Ovulation Induction in In-Vitro Fertilization

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Reproductive endocrinology, a relatively new subspecialty of obstetrics and Gynecology, came of age during the 1980s. The discipline has benefited greatly from substantial recent advances in reproductive biology and allied fields and technologic improvements in computers, ultrasonography, and surgical instrumentation. All of these developments have been applied to clinical practice at an unprecedented rate. [1]

Introduction
Reproductive endocrinology, a relatively new subspecialty of obstetrics and Gynecology, came of age during the 1980s. The discipline has benefited greatly from substantial recent advances in reproductive biology and allied fields and technologic improvements in computers, ultrasonography, and surgical instrumentation. All of these developments have been applied to clinical practice at an unprecedented rate. [1]

The work of Steptoe and Edwards resulted in the birth of the first in-vitro fertilization (IVF.) infant; the conception was the product of a spontaneous cycle. [2]

During their initial effort human menopausal gonadotropins (hMG) were utilized for ovarian stimulation to produce multiple follicles for ovum retrieval. [3]

Their technique using the spontaneous cycle was based on endocrine abnormalities and luteal phase defects (LPD) associated with stimulation. [4]

However, in view of the relatively low pregnancy rate, due not only to the presence of a single
Several groups of investigators again adopted the use of ovulation inducing agents. Indeed, these workers soon demonstrated that not only was it possible to produce pregnancies in cycles in which the patient received ovulation-inducing agents, but actually the percentage of patients successfully undergoing oocyte recovery and ultimately embryo replacement and pregnancy was substantially higher.

Accordingly, all established groups today rely on the use of ovulation-inducing agents to increase the number of preovulatory follicles, and thus, ultimately the number of embryos available for replacement.

**Ovarian simulation**

The first pregnancy obtained by IVF and embryo transfer was obtained using ovarian stimulation but it proved to be an ectopic pregnancy. The first full-term pregnancies were achieved with oocytes from unstimulated cycles. Subsequent studies, however, have shown that ovarian stimulation is associated with better results. Hence, most centres are now performing IVF and other assisted reproduction techniques in stimulated cycles. The following agents are used to stimulate the ovaries:

(i) clomiphene citrate, alone or in combination with hMG (concomitantly or sequentially),

(ii) hMG,

(iii) purified FSH, alone or in combination with hMG,

(iv) highly purified urinary FSH and

(v) recombinant FSH.

GnRH agonists are administered intra-nasally, s.c. or i.m., in the long, short or ultra short protocol and in combination with hMG and/or purified FSH.

GnRH antagonists are involved in the final steps of oocyte maturation, which is achieved by the administration of HCG or by the endogenous luteinizing hormone (LH) surge. Oocyte aspiration is performed 34-38 h after HCG injection or 26-28 h after the detection of an endogenous LH surge.

Follicular development can be monitored by serial hormonal measurements (oestradiol, LH, progesterone) and by ultrasonography. The use of these indices may optimize ovarian stimulation and lower the incidence of OHSS. On the other hand, the application of GnRH agonists requires less monitoring.

**Physiology**

**Spontaneous cycle**

A cohort of primordial follicles are continuously initiating follicular growth independent of gonadotropin stimulation. Once the growing follicle reaches the preantral stage, however, appropriate levels of gonadotropins, particularly follicle stimulating hormone (FSH) are required for development to the preovulatory stage. The presence of FSH induces an increase in estrogen production from the follicle and synergistically, the estrogen and FSH increase the FSH receptor content of the growing follicle.

In a spontaneous cycle the levels of FSH are rising immediately prior to and during menses.

The follicle that is at the appropriate preantral stage of development when the FSH begins increasing is selected to become the sole surviving or dominant follicle. As this soon-to-be dominant follicle begins growing and producing increasing amounts of estrogen, FSH production decreases through negative feedback, thus heralding the death (or atresia) of the less developed follicles. The role of ovulation-inducing agents for in vitro fertilization is to disturb this normal relationship by increasing the amounts of FSH available to follicles other than the dominant follicles and thus to increase the total number of follicles that reach the preovulatory stage.

**Clomiphene Citrate (CC)**

The single agent most commonly used for enhanced follicular recruitment for in-vitro fertilization has been clomiphene citrate.
Clomiphene citrate was first synthesized in 1956, introduced for clinical trials in 1960, and approved for clinical use in the United States in 1967. Clomiphene is available in 50 mg. tablets, under the trade names of Clomid, and Serophene. It is a mixture of two stereo-chemical isomers which have anti- and weak oestrogenic properties (the En and Zu isomers, respectively). The anti-oestrogenic properties effect ovarian activity via an increase in endogenous gonadotropin secretion from the pituitary. Current clinical preparations contain about 40% Zu and 60% En isomer.

