Ovarian Stimulation: Today and Tomorrow

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Abstract: In assisted reproductive technology, medications and ovarian stimulation play a crucial role. The availability of gonadotrophins and GnRH analogues has allowed the tailoring of several stimulation schemes. The two most commonly used gonadotrophin forms are urinary hMG and recombinant FSH in combination with GnRH agonists or GnRH antagonists. Cycles stimulate with recombinant FSH appear to have a higher risk of premature progesterone rise in the late follicular phase, if not triggered on time. Recently, corifollitropin alfa, a new long acting recombinant FSH was introduced which sustain multiple follicular growth for 7 days in women undergoing ovarian stimulation using GnRH antagonists. GnRH antagonist and agonist do have similar live birthrate. However, GnRH antagonists reduce significantly the risk of OHSS. Moreover, with the introduction of GnRH antagonists, it became possible to trigger ovulation with a bolus of a GnRH agonist as an alternative to hCG. The early OHSS is eliminated completely with the GnRH agonist trigger. However, due to an uncorrectable luteal phase deficiency, a poor clinical outcome is present, when GnRHa is used to trigger final oocyte maturation in IVF/ICSI antagonist protocols. Therefore, it has been suggested to cryopreserve the embryos and transfer in consecutive natural cycles. Future trials should aim to eliminate OHSS and multiple pregnancy rates by performing a single stimulation in a simplified corifolitropin alfa/GnRH antagonist cycle triggered by a GnRH agonist followed by Cryo-thawed SET in consecutive natural cycles. With this approach, the two major complications of COH for IVF could be eliminated without jeopardizing the outcome.

Keywords: Corifollitropin alfa, future of IVF, GnRH agonist, GnRH antagonist, gonadotropins, ovarian stimulation.

INTRODUCTION

Infertility affects up to 16.7% of couples of reproductive age [1]. In ART, medications play a crucial role in follicular stimulation and endometrial priming making it receptive for the embryos transferred into the uterine cavity. The availability of gonadotrophins and GnRH analogues has allowed the tailoring of several stimulation schemes, which have improved treatment outcome.

In the natural cycle, follicle growth is driven by a delicate interplay of FSH and LH that affects theca and granulosa cells, leading to the selection of a single dominant follicle through a series of feedback mechanisms [2, 3]. FSH drives the development of the granulosa cell compartment and is essential for follicle survival and differentiation.

Structurally, there is a great similarity between FSH and LH. They are both glycoproteins that share identical α-subunit and differ only in the structure of their β-subunit, which confer receptor specificity [4]. The synthesis of the β-subunit is the rate regulating step in gonadotropin biosynthesis [5]. The α-subunit consists of 92 aminoacids stabilized by 5 disulfide bonds, while the β-subunit contains of 118 amino acids and 5 sialic acid residues. Neither subunit has any intrinsic biologic activity by itself. The variation of the sialic acid component is responsible for the different half life of these hormones. Sialic acid prevents the hepatic clearance; thus, the greater the sialic acid component, the longer the half-life [6]. HCG, for with 20 salic acid residues, has the longest half-life (about 24 hours), whereas LH (1 to 2 sialic acid residues) has a very short half life (20-30 minutes) [6].

Gonadotrophins

Gonadotrophin therapy in various forms has been used to restore ovulation since the 1930s. Only after the introduction of IVF, gonadotrophins have been applied to stimulate multiple follicle development [7, 8].

The latter treatment overrides the physiologic selection of a single dominant follicle by extending the time during which serum follicle-stimulating hormone (FSH) concentrations remain above the threshold level required for follicular recruitment and ongoing maturation [9-11].

Today, the two most commonly used gonadotrophin forms are urinary hMG (which contains FSH and hCG with LH activity) and recombinant FSH (which contains just FSH) [12] in combination with GnRH agonists or GnRH antagonists to prevent premature LH surge.

The extraction and purification of postmenopausal urine were pioneered in the late 1940s to result in the production of hMG [13]. However, due to the contamination with non-specific co-purified proteins, severe hypersensivity and discomfort followed the injections. Through the 1960s the extraction process became more sophisticated and the side effects were reduced with the increase in the activity tenfold compared to early preparations.

