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Contemporary Pharmacological Manipulation in Assisted Reproduction

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Abstract

Follicle-stimulating hormone (FSH) treatment to induce follicular development in anovulating women and multiple follicular development for assisted conception has been incorporated in almost all reproductive treatment cycles in the form of either urinary, purified urinary or recombinant preparations. Besides improved tolerance and theoretically lower chances of infection by prions, the

latter may be more effective in terms of clinical pregnancy rates, FSH requirement and cost effectiveness. The low-dose, step-up protocol to induce monofollicular development, which is applied worldwide, has to compete with the equally effective but health economically beneficial step-down protocol. The long protocol using recombinant FSH 150 IU/day is advocated when using gonadotropin-releasing hormone (GnRH) agonists in *in vitro* fertilisation (IVF) or intracytoplasmatic sperm injection treatment. However, the current paradigmatic hyperstimulation came under scrutiny after the introduction of the GnRH antagonists, which allow milder and more convenient approaches with acceptable cancellation and pregnancy rates but lower requirements for FSH. Risk of ovarian hyperstimulation syndrome (OHSS) can be further eliminated if recombinant luteinising hormone (rLH) or GnRH agonists are used to trigger oocyte maturation and ovulation; the latter require pituitary responsiveness and are therefore excluded in agonist protocols.

FSH and LH are both required for appropriate folliculo- and steroidogenesis. In hypogonadotropic women, the addition of LH (human menopausal gonadotropin, human chorionic gonadotropin or rLH) is therefore obligate to achieve appropriate follicular growth and pregnancy. The role of LH in ovulation induction is still a matter of debate, although in GnRH agonistic protocols there seems to be a 'therapeutic window'; levels that are too high or too low have detrimental effects on IVF outcome.

To broaden the pharmaceutical armoury, recent efforts have been directed towards the development of novel GnRH antagonists and FSH preparations with optimal pharmacokinetic, pharmacodynamic and safety profiles. Alternative strategies with fewer adverse effects and higher benefit/cost ratios are under development. However, before the GnRH agonist is abandoned for the antagonist as standard therapy, the cause of the observed possible lower pregnancy rates with the latter need to be clarified. In addition, prospective studies investigating possible direct effects of GnRH analogues, optimal dose-finding studies and treatment regimens under different conditions, with or without pharmacological coadministration and for different indications, should be performed to optimise the efficacy and tailor treatment strategies to individual needs.

Since the discovery and isolation of gonadotropins (i.e. follicle-stimulating hormone [FSH], luteinising hormone [LH] and human chorionic gonadotropin [hCG]) and gonadotropin-releasing hormone (GnRH), an extensive variety of pharmacotherapeutic preparations have been developed in order to be used in ovulation induction and assisted reproductive therapy (ART). This paper focuses on the use of gonadotropins and GnRH analogues, and the new developments in this field. We provide insight into the different types of drugs used, their mechanism of action and how they manipulate the hypothalamic-pituitary-ovarian axis. We discuss the current situation in the use of pharmacological manipulation in assisted reproduction based on the development of various therapeutic strategies and comparative studies.

1. Gonadotropins

In the 65 years since the gonadotropin hormones were discovered,[1] FSH has attained a central role in contemporary infertility therapies. Clinical applications include the treatment of anovulatory infertility and controlled ovarian stimulation in women being treated with *in vitro* fertilisation (IVF). In addition,

FSH is used to treat male infertility caused by gonadotropin deficiency (hypogonadotropic hypogonadism). The extraction and purification of gonadotropins from postmenopausal urine was developed in Italy in the late 1940s. Although the first human menopausal gonadotropin (hMG) was developed in 1949, clinical interest in these compounds did not grow until the 1960s, when the first hMG-assisted live human birth was reported.[2] The first clinically available gonadotropin preparations contained a mixture of FSH and LH in a ratio of activity of 1 : 1, and a large number of urinary proteins. Early production techniques were crude and labour intensive, requiring up to 30L of urine to produce enough hMG for one treatment cycle. Improvements in purification techniques led to increasing relative amounts of the active ingredients and the first urine-derived preparation containing only urinary 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 (Metrodin®[1]). Although improvements in purity and specific activity were thus achieved, the large quantities of urine required and a massive increase in worldwide demand for FSH compounds for infertility treatment put increasing pressure on production and availability. In the 1990s, recombinant DNA technology led to the development and clinical introduction of human recombinant FSH (rFSH) by stable transfection of the common α- and the β-FSH subunit into Chinese hamster ovary cells. This development promised not only unlimited availability, but also improved purity and batch-to-batch consistency in comparison with urine-derived products. Since 1996, rFSH has been clinically available, in the form of follitropin-α (Gonal-F®) and follitropin-β (Puregon®).

More recently, recombinant hCG (rhCG) and recombinant LH (rLH) have been added to the clinical armoury for ovarian stimulation and assisted conception. Both LH and hCG are heterodimer glycoproteins, composed of two non-covalently linked subunits (α and β). For the production of rLH and rhCG, the human genes are also transfected into Chinese hamster ovary cells, encoding for the subsequent α and β subunits, producing lutropin-α (Luveris®)[3,4] and choriogonadotropin-α (Ovidrel®), by repeated chromatographic steps.

Recombinant technology has also opened the way towards the development of novel molecules with shorter- and longer-acting properties.

In this article, the role of native FSH, LH and hCG are reviewed, and the clinical applications of current gonadotropins preparations are discussed (see table I). Finally, some novel preparations soon to be clinically available are described.

1.1 What Does Native Follicle-Stimulating Hormone (FSH) Do?

FSH is a complex heterodimeric glycoprotein consisting of two subunits produced in the anterior lobe of the pituitary gland. While the role of FSH in early follicle development is unclear, late follicular development is FSH dependent. The great majority of human oocytes are destined to undergo atresia.[5,6] Only those follicles able to respond to stimulation by FSH and which gain increased FSH sensitivity will enter the final stage of development and ovulate.[7,8] As a result of the demise of the corpus luteum and a subsequent decrease in progesterone and estradiol production, FSH levels increase at the end of the luteal phase of the menstrual cycle.[9,10] The high FSH levels, which occur during the luteofollicular transition, give rise to continued growth of a limited number (or cohort) of follicles.[11] Subsequent development of this cohort during the follicular phase becomes dependent on continued stimulation by gonadotropins.[12] Each growing follicle possesses a threshold requirement for stimulation by circulating FSH.[13,14] This threshold level needs to be passed to ensure ongoing preovulatory development. In the normo-ovulatory cycle only one follicle will become responsive to FSH above this threshold and become capable of converting the theca cell-derived substrate androstenedione to estradiol by

1 The use of tradenames is for product identification purposes only and does not imply endorsement.

Table I. Gonadotropins commonly used in assisted reproductive therapy

Compound generic name	Active substrate	FSH : LH ratio	Route	Tradename Europe/USA
Follicle-stimulating hormone (FSH)				
Menotropin	uFSH and uLH	1 : 1	SC	HMG-Ferring, Humegon, Menogon, Pergonal, Merional/Reponex
		3 : 1		Normegon
Purified menotropin	uFSH and uLH	2 : 1	SC	Menopur
Urofollitropin	uFSH and uLH	1 : <0.01	SC	Metrodin, Follegon
Purified urofollitropin	uFSH and uLH	1 : <0.001	SC, IM	Metrodin HP/Fertinex, Bravelle
Follitropin-α	rFSH	100% FSH	SC	Gonal-F
Follitropin-β	rFSH	100% FSH	SC	Puregon/Follistim
Luteinising hormone (LH)				
Lutropin-α	rLH	100% LH	SC	Lhadi, Luveris
Human chorionic gonadotropin (hCG)				
Choriogonadotropin	uhCG		IM	Pregnyl, Profasi, Novarel
Choriogonadotropin-α	rhCG	100% hCG	SC	Ovidrel/Ovitrelle

IM = intramuscular; **rFSH** = human recombinant FSH; **rhCG** = recombinant hCG; **SC** = subcutaneous; **uFSH** = urinary FSH; **uhCG** = urinary hCG; **uLH** = urinary luteinising hormone.

induction of the aromatase enzyme. In response to negative feedback from rising estradiol and inhibin levels, the FSH level falls in the late follicular phase. The dominant follicle has increased sensitivity to the falling FSH level and continues growing. Those follicles that commence the latter stages of development after FSH levels start to fall undergo atresia (figure 1). The duration of this 'FSH window', during which FSH levels are above the threshold required to stimulate ongoing development, determines the number of follicles which can develop to the preovulatory stage.[15,16] These advanced stages of follicular development are open to therapeutic intervention with exogenous FSH.

