GnRH antagonists

Maria Elisabetta Coccia[*], Ciro Comparetto, Gian Luca Bracco, Gianfranco Scarselli

Department of Gynaecology, Perinatology, and Human Reproduction, University of Florence, Via Ippolito Nievo 2, 50129 Florence, Italy

Abstract

Ovarian stimulation is an important step in the success rate of in vitro fertilization (IVF) allowing multiple follicular growth, several oocytes and consequently more embryos. The combination of GnRH-antagonists (GnRH-ant) and gonadotrophins is now available for clinical use and represent a valid alternative to classical protocol with GnRH agonist. GnRH-antagonists induce a direct block of GnRH receptor with a rapid decrease in LH and FSH, preventing LH surge. Two protocols has been designed for assisted reproduction technology (ART) treatment: multiple-dose protocol and a single-dose. Both protocols are simply, efficacious, started in the late follicular phase and do not have side effects. A review of GnRH-antagonist applications in ART cycles are presented. Smaller doses of gonadotrophins, shorter stimulation period and lower ovarian hyperstimulation syndrome (OHSS) incidence are reported in literature using GnRH-antagonist compared to agonist. Triggering of ovulation, the use in polycystic ovarian syndrome (PCOS) and poor reponders patients are other interesting indication. Regarding to pregnancy rate and potentially adverse effects of drugs on endometrium or implantation needed more data.
© 2004 Elsevier Ireland Ltd. All rights reserved.

Keywords: GnRH-antagonist; Ovarian hyperstimulation; Assisted reproduction techniques

1. Introduction

The in vitro fertilization (IVF) process involves controlled ovarian stimulation (COS) to stimulate follicular growth. Gonadotrophin preparations, such as human menopausal gonadotrophin (hMG) or recombinant human follicle stimulating hormone (rhFSH), are usually used to stimulate the ovaries to produce oocytes. Human chorionic gonadotrophin (hCG) is then used to induce oocyte maturation and to trigger ovulation in stimulated cycles if appropriate.

Cancellation (i.e. not proceeding with treatment in that cycle) may be advised because of a poor response or excessive response to ovarian stimulation. In gonadotrophin treated cycles a premature luteinizing hormone (LH) surge and premature ovulation may require cancellation.

In fact, high LH levels have a negative role in IVF, so reduction in bioactive LH levels in the serum is desirable, particularly in the light of evidence associating raised LH levels in the follicular phase with adverse reproductive outcomes [1]:

- Reduced fertilization and pregnancy rates.
- Increased spontaneous abortion rate.
  Most specialist infertility clinics attempt to minimise

premature LH surges using pituitary down-regulation. To achieve this, gonadotrophin-releasing hormone agonists (GnRH-a) were introduced into IVF superovulation regimens in the late 1980s and have become established as a component of standard regimens in most centres worldwide.

The inclusion of GnRH-a in ovarian stimulation protocols for assisted reproduction technologies (ART) has resulted in significant improvements in outcome [2]:

- Cycle cancellation rates have decreased.
- Clinical pregnancy rates have increased.
  In fact, before GnRH-a became available, approximately 20% of stimulated cycles within an IVF program were cancelled due to premature LH surges. By using the GnRH-a to prevent LH surges via gonadotrope GnRH receptor (GnRH-R) down-regulation and desensitisation, this percentage decreased to about 2%, and concomitantly, the IVF and pregnancy rates (PR) per cycle initiated were increased.

Several treatment schedules currently are in use (long, short, or ultrashort protocol): the *long protocol*, in which the GnRH-a is begun in the luteal phase of the cycle preceding the treatment (stimulation) cycle and down-regulation occurs before the start of the gonadotrophin-stimulation treatment phase, is generally the most effective regimen and is presently the most frequently used protocol. However,

[*] Corresponding author. Fax: +39-05588431171.
E-mail address: cocciame@tin.it (M.E. Coccia).

Agonists

	1	2	3	4	5	6	7	8	9	10
GnRH	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH$_2$
Buserelin	1	2	3	4	5	D-Ser	7	8	9ethylamide	
Goserelin	1	2	3	4	5	D-Ser	7	8	9	AzGly
Leuprolin	1	2	3	4	5	D-Leu	7	8	9ethylamide	
Triptorelin	1	2	3	4	5	D-Trp	7	8	9	Gly-NH$_2$
Nafarelin	1	2	3	4	5	D-Nal	7	8	9	Gly-NH$_2$

Antagonists

Cetrorelix	D-Nal	D-Phe	D-Pal	4	5	D-Cit	7	8	9	D-Ala
Nal-Glu	D-Nal	D-Phe	D-Pal	4	Arg	D-Glu	7	8	9	D-Ala
Antide	D-Nal	D-Phe	D-Pal	4	NicLys	D-Niclys	7	Lys(iPr)	9	D-Ala
Ganirelix	D-Nal	D-Phe	D-Pal	4	5	D-hArg	7	hArg	9	D-Ala
AzalineB	D-Nal	D-Phe	D-Pal	4	Phe	D-Phe	7	Lys(iPr)	9	D-Ala
Degarelix	D-Nal	D-Cpa	D-Pal	4	Aph	D-Aph	7	Lys(iPr)	9	D-Ala

Fig. 1. Amino-acid sequences of GnRH analogues.

it has some disadvantages, such as hypoestrogenic side effects and an increase in the number of ampoules of FSH or hMG required for adequate stimulation [3].

Low doses of the native peptide delivered in a pulsatile manner to mimic that found in the hypothalamic portal vessels restore fertility in hypogonadal patients, and are also effective in treating cryptorchidism and delayed puberty. Administration of high doses of GnRH or agonist analogues causes desensitisation of the gonadotrope gland with consequent decline in gonadal gametogenesis and steroid and peptide hormone synthesis. This phenomenon finds extensive therapeutic application in clinical medicine in a wide spectrum of diseases and in IVF to avoid LH incraese. In addition, GnRH analogues could be used as new generation male and female contraceptives in conjunction with steroid hormone replacement.

GnRH-antagonists (GnRH-ant) inhibit the reproductive system through competition with endogenous GnRH for the receptor and, in view of their rapid effects, are being increasingly used for the above mentioned applications.

2. Pharmacology

GnRH is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly.NH2) characterized in 1971 by Schally et al. [4] which is produced from a precursor polypeptide in hypothalamic neurons and secreted in a pulsatile manner to stimulate the secretion of LH and FSH via its interaction with the receptor on gonadotropes.

GnRH amino acids with crucial functions are at positions 1, 2, 3, 6, and 10. Position 6 is involved in enzymatic cleavage, positions 2 and 3 in gonadotrophin release, and positions 1, 6, and 10 are important for the three-dimensional structure.

Synthetic analogues of GnRH with a deletion or substitution of the histidine in position 2 have been shown to be competitive antagonists of the native hormone by means of their ability to bind to, but not activate, the GnRH-R. Further substitutions at positions 1, 3, 6, 8, and 10 in the molecule

have resulted in progressive increases in antagonistic potency and improved physical–chemical characteristics.Thus, the structures of the antagonists, unlike the agonists, which only differ for one amino acid (residue 6), substantially differ from that of GnRH. Five of the 10 amino acids are unnatural and of D configuration (Fig. 1).

The first generation of GnRH-ant had the disadvantage of producing adverse side effects which were constituted above all by anaphylactic reactions due to histamine release. The structural combination of a hydrophobic N-terminus (residues 1–3) and a basic/hydrophilic C-terminus (residues 6 and 8) was thought to be responsible for some of these reactions encountered also, even if to a lesser extent, with the second generation of GnRH-ant. This side effect was greatly reduced in the third generation by substituting the appropriate combination of amino acids at positions 5, 6, and 8 (Fig. 2).

To the third generation of GnRH-ant belong cetrorelix and ganirelix, which competitively inhibit the secretion of LH and FSH from the pituitary gland and oestradiol (E2), by blocking the binding of GnRH to pituitary GnRH-R with a dose-dependent mechanism that is maintained throughout continuous treatment and is reversible after treatment discontinuation [5–9]. In IVF the minimal effective doses of these drugs to prevent LH surges are 0.25 mg per day for the multiple-dose protocol and 3 mg for the single-dose protocol, administered via the subcutaneous (SC) route.

Cetrorelix has a low histamine-releasing potential. In infertile women treated with cetrorelix pituitary
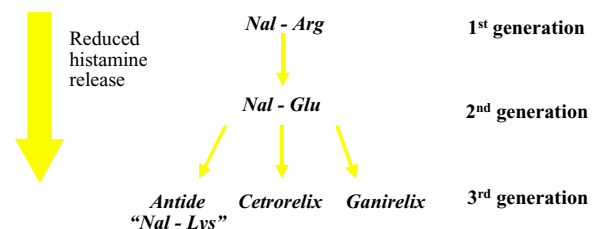
Fig. 2. GnRH antagonist evolution.

responsiveness was preserved [10]. During GnRH-ant administration, an escalating dose regimen of LH replacement is optimal for maintenance of the structure and functional life span of the primate corpus luteum [11]. Impairment of corpus luteum function during cycles stimulated with hMG appears to be less with cetrorelix than that associated with gonadorelin analogues [12,13].