The use of monoclonal antibodies in the 1980s enabled further purification to be achieved by specifically selecting FSH out from the bulk hMG [14]. A significantly reduced hypersensivity was reported. Furthermore, it could be admin-

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istered subcutaneously. However, with the increased use of gonadotrophins, the future of infertility therapy clearly relied on the capacity to produce sufficient quantities of gonadotrophins to meet the ever increasing worldwide demand. It was estimated that to satisfy this demand, it was necessary to collect and process about 120 million liters of postmenopausal urine per year. Moreover, the collection of such huge amounts of urine would make it almost impossible to maintain the quality and safety parameters of the past [15]. Therefore, the manufacturing of gonadotrophin compounds using recombinant technologies became an important challenge. Genetic engineering was used to produce biosynthetic preparations. The genes for the alfa and beta subunits of FSH were incorporated into the vectors which then were introduced into cells from a Chinese hamster ovary cell line. The result was an unlimited supply of highly stable therapeutic preparation with a high specific activity [16].

Over the past four decades, FSH products have evolved from menotropin, also known as human menopausal gonadotropin (which consists of FSH, luteinizing hormone (LH) and urinary proteins), to urofollitropin (a purified FSH preparation of menotropin from which LH, but not the urinary proteins, has been removed), to urofollitropin highly purified (HP) (i.e. urofollitropin from which most of the urinary proteins have been removed), to the follitropin produced *in vitro* by recombinant DNA technology (rFHS). The purity and batch-to-batch consistency of rFSH make it an attractive alternative to urinary FSH (uFSH). (17).

Moreover, orally active non-peptide mimetics of luteinizing hormone and FSH are currently being developed. However, no data on the administration to humans have been published to date, and only scarce data on *in vitro* and animal experiments are available.

GnRH Analogues

For over 20 years, GnRH agonists have been used to prevent the midcycle luteinizing hormone (LH) surge that results from multiple follicular development. In the long protocol, GnRH agonist treatment is initiated in the midluteal phase or on Day 1 of the cycle. However, day 1 is not preferable in long agonist cycles, due to the increased risk of cyst formation.

GnRH agonist administration causes an initial flare of gonadotrophins, followed by down-regulation of GnRH receptors and a consequent reduction in the release of gonadotrophins, which in turn leads to inhibition of androgen and estrogen production. Pituitary desensitization is usually achieved after ~2 weeks of treatment, after which ovarian stimulation with exogenous gonadotrophins can begin [18].

In the short GnRH agonist treatment, GnRH agonist is initiated on day one of he cycle, followed by gonadotropin stimulation the following day. However, on the basis of clinical pregnancy rate per cycle started, the long GnRH agonist protocol is superior over the short GnRH agonist protocols (19).

GnRH antagonists, introduced in assisted reproductive technologies at the beginning of this decade, are able to inhibit premature LH surges during ovarian stimulation [20].

In contradiction with GnRH agonists, GnRH antagonists cause immediate and rapid suppression of gonadotrophin production. They are therefore administered only when there is a risk of a premature rise in LH usually between Days 5 and 7 of stimulation. This avoids the initial gonadotrophin flare and subsequent pituitary down-regulation associated with GnRH agonists [21-23].

Comparing the cycle parameters of GnRH agonist versus GnRH antagonists, it has been demonstrated that the treatment cycle is significantly shorter with GnRH antagonist than with GnRH agonist co-treatment [24] due to different ways of suppression. Moreover, GnRH antagonist treatment does not produce an initial flare of gonadotrophins, which may cause ovarian cysts [25].

Furthermore, GnRH antagonist treatment is not associated with the profound hypo-estrogenaemia seen with GnRH agonist co-treatment. Profoundly suppressed estrogens may be associated with side effects that include weight gain, headache, hot flushes, night sweats, mood swings, breast tenderness, abdominal pain, diarrhea and nausea [26]. Last but not least, one of the most serious complications of ovarian stimulation is severe ovarian stimulation syndrome (OHSS), a potentially life-threatening condition characterized by ovarian enlargement, pleural effusion, ascites, oliguria, haemoconcentration and thromboembolism. Death due to OHSS is rare, with a mortality rate estimated at 1:400 000-1:500 000 stimulated cycles [27].

However, OHSS remains a source of significant and distressing morbidity. Overall, OHSS results in hospitalization in ~2.1% of patients treated with GnRH antagonists [28]. However, in patients treated with GnRH agonists, the hospital admission for OHSS will be increased by 54% compared with antagonists (odds ratio 0.46, 95% CI 0.26-0.82; $P = 0.01$) [29].

In a long GnRH-agonist protocol there is flexibility in the starting day of gonadotrophin stimulation, which is lacking in the GnRH-antagonist protocol. This flexibility is beneficial for both the patients' convenience in controlling their time of stimulation and for the IVF units for controlling their workload. To facilitate the planning of the initiation of exogenous gonadotropins in a GnRH antagonist cycle, independent of the menstrual period, oral contraceptive (OCP) pretreatment has been suggested. However, in a recent meta analysis, the ongoing pregnancy rate per randomized woman was found to be significantly lower in patients with OCP pretreatment (30).

