacur HA, et al: Disappearance of exogenously administered human chorionic gonadotropin. *Fertil Steril* 52:398–400, 1989.
249. Chandrasekher YA, Hutchison JS, Zelinski-Wooten MB, et al: Initiation of periovulatory events in primate follicles using recombinant and native human luteinizing hormone to mimic the midcycle gonadotropin surge. *J Clin Endocrinol Metab* 79:298–306, 1994.
250. 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 hyperstimulation for in vitro fertilization. *J Clin Endocrinol Metab* 87:709–715, 2002.
251. Simon C, Garcia Velasco JJ, Valbuena D, et al: Increasing uterine receptivity by decreasing estradiol levels during the preimplantation period in high responders with the use of a follicle-stimulating hormone step-down regimen. *Fertil Steril* 70:234–239, 1998.
252. Humaiden P, Kol S, Papanikolaou EG, et al: GnRH agonist for triggering of final oocyte maturation: time for a change of practice? *Hum Reprod Update* 17:510–514, 2011.
252a. Engmann L, Benadiva C, Humaiden P: GnRH agonist trigger for the induction of oocyte maturation in GnRH agonist IVF cycles. A SWOT analysis. *Reprod Biomed Online* 32:274–285, 2016.
253. The European Recombinant LH Study Group: Human recombinant luteinizing hormone is as effective as, but safer than, urinary human chorionic gonadotropin in inducing final follicular maturation and ovulation in in vitro fertilization procedures: results of a multicenter double-blind study. *J Clin Endocrinol Metab* 86:2607–2618, 2001.
254. Humaidan P, Bredkjaer HE, Bungum L, et al: GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. *Hum Reprod* 20:1213–1220, 2005.
255. Griesinger G, Kolibianakis EM, Papanikolaou EG, et al: Triggering of final oocyte maturation with gonadotropin-releasing hormone agonist or human chorionic gonadotropin. Live birth after frozen-thawed embryo replacement cycles. *Fertil Steril* 88:616–621, 2007.
256. Engmann L, Diluigi A, Schmidt D, et al: The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril* 89:84–91, 2008.
257. Jones HW: What has happened? Where are we? *Hum Reprod* 11:7–24, 1996.
258. Donderwinkel PF, Schoot DC, Pache TD, et al: Luteal function following ovulation induction in polycystic ovary syndrome patients using exogenous gonadotrophins in combination with a gonadotrophin-releasing hormone agonist. *Hum Reprod* 8:2027–2032, 1993.
259. Smitz J, Erard P, Camus M, et al: Pituitary gonadotrophin secretory capacity during the luteal phase in superovulation using GnRH-agonists and HMG in a desensitization or flare-up protocol. *Hum Reprod* 7:1225–1229, 1992.
260. Penzias AS: Luteal phase support. *Fertil Steril* 77:318–323, 2002.
261. Elter K, Nelson LR: Use of third generation gonadotropin-releasing hormone antagonists in in vitro fertilization-embryo transfer: a review. *Obstet Gynecol Surv* 56:576–588, 2001.
262. van der Linden M, Buckingham K, Farquhar C, et al: Luteal phase support for assited reproduction cycles. *Cochrane Database Syst Rev* (5):CD009154, 2011.
263. Fatemi HM, Popovic-Todorovic B, Papanikolaou E, et al: An update of luteal phase support in stimulated IVF cycles. *Hum Reprod Update* 13:581–590, 2007.
264. Ragni G, Vegetti W, Baroni E, et al: Comparison of luteal phase profile in gonadotrophin stimulated cycles with or without a gonadotrophin-releasing hormone antagonist. *Hum Reprod* 16:2258–2262, 2001.
265. Albano C, Grimbizis G, Smitz J, et al: The luteal phase of nonsupplemented cycles after ovarian superovulation with human menopausal gonadotropin and the gonadotropin-releasing hormone antagonist Cetrorelix. *Fertil Steril* 70:357–359, 1998.
266. de Jong D, Macklon NS, Fauser BC: A pilot study involving minimal ovarian stimulation for in vitro fertilization: extending the "follicle-stimulating hormone window" combined with the gonadotropin-releasing hormone antagonist cetrorelix. *Fertil Steril* 73:1051–1054, 2000.
267. Beckers NG, Macklon NS, Eijkemans MJ, et al: Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in in vitro fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. *J Clin Endocrinol Metab* 88:4186–4192, 2003.