Ganirelix is a potent synthetic third generation GnRH-ant with only minimal histamine-releasing properties and high aqueous solubility. The molecule has a ninefold higher receptor binding affinity ($K_D = 0.4$ nM) as compared to GnRH ($K_D = 3.6$ nM) [14]. It has been generally well tolerated in clinical trials.

Degarelix (FE200486) is a member of a new class of long-acting GnRH-ant. At single SC injections of 0.3–10 µg/kg in rats, degarelix produced a dose-dependent suppression of the pituitary–gonadal axis as revealed by the decrease in plasma LH and testosterone (T) levels. Duration of LH suppression increased with the dose: in the rat, significant suppression of LH lasted 1, 2, and 7 days after a single SC injection of degarelix at 12.5, 50, or 200 µg/kg, respectively. Degarelix fully suppressed plasma LH and T levels in the castrated and intact rats as well as in the ovariectomized (OVX) rhesus monkey for more than 40 days after a single 2 mg/kg SC injection. In comparative experiments, degarelix showed a longer duration of action than the GnRH-ant abarelix, ganirelix, cetrorelix, and azaline B.

The in vivo mechanism of action of degarelix was consistent with competitive antagonism, and the prolonged action of degarelix was paralleled by continued presence of radioimmunoassayable degarelix in the general circulation. In contrast to cetrorelix and similarly to ganirelix and abarelix, degarelix had only weak histamine-releasing properties in vitro. These results demonstrate that the unique and favourable pharmacological properties of degarelix make it an ideal candidate for the management of sex steroid-dependent pathologies requiring long-term inhibition of the gonadotropic axis [15].

3. Mechanism of action

The clinical usefulness of the GnRH-a drugs is based on their ability to reversibly block pituitary gonadotrophin secretion, thereby preventing a premature surge of LH, which causes luteinization and disruption of normal follicle and oocyte development, a situation that was frequently observed with gonadotrophin-only stimulation protocols. Recent UK guidelines on infertility management recommend the routine use of gonadorelin analogues in IVF [16].

GnRH plays a crucial role in controlling the ovarian cycle in women. By modification of the molecular structure of this decapeptide, analogues were synthesized with agonistic or antagonistic effects on the gonadotrophic cells of the anterior pituitary gland. The mechanisms of action of GnRH-ant and of agonists is completely different.
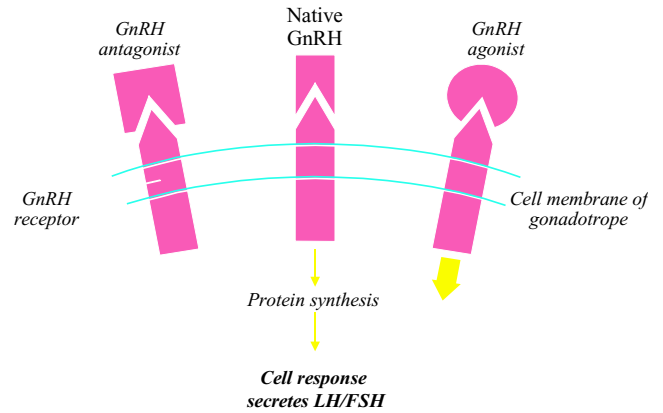

Fig. 3. GnRH analogues: mode of action.

The agonists, after an initial stimulatory effect (*flare-up*), lead to desensitisation of the gonadotrophic cells and a reduction in the number of GnRH-R on the cell membrane (*down-regulation*), thereby reducing the release of FSH and LH, which in turn leads to inhibition of androgen and oestrogen production, while the antagonists produce an immediate effect by competitive blockade of the GnRH-R (Figs. 3–7) [17].

After administration of GnRH-ant, the serum levels of FSH and LH decrease within hours. Nevertheless, the adenohypophysis maintains its responsiveness to a GnRH stimulus (*pituitary response*) after pre-treatment with an antagonist. Due to competitive blockage of GnRH-R by antagonist administration, LH (and to a lesser extent FSH) levels drop rapidly. Moreover, pituitary function normalizes immediately following cessation of medication. This different pharmacological mechanism of GnRH-ant makes possible new approaches to ovarian stimulation and to the therapy of sex steroid dependent diseases. The direct and rapid action of GnRH-ant, the dose-dependent suppression of LH and FSH and the rapid restoration of hypophyseal function after cessation of the use of antagonists may shorten and simplify IVF, with less chance of side effects or complications. Antagonists can usefully be applied for other gynaecological indications such as the polycystic ovarian
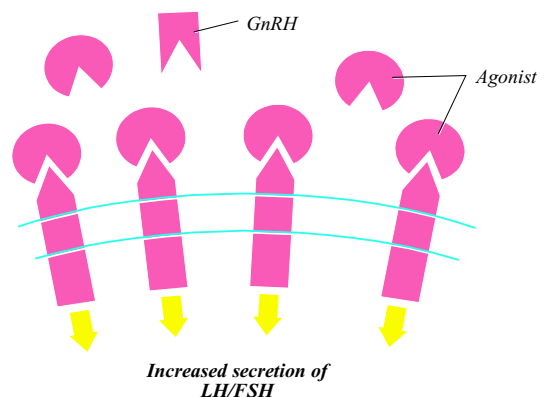

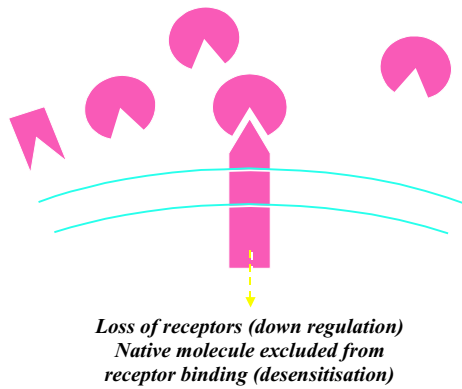Fig. 4. Agonist—initial phase: stimulation.

Loss of receptors (down regulation)
Native molecule excluded from
receptor binding (desensitisation)

Fig. 5. Agonist—chronic administration: suppression.

GnRH

Antagonist

Receptor blocked no micro-aggregation

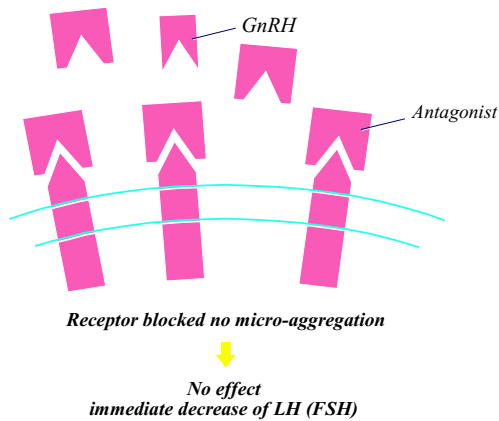*No effect*
immediate decrease of LH (FSH)

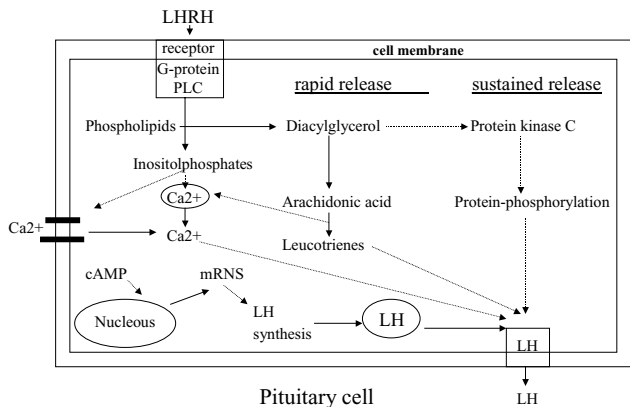Fig. 6. Antagonist—initial: immediate suppression.

Fig. 7. GnRH analogues: mechanism of action.

syndrome (PCOS). The possibilities of profitable long term treatment will increase considerably if it proves possible to develop a sustained action formulation [18,19].

4. Pharmacokinetics

GnRH is short-lived with a plasma half-life of 2–5 min due to rapid enzymatic degradation by peptidases which interact with peptide in position 6; small amounts of the unchanged drug (4%) appear in urine.