However, the planning of GnRH antagonist cycle is also feasible without OCP pretreatment, as suggested by Fanchin *et al.* (31). Luteall estradiol administration reduces the pace of growth, improves size homogeneity of antral follicles and facilitates the planning in GnRH antagonist cycles.

Outcome

Recently, two meta-analyses demonstrated a better outcome in terms of the live birth rate when hp-hMG was used for ovarian stimulation compared with rFSH in the GnRH agonist long protocol [32, 33]. However, a similar outcome was observed for hp-hMG and rFSH when used for stimulation in GnRH antagonist cycles [34]. An interesting factor

observed in cycles stimulated with rFSH, independently with which GnRH analogue administered, is the fact that towards the end of the follicular phase, in some cycles a significant increase of progesterone can be observed. This observation is related to the size of the follicles and estradiol values towards the end of the follicular phase [35, 36, 37]. It has been demonstrated that in rFSH/antagonist cycles, the hCG for the final oocyte maturation should be administered as soon as 3 follicles with a diameter of 17 mm are present. By postponing the hCG administration, the pregnancy rate will decrease significantly due to the premature progesterone rise and decrease of endometrial receptivity [35]. Recently, it was demonstrated that premature progesterone rise on the day of hCG administration in GnRH antagonist/rFSH stimulated cycles does have an negative impact on endometrial gene expression, jeopardizing the potential of implantation [38].

The largest trials ever comparing rFSH versus hMG in the GnRH agonist long protocol, is the Merit trial [39]. Interestingly enough, in that trial, the cycles with rFSH were triggered at a moment where significantly more follicles of ≥ 17mm were present as compared with hMG cycles [39]. It means that hMG cycles were triggered at a significantly smaller size as compared to rFSH cycles. As a corollary of this, higher serum progesterone values were present in the rFSH group as compared to the hMG group. Moreover, Bosch et al, observed higher serum progesterone levels in the rFSH group as compared to hMG in GnRH antagonist cycles [34].

From these findings, one could conclude that timing of triggering in rFSH cycles is crucial and that at latest, as soon as 3 follicles of 17 mm are present, the HCG for final oocyte maturation should be administered. Moreover, there is need for further research to determine whether earlier triggering would improve the outcome by preventing the premature LH rise during the luteal phase.

New Developments for Ovarian Stimulation

Corifollitropin Alfa

Due to the relatively short half-life of rec-FSH preparations (32+/-12 hours) [40] and rapid metabolic clearance, daily FSH injections are required to maintain the FSH concentration above the FSH threshold. The daily subcutaneous administration of the FSH preparations causes considerable discomfort to the patient [41].

The availability of longer-acting FSH analogue would thus allow the development of new, more convenient treatment regimens with the increase in injection-free interval.

By alteration of key proteins and carbohydrate regions in the ß-subunits of FSH and LH, new forms of gonadotrophin agonists and antagonists have been created by recombinant DNA technology [42]. FSH has a relatively short half-life, and hCG has a relatively long half-life. The long half-life of hCG is in part due to the presence of four serine O-linked oligosaccharides attached to an extended hydrophilic carboxyterminus.

Site-directed mutagenesis and gene transfer techniques fused the carboxyterminal extension of hCGß (CTP) to the 3' end of the FSH coding sequence creating the recombinant FSH-CTP fusion protein with a prolonged circulating half-life but keeping the biological in-vivo activity of FSH. The new recombinant FSH CTP was designated corifollitropin alfa.

Recent trials demonstrated that one single injection of corifollitropin alfa is able to initiate and sustain multiple follicular growth for 7 days in women undergoing ovarian stimulation for IVF/ICSI using GnRH antagonists or long GnRH agonist [43, 44, 45]. This will simplify ovarian stimulation requiring daily injections.

Low Dose hCG in the Late Follicular Phase

For many years, FSH was considered the only stimulatory factor needed for ovulation induction, acting through specific receptors present on granulosa cells of ovarian follicles. The role of LH, the other critical hormone involved in the control of the human menstrual cycle, is critical for dominant follicle selection and maturation at a time of declining FSH levels, somewhat later in the follicular phase. Granulosa cells of larger follicles (i.e. follicles of >10 mm in diameter) become responsive to LH through the expression of LH/hCG receptors induced by FSH and estrogens, thus making them sensitive to LH activity stimulation. A corollary of these findings is the capacity of LH activity to stimulate follicle function and growth independently of FSH administration, once LH receptors are expressed by granulosa cells. In a GnRH agonist protocol, it was demonstrated that the administration of low dosages of hCG can be successfully applied in patients undergoing ART to substitute for recombinant FSH in the final days of ovarian stimulation [46]. Recently, a comparable protocol was successfully used in a GnRH antagonist protocol [47]. Low-dose HCG supplementation may improve pregnancy rates in antagonist protocols. Overall, these low-dose HCG-supplemented protocols are a cost-effective strategy [48].