268. Tavaniotou A, Albano C, Smitz J, et al: Comparison of LH concentrations in the early and mid-luteal phase in IVF cycles after treatment with HMG alone or in association with the GnRH antagonist Cetrorelix. *Hum Reprod* 16:663–667, 2001.
269. Deleted in review.
270. Schenker JG, Ezra Y: Complications of assisted reproductive techniques. *Fertil Steril* 61:411–422, 1994.
271. Boivin J, Domar AD, Shapiro DB, et al: Tackling burden in ART: an integrated approach for medical staff. *Hum Reprod* 27:941–950, 2012.
272. Olivennes F, Howles CM, Borini A, et al: Individualizing FSH dose for assisted reproduction using a novel algorithm: the CONSORT study. *Reprod Biomed Online* 22:S73–S82, 2011.
273. Nardo LG, Fleming R, Howles CM, et al: Conventional ovarian stimulation no longer exists: welcome to the age of individualized ovarian stimulation. *Reprod Biomed Online* 23:141–148, 2011.
274. Fauser BC, Diedrich K, Devroey P: Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation. *Hum Reprod Update* 14:1–14, 2008.
275. Mahdavi A, Pejovic T, Nezhat F: Induction of ovulation and ovarian cancer: a critical review of the literature. *Fertil Steril* 85:819–826, 2006.
276. Edwards RG, Lobo R, Bouchard P: Time to revolutionize ovarian stimulation. *Hum Reprod* 11:917–919, 1996.
277. Pennings G, Ombelet W: Coming soon to your clinic: patient-friendly ART. *Hum Reprod* 22:2075–2079, 2007.
278. Lundin K, Bergh C: Cumulative impact of adding frozen-thawed cycles to single versus double fresh embryo transfers. *Reprod Biomed Online* 15:76–82, 2007.
279. Zeleznik AJ, Kubik CJ: Ovarian responses in macaques to pulsatile infusion of follicle-stimulating hormone (FSH) and luteinizing hormone: increased sensitivity of the maturing follicle to FSH. *Endocrinology* 119:2025–2032, 1986.
280. 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* 88:166–173, 2003.
281. de Klerk C, Heijnen EM, Macklon NS, et al: The psychological impact of mild ovarian stimulation combined with single embryo transfer compared with conventional IVF. *Hum Reprod* 21:721–727, 2006.
282. Verberg MF, Eijkemans MJ, Heijnen EM, et al: Why do couples drop-out from IVF treatment? A prospective cohort study. *Hum Reprod* 23:250–255, 2008.

283. Fauser BC, Nargund G, Andersen AN, et al: Mild ovarian stimulation for IVF: 10 years later. *Hum Reprod* 25:2678–2684, 2010.
284. Filicori M, Cognigni GE, Gamberini E, et al: Efficacy of low-dose human chorionic gonadotropin alone to complete controlled ovarian stimulation. *Fertil Steril* 84:394–401, 2005.
285. Thuessen LL, Loft A, Egeberg AN, et al: A randomized controlled dose-response pilot study of addition of hCG to recombinant FSH during controlled ovarian stimulation for in vitro fertilization. *Hum Reprod* 27(10):3074–3084, 2012.
286. Haaf T, Hahn A, Lambrecht A, et al: A high oocyte yield for intracytoplasmic sperm injection treatment is asociated with an increased chromosome error date. *Fertil Steril* 91:733–738, 2009.
287. Xu YW, Peng YT, Wang B, et al: High follicle-stimulating hormone increases aneuploidy in human oocytes matured in vitro. *Fertil Steril* 95:99–104, 2011.
288. Munne S, Magli C, Adler A, et al: Treatment-related chromosome abnormalities in human embryos. *Hum Reprod* 12:780–784, 1997.
289. Katz-Jaffe MG, Trounson AO, Cram DS: Chromosome 21 mosaic human preimplantation embryos predominantly arise from diploid conceptions. *Fertil Steril* 84:634–643, 2005.
290. Baart EB, Martini E, Eijkemans MJ, et al: Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 22:980–988, 2007.
291. Laven JS, Imani B, Eijkemans MJ, et al: New approach to polycystic ovary syndrome and other forms of anovulatory infertility. *Obstet Gynecol Surv* 57:755–767, 2002.
292. Imani B, Eijkemans MJ, de Jong FH, et al: Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab* 85:676–682, 2000.
293. Hunault CC, Laven JS, van Rooij IA, et al: Prospective validation of two models predicting pregnancy leading to live birth among untreated subfertile couples. *Hum Reprod* 20:1636–1641, 2005.