The pharmacokinetics and pharmacodynamics of GnRH-ant *cetrorelix* following single and multiple SC administration of different doses was investigated in healthy female volunteers who received either 0.25, 0.50, or 1.00 mg cetrorelix, in a first menstrual cycle as single-dose and in a second cycle as multiple-dose (daily between cycle days 3 and 16).

After single administration of each dose, maximum cetrorelix concentrations (C_{max}) were reached after 1 h, and C_{max} and area under the concentration (AUC) time curve increased linearly with the dose. The median terminal half-life ranged from 5 to 10 h in the three different dose groups. FSH, LH, E2, and progesterone (P) concentrations were suppressed, with a nadir at 6–12 h after cetrorelix administration.

During multiple administration, C_{max} and AUC also showed dose-linearity. The median terminal half-life of cetrorelix varied between 20 and 80 h. A dose-dependent suppression of FSH, LH, and E2 concentrations was observed during treatment. After multiple administration, ovulation was delayed for 5, 10, and 13 days in the 0.25, 0.50, and 1.00 mg dose groups, respectively [7].

The bioavailability of cetrorelix after SC injection is about 85%. It has linear pharmacokinetics, is 85% protein bound, and has a terminal elimination half-life after SC administration of about 30 h [5,7].

The development of pharmacokinetic and pharmacodynamic models for the LH suppression and subsequent shift in LH surge and FSH by cetrorelix in women was also made in a placebo-controlled study. Single SC doses of 1, 3, and 5 mg of cetrorelix were given on day 8 of the natural menstrual cycle. Cetrorelix pharmacokinetics were described by a 2-compartment model with a terminal half-life of 56.9 ± 27.1 h. Mean shift in LH surge was by 4.1, 7.5, and 9.3 days with the 1, 3, and 5 mg doses, respectively. An indirect response sigmoid E_{max} model was developed for the suppression of LH and the shift in the LH surge. The inhibitory concentration of 50% (for LH suppression) and median effective concentration (for surge shift) estimates were 3.6 and 1.6 ng/ml, respectively. The suppression of FSH was described by a similar E_{max} model, with an inhibitory concentration of 50% of 7.25 ng/ml [20].

Regarding *ganirelix*, the absolute bioavailability after a single SC injection was assessed in a randomised, crossover, pharmacokinetic study (phase I clinical research unit). Healthy female volunteers of reproductive age were submitted to two separate injections of 0.25 mg of ganirelix, one SC and one intravenously (IV), with a washout period of 1 week between injections. The mean concentration-time profile after SC administration was comparable to that after IV administration. The mean ($\pm$standard deviation (S.D.)) peak concentration and time of occurrence after SC administration were 14.8 ± 3.2 and 1.1 ± 0.3 h, respectively. The mean ($\pm$S.D.) half-lives after IV administration and SC

administration were highly similar (12.7 ± 3.7 and 12.8± 4.3 h, respectively). Mean (±S.D.) AUC 0-infinity values of 105 ± 11 and 96 ± 12 ng (ml/h) were calculated for IV administration and SC administration, respectively, resulting in an absolute mean (±S.D.) bioavailability of 91.3% ± 6.7%. Both treatments were well tolerated. In conclusion, ganirelix is absorbed rapidly and extensively after SC administration, resulting in a high absolute bioavailability of >90% [21].

The dose-proportionality and pharmacodynamic properties of multiple-doses of ganirelix were assessed in a randomised, parallel, pharmacokinetic, and pharmacodynamic study (phase I clinical research unit) on healthy female volunteers of reproductive age. SC injections of 0.125, 0.25, or 0.50 mg of ganirelix were given once daily for 7 days. Steady-state levels were reached between days 2 and 3. Peak concentrations, which occurred approximately 1 h after dosing, increased in a dose-proportional manner and averaged 5.2, 11.2, and 22.2 ng/ml for the 0.125, 0.25 and 0.50 mg doses, respectively. Corresponding mean values for the AUC over one dosing interval (24 h) were 33, 77.1, and 137.8 ng (ml/h), respectively. After the last 0.25 mg dose of ganirelix, serum LH, FSH, and E2 concentrations were maximally decreased (by 74, 32, and 25% at 4, 16, and 16 h after injection, respectively). Serum hormone levels returned to pre-treatment values within 2 days after the last injection. In conclusion, the pharmacokinetics of ganirelix were dose-proportional within the dose range studied. Multiple injections resulted in immediate suppression of gonadotrophins, which was rapidly reversed after treatment discontinuation [22].

5. Indications/contraindications

5.1. Assisted reproduction technologies

One of the first reports on the use of GnRH-ant in gynaecology is the study by Felberbaum et al. (1995), who tested the applicability of the GnRH-ant cetrorelix within COH to avoid the premature LH surge. Patients suffering from tubal infertility were stimulated for IVF by hMG and concomitant administration of cetrorelix in different dosages (3, 1 and 0.5 mg). No premature LH surge could be observed. The authors concluded that short term administration of the GnRH-ant avoids the occurrence of a premature LH surge [23].

In a pilot study, to assess the ability of a single injection of the GnRH-ant cetrorelix to prevent premature LH surges in an IVF-ET program when administered on a fixed day in the late follicular phase, these findings were confirmed [24].

The use of GnRH-ant has been studied in *ART* as part of the COS procedure in healthy female partners of infertile couples. The primary endpoint in most studies was prevention of premature LH surge and consequent ovulation in order to allow the follicles to mature for planned oocyte collection, although the definition varied between different study protocols.

Two protocols for ART cycles were designed which were widely used in COS in several phase II and III studies as well as in clinical practice since the GnRH-ant cetrorelix and ganirelix are available on the market: cetrorelix was applied in single- and multiple-dose protocols; ganirelix was used until now only according to the multiple-dose protocol. The single-dose protocol allies simplicity and efficacy, while the multiple-dose protocol is efficient and could reduce monitoring of the cycle, though compliance is mandatory.An open-label non randomised clinical study on normal human volunteers in an academic research center was conducted to determine if daily SC doses of ganirelix could suppress and maintain E2 ≤ 30 pg/ml, the serum profiles of LH and FSH during and after cessation of treatment, the time-course of the resumption of normal ovarian function after ganirelix cessation, and to identify side effects of daily treatment. Ganirelix treatment rapidly decreased serum levels of gonadotrophins and E2 after both 1 and 2 mg administration. Twenty-four hours after the first dose of ganirelix, E2 decreased from a mean of 50 ± 8 and 67 ± 11 pg/ml at baseline to 25 ± 4 and 20 ± 3 in the 1 and 2 mg groups, respectively. E2 remained suppressed (mean levels <26 pg/ml) on all subsequent 7 days of ganirelix dosing in both groups. After the final dose of ganirelix, there was a rapid return of ovarian function in all volunteers. All women had P levels indicative of ovulation in the subsequent cycle, and the mean number of days from the final ganirelix dose to the next menses was 25.8 ± 2.1 and 27.3 ± 1.6 in the 1 and 2 mg groups, respectively. The Authors conclude that daily ganirelix administration is effective in suppressing the pituitary–gonadal axis and has a side effect profile that should be well tolerated [25].

Another pilot study was designed to determine if GnRH-a could induce a LH surge in patients where a GnRH-ant was used to prevent premature spontaneous LH surge in women treated with ovarian stimulation and intrauterine insemination (IUI) for idiopathic infertility. A LH and FSH surge as well as a P rise were obtained in the patients studied. A GnRH-a successfully induced a LH surge after GnRH-ant administration [26].

In a further study, subtle serum P rise (≥1.1 ng/ml) during the late follicular phase was reported for the first time in patients using cetrorelix, in combination with hMG for ovarian stimulation prior to intracytoplasmic sperm injection (ICSI). In 20% of the patients serum P levels were ≥1.1 ng/ml. The cycle characteristics of the patients were similar in both groups. No premature endogenous LH surge occurred and the serum LH concentrations were constantly low during the follicular phase. The 17-β E2 and FSH exposure were higher in cycles with premature luteinization. The greater E2 and FSH exposure confirm that one of the possible factors inducing subtle serum P rise is the increased E2- and FSH-induced LH receptivity in granulosa cells [27].

The first attempt to treat imminent ovarian hyperstimulation syndrome (OHSS) by using a GnRH-ant was made in 1998 [28]. Moreover, in 1998 a case report described the first established pregnancy after the use of the GnRH-ant ganirelix to prevent a premature LH surge during ovarian hyperstimulation with rhFSH. The pregnancy progressed normally and ended with the birth of a healthy boy and a girl after an elective caesarean section at gestational age of 37 weeks [29].

Ongoing pregnancies have been also previously achieved in older recipients with *natural cycle oocyte donation* from young donors using a GnRH-ant, with hMG and hCG to complete oocyte maturation. This provides a new alternative to ovarian stimulation for both oocyte donation and routine IVF [30].