There are problems associated with CC use:

- Its effects are long-lasting. After a standard five-day course of treatment (100 mg daily, starting between the third and the fifty day of spontaneous or induced bleeding), binding activity was detected on day 14 and, in some patient, on day 22 of the cycle.
- The Zu (but not En) isomer is long acting. Significant plasma concentrations of the Zu isomer were detected up to one month after treatment.
- The En isomer is the active component in initiating follicular development. The Zu isomer does not significantly affect the number of follicles present, or oestradiol or luteal phase progesterone.
- There is a reported higher incidence of subclinical loss in clomiphene citrate-induced pregnancies compared to the normal population.
- The induced increase in luteinsing hormone (LH) secretion can far exceed that for follicle stimulating hormone (FSH), which is further exaggerated in a polycystic ovaries (PCO) patient. High LH has been associated with miscarriage, which in a PCO patient can be best corrected by the use of a gonadotropin-releasing hormone (GnRH) agonist.
- There is a high reported incidence of luteinised unruptured follicle (LUF) syndrome in patients with unexplained infertility. The anti-oestrogenic effects are at the level of the cervix and endometrium.
- There is an increased incidence of ectopic pregnancies in in vitro fertilisation (IVF).
- There is substantial literature support for a possible direct adverse effect at the level of the rat, rabbit and human oocyte.

**Clomiphene citrate in IVF**

Saunders et al (1992) have associated the use of clomiphene citrate in superovulation cycles with a higher miscarriage rate than GnRH agonist. Corson and Batzer (1986) and Cohen et al (1986) have suggested a relationship between clomiphene citrate use and ectopic pregnancy. However, Grab et al (1992) believe that the higher rate of ectopic pregnancies in patients treated with clomiphene citrate is more likely to be associated with the diagnosis of infertility. Harrison et al (1993) have reported a trend towards an increasing ectopic pregnancy rate with increasing daily doses of clomiphene citrate. (This could be related to clomiphene citrate effects on tubal transport). They state that, although clomiphene citrate “remains a valuable tool in the treatment of infertility ... until its does-effect relationship with pregnancy loss is clarified, it would seem prudent to use the minimum possible dose”.

Gonen and Casper (1990) found that the endometrium was thinner following the use of clomiphene citrate with hMG compared to hMG alone in IVF patients who had a thin endometrium in a previous clomiphene citrate/hMG cycle. This may be due to the anti-oestrogenic effect of clomiphene citrate on the endometrium.

A report in 1983 demonstrated that 50 mg. per day of CC, when given on cycle days 5 - 9 produced statistically identical degrees of enhanced follicular recruitment (size and number) compared with higher dosage.

In 1995 Benadiva et al reported that: selected patients who failed previous IVF attempts...
with gonadotropins with or without GnRH analogues may benefit from the addition of CC to their ovarian stimulation protocol (45).

In 1998 an open randomized study of IVF in natural cycles or with clomiphene citrate (CC) in the more fertile younger patients and those with normal ovulatory function was done, and the authors were concluded that CC was an acceptable alternative to GnRH-a and FSH yielding a comparable success rate per embryo transfer, but with a low twin rate and if patients accept the increased cycle cancellation rates (40% in natural and 20% in CC cycles), CC may replace GnRH a in selected patient groups in clinics with otherwise high implantation rates, whereas natural cycles IVF seems to be too inefficient for routine use. A negative anti-oestrogenic effect of CC on Oocyte fertilization, embryo development or implantation rates was not detected (46).

**Effect on human oocytes and embryo development**

More information is now available on the effects of clomiphene citrate on gametes. Yoshimura et al (1988) (47) demonstrated no effect of clomiphene citrate administrated to perfused rabbit ovaries on either ovulation or fertilisation rates, but a significant reduction in the number offspring resulting from embryo transfer. Administration of oestrogen to the perfusate reversed this effect, suggesting that the anti-oestrogenic effects of clomiphene citrate may affect post-fertilisation development. Clomiphene citrate also decreases the fertilisation rate in mouse oocytes (48). In the human, Oelsner et al (1987) (49) have measured high concentrations of clomiphene citrate isomers, particularly the Zu isomer, in follicular fluid obtained at the time of oocyte recovery in women undergoing IVF using clomiphene citrate for superovulation (49). They reported a direct relationship between the rate of degeneration of blastocysts and the concentration of clomiphene citrate. Wramsby et al (1987) (50) reported a 50% incidence of abnormal chromosome karyotype in 23 human oocytes obtained at laparoscopy from women treated with clomiphene citrate (50). While reports to date are largely preliminary, they suggest that clomiphene citrate has a widespread effect which may help to explain the low pregnancy rate. (49)

**Safety**

**Side-effects**

Minor side-effects do occur, but they rarely interfere with treatment. About 10% of women complain of hot flushes during administration; the concomitant administration of oestrogen does not alleviate these (51). Among almost 4,000 women reviewed by Kistner (1968) (52) less than 2% complained of other minor side-effects such as nausea, vomiting, breast tenderness, dizziness, mild skin reaction and reversible hair loss (52). Some women (1.6%) noted mild visual disturbances which resolved once the drug was withdrawn.