How to Avoid Complications after Ovarian Stimulation for IVF/ICSI

The two major complications observed in modern IVF are the occurrence of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies.

In the natural cycle, ovulation is induced by the mid-cycle surge of LH (and FSH) from the pituitary, elicited by an increasing late follicular level of estradiol. As a substitute for the endogenous LH to induce final oocyte maturation, exogenous hCG (5.000-10.000 IU) was successfully introduced more than 50 years ago. Sharing the same α subunit and 81% of the aminoacid residues of the β subunit, LH and hCG bind to the same receptor, the LH/hCG receptor [49]. The ovulatory dose of hCG supports the corpora lutea for 7-10 days, after which hCG is cleared from the circulation [50, 51] and the corpora lutea will from now on be totally dependent on the endogenous LH secretion by the pituitary and the possible hCG production from an implanting embryo. Importantly, the significantly longer half-life of hCG as compared to LH, leads to a prolonged luteotrophic effect, development of multiple corpora lutea, and raised serum levels of estradiol and progesterone throughout the luteal phase [52], which increases the risk of OHSS [53].

GnRHa had previously been shown to effectively stimulate ovulation and final oocyte maturation [52, 54] and this concept gained some interest in the late eighties and early nineties. GnRHa displaces the GnRH antagonist from the GnRH receptor in the pituitary, which will induce an initial secretion of LH and FSH (flare-up), similar to that of the natural cycle, prior to down-regulation of the receptor. However, with the introduction of GnRHa for pituitary down-regulation prior to IVF/ICSI treatment in order to avoid a premature endogenous LH surge [55, 56], this concept was clearly not applicable, as the simultaneous use of GnRHa for down-regulation and triggering of final oocyte maturation is not possible.

When GnRH antagonist protocols were introduced for the prevention of a premature LH surge [21, 57, 58], it became possible again to trigger ovulation with a bolus of a GnRHa as an alternative to hCG. Importantly, there are significant differences between the GnRHa induced surge of gonadotrophins and that of the natural cycle. The LH surge of the natural cycle is characterized by three phases with a total duration of 48 hours [59] as compared to the GnRHa induced LH surge which consists of two phases with a duration of ~ 24-36 hours [52]. This leads to a significantly reduced total amount of gonadotrophins (LH and FSH) released from the pituitary when GnRHa is used to trigger final oocyte maturation [52, 54], which *per se* could induce a defective luteal phase [60, 61]. An advantage of GnRHa triggering could be the simultaneous induction of a surge of FSH like in the natural cycle. Although the role of the mid cycle FSH surge is not clear, it has been shown to promote LH receptor formation in the luteinizing granulosa cells securing the function of the CL during the luteal phase in addition to nuclear maturation and cumulus expansion [62-65].

Furthermore, the OHSS rate is significantly reduced or even eliminated [66-68] when GnRHa is used to trigger ovulation as compared to hCG.

However, a poor clinical outcome is present, when GnRHa is used to trigger final oocyte maturation in IVF/ICSI antagonist protocols, due to an uncorrectable luteal phase deficiency, most probably due to a lack of LH/hCG. The negative impact of agonist trigger seems to be on the endometrium and not the oocyte/embryo quality [69].

Therefore, it has been suggested to cryopreserve the embryos and transfer in consecutive natural cycles. Limited evidence is available regarding the optimal preparation of the endometrium for implantation in FET cycles. Recently, it was demonstrated that for patients with a regular cycle, FET in a spontaneous natural cycle has high implantation and ongoing pregnancy rates [70].

CONCLUSIONS

It can be concluded that rFSH and HMG seem to have comparable results in GnRH antagonist cycles. However, the time to trigger and progesterone levels during the follicular phase are crucial and there is a need for further RCT to determine the best time to trigger. Future trials should aim to eliminate OHSS and multiple pregnancy rates by performing a single stimulation in a simplified corifolitropin alfa/GnRH antagonist cycle triggered by a GnRH agonist followed by Cryo-thawed SET in consecutive natural cycles. With this approach, the two major complications of COH for IVF could be eliminated without jeopardizing the outcome.

ABBREVIATIONS

ART	=	Assisted reproductive technology
FSH	=	Follicle stimulation hormone
HMG	=	Human menopausal gonadotrophi
LH	=	Luteinizing hormone
GnRH	=	Gonadptropin releasing hormone
hCG	=	Human chorionic gonadotropin
SET	=	Single embryo transfer

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