GnRH-ant are used also in *embryo cryopreservation* programs. Therapeutic regimens for the treatment of malignant disease, in fact, may compromise future fertility. One approach to circumvent this is the cryopreservation of embryos created before treatment for the malignancy.

Conventional regimens using GnRH-a are time consuming, requiring pituitary down-regulation before gonadotrophin administration, thus the duration of treatment is approximately 20–30 days. GnRH-ant do not cause an initial stimulation of gonadotrophin secretion and can thus be administered during the later stages of follicular maturation to prevent premature luteinization and ovulation The duration of ovulation induction/IVF treatment is thus reduced. These are potential uses and advantages of a GnRH-ant in ovulation induction/IVF when the need for immediate initiation of treatment and its duration are critical factors [31].

A retrospective data analysis was made to compare the pregnancy rates of *frozen–thawed 2-pronucleate (2PN) oocytes* obtained either in a long protocol or in an antagonist protocol and ovarian stimulation with either hMG or rhFSH on infertile couples who underwent a transfer of cryopreserved 2PN oocytes. Implantation rates in the freeze–thaw cycles were 5.6% (hMG) and 3.8% (rhFSH) with 2PN oocytes from the long protocol and 7% from the antagonist cycles, irrespective of whether hMG or rhFSH was used. PR were similar independent of whether they resulted from the long-protocol cycles with hMG (15.4%) and rhFSH (13.1%) or from the antagonist protocol cycles with hMG (25.0%) and rhFSH (17.5%). The potential to implant is independent of the GnRH analogue and gonadotrophin chosen for the collection cycle when previously cryopreserved 2PN oocytes were replaced after thawing in the cleavage stage [32].

IVF patients *older than 40 years* undergoing IVF-ET cycles were examined to determine if the use of a mid-cycle GnRH-ant provided better clinical outcomes and lower cancellation rates. In the past, COS in women ≥ 40 years was performed with FSH/hMG only and no GnRH-a or -ant (group I). In subsequent times, following the release of ganirelix, all women ≥ 40 years were stimulated with FSH/hMG + ganirelix (group II). Outcomes of IVF cycles prior to ganirelix were compared to results after its introduction. Cancellation rates were significantly lower in group II (16%) as compared to group I (67%) ($P < 0.05$). In patients with oocytes retrieved, group II had a significantly higher number of recovered oocytes (7.7 ± 0.8 versus 5.3 ± 0.7, $P < 0.05$). However, the number of embryos transferred, cumulative embryo scores, implantation rates and ongoing PR did not differ significantly between groups. Although these results were preliminary, the addition of GnRH-ant avoided ovarian suppression at the start of COH and prevented the premature LH surge at mid-cycle. Thus, more patients attempting IVF underwent oocyte retrieval, although clinical outcomes could not necessarily be improved [33].

With GnRH-ant, *soft stimulation protocols* on the basis of clomiphene pre-treatment should be possible as the pituitary remains fully sensitive at the beginning of the cycle. A prospective trial was carried out on patients undergoing IVF treatment using the multiple-dose GnRH-ant protocol (cetrorelix), clomiphene citrate (CC), and either hMG or rhFSH. Both treatment groups, hMG and rhFSH, yielded comparable results concerning gonadotrophin dose, stimulation days, and PR. A mean number of 6.34 ± 4.4 metaphase II oocytes was retrieved and a mean number of 2.45 ± 0.65 embryos was transferred. However, the overall rate of premature LH surges was 21.5% (defined as measurement of LH > 10 IU/l and $P > 1$ ng/ml) which is unacceptable for clinical practice. Increasing the daily cetrorelix dose from 0.25 to 0.5 mg might decrease the number of premature LH surges. Soft stimulation protocols with clomiphene should be used cautiously [34].

Several Authors have suggested that increased plasma LH levels have deleterious effects on the fertility of women with *PCOS*. Indeed, fewer spontaneous pregnancies with more miscarriages are observed when plasma LH levels are high. ART such as IVF have provided other clues to the role of the LH secretory pattern in women with PCOS. The number of oocytes retrieved, the fertilization rate, and the cleavage rate are lower in PCOS patients undergoing IVF and this is inversely correlated with FSH/LH ratio. These abnormalities are corrected when endogenous secretion of LH is suppressed. On the other hand, implantation and PR after IVF are similar to those observed in control women.

New GnRH-ant are devoid of side effects and suppress LH secretion within a few hours without a flare-up effect. This action lasts for 10–100 h. When GnRH-ant are associated with IV pulsatile GnRH, this combination both suppresses the effect of endogenous GnRH and because of the competition for GnRH-R restores a normal frequency of LH secretion.

In conclusion, the combination of GnRH-ant and GnRH pulsatile treatment can re-establish normal LH secretory pattern in patients with PCOS. The failure to induce ovulation with this regimen suggests the existence of an inherent ovarian defect in women with PCOS [35].

GnRH-ant have been proven safe and effective, with no adverse effects on offspring in animal studies. Careful study of *pregnancy outcome* in humans is mandatory. A preliminary report includes follow-up data of patients treated with the GnRH-ant ganirelix during ovarian stimulation for IVF or ICSI. Patients were randomised in a multicentre, double-blind, dose-finding study of ganirelix, at six different doses ranging from 0.0625 to 2 mg. Follow-up of the pregnant patients revealed 9% of miscarriages. The mean gestational age was 39.4 weeks for singleton pregnancies, and 36.6 weeks for multiple pregnancies. A birth weight <2500 g was reported for 8.7 and 54.2% of the infants resulting from singleton and twins delivery, respectively. One major congenital malformation was diagnosed; a boy with Beckwith–Wiedemann syndrome (exomphalos and macroglossia). Seven minor malformations were reported among five infants. In this first follow-up study, the incidence of adverse obstetrical and neonatal outcome was comparable with reported incidences for IVF-ET pregnancies [36].

When using GnRH-ant in COS, ovulation or maturation of the oocyte can be induced by a variety of drugs, e.g. native GnRH, rhLH, or short-acting GnRH-a. Short-acting GnRH-a were recommended for *triggering ovulation* in cases with a high risk of developing OHSS.

Since it is evident that GnRH is required to initiate the LH surge and the E2 rise, a single administration of GnRH-ant during the late follicular phase delays the LH surge. Studies showed that a single SC administration of 3 or 5 mg of cetrorelix in the late follicular stage was sufficient to prevent the LH surge for 617 days. This phenomenon can be used in *high responder patients* who are at risk for OHSS. The question whether this delay has any effect on oocyte quality and maturation still remains unanswered.

Overall, there are four uses for GnRH-ant:

(1) using short-acting GnRH-a for triggering ovulation in cases in which the GnRH-ant is part of the protocol for ovarian stimulation; rhLH and native GnRH could also be used as triggers of LH surge;
(2) delaying the LH surge in cases of risk to OHSS by treatment with GnRH-ant;
(3) to administer GnRH-ant during the luteal phase to decrease the activity of corpora lutea;
(4) in PCOS with elevated LH the LH/FSH ratio can be corrected with the injection of GnRH-ant prior to and during ovarian stimulation [37].

Another report described the use of 0.2 mg triptorelin to trigger ovulation in patients who underwent COH with rhFSH and concomitant treatment with the GnRH-ant ganirelix for the prevention of premature LH surges. All patients were considered to have an increased risk for developing OHSS (at least 20 follicles ≥11 mm and/or serum E2 at least 3000 pg/ml). On the day of triggering the LH surge, the mean number of follicles ≥11 mm was 25.1 ± 4.5 and the median serum E2 concentration was 3675 pg/ml (range 2980–7670 pg/ml).

After GnRH-a injection, endogenous serum LH and FSH surges were observed with median peak values of 219 and 19 IU/l, respectively, measured 4 h after injection. The mean number of oocytes obtained was 23.4 ± 15.4, of which 83% were mature (metaphase II). None of the patients developed any signs or symptoms of OHSS. So far, four clinical pregnancies have been achieved from the embryos obtained during these cycles, including the first birth following this approach. It is concluded that GnRH-a effectively triggers an endogenous LH surge for final oocyte maturation after ganirelix treatment in stimulated cycles. These preliminary results suggest that this regimen may prove effective in triggering ovulation and could be said to prevent OHSS in high responders [38].

The use of cetrorelix in conjunction with CC and gonadotrophin has been assessed in IVF/gamete intra-fallopian transfer (GIFT) cycles for *poor responders*. Group I included difficult responders (24 cycles) with no live birth in previous IVF cycles with GnRH-a. Group II included patients (7 cycles) with polycystic ovaries. The treatment protocol involved a daily dose of CC 100 mg for 5 days and gonadotrophin injections from cycle day 2. Cetrorelix 0.25 mg per day was started when the leading follicle reached 14 mm. The outcome in both groups was favourable compared to previous treatment with GnRH-a. In group I the abandoned cycle rate was 29% versus 57% ($P = 0.06$). More oocytes were produced (6.4 versus 4.7 oocytes/cycle) at a lower dose of FSH (709 versus 1163 IU/oocyte; $P = 0.08$) and two live births resulted (11.8%). In group II fewer oocytes were produced (10.2 versus 14.5 oocytes/cycle), using a lower dose of gonadotrophin (170 versus 189 IU/oocyte) and resulted in one ongoing pregnancy. No patients experienced OHSS [39].