294. van Santbrink EJ, Eijkemans MJ, Laven JS, et al: Patient-tailored conventional ovulation induction algorithms in anovulatory infertility. *Trends Endocrinol Metab* 16:381–389, 2005.
295. Imani B, Eijkemans MJ, Faessen GH, et al: Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. *Fertil Steril* 77:83–90, 2002.
296. van Santbrink EJ, Eijkemans MJ, Macklon NS, et al: FSH response-dose can be predicted in ovulation induction for normogonadotropic anovulatory infertility. *Eur J Endocrinol* 147:223–226, 2002.
297. Deleted in review.
298. Templeton A, Morris JK, Parslow W: Factors that affect outcome of in-vitro fertilisation treatment. *Lancet* 348:1402–1406, 1996.
299. te Velde ER, Pearson PL: The variability of female reproductive ageing. *Hum Reprod Update* 8:141–154, 2002.
300. Scott RT, Hofmann GE: Prognostic assessment of ovarian reserve. *Fertil Steril* 63:1–11, 1995.
301. Scott RT, Toner JP, Muasher SJ, et al: Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. *Fertil Steril* 51:651–654, 1989.
302. Broekmans FJ, Kwee J, Hendriks DJ, et al: A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 12:685–718, 2006.
303. Bancsi LF, Broekmans FJ, Mol BW, et al: Performance of basal follicle-stimulating hormone in the prediction of poor ovarian response and failure to become pregnant after in vitro fertilization: a meta-analysis. *Fertil Steril* 79:1091–1100, 2003.
304. Bancsi LF, Broekmans FJ, Eijkemans MJ, et al: Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertil Steril* 77:328–336, 2002.
305. de Vet A, Laven JS, de Jong FH, et al: Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 77:357–362, 2002.
306. Nelson SM, Anderson RA, Broekmans FJ, et al: Anti-mullerian hormone: clairvoyance or crystal clear? *Hum Reprod* 27:631–636, 2012.
307. Popovic-Todorovic B, Loft A, Lindhard A, et al: A prospective study of predictive factors of ovarian response in 'standard' IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram. *Hum Reprod* 18:781–787, 2003.
308. Popovic-Todorovic B, Loft A, Bredkjaeer HE, et al: A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a 'standard' dose of 150 IU/day in 'standard' patients undergoing IVF/ICSI treatment. *Hum Reprod* 18:2275–2282, 2003.
309. Lintsen AM, Pasker-de Jong PC, de Boer EJ, et al: Effects of subfertility cause, smoking and body weight on the success rate of IVF. *Hum Reprod* 20:1867–1875, 2005.
310. Olivennes F, Trew G, Borini A, et al: RCT of the CONSORT algorithm for individualised dosing of follitropin alfa. *Reprod Biomed Online* 30:248, 2015.
311. Pandian Z, Gibreel A, Bhattacharya S: In vitro fertilisation for unexplained subfertility. *Cochrane Database Syst Rev* (4):CD003357, 2012.
312. Gleicher N: Cost-effective infertility care. *Hum Reprod Update* 6:190–199, 2000.
313. Collins J: An international survey of the health economics of IVF and ICSI. *Hum Reprod Update* 8:265–277, 2002.
314. Jain T, Harlow BL, Hornstein MD: Insurance coverage and outcomes of in vitro fertilization. *N Engl J Med* 347:661–666, 2002.
315. Stevenson RC, McCabe CJ, Pharoah PO, et al: Cost of care for a geographically determined population of low birthweight infants to age 8–9 years. I. Children without disability. *Arch Dis Child Fetal Neonatal Ed* 74:F114–F117, 1996.
316. Henderson J, Hockley C, Petrou S, et al: Economic implications of multiple births: inpatient hospital costs in the first 5 years of life. *Arch Dis Child Fetal Neonatal Ed* 89:F542–F545, 2004.
317. Polinder S, Heijnen EM, Macklon NS, et al: Cost-effectiveness of a mild compared with a standard strategy for IVF: a randomized comparison using cumulative term live birth as the primary endpoint. *Hum Reprod* 23:316–323, 2008.
318. Chen ZJ, Shi Y, Sun Y, et al: Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. *N Engl J Med* 375(6):523–533, 2016.
319. Greb RR, Grieshaber K, Gromoll J, et al: A common single nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major determinant of length and hormonal dynamics of the menstrual cycle. *J Clin Endocrinol Metab* 90:4866–4872, 2005.