Two other major side-effects of clomiphene citrate administration are those associated with ovarian stimulation and ovulation induction. Clomiphene citrate induces multiple follicular development, and ovarian hyperstimulation can occur. It occurs less often, however, than following ovulation induction with conventional gonadotropin therapy, although chronic, low-dose, gonadotropin protocols reportedly cause significantly less ovarian hyperstimulation syndrome and multiple births (53). Rust et al (1974) (54) reported ovarian cysts in 6.7% of women studied (54). The duration of therapy is probably more important than the dose of clomiphene citrate used (55) cysts usually resolve spontaneously in a few weeks, and cases of full-blown hyperstimulation with nausea, vomiting, ascites and hydrothorax are rare (57) (58). Additionally, bilateral adnexal torsion has been reported after CC therapy (55). As a consequence of multiple follicular development, multiple pregnancy does occur after ovulation induction with CC (6-7% (59), 17.8% (60)). While the majority are twin pregnancies, triplets and higher multiples have been reported.

A recent study, which considered 3837 women treated for infertility, between 1974 and 1985, has highlighted that long-term CC use may increase the risk of ovarian cancer (61), however as only eight women with cancer were identified in this study, more powerful studies are needed to confirm or refutes these results. The pregnancy rate in both long term (>12 cycles) and short-term CC users was similar. Thus it is recommended that a patient’s cause of infertility should be reassessed if she has not conceived after a maximum of six treated cycles (61).

**Human menopausal gonadotropin (hMG)**

**hMG Alone**

**Low Dose**

The principal experience with the “physiologic” use of hMG for follicular recruitment comes from the
Eastern Virginia Medical School. Those investigators adapted their extensive experience with hMG for ovulation induction in anovulatory women to its use for enhanced follicular recruitment. Typically, two ampules of hMG were administered daily beginning on the third or fifth cycle day, depending upon the length of the preceding cycle. Based on the clinical response of the patients (cervical mucus changes and vaginal cytology) as well as the measured levels of serum estradiol, hMG administration was continued until the appropriate degree of follicular development was achieved, at which time the preovulatory dose of hCG was given. In their initial experience with this regimen, 2.0 and 2.4 oocytes were recovered per patient undergoing laparoscopy.

**High Dose**

In an attempt to further increase the number of oocytes obtained per patient, a group at Yale University pioneered the use of relatively high doses of hMG for enhanced follicular recruitment. These investigators administered 3 ampules per day of hMG from cycle days 3 through 7, followed by a stepwise increase in the hMG dosage from cycle day 8 until there were at least two follicles 16-18 mm in mean diameter, at which time hCG was administered. Using this regimen, they reported the recovery of mean of 3.2 oocytes per patient undergoing laparoscopy. In a randomized comparison of high-dose hMG alone compared with 50 mg of clomiphene citrate per day for cycle days 5 through 9, they recovered in the hMG group a mean of 4.6 oocytes per patient undergoing laparoscopy (Table 1). The patients in the hMG group received 4 ampules per day of hMG, beginning on cycle day 3 and continuing until the day before hCG administration. This resulted in a marked increase in the measured levels of FSH, particularly when compared with the levels seen in spontaneous cycles. In this study hCG was administered on the evening of the day that there were at least two follicles greater than or equal to 16 mm in mean diameter.

When the length of the luteal phase in the patients receiving clomiphene alone was compared with the luteal length in the hMG patients, there was a highly significant shortening among the hMG group (Table 2). Edwards and co-workers (1980) had previously noted an inverse correlation between the peak follicular phase estrogen secretion and luteal length in a group of patients receiving high doses of hMG. They postulated that the large amount of estrogen produced by the multiple follicles developing in response to the ovarian “hyperstimulation” interfered with subsequent corpus luteum function.