Patients with a poor response in previous treatment cycles were included in another study. They were divided into two groups: group I received ovarian stimulation for 20 cycles, without the addition of either GnRH-a or -ant; while group II patients received ovarian stimulation for 20 cycles, including the administration of a GnRH-ant (cetrorelix, 0.25 mg daily) during the late follicular phase. There was no statistically significant difference between the groups for mean age, duration of infertility, baseline FSH concentration, cancellation rate, number of ampoules of gonadotrophin used, number of mature oocytes retrieved, E2 concentrations on the day of injection of hCG, fertilization rate, and number of embryos transferred. The clinical pregnancy and implantation rates in group II appeared higher than in group I, but were not significantly different (20 and 13.33% compared with 6.25 and 3.44%, respectively) [40].

5.2. Other indications

For *male hormonal contraception*, combined administration of GnRH-ant and androgens effectively suppresses spermatogenesis to azoospermia [41].

Treatment with GnRH-a of *uterine myoma*, *endometriosis*, and some hormone-dependent *cancers*, such as breast, ovarian, endometrial, and prostate cancer, seems to have a beneficial effect. The use of GnRH-ant, which cause an immediate and dose-related inhibition of LH and FSH by competitive blockade of the receptors, is much more advantageous. Clinical trials in patients are currently in progress and have already shown the usefulness of this new treatment modality [42,43].

5.3. Contraindications

GnRH-ant are contra-indicated in pregnancy and lactation, postmenopausal women, patients with moderate to severe renal or hepatic impairment, and those with hypersensitivity to these drugs, extrinsic peptide hormones, or mannitol. To date no clinically significant drug interactions have been documented.

6. Dosage and administration

The peptide agonists and antagonists currently available require parenteral administration, typically in the form of long-acting depots. A new generation of non-peptide GnRH-ant are beginning to emerge which should allow oral administration and, therefore, may provide greater flexibility of dosing, lower costs and increased patient acceptance [44].

Two different protocols have been investigated (Figs. 8 and 9):

(1) in multiple-doses regimen (the *Lubeck protocol*), small doses of antagonist (0.25 mg once daily by SC injection into the lower abdominal wall) are injected in the morning, starting on day 5 or 6 of ovarian stimulation with gonadotrophins (or each evening starting on day 5 of ovarian stimulation) and continue throughout gonadotrophin treatment, including day of ovulation induction (or evening before ovulation induction) with hCG;

(2) in the single-dose regimen (the *French protocol*), one injection of a larger dose (3 mg by SC injection into the lower abdominal wall) is proposed in the late follicular phase on day 7 of ovarian stimulation with gonadotrophins. If follicle growth does not allow ovulation
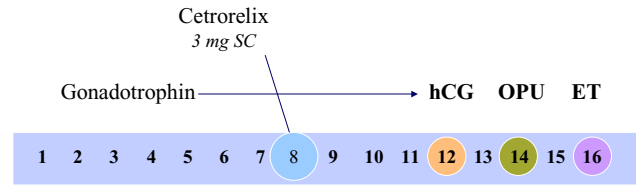

Fig. 8. The "Lubeck Protocol".

Fig. 9. The "French Protocol".

induction on the 5th day after injection, an additional dose of 0.25 mg once daily until the day of ovulation induction is suggested.

The remaining levels of endogenous LH appear to be sufficient in the multiple-doses protocol. In the single an E2 drop is observed in some patient following the 3 mg injection of cetrorelix. This drop is related to the LH decrease. Its adverse effect on IVF results is not demonstrated [45].

7. Advantages/adverse effects

7.1. Advantages

The use of GnRH-ant offers several potential advantages over gonadorelin analogues. Instead of down-regulation and desensitisation, the GnRH-R on the cell membrane are blocked. There is no initial stimulation (flare-up), the treatment period can be shorter, and there is a suggestion that lower doses of gonadotrophins may be required [5,18]. The main advantages for women treated with cetrorelix are the avoidance of hormonal withdrawal side effects (e.g. hot flushes) and the convenience of the dosage regimen including the fact that:

- no pre-treatment is required before gonadotrophin usage;
- results of clinical trials to date suggest that with GnRH-ant much shorter treatment regimens;
- with fewer injections and possibly less gonadotrophin can achieve good clinical results [46];
- fertilization rates of >60% as well as clinical PR of about 30% per transfer sound most promising;
- E2 secretion is not compromised by the GnRH-ant using rhFSH for COH;
- the incidence of a premature LH surge is far below 2% while the pituitary response remains preserved, allowing the induction of ovulation by GnRH or GnRH-a [47,48];
- cetrorelix is being promoted on the basis that it eliminates the need for long term down-regulation, fits into the natural menstrual cycle, reduces the risk of OHSS, and avoids hormonal withdrawal symptoms. These claims can be supported by published data.

Cetrorelix is more expensive than agonists used in COS although there may be some economic benefit in terms of fewer clinic attendances and time off work for the patient.
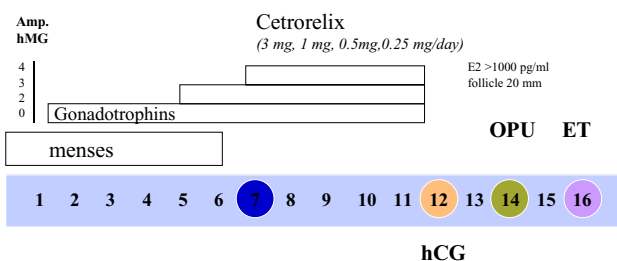
More data are needed before its relative cost-effectiveness can be determined.

7.2. Adverse effects

The most common adverse events are mild transient local injection site reactions, e.g. erythema, itching, and swelling, which may occur. Occasionally asthenia, nausea, malaise, headache, and fatigue have been reported [5,49].

A prospective, randomised phase III clinical trial was conducted to study the influence of an GnRH-ant protocol (cetrorelix) and the use of rhFSH on the development of leukocytosis, compared to the use of urinary hMG (uhMG) on patients undergoing IVF/ICSI treatment following COS using a multiple-dose protocol and the GnRH-ant cetrorelix. Statistically significant increase in white blood cell (WBC) count in the hMG group from the start of stimulation to the mid-luteal phase were detected. No statistically significant increase in the rhFSH group, but only a trend towards higher values was observed. The development of a leukocytosis in COS did not depend on the protocol used [50].

There is a wide variety of functional and morphological effects of GnRH analogues on the ovary. The sometimes paradoxical effects indicate that a variety of factors may be involved in the various processes. Those factors are:

- the type and dose of the analogue;
- the different regimens of administration;
- ovarian status at the time of exposure;
- ovarian cell types in in vitro systems;
- hormonal pre-treatment of these cultures;
- the type of hormonal stimulation added to the in vitro culture;
- further methodological differences in the experiments;
- physiological variations in GnRH-R abundance which depends on species and/or timing in the cycle.

With the increasing number of patients using GnRH analogues in ART, there will be an increasing number of pregnancies exposed to these drugs. So far, there does not appear to be an increased risk of birth defects or pregnancy wastage in human pregnancies exposed to daily low-dose GnRH-a therapy in the first weeks of gestation [51].

8. Efficacy

Open studies in more than 100 women confirmed that cetrorelix as a single-dose (3 or 5 mg) or in a range of multiple-doses significantly suppressed LH and prevented the LH surge [24,52–55]. Two non-randomised dose finding studies evaluated the minimal effective single-dose of cetrorelix and the efficacy of multiple-doses. Cetrorelix 3 mg was compared with 2 mg in women undergoing IVF. Treatment was administered on day 8 of cycle. There was one LH surge and LH was suppressed for a shorter time in the 2 mg group. IVF results were comparable [56]. In another comparison of cetrorelix 0.1, 0.25, or 0.5 mg daily in the multiple-dose regimen, the minimal effective dose was found to be 0.25 mg daily [57].