1.2 FSH in Clinical Practice

1.2.1 FSH in Anovulatory Infertility

The aim of ovulation induction in anovulatory women is the formation and ovulation of a single dominant follicle. In order to achieve this, specific treatment and monitoring protocols are needed. While several approaches to ovulation induction with gonadotropins have been described, the two most frequently used in clinical practice are the low-dose step-up and the step-down protocols. The initially described 'standard' step-up protocol had a starting dose of FSH 150 IU/day. However, this regimen was associated with a high complication rate. Multiple pregnancy rates of up to 36% were reported, while ovarian hyperstimulation occurred in up to 14% of treatment cycles.[16] As a result, this protocol has been largely abandoned.

A low-dose, step-up protocol[18] 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. In this protocol, the initial subcutaneous or intramuscular dose of FSH is 50–75 IU/day and the dose is increased if, after 14 days, no response is observed on ultrasonography (and serum estradiol monitoring). Increments of 37.5IU are then given at weekly intervals up to a maximum of 225 IU/day. The detection of an ovarian response is an indication to continue the current dose until hCG can be given to trigger ovulation. In a series describing outcomes using the low-dose, step-up regimen in 225 women with polycystic ovary syndrome (PCOS) treated over a 10-year period, ovulation rates of 72% per cycle were reported; 45% of these women conceived as a result of ovulation induction.[19]

The low-dose, step-up protocol is associated with a lower incidence of multiple folliculogenesis and hyperstimulation than the standard protocol[20] and pregnancy rates appear similar.[21] However, the

results of the low-dose, step-up protocol are negatively influenced by obesity,[19] age[22] and insulin resistance.[23] More recent studies focusing on further reducing the starting dose have reported the feasibility of commencing with 50IU[24] and 37.5IU.[25] However, while ovulation can be achieved with this approach, the stimulation period may be further extended.[25]

Step-down protocols are aimed at rapidly achieving the FSH threshold for stimulating follicle development. Current regimens normally begin therapy with 150 IU/day started shortly after a spontaneous or progesterone-induced bleeding. This dose is continued until a dominant follicle (≥10mm) is seen on transvaginal ultrasonography. The dose is then decreased to 112.5 IU/day followed by a further decrease to 75IU 3 days later, which is continued until hCG is administered to induce ovulation.[26] Should no ovarian response be observed after 3–5 days, the FSH dose can be further increased. For some patients an initial dose of 150 IU/day may be too high, reflecting major individual differences in the FSH threshold. The appropriate starting dose may be determined by using the low-dose step-up regimen for the first treatment cycle in order to assess the individual FSH response dose[27] or by applying a formula for the calculation of the individual response dose.[27,28]

Experience with the step-down protocol in a series of 82 women suggested that the duration of treatment and total gonadotropin dosage were reduced compared with the low-dose, step-up protocol. Moreover, monofollicular growth was more frequently achieved.[29] These findings were subsequently confirmed by a prospective, randomised comparison of low-dose, step-up and step-down regimens.[30] The clinical benefits of a more physiological means of stimulating follicle development were reflected in an incidence of monofollicular cycles of 88% compared with 56% observed in women treated with the step-up regimen, presumably reducing the risk of multiple pregnancy and hyperstimulation. Potential health-economic benefits were also apparent since those treated with the step-down regimen required a mean duration of treatment of just 9 days, as opposed to 18 days in women treated with the low-dose, step-up regimen. A recent multicentre, randomised study comparing the step-up versus step-down protocol using rFSH reported a shorter duration of stimulation when the step-down protocol was used.[31] The cumulative rate of clinical gestations did not differ between the two groups but, in contrast with the findings of an earlier single-centre study,[30] the step-up protocol was associated with a higher rate of monofollicular development and a lower rate of ovarian hyperstimulation. These differences may reflect the necessity for increased skill and care in monitoring step-down stimulation cycles, which is easier to ensure in a single-centre setting.

The balance between success and complications resulting from ovulation induction is dependent on many factors, including patient characteristics, gonadotropin preparations and dose regimens used, the intensity of monitoring ovarian response to stimulation and willingness to cancel the cycle in case of hyper-response. Cumulative success rates of ovulation induction are reported to be around 75%,[32] with a coinciding incidence of multiple pregnancies of <10% and of ovarian hyperstimulation syndrome (OHSS) of <2%.

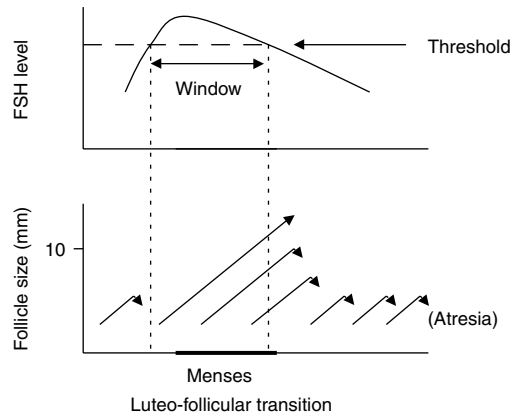

Fig. 1. In the spontaneous normo-ovulatory cycle, the intercycle rise in serum follicle-stimulating hormone (FSH) levels exceeds the threshold for recruitment of a cohort of follicles for further development. The number of follicles recruited is determined by the duration ('window') for which the serum FSH is above the threshold at which recruitment occurs (adapted from Macklon and Fauser,[17] with permission from Cambridge University Press).

The degree to which the type of FSH compound employed may influence outcomes in ovulation induction remains the subject of controversy. A meta-analysis comparing the effectiveness of daily uFSH with daily hMG in a step-up regimen for inducing ovulation in women with polycystic ovary syndrome (PCOS) who had not responded to clomifene (clomifene citrate) demonstrated no difference in pregnancy rates per treatment cycle.[33] However, women given FSH were less likely to have moderately severe or severe OHSS. Subsequent studies remained consistent with these conclusions but have suggested a less marked difference in the risk of OHSS.[34] With respect to rFSH, a multicentre, prospective trial found that the cumulative ovulation rates were comparable with those achieved with purified uFSH (95% after three cycles).[35] The total dose of rFSH needed and duration of treatment was less, and the complication rates were similar. In a meta-analysis of randomised, controlled trials comparing rFSH with uFSH for ovulation induction in women with clomifene-resistant PCOS, no significant differences were demonstrated for the ovulation rate (odds ratio [OR]: 1.19, 95% CI 0.78–1.80). Moreover, the ORs for pregnancy rate (0.95, 95% CI 0.64–1.41), miscarriage rate (1.26, 95% CI 0.59–2.70), multiple pregnancy rate (0.44, 95% CI 0.16–1.21) and OHSS (1.55, 95% CI 0.50–4.84) showed no significant difference between rFSH and uFSH.[36]

Purified uFSH has some LH activity but rFSH does not. Experience with rFSH in hypogonadotropic hypogonadal women (WHO class 1) indicates that those women who have very low serum LH levels (<0.5 IU/L) need exogenous LH activity (derived from LH or hCG) to maintain adequate estradiol biosynthesis and follicle development.[37]

1.2.2 FSH in Assisted Conception

In contrast with ovulation induction regimens, where the aim is to induce monofollicular development in an anovulatory woman, FSH is applied in assisted conception to obtain large numbers of oocytes for IVF and subsequent selection of embryos for intrauterine transfer. Higher doses of gonadotropins are therefore administered. The most commonly applied regimen is the 'long protocol' where gonadotropins are administered following down-regulation with a GnRH agonist for the prevention of premature luteinisation (see section 2.2.1). The question of what is the optimal dose of FSH required to stimulate the development of adequate numbers of follicles without causing the potentially dangerous OHSS has been the subject of a number of recent studies.

The most frequently applied starting doses in normo-ovulatory women undergoing ovarian hyperstimulation for IVF is 150 or 225 IU/day. In one study comparing these two starting doses of rFSH in combination with the GnRH antagonist cetrorelix in women undergoing ovarian hyperstimulation for IVF or intracytoplasmatic sperm injection (ICSI), the number of oocytes retrieved was significantly higher in the 225IU group (11.0 ± 4.6 vs 9.1 ± 4.4, $p = 0.024$), but the ongoing pregnancy rates per started cycle and per embryo transfer did not differ significantly between the groups.[38] Similar results were reported in another study in which 138 patients received rFSH at a dose of 150 or 250 IU/day. The mean number of oocytes retrieved was 9.1 in the 150IU group compared with 10.6 in the 250IU group.[39] In women between 30 and 39 years of age a decline in oocytes obtained was observed, but this was not overcome by an augmented daily dose of rFSH from 150 to 250IU. Moreover, ongoing pregnancy rates did not differ significantly between the two study groups. Starting doses <150 IU/day have also been examined. In a study of 179 women randomised to receive rFSH at a dose of either 100 or 200 IU/day, fewer oocytes were obtained after the lower dose (5.7 vs 12.0, $p < 0.001$).[40] However, while no differences in vital pregnancy rates per started cycle (16.9% vs 19.2%) or per embryo transfer (19.3% vs 26.2%) were observed, the number of cancelled cycles was higher in the 100IU group. Thus, the collective evidence to date would suggest that 150 IU/day is an appropriate starting dose for most women undergoing ovarian hyperstimulation for IVF with rFSH as part of a long GnRH agonist or GnRH antagonist protocol.

With the clinical introduction of GnRH antagonists, the opportunity has arisen to look again at current paradigms for ovarian hyperstimulation. Contemporary protocols aim at achieving maximal numbers of oocytes. However, the disadvantages of this approach, which include the risks of ovarian hyperstimulation, expense, inconvenience and adverse effects for the patient, are becoming increasingly recognised and alternative less aggressive approaches are now being investigated.[41,42] The availability of GnRH antagonists has opened the way to the development of novel regimens, in which the endogenous production of FSH can simply be supplemented by lower doses of FSH, thus extending the FSH 'window' and enabling a number of oocytes to achieve dominance (figure 2).[17,43,44] Following the publication of a pilot study indicating the feasibility of this approach in clinical IVF,[45] a prospective, randomised study comparing this approach with the long protocol indicated that while fewer oocytes may be obtained, the pregnancy rate per started cycle is not reduced.[46] Ongoing studies are assessing the cost/benefit aspects of milder approaches to ovarian stimulation for FSH.

Since the first pregnancies resulting from the use of rFSH were reported in the early 1990s, several

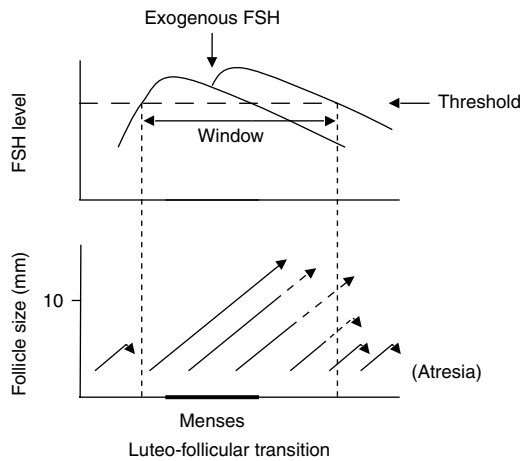

Fig. 2. When the duration of the follicle-stimulating hormone (FSH) 'window' is extended by the administration of exogenous FSH in the mid-late follicular phase, more follicles may achieve dominance, leading to the multiple dominant follicle selection that is appropriate for *in vitro* fertilisation treatment (adapted from Macklon and Fauser,[17] with permission from Cambridge University Press).

controlled trials comparing rFSH with uFSH have been published. One study of 123 women found that the two preparations were equally effective.[47] However, in three larger studies of 981,[48] 235[49] and 496 women,[50] the dose of a different rFSH preparation and the duration of rFSH treatment needed to stimulate follicle development were less and more oocytes were recovered, implying greater efficacy for rFSH. More recently, a total of 18 studies comparing rFSH with uFSH preparations were subject to a meta-analysis in which the clinical pregnancy per cycle started was the primary focus. The overall OR was 1.21 (95% CI 1.04–1.42) in favour of rFSH. This equates with a 3.7% (95% CI 0.8–6.7) absolute increase in clinical pregnancy rate for patients stimulated with rFSH compared with those stimulated with uFSH. Moreover, the total FSH dose was lower by 406IU (95% CI 185–627) with rFSH.[51,52] In contrast, a recent meta-analysis of five studies comparing the effectiveness of hMG and rFSH in IVF following down-regulation indicated higher clinical pregnancy rates with hMG (relative risk [RR] 1.22, 95% CI 1.03–1.44].[53] No differences in ongoing clinical pregnancy rates were observed.

Inconsistency in the results of randomised, controlled trials and meta-analyses in this commercially sensitive area may be due to chance and are to be expected given differences in methodology, participants and clinical settings.[54] The debate as to whether rFSH is more efficacious then uFSH in IVF treatment is unlikely to be resolved in the near future. In practice, the choice of preparation is based increasingly on other factors, such as relative cost, purity, batch variance, side effects and possible long-term risks.[55]

OHSS is the most commonly reported serious adverse event. No statistically significant differences in the incidence of OHSS have been found between uFSH and rFSH preparations. Pooled data from 14 comparative trials (including four that reported no cases of OHSS) in the meta-analysis by Daya and Gunby[51] indicated that the incidence of OHSS was 2.0% in the rFSH group and 1.4% in the uFSH group (RR 1.50, 95% CI 0.88–2.58).

In terms of cost effectiveness, recent studies have indicated benefits with rFSH over uFSH. The increased yield of mature oocytes, requiring a lower total FSH dose and a shorter period of treatment, combined with a higher clinical pregnancy rate per started cycle would certainly indicate that rFSH is likely to be a more cost-effective option than uFSH. However, rFSH remains considerably more expensive than uFSH. In a recent study addressing this issue, Markov decision analysis was used to model the outcomes of a large number of ART cycles using rFSH and uFSH.[56] Analysing 5000 stimulated cycles on a Markov cohort of 100 000 patients, the number of pregnancies achieved was predicted to be significantly higher with rFSH than with uFSH (40 575 vs 37 358, $p < 0.0001$). The cost per successful pregnancy calculated within the context of the UK National Health Service was €5906 ± 455 in the rFSH group and €6060 ± 547 in the uFSH group ($p < 0.0001$). Fewer cycles of treatment were required to achieve an ongoing pregnancy with rFSH (3.83 vs 4.29). Applying this approach to the context of the US health system, the same group showed that rFSH was more cost effective than uFSH. A recent analysis of the mean cost of achieving an ongoing pregnancy was carried out in the context of a randomised trial of highly purified hMG versus rFSH.[57] While the two preparations appeared to be equally effective, hMG was associated with a significantly lower cost per pregnancy of £10 781 (95% CI 9056–12 919) compared with £11 883 (95%CI 11 883–17 891) with rFSH.

The two clinically available rFSH preparations, follitropin-α and follitropin-β have been compared in a recent prospective study in 492 women.[58] No statistically significant differences were found for numbers of follicles on the day of hCG administration, numbers of oocytes retrieved and clinical pregnancy rate per started cycle.