A multicentre, double-blind, randomised dose-finding study of Org 37462 (ganirelix) was conducted in women undergoing ovarian stimulation with rhFSH to establish the minimal effective dose preventing premature LH surges during ovarian stimulation. Serum Org 37462 concentrations increased in a linear dose-proportional manner, whereas serum LH and increases of E2 fell with increasing Org 37462 dose. During Org 37462 treatment, serum LH concentrations ≥ 10 IU/l were observed in the lowest dose groups with incidences of 16% (0.0625 mg), 9% (0.125 mg), and 1.4% (0.25 mg). On the day of hCG, the number of follicles ≥ 11, ≥ 15, and ≥ 17 mm were similar in the six dose groups, whereas serum E2 concentrations were highest in the 0.0625 mg group (1475 pg/ml) and lowest in the 2 mg group (430 pg/ml).

The median daily dose of rhFSH was between 150 and 183 IU and the overall median duration of Org 37462 treatment was approximately 5 days in the six treatment groups. Overall, Org 37462 treatment appeared to be safe and well tolerated. The mean number of recovered oocytes and good-quality embryos was similar in all dose groups and ranged from 8.6 to 10.0 and 2.5–3.8, respectively. The mean number of replaced embryos in the different dose groups ranged from 2.3 to 2.7. The implantation rate was highest in the 0.25 mg group (21.9%) and lowest in the 2 mg group (1.5%). The early miscarriage rates (first 6 weeks after ET) were 11.9 and 13% in the 1 and 2 mg group, respectively, whereas in the other dose groups this incidence was zero (0.0625%) up to a maximum of 3.7% (0.5 mg group). The vital PR (with heart activity) at 5–6 weeks after ET was highest in the 0.25 mg group, i.e. 36.8% per attempt and 40.3% per transfer, and resulted in an ongoing PR 12–16 weeks after ET of 33.8% per attempt and 37.1% per transfer. In conclusion, a daily dose of 0.25 mg Org 37462 prevented LH surges during ovarian stimulation and resulted in a good clinical outcome [58].

In another trial, a significant drop in plasma LH concentration was observed within a few hours of the first administration of GnRH-ant (cetrorelix) ($P < 0.005$). Moreover, no LH surge was detected at any point in the treatment period in any of the patients. A mean E2 concentration of 2111 ± 935 ng/l was observed on the day of the hCG administration, indicating normal folliculogenesis. Like LH, P concentration also dropped within a few hours of the first administration of cetrorelix ($P < 0.005$) [53].

A study was performed to evaluate the effect of GnRH-ant (Nal-Glu) administration in women after the beginning of the LH surge. Twenty-four hours after administration of the antagonist, the LH surge had been interrupted in all subjects. LH levels fell by 68.5%, E2 by 42%, and FSH by 53.2% [59].

Several studies have directly compared these new stimulation protocols against the long GnRH-a protocol.

A *multi-centre phase III randomised study* compared cetrorelix and buserelin in the prevention of LH surge during ovarian stimulation in IVF/ICSI cycles. The intention to treat response rate, defined as the number of women reaching hCG injection day in the absence of LH surge, was 96.3% for cetrorelix and 90.6% for buserelin. In the cetrorelix group significantly fewer hMG ampoules were administered and the mean number of hMG stimulation days was significantly less ($P < 0.01$). The incidence of premature luteinization with cetrorelix was 1.6%. On the day of hCG administration, more follicles of a small diameter were observed with buserelin ($P = 0.02$) and the mean serum E2 concentration was significantly higher with buserelin ($P < 0.01$). Fertilization and PR were similar in the two groups. The mean number of treatment days was 5.7 days for cetrorelix and 26.6 days for buserelin. The incidence of OHSS World Health Organization (WHO) grades II and III was significantly higher with buserelin (6.5% versus 1.1%, $P = 0.03$) [60].

Another multicenter randomised, prospective study was conducted to confirm the value of a single-dose of 3 mg of cetrorelix versus triptorelin depot in preventing the occurrence of premature LH surges. No LH surge occurred after cetrorelix administration. The patients in the cetrorelix group had a lower number of oocytes and embryos. The percentage of mature oocytes and fertilization rates were similar in both groups, and the PR were not statistically different. The length of stimulation, number of hMG ampoules administered, and occurrence of the OHSS were lower in the cetrorelix group. Tolerance of cetrorelix was excellent [61].

A prospective, randomised study was performed to compare the efficiency of hormonal stimulation for IVF in either the long luteal protocol, using the GnRH-a buserelin, or the multiple-dose GnRH-ant protocol, using the GnRH-ant cetrorelix. The incidence of WHO grade II and grade III OHSS was significantly lower in the cetrorelix than in the buserelin group (1.1% versus 6.5%, $P = 0.03$). The follicle maturation was more homogeneous in the cetrorelix protocol, with less small follicles on the day of hCG administration but a similar number of oocyte cumulus complexes retrieved. The PR per cycle were not significantly different in the cetrorelix and buserelin protocol (22% versus 26%). So, the cetrorelix multiple-dose protocol is advantageous compared to the long protocol regarding the incidence of OHSS, a potentially life threatening complication of COS [62].

A *meta-analysis* was performed to evaluate whether there is a reduction in cases of OHSS and/or a reduction in PR. There was a significant reduction of OHSS cases in the cetrorelix studies (odds ratio, OR, 0.23; 95% confidence interval, CI, 0.10–0.54), but no reduction for ganirelix (OR 1.13; 95% CI 0.24–5.31). The incidence of OHSS WHO grade III cases was reduced in the cetrorelix protocols as compared to the long protocol to a nearly significant degree (OR 0.26; 95% CI 0.07–1.01), while ganirelix did not reduce the incidence of OHSS WHO grade III at all (OR 1.08; 95% CI 0.27–4.38). The PR per cycle was significantly lower in the ganirelix protocols than in the long protocol (OR 0.76; 95% CI 0.59–0.98). The studies using cetrorelix showed quite similar, not significantly different results for the antagonist and the long protocol groups for the PR per cycle (OR 0.91; 95% CI 0.68–1.22). From the data one can conclude that cetrorelix but not ganirelix will reduce the incidence of cases of OHSS and that cetrorelix but not ganirelix will result in the same PR as the long protocol [63].

Randomised controlled studies comparing different protocols of GnRH-ant with GnRH-a in assisted conception cycles were included in a *Cochrane review*. In comparison to the long protocol of GnRH-a, the overall OR for the prevention of premature LH surges was 1.76 (95% CI 0.75, 4.16), which is not statistically significant. There were significantly fewer clinical pregnancies in those treated with GnRH-ant (OR 0.78, 95% CI 0.62, 0.97). The absolute treatment effect (ATE) was calculated to be 5%. The number needed to treat (NNT) was 20. There was a statistically significant reduction in incidence of severe OHSS (relative risk, RR, 0.36, 95% CI 0.16, 0.80) using antagonist regimens as compared to the long GnRH-a protocol.

The reviewer's conclusions were the following:

- the new fixed GnRH-ant protocol (i.e. with antagonist start fixed on day 6 of gonadotrophin-stimulation) is a short and simple protocol with a significant reduction in incidence of severe OHSS but a lower PR compared to the GnRH-a long protocol;
- there is a non significant difference between both protocols regarding prevention of premature LH surge;
- the clinical outcome may be further improved by developing more flexible antagonist regimens taking into account individual patient characteristics; the GnRH-ant flexible regimen should be the area of research in the near future [64,65].

8.1. Implications

GnRH-ant's fixed protocol facilitates short and simple protocol for ovarian stimulation in assisted conception. However, in view of the available data, the GnRH-ant regimens have been associated with a slightly lower pregnancy and implantation rate than the established GnRH-a protocols. This remains the biggest hurdle to their more general acceptance. Differences in serum E2 patterns preceding oocyte retrieval are the most likely contributing factor [47]. So, counseling subfertile couples necessitates before recommending change from GnRH-a to -ant. Cost effectiveness analysis should be carried out to evaluate the difference between the two protocols regarding cost per pregnancy.

The use of a fixed protocol that starts GnRH administration on a fixed day of the cycle with a fixed dose should be re-evaluated because it causes planning problems within the

centres. To overcome some use programming cycle with oral contraceptives but it has an immediate negative effect on the duration of the treatment. Impact of GnRH-ant on the endometrium and subsequent implantation potential should be examined. Other limits are related to the following issues.

- Multicentre trials have significant difference between centres.
- Variation on starting dose.
- Optimal antagonist treatment has not been established.

The future research should focus on:

- safety aspect on ovary-oocytes granulosa cells and embryo;
- lower implantation rate with higher dose of ganirelix which is possibly related to:
  ○ direct effect on embryos;
  ○ endometrium.

9. Conclusions

GnRH analogues have made possible new approaches to the treatment of some hormone-dependent cancers and diseases and conditions which result from inappropriate sex hormone levels. In the fields of both gynaecology and oncology, the development of sustained delivery depot systems has played a key role in the clinical use of GnRH-a and is also essential for the GnRH-ant.

GnRH-a have been employed in IVF-ET programs to prevent a premature rise in LH and various results suggest that the use of antagonist cetrorelix in assisted reproduction procedures, could be even more advantageous.