1.3 What Does Native Luteinising Hormone (LH) Do?

LH is essential for timing of ovulation, the regulation of target tissue responses and the production of steroid hormones. In a natural cycle, a sudden and profound midcycle peak of LH and FSH levels (LH and FSH surge, lasting for about 48 hours) is observed, which is regulated by a complex mechanism that integrates multiple neurotransmitters and sex steroids.[59] Estrogens play important functional roles in the reproductive system by, among other functions, stimulating cervical mucous production and endometrial proliferation. Persistently elevated oestrogen levels in combination with a small, but distinct, rise in progesterone, which results in increased pituitary sensitivity for GnRH and a substantial rise of GnRH,[60] induce the LH surge. Gonadotropin surge attenuating factor has also been implicated in the regulation of the LH surge timing and amplitude.[61] The LH surge is obligate for the resumption of meiotic maturation of the oocyte, rupture of the dominant follicle resulting in the release of the oocyte, and luteinisation of thecal and granulose cells resulting in the formation of the corpus luteum, providing support for the early stages of pregnancy.

During follicular development, LH has a synergistic action with FSH. Theca cells are stimulated by LH to convert cholesterol into androstenedione and testosterone by cytochrome P450 side chain cleavage oxidases and 3β-hydroxy-steroid dehydrogenase. Aromatase activity in the granulosa cells is induced by FSH, and converts androstenedione and testosterone into estrone and estradiol. The involvement of two cell types (granulosa and theca cells) and two hormones (LH and FSH) to produce estrogens from cholesterol has led to the concept of the 'two cell, two gonadotropin' theory.[62] *In vivo* evidence for this concept has grown in recent years. In 1992, Schoot et al.[63] described the effects of rFSH administration on a woman with isolated gonadotropin deficiency as a result of a previous hypophysectomy. For the first time, the effects of FSH alone could be studied in the absence of endogenous and exogenous LH. While multiple follicular growth was induced, estradiol levels remained low and no pregnancy ensued. Subsequent case reports confirmed these findings.[64,65]

Recent evidence points to a central role for LH in monofollicular selection and dominance in the normal ovulatory cycle. As described in section 1.1,

despite falling FSH levels in the late follicular phase, the dominant follicle continues to develop. One of the actions of FSH is the induction of LH receptors on granulosa cells. While granulosa cells from early antral follicles respond only to FSH, those from mature follicles, possessing receptors to both gonadotropins, are responsive to both FSH and LH. The maturing dominant follicle may become less dependent on FSH because of the ability to respond to LH levels, which remain relatively constant during the follicular phase.[66] Elegant studies employing rLH in down-regulated women have indicated that although follicles of ≥14mm require gonadotropic stimulation for continued estradiol production, they are responsive to either FSH or LH.[67] In contrast, less mature follicles which do not express LH receptors undergo atresia as a consequence of falling FSH levels.

1.4 LH and Human Chorionic Gonadotropin (hCG) in Clinical Practice

Both LH and hCG are heterodimeric glycoproteins with identical α subunits. The major differences between the two hormones include the sequence of the β subunit, the regulation of their secretion, their receptor affinity and their clearance.[68,69] Until recently, hMG, a urinary extract containing a fixed combination of LH and FSH, was the only source of exogenous LH. Therefore, exogenous hCG (which activates the LH receptor as a result of structural and biological similarities to LH, and which is easy to extract from urine of pregnant women) has been used for decades to replace endogenous LH during FSH stimulation protocols but is associated with a number of disadvantages. Currently, human gene products like rLH and rhCG are available for pharmacological application.

The success in 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 and IVF.

1.4.1 LH in Anovulatory Infertility

The treatment of hypogonadotropic women with FSH alone leads to follicular development but not pregnancy.[63,64] 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. rLH now offers the possibility for a more sophisticated and individualised approach to treatment. Recent studies have demonstrated the safety and appropriate dose required to effect follicle development and subsequent pregnancy.[70] It has been established that resting levels of 1–10 IU/L should be sufficient to provide maximal stimulation to thecal cells.[71] In a recent study of hypogonadotropic women undergoing treatment with rFSH and rLH, a dose of 75 IU/day of rLH 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.[72]

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.[73]

More recently, however, the demonstration of the importance of LH in optimising oocyte quality has reopened the debate as to the role of LH in ovulation induction.[74] Supplementation of LH activity may offer advantages in some patients by hastening large follicle development and therefore shortening the duration of treatment.[75] Moreover, the work of Sullivan et al.[67] referred to a potential therapeutic role for LH in affecting monofollicular stimulation, as part of a sequential ovarian stimulation protocol following initiation with rFSH.

As the availability of recombinant gonadotropins leads to increasing knowledge of the processes of follicular development and selection, further improvements in the efficacy and safety of ovulation induction may follow.

1.4.2 LH/hCG in Assisted Conception

Although there is clear evidence for the crucial role played by LH in normal follicular development, the necessity for LH supplementation in ovarian hyperstimulation for IVF remains a contentious issue. Excessive suppression of LH levels following down-regulation with GnRH agonists has been associated with a detrimental effect on outcome of IVF.[76-78] On the other hand, a number of studies comparing uFSH with hMG which measured LH levels suggested that resting levels of LH following down-regulation are sufficient to support development and maturation of follicles and oocytes in normogonadotrophic women.[79,80] In one recent study, no significant differences in either performance or clinical outcome among groups of patients with varying degrees of LH suppression were observed.[81] However, another recent study using GnRH agonists suggested that LH levels that are too low or too high on stimulation day 8 for IVF can have a negative impact on fertilisation and clinical pregnancy rates.[82] Taken together, recent studies suggest that midfollicular LH levels <0.5 IU/L are likely to be detrimental to IVF outcome. Therefore, there may be a role for LH supplementation in some patients undergoing ovarian stimulation for IVF. Moreover, supplemental LH may offer a new strategy for dealing with poor response to ovarian stimulation with rFSH.

Recent studies in IVF patients have confirmed the earlier observed ability of LH activity alone to complete follicle and oocyte maturation. In a study using hCG to provide LH activity, Filicori et al.[83] showed that low-dose hCG can stimulate the growth and support the maturation of larger follicles in the absence of FSH administration. In addition, LH activity without FSH appeared to cause a selective reduction in the number small preovulatory follicles. This work has opened new possibilities for further refining ovarian stimulation regimens for IVF, allowing sufficient stimulation for successful IVF treatment while reducing the risks associated with excessive multifollicular development.[83]

1.4.3 LH/hCG to Trigger Ovulation or Resumption of Meiotic Division

The most used substitute for the midcycle LH surge in ovulation induction and ART is hCG. However, hCG may be an additional promoting factor for the often observed dangerous complication of OHSS in ovarian hyperstimulated cycles, which may be explained by the longer half-life of hCG compared with LH.[68,69] To avoid this serious event, alternative ways for triggering ovulation are under development, using rLH, native GnRH or a GnRH agonist (see section 2.2.3). Three prospective, randomised, controlled trials (with 84, 190 and 297 patients, respectively), between rhCG and uhCG implicated rhCG to be as efficacious as uhCG in terms of IVF outcome, with the benefit of improved local tolerance.[84-86] In a prospective, randomised trial, 259 infertile women were treated with either uhCG or rLH in doses of 5000, 15 000, 30 000 or 15 000IU plus 10 000IU 3 days later.[87] A single dose of rLH was as effective in inducing final follicular maturation and early luteinisation in IVF as uhCG 5000IU, but with a significant reduction in OHSS. The dose with the highest efficacy to safety ratio was between rLH 15 000 and 30 000IU.[87] Moreover, less intense circulatory changes were observed after rLH 5000IU than after uhCG 5000IU.[88] Altogether, rLH seems to be an attractive alternative for hCG with equal efficacy, but reduced risk for OHSS and improved local tolerance.

1.5 Safety Aspects of Gonadotropins

An advantage offered by rFSH preparations over urinary products is improved tolerability. The absence of contaminating urinary, nonhormonal proteins reduces the (rare) complication of local reactions to exogenous gonadotropins at the injection site.[89] Furthermore, rFSH used in clinical trials was reported to be well tolerated by both patients undergoing ART and anovulatory patients treated for ovulation induction; the most common adverse event was associated with treatment is reaction at the injection sites.

In terms of long-term risks, urinary preparations are associated with a theoretical risk of transmission of prion proteins, which have been identified in human urine.[90] Although infections by urine prions in humans and animals has not been reported, and in 40 years of use no such infections have been identified, the risk of prion disease such as new variant Creutzfeldt-Jakob disease has been deemed by some to be sufficient to advise against the use of uFSH, uLH or uhCG.[91] However, others consider the risk to be minimal and not in itself a reason to prescribe recombinant preparations over urinary ones.[92]

Moreover, studies of follitropin-α and follitropin-β suggest that the incidence of OHSS is similar with both products, while a number of small studies have suggested that the incidence of local reactions following injection of follitropin-α may be lower than with follitropin-β.[58]

1.6 New Developments With Gonadotropins

The degree of purity and batch-to-batch consistency in activity achieved in the production of rFSH allows improved consistency in dose from day-to-day and cycle-to-cycle that was difficult to achieve with urine-derived products. However, the currently applied system of determining activity means that in the clinical setting variations in activity may be observed. The bioactivity of uFSH and rFSH compounds is determined by an *in vivo* bioassay which is cumbersome and subject to such variation that an ampoule labelled to contain 75IU may range in true activity from FSH 50 to 120IU. The constant relationship between FSH mass (μg) and biological activity (IU) has allowed the development of a filled by mass rFSH product which promises greater control and consistency in optimising the dose for an individual patient. In a double-blind study, in which 131 patients undergoing ART were randomised in rFSH batches filled by IU (FbIU) or filled by mass (FbM), improved consistency in clinical outcome was observed in the FbM group.[93]

Longer-acting rFSH preparations are also undergoing clinical studies. The half-life of rFSH, around 34 hours, is not markedly different from that of urine-derived products. As a result, daily injections are required in order to cause ovarian stimulation. Manipulation of the rFSH molecule may alter its pharmacokinetic properties. FSH is a member of the gonadotropin/thyrotropin hormone family, which is characterised by a heterodimeric structure consisting of a common α subunit and a hormone-specific β subunit.[94] The β subunit of the pregnancy hormone hCG is distinctly different from the other hormones in this family owing to an extension at the carboxyl end, that is, a C-terminal peptide (CTP) with four O-linked oligosaccharides. Analysis of the β-hCG coding sequence suggests that this extension results from the loss of the termination codon of the ancestral LH.[95] Both hCG and LH bind to the same receptor and exhibit comparable bioactivity *in vitro*. The CTP extension of the β subunit of hCG has been shown to be responsible for the reduced clearance and resulting major enhancement of *in vivo* bioactivity. In an attempt to create a long-acting FSH agonist preparation, chimeric genes containing the sequence encoding the CTP of β-hCG fused with rFSH were constructed.[96] The first human exposure in hypogonadal males showed that rFSH-CTP could be administered safely and showed an extended half-life of 95 hours.[97] Recently, the half-life of rFSH-CTP in women has been shown to be dose dependent. The elimination half-life was around twice that of rFSH.[98] Clinical studies are now ongoing in both IVF and ovulation induction patients, and the first ongoing pregnancy using rFSH-CTP in IVF treatment has recently been reported.[99]

2. Gonadotropin-Releasing Hormone (GnRH) and its Analogues

Thirty years after the discovery of the amino acid sequence of GnRH, also known as gonadorelin or LH-releasing hormone (LHRH), agonistic and antagonistic analogues were introduced in assisted reproduction. After an initial short period of gonadotropin hypersecretion, continuous administration of GnRH or its agonists cause desensitisation, resulting in a state of chemical hypophysectomy by a mechanism still far from understood.[100] GnRH antagonists cause an immediate and rapid, reversible suppression of gonadotropin secretion, by competitive

occupancy of the GnRH receptor. The development of agonists with good clinical safety was relatively simple by just changing one or two amino acids. The potency was increased by the replacement of glycine at position 6 by D amino acids and the replacement Gly-NH_2 at the C-terminus by NH_2-ethylamide binding to the proline at position 9, resulting in nonapeptides.[101] GnRH agonists have the same effect on gonadotropin release after binding the type I receptor as native GnRH. The main difference of GnRH agonists used in clinical practice compared with native GnRH is that the half-life and bioavailability are prolonged as a result of increased lipophilicity.

It took almost 30 years to obtain an antagonist with an acceptable pharmacokinetic, safety and commercial profile. The first-generation antagonists, containing replacements for His and Trp at position 2 and 3, respectively, had low suppressive activities. The potency in the second generation was increased after the incorporation of a D-amino acid at position 6, but resulted in increased anaphylactic reactions due to the increased histamine-releasing activity. These problems were resolved in the third generation by the replacement of D-Arg at position 6 by D-ureidoalkyl amino acids.[102]

The reversible state of chemical hypophysectomy, which can be achieved by both types of analogues, offers an important pharmacological tool which can be used to manipulate hormone secretion in a broad variety of disorders. The most promising indication for GnRH analogues was the use in controlled ovarian hyperstimulation (COH) protocols for the prevention of a premature LH surge. In both the natural and stimulated cycle, IVF treatment was hampered by the occurrence of premature luteinisation and ovulation. Placebo-controlled studies of GnRH agonists revealed that, without an agonist, there was a premature LH increase that lead to the cancellation of the IVF cycle in about 20% of women.[103,104] Preventing the LH surge is clearly beneficial. Moreover, the oocyte yield is higher with more embryos, allowing better selection leading to an increase in pregnancy rate.[105] In 1984, GnRH agonists were introduced in IVF;[106] thereafter, many treatment regimens have been developed. In most centres the long agonist protocol has become the standard procedure. The immediate and dose-dependent suppressive action of antagonists provides a convenient alternative to agonists. Their administration results in the suppression of LH (about 70%) and FSH (about 30%) serum levels after approximately 6 hours. GnRH agonists may be preferred in indications in which long-term, profound suppression of the endogenous gonadotropin release is desired. Conversely, GnRH antagonists are preferred if the initial gonadotropin surge caused by GnRH agonists is undesirable.

2.1 What Does Native GnRH Do?

Since the isolation and characterisation of the ten amino acids of GnRH type I by the groups of Schally and Guillemin,[107,108] many GnRH types have been isolated in different species.[109] In humans, so far three types of GnRH have been isolated. Compared with GnRH type I, types II and III have three and two different amino acids, respectively. GnRH type II has been found to be identical to chicken GnRH and GnRH type III is equal to salmon GnRH.[110] The genes for human GnRH type I and II are identified on chromosome 8 (8p11.2–p21) and 20 (20p13), respectively.[111,112] The gene locus of the third isoform has not been identified yet. The physiological meaning of the multiple isoforms in the human brain has not yet been elucidated, nor has the distribution of different types of GnRH receptors in the different tissues. GnRH type I is the classical hypothalamic reproductive neuroendocrine factor, which is synthesised in the diencephalon and then transported by the axons to the neuronal terminal. There it is released in a pulsatile fashion into the capillaries of the pituitary-portal circulation. Thereafter, it binds to the GnRH receptors in the pituitary gonadotropic cells after which it stimulates the release of LH and FSH in an orderly way, which is crucial for the control of normal gonadal function.

2.2 GnRH Agonists in Clinical Practice

GnRH agonists may be delivered intranasally or subcutaneously. Subcutaneous formulations induce

more stable serum levels, and the inter- and intra-individual variability is less than with intranasal formulations. In addition, short- and long-acting or slow-releasing subcutaneous formulations are available.[113] Although depot preparations seem to be more convenient for the patient since this requires fewer injections, some concern has been proposed about the possible interference with the luteal phase and embryo development.[113] In terms of IVF outcome, no clear beneficial effect can be advocated for long-acting products. Therefore, in general, short-term agonists will be prescribed to minimise any compromising effect on an emerging pregnancy.