Two protocols for ART cycles were designed: the *Lubeck protocol* (single-dose) allies simplicity and efficacy, while the *French protocol* (multiple-dose) is efficient and could reduce monitoring of the cycle, though compliance is mandatory. Both protocols using GnRH-ant were associated with the need for a smaller dose of gonadotrophin, a shorter stimulation period and a lower incidence of OHSS, albeit with statistically comparable PR. A trend is observed in all studies showing a lower PR in GnRH-ant cycles as compared with GnRH-a cycles. The role of the lower number of embryos, and the potential adverse effects of GnRH-ant on endometrium or follicle must be studied. More cycles using GnRH-ant are necessary to confirm their equivalent PR. There is room for improvement in both protocols with regard to scheduling, antagonist dose level and the timing of its administration.

Until further studies have been conducted, luteal support seems to remain mandatory. Perinatal outcome appears similar to that with other stimulation regimens. Triggering of ovulation can be obtained with GnRH-a for patients at risk of OHSS. With regard to GnRH-ant, questions remain regarding PR, the indications of their use in patients with PCOS or poor responders, and in ovarian stimulation outside IVF [66,67].

10. Condensation

This article represents an extensive review of all actual GnRH-antagonist applications.

References

[1] Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS. Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. BMJ 1988;297(6655):1024–6.

[2] Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. Fertil Steril 1992;58(5):888–96.

[3] Barlow DH. GnRH agonists and in vitro fertilization. J Reprod Med 1998;43(3 Suppl):245–51.

[4] Schally AV, Nair RM, Redding TW, Arimura A. Isolation of the luteinizing hormone and follicle-stimulating hormone-releasing hormone from porcine hypothalami. J Biol Chem 1971;246(23): 7230–6.

[5] Committee for Proprietary Medicinal Products European Public Assessment Report (EPAR). Cetrotide. The European Agency for the Evaluation of Medicinal Products, 13 April 1999 CPMP/2979/98.

[6] Gonzalez-Barcena D, Buenfil MV, Procel EG, et al. Inhibition of luteinising hormone, follicle-stimulating hormone, and sex-steroid levels in men and women with a potent antagonist analogue of luteinising hormone-releasing hormone, cetrorelix SB-75. Eur J Endocrinol 1994;131:286–92.

[7] Duijkers IJM, Klipping C, Willemsen WNP, et al. Single and multiple dose pharmacokinetics and pharmacodynamics of the gonadotrophin-releasing hormone antagonist cetrorelix in healthy female volunteers. Hum Reprod 1998;13:2392–8.

[8] Sommer L, Zanger K, Dyong T, et al. Seven day administration of the gonadotrophin-releasing hormone antagonist cetrorelix in normal cycling women. Eur J Endocrinol 1994;131:280–5.

[9] Leroy I, d'Acremont MF, Brailly-Tabard S, Frydman R, de Mouzon J, Bouchard P. A single injection of gonadotrophin-releasing hormone GnRH antagonist cetrorelix postpones the luteinising hormone LH surge: further evidence for the role of GnRH during the LH surge. Fertil Steril 1994;62:461–7.

[10] Felberbaum RE, Reissmann T, Kupker W, et al. Preserved pituitary response under ovarian stimulation with HMG and GnRH antagonist cetrorelix in women with tubal infertility. Eur J Obstet Gynaecol Reprod Biol 1995;61:151–61.

[11] Duffy DM, Stewart DR, Stouffer RL. Titrating luteinizing hormone replacement to sustain the structure and function of the corpus luteum after gonadotropin-releasing hormone antagonist treatment in rhesus monkeys. J Clin Endocrinol Metab 1999;84(1):342–9.

[12] Albano C, Grimbizis G, Smitz J, et al. The luteal phase of nonsupplemented cycles after ovarian superovulation with human menopausal gonadotrophin and the gonadotrophin-releasing hormone antagonist cetrorelix. Fertil Steril 1998;70:357–9.

[13] Lin Y, Kahn JA, Hillensjo T. Is there a difference in the function of granulosa-luteal cells in patients undergoing in-vitro fertilisation either with gonadotrophin-releasing hormone agonist or gonadotrophin-releasing hormone antagonist? Hum Reprod 1999; 14:885–8.

[14] Nelson LR, Fujimoto VY, Jaffe RB, Monroe SE. Suppression of follicular phase pituitary-gonadal function by a potent new gonadotropin-releasing hormone antagonist with reduced histamine-releasing properties (ganirelix). Fertil Steril 1995;63(5):963–9.

[15] Broqua P, Riviere PJ, Conn PM, Rivier JE, Aubert ML, Junien JL. Pharmacological profile of a new, potent, and long-acting gonadotropin-releasing hormone antagonist: degarelix. J Pharmacol Exp Ther 2002;301(1):95–102.

[16] Royal College of Obstetricians and Gynaecologists. The management of infertility in tertiary care, UK, Evidence-Based Clinical Guideline 2000;6.

[17] Kiesel L, Runnebaum B. Gonadotropin releasing hormone and analogs. Physiology and pharmacology. Gynakol Geburtshilfliche Rundsch 1992;32:22–30.

[18] Reissmann TH, Felberbaum R, Diedrich K, Engel J, Comaru-Schally AM, Schally AV. Development and applications of luteinising hormone-releasing hormone antagonists in the treatment of infertility: an overview. Hum Reprod 1995;10:1974–81.

[19] Fauser BC, Laven JS, de Jong D, Macklon NS. Gonadotrophin-releasing hormone antagonists: application in ovary-stimulating and sex-steroid dependent disorders. Ned Tijdschr Geneeskd 2000;144(8):370–4.

[20] Nagaraja NV, Pechstein B, Erb K, Klipping C, Hermann R, Niebch G, et al. Pharmacokinetic and pharmacodynamic modeling of cetrorelix, an LH-RH antagonist, after subcutaneous administration in healthy premenopausal women. Clin Pharmacol Ther 2000;68(6):617–25.

[21] Oberye JJ, Mannaerts BM, Kleijn HJ, Timmer CJ. Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Antagon/Orgalutran). Part I. Absolute bioavailability of 0.25 mg of ganirelix after a single subcutaneous injection in healthy female volunteers. Fertil Steril 1999;72(6):1001–5.

[22] Oberye JJ, Mannaerts BM, Huisman JA, Timmer CJ. Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Antagon/Orgalutran). Part II. Dose-proportionality and gonadotropin suppression after multiple doses of ganirelix in healthy female volunteers. Fertil Steril 1999;72(6):1006–12.

[23] Felberbaum R, Reissmann T, Zoll C, Kupker W, al-Hasani S, Diedrich C, et al. GnRH antagonists in gynecology: initial results within the scope of controlled ovarian hyperstimulation. Gynakol Geburtshilfliche Rundsch 1995;35(Suppl 1):113–7.

[24] Olivennes F, Fanchin R, Bouchard Ph, Taieb J, Selva J, Frydman R. Scheduled administration of a gonadotrophin-releasing hormone antagonist cetrorelix on day 8 of in-vitro fertilization cycles: a pilot study. Hum Reprod 1995;10:1382–6.

[25] Nelson LR, Fujimoto VY, Jaffe RB, Monroe SE. Suppression of follicular phase pituitary-gonadal function by a potent new gonadotropin-releasing hormone antagonist with reduced histamine-releasing properties (ganirelix). Fertil Steril 1995;63(5):963–9.

[26] Olivennes F, Fanchin R, Bouchard P, Taieb J, Frydman R. Triggering of ovulation by a gonadotropin-releasing hormone (GnRH) agonist in patients pretreated with a GnRH antagonist. Fertil Steril 1996;66(1):151–3.

[27] Ubaldi F, Albano C, Peukert M, Riethmuller-Winzen H, Camus M, Smitz J, et al. Subtle progesterone rise after the administration of the gonadotrophin-releasing hormone antagonist cetrorelix in intracytoplasmic sperm injection cycles. Hum Reprod 1996;11(7):1405–7.

[28] de Jong D, Macklon NS, Mannaerts BM, Coelingh Bennink HJ, Fauser BC. High dose gonadotrophin-releasing hormone antagonist (ganirelix) may prevent ovarian hyperstimulation syndrome caused by ovarian stimulation for in vitro fertilization. Hum Reprod 1998;13(3):573–5.

[29] Itskovitz-Eldor J, Kol S, Mannaerts B, Coelingh Bennink H. First established pregnancy after controlled ovarian hyperstimulation with recombinant follicle stimulating hormone and the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462). Hum Reprod 1998;13(2):294–5.

[30] Meldrum DR, Rivier J, Garzo G, Wisot A, Stubbs C, Hamilton F. Successful pregnancies with unstimulated cycle oocyte donation using an antagonist of gonadotropin-releasing hormone. Fertil Steril 1994;61(3):556–7.