GnRH agonist dosages used in IVF are derived from treatment schedules used in disseminated prostate cancer,[114] in which complete gonadal suppression is necessary. In ART, GnRH analogues are used to prevent a premature LH surge, for which it may be enough to suppress gonadotropin secretion only partially. Using lower dosages could have some advantages, such as avoidance of a direct effect of agonists on the ovary, oocyte, embryo and endometrium. There are some comparative studies, which indicate that the daily dose of agonists used in IVF may be decreased without compromising the results.[115,116] Only one prospective, randomised, double-blind, placebo-controlled, single-centre, dose-finding study has been performed in IVF for a GnRH agonist (triptorelin); 240 patients were included. The dosage necessary for suppressing the spontaneous LH surge was only 15–50% (15–50μg) of the dose needed for the treatment of prostate cancer (100μg), which is usually used in IVF.[104] Halving the dose of a daily administered GnRH agonist at the beginning of the stimulation has been successfully performed in normal[116] and poor responders,[117,118] without adverse effects on the quality of ovarian response to stimulation. In conclusion, it is very likely that the dosage of the commonly used GnRH agonists is far too high. In fact, dose-finding studies should be conducted before proper comparative studies between various GnRH agonists and between various GnRH agonists and antagonists in IVF can be performed. The GnRH analogues commonly used in assisted reproduction are described in table II.

2.2.1 GnRH Agonists in Assisted Conception

The use of GnRH agonists results in lower cancellation rates as a result of prevention of the premature LH surge, improved follicular recruitment with a larger number of oocytes recovered and improvement in routine organisation of assisted reproduction.[122] Many treatment schedules with the use of GnRH agonists in ART, particularly in ovarian hyperstimulation IVF/ICSI treatments, have been designed. The duration and initiation of agonist administration before the start of the actual ovarian stimulation varies widely. Initiation of the agonist treatment may either be in the early follicular or midluteal phase of the preceding cycle. This cycle may be a spontaneous cycle or an artificial one under the influence of the administration of progestogens and/or estrogens.

Table II. Gonadotropin releasing hormone (GnRH) analogues commonly used in assisted reproductive therapy (ART)

Generic drug name	Type	Minimal effective dose to prevent a LH surge[a]	Route	Brand name Europe/USA	References
GnRH agonists					
Leuprolide	Nonapeptide	NA	SC	Lucrin/Lupron	
Buserelin	Nonapeptide	NA	SC, IN	Buserelin/Suprefact	
Nafarelin	Decapeptide	NA	SC, IN	Synarel	
Triptorelin	Decapeptide	25 μg/day	SC	Decapeptyl/Trelstar depot	104
GnRH antagonists					
Cetrorelix	3rd generation	0.25 mg/day or 1 × 3mg	SC	Cetrotide	119,120
Ganirelix	3rd generation	0.25 mg/day	SC	Orgulatran/Antagon	121

a If coadministered only with follicle-stimulating hormone and/or luteinising hormone for in controlled ovarian hyperstimulation in ART.

GnRH = gonadotropin-releasing hormone; **IN** = intranasal; **LH** = luteinising hormone; **NA** = not assessed; **SC** = subcutaneous.

In the long or desensitisation protocol the agonist starts in the follicular phase or in the early, mid- or late-luteal phase in the preceding cycle until hCG administration. Stimulation with gonadotropins is started when pituitary and ovarian suppression has been achieved. A meta-analysis comparing ultra-short, short and long IVF protocols showed a higher number of oocytes retrieved and higher pregnancy rates with the long protocol, although more ampoules of gonadotropins were needed.[123] In terms of gonadotropin suppression and number of retrieved oocytes, the midluteal phase of the preceding cycle is the optimal moment for the initiation of the GnRH agonist, compared with the follicular, early or late luteal phase.[124-126] A major advantage of the long protocol of GnRH agonist administration is the contribution to the planning of the ovum pick-up (OPU), since both the initiation of exogenous gonadotropins after pituitary desensitisation and the administration of hCG can be delayed without any detrimental effect on IVF outcome.[127,128]

The short or flare-up protocol combines GnRH agonist therapy, started at cycle day 2, with gonadotropins initiated 1 day later. The immediate stimulatory action of the GnRH agonist serves as the initial stimulus for follicular recruitment. Adequate follicular maturation is on average reached in 12 days, which should allow enough time for sufficient pituitary desensitisation in order to prevent any premature LH surges.[129] The use of a long protocol in poor responders has been found to result in reduced ovarian responses to hormonal stimulation.[130] The short GnRH agonist protocol has been proposed as a better stimulation protocol for poor responders. The initial stimulatory effect of GnRH agonist on pituitary hormone levels may improve the ovarian response.[131] On the other hand, this short protocol might increase gonadotropins in the early phase, which induces enhanced ovarian androgen release and that is associated with declined oocyte quality and reduced ongoing pregnancy rates compared with the long protocol.[132] Nevertheless, experience to date shows that the short protocol has an important role in the treatment of poor responders.[133]

In the ultra-short protocol, the agonist is given during a period of 3 days in the early follicular phase. At the second day of agonist administration stimulation with gonadotropin is started.[134]

In the 'long-short', 'early cessation' or 'discontinuation-long' protocol, several investigators have tried to shorten the duration of GnRH agonist administration by early cessation. The agonist is started mid-luteal in the preceding cycle and discontinued during, or even before, the FSH treatment is started. Several prospective, randomised, controlled studies have been performed comparing the 'early cessation' and the 'long' protocols.[135-138] Increased hMG/FSH requirement and cancellation rates were reported after early cessation in 137 normo-ovulating IVF patients,[135] but the opposite was found in a recent study which included 230 normo-ovulating IVF patients,[136] although pregnancy rates were the same in both studies.[135,136] The paradoxical dip in serum LH levels after early cessation, leading to significantly lower estradiol levels on the day of hCG, may have a deleterious effect on oocyte quality.[135,136] The early discontinuation protocol may improve ovarian responsiveness based on a hypothetical effect on the ovary and was, therefore, tested in poor responders. However, this approach reported no further advantages compared with the long protocol to these patients in terms of pregnancy and implantation rates,[137,138] although the number of retrieved oocytes were significantly higher and the amount of required gonadotropins were reduced after early cessation.[138] In conclusion, the currently available data are in general not in favour of an 'early cessation' protocol but it may have some beneficial effects in poor responders.

'Coasting' may be defined as a treatment regimen whereby gonadotropin therapy is discontinued while continuing the GnRH agonist. This delay by a variable number of days in administering hCG injection is to trigger oocyte maturation prior to oocyte retrieval until lower estradiol levels are attained. It has been suggested that this approach prevents severe OHSS by reducing FSH-stimulated granulosa cell proliferation and consequently reduction of available granulasa cells for luteinisation.[139] This

allows continued follicular growth and maturation while reducing the risk of OHSS.[140] However, in a recent systematic review it was concluded that there is a lack of randomised, controlled trials comparing 'coasting' with no 'coasting'.[141] Only one prospective, comparative trial in 60 IVF patients showed a similar incidence of moderate and severe OHSS whether 'coasting' was applied or not.[142]

GnRH agonists are not frequently used during intrauterine insemination (IUI) since a longer pretreatment period is required and the risk of multiple pregnancies is increased because of increased follicular growth. Two prospective, randomised studies on this subject reported similar effects on cycle fecundity or live-birth rates between patients treated with hMG alone or in conjunction with GnRH agonists, although hMG requirement was increased in the agonist group.[143,144]

2.2.2 GnRH Agonists in Anovulatory Infertility

In the early 1980s, the first studies using GnRH agonist in combination with hMG for anovulatory patients with PCOS showed optimistic results: seven of eight patients conceived.[145] Nevertheless, subsequent prospective, randomised studies indicated that GnRH agonists provide no benefit over hMG therapy alone and did not reduce the tendency of the polycystic ovary to multifollicular development, cyst formation or OHSS.[146] It has even been shown that the addition of GnRH agonists in low-dose, step-up protocols may cause multiple follicular growth, probably by interfering in the integrity of the hypothalamic-pituitary-ovarian axis.[147] GnRH agonists have also been employed to enhance responsiveness of patients with PCOS to other ovulation-induction drugs such as pulsatile GnRH. However, rates of ovulation were disappointing and miscarriage rates were as high as 45%.[148,149] In a systematic review, it was assessed that an ovulation induction protocol with a GnRH agonist as an adjunct to FSH/hMG compared with FSH/hMG alone did not improve pregnancy and OHSS rates, and should therefore not be recommended as a standard treatment for this patient group.[150]

2.2.3 GnRH Agonists to Trigger Ovulation or Resumption of Meiotic Division

In accordance to the assumed midcyle endogenous GnRH rise in the natural cycle,[60] GnRH analogues may be used as an alternative way for hCG to trigger the endogenous LH and FSH surges, and subsequent final maturation of the oocytes and ovulation.[151,152] Since hCG is believed to contribute to the occurrence of the OHSS, because of its prolonged circulating half-life compared with native LH, this strategy seems to be an attractive alternative to prevent OHSS.

In the early 1990s, it was shown that single-dose GnRH agonists administrated in IVF patients undergoing COH were able to induce an endogenous rise in both LH and FSH levels, leading to follicular maturation and pregnancy.[153,154] Mean serum LH and FSH levels rose over 4–12 hours and were elevated for 24–34 hours after administration of a GnRH agonist compared with approximately 6 days of elevated LH levels after hCG 5000IU administration. The capacity of a single administration of a GnRH analogue to trigger follicular rupture in anovulatory women or in preparation for IUI has been well established and seems to induce lower OHSS rates with comparable or even improved results, despite short luteal phases, than with hCG cycles.[151,152,155]

Interest for this approach was lost during the 1990s because GnRH agonists were introduced in ovarian hyperstimulation protocols to prevent premature luteinisation by pituitary desensitisation, precluding stimulation of the endogenous LH surge. However, interest returned after the recent introduction of GnRH antagonist protocols in which pituitary responsiveness is preserved.

This new concept of triggering final oocyte maturation after GnRH antagonist treatment by a single GnRH agonist injection was successfully tested in patients undergoing COH for IUI[156] and in high responders for IVF.[157] None of these patients developed OHSS. The efficacy and success of this new treatment regimen was established in a prospective, multicentre trial in which 47 patients were randomised to receive either triptorelin 0.2mg, leuprorelin 0.5mg or hCG 10 000IU.[158] The LH surges peaked

at 4 hours after agonist administration and returned to baseline after 24 hours, the luteal phase steroid levels were also closer to the physiological range compared with the hCG groups. In terms of triggering the final stages of oocyte maturation, similar outcomes were observed in all groups as demonstrated by the similar fertilisation rates and oocyte quality.[158] A prospective, randomised study in 105 stimulated IUI cycles with a GnRH antagonist in patients with clomifene-resistant PCOS, showed statistically significantly more clinical pregnancies after ovulation triggering by a GnRH agonist than with hCG (28.2% vs 17% per completed cycle, respectively).[159] Thus, this new approach of ovulation triggering seems to be an attractive alternative to hCG in ART if administered in GnRH antagonist-treated cycles, with lower OHSS and similar or improved IVF outcome.

2.3 GnRH Antagonists in Clinical Practice

2.3.1 GnRH Antagonists in Assisted Conception

GnRH antagonists have been recently introduced in clinical practice for COH in ART cycles to prevent premature luteinisation. So far, cetrorelix (Cetrotide®) and ganirelix (Orgulatran® or Antagon®) are the two registered antagonists for this indication. Because of the acute gonadotropin suppressive activity of these agents, GnRH antagonists may be administered at any time during the follicular phase of a treatment cycle to prevent a premature LH surge. Several studies have been performed to determine the minimally effective or optimal dose, and treatment schedule in IVF patients.[119-121]

Two general approaches have emerged. In the single dose protocol, one injection of cetrorelix 3mg (ganirelix is not provided in a depot formulation) is administered in the late follicular phase on stimulation day 8 or 9 is sufficient to prevent a LH surge, although in slow responders a repeat injection may be needed.[120] In the multiple dose antagonist protocol cetrorelix or ganirelix 0.25mg is given daily from the sixth or seventh day of gonadotropin stimulation onward, including the day of hCG administration.[119,121,160]

Four large industry-sponsored prospective, multicentre clinical trials comparing daily antagonist injections with long agonist protocols in IVF patients undergoing COH were reported.[161-164] With an antagonist, the duration of gonadotropin treatment is shortened by 1–2 days and slightly fewer follicles are seen at the time of hCG injection compared with an agonist. Therefore, the number of recovered oocytes tends to be lower. A likely explanation is that long agonist protocols extend the duration of the 'FSH window' by suppressing the intercycle FSH rise. In these studies, no significant difference was found with respect to percentages of metaphase II oocytes, fertilisation rates and number of good quality embryos. Pregnancy rates were high in both groups in all four studies but in every one the rate was lower in the antagonist group.

A meta-analysis of five large randomised trials shows an overall significantly lower rate of pregnancy of 5% (OR: 0.75, 95% CI 0.62–0.97). This meta-analysis also included the study that compared a single-dose analogue regimen with different gonadotropin starting doses as an additional variable.[165] It has been hypothesised that the lower pregnancy rates may be a consequence of the currently advised treatment regimen. It has also been suggested that the larger numbers of oocytes and embryos with agonists allow better selection, although the numbers of good quality embryos do not seem to be different. The antagonist was started on a fixed day of stimulation (day 6) in these studies, which may be too early for some patients, and may lead to a diminished number and quality of oocytes.[161-165]

More flexible alternative regimens are currently under development. Prospective studies comparing the fixed antagonist protocol with a flexible protocol, in which the daily antagonist administration is started when at least one follicle has reached a size of 14mm, showed no differences in IVF outcome except that the dose of GnRH antagonist was reduced in the flexible protocol.[166,167] Moreover, one study even reported more oocytes per OPU despite lower FSH requirements in the flexible protocol than with the fixed protocol.[168]

Concerning OHSS, the results of the four comparative studies are inconclusive. Although three studies show decreased OHSS incidence with the antagonist,[161-163] one study showed a higher incidence of OHSS.[164] The meta-analyses, which compared the five large comparative studies, showed significantly lower OHSS after GnRH antagonists than after GnRH agonists (RR 0.36, 95% CI 0.16–0.80).[165] Recently, in a prospective, randomised study, higher vascular endothelial growth factor mRNA and protein levels were expressed in IVF patients undergoing COH treated with GnRH agonists versus antagonists.[169] This provides a hypothetical biological explanation for the clinical experiences of the lower observed OHSS rates in antagonist cycles.

Retrospective comparison of pregnancy rates after transfer of frozen-thawed two-pronucleate oocytes obtained either in long agonistic protocols or in antagonist protocols, showed no differences in implantation, pregnancy and abortion rates.[170,171]

On a theoretical basis, the use of GnRH antagonists may be preferred above GnRH agonists in poor responders. First, GnRH antagonists are given in the late follicular phase of COH and are not involved in the early period of folliculogenesis, which may be critical for poor responders with a limited cohort of follicles. Secondly, FSH levels are less 'over-suppressed' by GnRH antagonists than with GnRH agonists in long protocols, probably leading to less exogenous gonadotropin being required. Finally, some investigators assume that GnRH agonists may have a direct deleterious effect on the ovary, which could be especially important for patients who are poor responders.[172] In a small prospective, comparative study, Akman et al.[173] evaluated the effect of a GnRH antagonist in IVF cycles versus IVF cycles without any GnRH analogue addition in 40 poor responders. There were no statistical differences with regard to cancellation rate, IVF outcome and FSH requirement.[173] In an additional small, prospective, randomised trial comparing an agonistic flare-up protocol and antagonistic multiple-dose protocol in 48 poor responders in IVF, no differences could be found either.[174] A small, matched, case-control study indicated that slightly fewer ampoules of FSH were needed in antagonist- versus agonist-treated poor responders in IVF.[175] To establish these results and to advocate a particular treatment regimen in poor responders, further randomised studies with larger sample sizes are required.

Accurate prospective trials comparing GnRH antagonists with agonists in patients with PCOS, undergoing IVF, have not yet been published.

On theoretical grounds, prevention of excess LH secretion in gonadotropin-stimulated IUI might be beneficial. One inconclusive study has been published with regard to the use of a GnRH antagonist in IUI.[176] This indication obviously requires further study before general introduction.