[31] Anderson RA, Kinniburgh D, Baird DT. Preliminary experience of the use of a gonadotrophin-releasing hormone antagonist in ovulation induction/in-vitro fertilization prior to cancer treatment. Hum Reprod 1999;14(10):2665–8.

[32] Seelig AS, Al-Hasani S, Katalinic A, Schopper B, Sturm R, Diedrich K, et al. Comparison of cryopreservation outcome with gonadotropin-releasing hormone agonists or antagonists in the collecting cycle. Fertil Steril 2002;77(3):472–5.

[33] Chang PL, Zeitoun KM, Chan LK, Thornton 2nd MH, Sauer MV. GnRH antagonist in older IVF patients. Retrieval rates and clinical outcome. J Reprod Med 2002;47(4):253–8.

[34] Engel JB, Ludwig M, Felberbaum R, Albano C, Devroey P, Diedrich K. Use of cetrorelix in combination with clomiphene citrate and gonadotrophins: a suitable approach to "friendly IVF"? Hum Reprod 2002;17(8):2022–6.

[35] Lubin V, Charbonnel B, Bouchard P. The use of gonadotrophin-releasing hormone antagonists in polycystic ovarian disease. Baillieres Clin Obstet Gynaecol 1998;12(4):607–18.

[36] Olivennes F, Mannaerts B, Struijs M, Bonduelle M, Devroey P. Perinatal outcome of pregnancy after GnRH antagonist (ganirelix) treatment during ovarian stimulation for conventional IVF or ICSI: a preliminary report. Hum Reprod 2001;16(8):1588–91.

[37] Ron-El R, Raziel A, Schachter M, Strassburger D, Kasterstein E, Friedler S. Induction of ovulation after gnRH antagonists. Hum Reprod Update 2000;6(4):318–21.

[38] Itskovitz-Eldor J, Kol S, Mannaerts B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report: short communication. Hum Reprod 2000;15(9):1965–8.

[39] Craft I, Gorgy A, Hill J, Menon D, Podsiadly B. Will GnRH antagonists provide new hope for patients considered 'difficult responders' to GnRH agonist protocols? Hum Reprod 1999;14(12):2959–62.

[40] Akman MA, Erden HF, Tosun SB, Bayazit N, Aksoy E, Bahceci M. Addition of GnRH antagonist in cycles of poor responders undergoing IVF. Hum Reprod 2000;15(10):2145–7.

[41] Behre HM, Kliesch S, Lemcke B, von Eckardstein S, Nieschlag E. Suppression of spermatogenesis to azoospermia by combined administration of GnRH antagonist and 19-nortestosterone cannot be maintained by this non-aromatizable androgen alone. Hum Reprod 2001;16(12):2570–7.

[42] Reissmann T, Schally AV, Bouchard P, Riethmiiller H, Engel J. The LHRH antagonist cetrorelix: a review. Hum Reprod Update 2000;6(4):322–31.

[43] Albano C, Platteau P, Devroey P. Gonadotropin-releasing hormone antagonist: how good is the new hope? Curr Opin Obstet Gynecol 2001;13(3):257–62.

[44] Millar RP, Zhu YF, Chen C, Struthers RS. Progress towards the development of non-peptide orally-active gonadotropin-releasing hormone (GnRH) antagonists: therapeutic implications. Br Med Bull 2000;56(3):761–72.

[45] Olivennes F. LH and GnRH antagonists. J Gynecol Obstet Biol Reprod (Paris) 2002;31(2 Pt 2):1S25–7.

[46] Felberbaum R, Diedrich K. Ovarian stimulation for in-vitro fertilization/intracytoplasmic sperm injection with gonadotrophins and gonadotrophin-releasing hormone analogues: agonists and antagonists. Hum Reprod 1999;14(1):207–21.

[47] Gordon K. Gonadotropin-releasing hormone antagonists implications for oocyte quality and uterine receptivity. Ann NY Acad Sci 2001;943:49–54.

[48] Diedrich K, Ludwig M, Felberbaum RE. The role of gonadotropin-releasing hormone antagonists in in vitro fertilization. Semin Reprod Med 2001;19(3):213–20.

[49] Gillies PS, Faulds D, Balfour JA, Perry CM. Ganirelix Drugs 2000;59(1):107–11.

[50] Ludwig M, Strik D, Felberbaum R, Al-Hasani S, Diedrich K. No significant leukocytosis under controlled ovarian stimulation using the LHRH antagonist Cetrorelix and recFSH. Eur J Obstet Gynecol Reprod Biol 2000;89(2):177–9.

[51] Hanssens RM, Brus L, Cahill DJ, Huirne JA, Schoemaker J, Lambalk CB. Direct ovarian effects and safety aspects of GnRH agonists and antagonists. Hum Reprod Update 2000;6(5):505–18.

[52] Felberbaum RE, Reissmann T, Kupker W, et al. Preserved pituitary response under ovarian stimulation with HMG and GnRH antagonist cetrorelix in women with tubal infertility. Eur J Obstet Gynaecol Reprod Biol 1995;61:151–61.

[53] Albano C, Smitz J, Camus M, Bennink HC, Van Steirteghem AC, Devroey P. Hormonal profile during the follicular phase in cycles stimulated with a combination of human menopausagonadotrophin and gonadotrophin-releasing hormone antagonist cetrorelix. Hum Reprod 1996;11:2114–8.

[54] Diedrich K, Diedrich C, Santos E, et al. Suppression of the endogenous luteinising hormone surge by the gonadotrophin-releasing hormone antagonist cetrorelix during ovarian stimulation. Hum Reprod 1994;9:788–91.

[55] Olivennes F, Fanchin R, Bouchard PH, et al. The single or dual administration of the gonadotrophin-releasing hormone antagonist cetrorelix in an in vitro fertilization—embryo transfer programme. Fertil Steril 1994;62:468–76.

[56] Olivennes F, Alvarez S, Bouchard P, Fanchin R, Salat-Baroux J, Frydman R. The use of a GnRH antagonist Cetrorelix in a single dose protocol in IVF-embryo transfer: a dose finding study of 3 mg versus 2 mg. Hum Reprod 1998;13:2411–4.

[57] Albano C, Smitz J, Camus M, Riethmuller-Winzen H, Van Steirteghem A, Devroey P. Comparison of different doses of gonadotrophin-releasing hormone antagonist cetrorelix during controlled ovarian hyperstimulation. Fertil Steril 1997;65:917–22.

[58] The Ganirelix Dose-Finding Study Group. A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). Hum Reprod 1998;13,(11):3023-31.

[59] Christin-Maitre S, Olivennes F, Dubourdieu S, Chabbert-Buffet N, Charbonnel B, Frydman R, et al. Effect of gonadotrophin-releasing hormone (GnRH) antagonist during the LH surge in normal women and during controlled ovarian hyperstimulation. Clin Endocrinol (Oxf) 2000;52(6):721–6.

[60] Albano C, Felberbaum RE, Smitz J, et al. Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone LHRH-antagonist cetrorelix and the LHRH-agonist buserelin. Hum Reprod 2000;153:526–31.

[61] Olivennes F, Belaisch-Allart J, Emperaire JC, Dechaud H, Alvarez S, Moreau L, et al. Prospective, randomized, controlled study of in vitro fertilization-embryo transfer with a single dose of a luteinizing hormone-releasing hormone (LH-RH) antagonist (cetrorelix) or a depot formula of an LH-RH agonist (triptorelin). Fertil Steril 2000;73(2):314–20.

[62] Ludwig M, Felberbaum RE, Devroey P, Albano C, Riethmuller-Winzen H, Schuler A, et al. Significant reduction of the incidence of ovarian hyperstimulation syndrome (OHSS) by using the LHRH antagonist Cetrorelix (Cetrotide) in controlled ovarian stimulation for assisted reproduction. Arch Gynecol Obstet 2000;264(1):29–32.

[63] Ludwig M, Katalinic A, Diedrich K. Use of GnRH antagonists in ovarian stimulation for assisted reproductive technologies compared to the long protocol. Meta-analysis. Arch Gynecol Obstet 2001;265(4):175–82.

[64] Al-Inany H, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. Cochrane Database Syst Rev 2001;(4):CD001750.

[65] Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod 2002;17(4):874–85.

[66] Olivennes F, Fanchin R, Ledee N, Righini C, Bouchard P, Frydman R. GnRH antagonists in IVF. J Gynecol Obstet Biol Reprod (Paris) 2001;30(7 Part 1):657–62.

[67] Olivennes F, Cunha-Filho JS, Fanchin R, Bouchard P, Frydman R. The use of GnRH antagonists in ovarian stimulation. Hum Reprod Update 2002;8(3):279–90.