2.3.2 GnRH Antagonist in Ovulation Induction

Triggering ovulation with GnRH agonists in GnRH antagonist-treated cycles is discussed in section 2.2.2. The coadministration of pulsatile GnRH to induce ovulation in GnRH antagonist-treated cycles provides a new treatment option but clinical data on its use are scanty. Studies in monkeys showed that blockade of the endogenous GnRH with an antagonist can be reversed by exogenously administered pulsatile GnRH, which restored gonadotropin secretion and ovulation.[177] In a very small pilot study, the same combination in PCOS patients normalised gonadotropin secretion but failed to induce ovulation.[178] In a small, noncontrolled study in 20 patients with PCO, it was indicated that daily ganirelix 0.25mg coadministered with rFSH 75 or 150IU might be an effective protocol for ovulation induction in these patients but prospective, randomised trials are warranted to confirm these preliminary findings.[179]

2.4 Safety Aspects of GnRH Analogues

Although GnRH analogues have acquired an important place in ART, caution remains with regard to the use of these drugs. The safety aspects of GnRH analogues, related to direct effects on extrapituitary structures such as the ovary, the oocyte, granulosa cells and the embryo, are a matter of debate, since the discovery of extrapituitary GnRH receptors in

humans.[180,181] GnRH and GnRH receptors are expressed in developing mouse embryos at the mRNA and protein levels. The incubation of the embryos with a GnRH agonist enhanced the preimplantation embryonic development in a dose-dependent way, whereas a GnRH antagonist could completely block this development.[182] Recently, it has been demonstrated that GnRH mRNA and GnRH proteins are produced in the human fallopian tube during the luteal phase of the menstrual cycle at the same time spermatozoa and oocytes are deposited in the oviduct to promote their union and nurture the resultant zygotes and early embryos.[183] These results underline the high priority for the safety aspects of GnRH analogues and stress the need for prospective, comparative studies concerning *in vivo* effects of the GnRH analogues on fertilisation, early embryonic development and implantation in humans, before the agonist should be abandoned for antagonists in general practice.[184]

On the basis of the inverse association between implantation rates and ganirelix dose in the higher dosage groups in the large dose-finding study,[121] the possibility of direct effects of antagonists on human embryos is of concern. However, this adverse effect was not observed on the freeze-thaw embryos of these cycles, suggesting that there is no direct negative effect of the GnRH antagonist on the quality of oocytes and embryos, but perhaps on the endometrium.[185] In accordance with the reported association between low LH levels (<0.5 IU/L) and lower ongoing pregnancy rates in IVF cycles,[77] as discussed in section 1.4.2, LH levels are thought to have a role in the lower pregnancy rates in GnRH antagonist-treated cycles, since these cycles often lead to extensive suppression of endogenous LH activity if combined with rFSH administration. However, in a large retrospective study in which patients with the latter regimen were divided in two groups based on the LH levels on the day of hCG administration, with a cut-off level of 0.5 IU/L, no differences were found in pregnancy rates and outcome.[186] Although, as discussed in section 1.4, it has been recently proposed that it might be more appropriate to look at an LH 'window' instead of a single LH cut-off level, since there seems to be a 'threshold' LH level, below which estradiol production is not adequate, and a 'ceiling' level, above which LH may be detrimental to follicular development.[187]

There does not appear to be an increased risk of birth defects or pregnancy wastage in human pregnancies exposed to daily low-dose GnRH agonist therapy in the first weeks of gestation. Obviously, there are no controlled trials on this subject. Although several authors claim normal outcome of pregnancy after inadvertent administration of a GnRH agonist during early pregnancy, Lahat et al.[188] reported a high incidence of attention deficit hyperactivity disorder in long-term follow-up of children inadvertently exposed to GnRH agonists early in pregnancy.

The follow-up data on pregnancy, birth and neonatal outcome of 227 children born after IVF or ICSI cycles in which cetrorelix was used, showed no abnormal results compared with outcome after commonly used long agonist protocols.[189] The safety of the novel antagonistic protocol was established on the hand of the similar follow-up results, collected during clinical development trials, of 340 ongoing pregnancies after ganirelix and 134 after a long agonist protocol.[190]

2.5 New Developments With GnRH Analogues

The availability of GnRH antagonists with good clinical safety on the commercial market provides an important alternative for the extensively used GnRH agonists for a variety of indications. As experience with these drugs increases, new protocols can be developed. Additional prospective, comparative studies should be performed to establish the optimal treatment regimen for the different indications. The application of the different regimens should be fine-tuned and adapted to the individual needs and properties of specific patient groups. For example, the minimal stimulation protocols which regained interest after the introduction of the GnRH antagonists (as discussed in section 1.2.2) might be specifically attractive for high responders or in patients in

which the ovarian reserve is not thought to be diminished (e.g. if IVF is indicated because of tubal dysfunction). The same can be said for the application of 'natural' cycle IVF with late follicular phase GnRH antagonist administration, which is a convenient and inexpensive procedure.[191,192]

It is important to realise that the use of GnRH analogues for different indications or in different treatment schedules requires adjusted dose-finding studies. The pituitary responsiveness depends on the timing of administered GnRH analogues, but also on the coadministered medication. In soft-stimulation IVF cycles with clomifene, 21.5% premature LH surges occurred despite daily cetrorelix 0.25mg administration[193] and elevated LH levels during the follicular and luteal phase[194] were observed, indicating that the required antagonist dose in this protocol is above 0.25mg/day. Alternate-day administration of 0.25mg antagonist in IVF/ICSI patients undergoing COH resulted in 5.8% premature ovulations, which seems to be an acceptable alternative.[195]

To facilitate planning the starting moment of an antagonist cycle, independent of the menstrual period, oral contraceptive (OC) pre-treatment has been evaluated in a few small prospective studies, in which the antagonist was started 3 days after OC withdrawal. OC pre-treatment induced a longer stimulation period with more oocytes, lower LH levels and lower pregnancy rates than the antagonist cycles.[196] The cause for longer simulation period and more oocytes recovered might be the oestrogen-induced suppression of the intercycle FSH rise and, subsequently, the prevention of some sensitive follicles being selected, resulting in a more synchronised and larger cohort, allowing an increased 'FSH window'.[197] Therefore, this strategy is hypothesised to be an advantage for poor responders. An uncontrolled, prospective study showed a mean number of six oocytes in 68 poor responders and a high clinical pregnancy rate (31% per cycle).[198] However, large prospective, randomised controlled studies are warranted to draw solid conclusions on this topic.

New antagonists are under development. Abarelix and degarelix are long-acting formulations which have been recently developed to be used for the management of sex steroid-dependent pathologies, in which the avoidance of the initial flare effect might be preferred over the currently used GnRH agonists.[199,200] Expected long-term developments are nonpeptide orally active GnRH analogues.[201]

The debate on the possible cause of the lower implantation rates after GnRH antagonist cycles compared with GnRH agonist cycles triggered a new field of research which focuses on the possible direct effects of GnRH analogues on fertilisation, early embryonic development and endometrium. The existence of the recently discovered additional GnRH receptor (type II) in mammals could have a role in the confusing and inconclusive results of many studies on direct extrapituitary effects of GnRH agonists and antagonists as reviewed by Janssens et al.,[181] since most studies assumed to deal with only one GnRH receptor. The type II receptor is equal to the chicken GnRH receptor, displays different affinity for the different analogues, and has different effects on gonadotropin release after binding by a GnRH agonist or antagonist.[202] The effect on gonadotropin release of GnRH antagonists after binding to the type I receptor or the type II receptor is also different.[203] Additional studies are under development to gain insight in the functional properties of the GnRH receptors and their impact on GnRH analogue binding.[204]

The different effect of analogues on the different GnRH receptors and their tissue specific distribution should be clarified before rational choices for clinical application of specific analogues in specific indications can be made, and to gain insight in possible extrapituitary side effects.

3. Conclusion

To broaden the pharmaceutical armoury, recent efforts have been directed towards the development of novel GnRH antagonists, FSH and LH preparations with optimal pharmacokinetic, pharmacodynamic and safety profiles in addition to the available GnRH agonists and urinary gonadotropins. Alternative strategies with fewer adverse effects and higher benefit to cost ratios are under development. Pro-

spective studies investigating possible direct effects of GnRH analogues, optimal dose-finding studies and treatment regimens under different conditions, in different types of patients, with or without pharmacological coadministration and for different indications, should be performed to optimise the efficacy and tailor treatment strategies to individual needs.

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