**Clomiphene/hMG Combination**

Clomiphene and hMG were used in order to maximize the recovery of fertilizable oocytes while minimizing the degree of ovarian hyperstimulation and its associated detrimental effect on the length of the luteal phase. In a prospective, randomized comparison of clomiphene alone (50 mg/day, cycle days 5-9) against the same regimen of clomiphene plus 2 ampules/day of hMG given on cycle days 6, 8, and 10, there was a statistically significant increase in the number of follicles developing per patient and the number of oocytes recovered per patient, and an increased, but not statistically increased, number of embryos transferred to each patient. In that study a mean of 2.8 oocytes were obtained per patient in the combination group (Table 3). Interestingly, in that study there was no statistical difference in the measured levels of FSH between the two patient groups. However, by the time the daily blood samples were obtained, the additional FSH from the previous day’s hMG injection was probably cleared, in view of the approximately 3-hour half-life of FSH. Other groups have also reported the use of differing combinations of clomiphene and hMG for enhanced follicular recruitment. Lopata (1983) reported recovering a mean of 4.6 oocytes/laparoscopy from patients who had received several different regimens of concurrent or sequential clomiphene and hMG.

A group at the University of Southern California (1983) reported the development of 4.5 follicles per patient, the transfer of 2.4 embryos per patient, and one pregnancy in a group of 13 patients receiving a combination of 150 mg/day of clomiphene on cycle days 3-7 and 2 ampules/day of hMG on cycle days 3, 5, and 7-11. Mandelbaum et al. (1983) reported a mean of 3.5 follicles per patient and three successful pregnancies in an unspecified number of patients receiving a combination of clomiphene, 100-150 mg/day, on days 5-9 and hMG, 2-3 ampules/day, on days 6, 8, and 10. Another trial used 50 mg of clomiphene per day on days 5-9, plus 1 ampule of hMG per day on cycle days 5-9, and continued the hMG at a dose of 1-3 ampules/ day based on peripheral estradiol values and the follicular size, number, and growth rate. This regimen has resulted in recovery of a mean of 3.4 oocytes per patient and a 20% clinical pregnancy rate per laparoscopy.
In a retrospective study of 813 oocyte retrieval-embryo transfer cycles in women with normal follicle stimulating hormone and luteinizing hormone concentrations, the relationship between the amount of human menopausal gonadotropin (hMG) used for ovarian stimulation and treatment outcome was investigated. Patients were divided into three groups: group A patients (495 cycles) required < 40 ampoules of hMG and had a predicted probability for pregnancy of 25% per embryo transfer; group B patients (165 cycles) required 41-77 ampoules per cycle, with a predicted probability rate for pregnancy of 5-25% per embryo transfer; and group C patients (153 cycles) required > 77 ampoules of hMG and the predicted probability for pregnancy was < 5% per embryo transfer. Groups C and A differed significantly (P < 0.005). The mean oestradiol concentration on the day of HCG administration in group C was 6412 pmol/L, and the mean number of eggs retrieved was seven. The highest success rates were found when up to 2.5 ampoules of hMG were required for each egg or 4.4 ampoules for each embryo. The lowest rates were obtained when > 4.8 ampoules of hMG were necessary for each oocyte or > 9.6 ampoules for each embryo (P < 0.005) (72).

Gonadotropin releasing hormone analogue (GnRHa)
The introduction of gonadotropin releasing hormone against (GnRHa) prior to and concomitant with human menopausal gonadotropin stimulation has provided one anticipated and one unexpected advantage (73) (74). It eliminates the possibility of premature LH surges and in addition it has provided some increase in the success rate of IVF. The GnRH agonist given either by subcutaneous injection or by nasal spray, can in prescribed doses, cause a down regulation of the pituitary instead of the normal stimulatory effect. FSH and LH secretion are decreased and ovarian follicle activity follows suit. The waves of oocytes that begin growth are inhibited. Therefore, when stimulation with pergonal is initiated, the ovary is in a resting state. It is uncertain why this may confer an advantage during IVF. In addition to preventing premature LH surges and premature luteinization (and progesterone production), it may decrease LH stimulation of ovarian androgen production (which can interfere with follicular development) (10) (22).

Hypothalamic GnRH plays a critical role in the neurohormonal control of reproduction by stimulating the secretion of the pituitary gonadotropins LH and FSH., which support the development of gonads, gametogenesis and the production and release of gonadal steroids. At the pituitary level, GnRH interacts with specific G protein-coupled receptors located on the surface of gonadotrophs and triggers the generation of an array of second messengers and the activation of several intracellular pathways, to regulate in an integrated manner the synthesis and release of gonadotropins. These include the activation of phosphoinositidase C with the ensuing production of diacylglycerol and inositol-trisphosphate, which are responsible for the activation of protein kinase C and the mobilization of intracellular Ca2+ respectively. GnRH also induces the activation of phospholipases D and A2, production of cAMP and cGMP and, under certain circumstances the activation of tyrosine kinases and the MAP kinase cascade. In addition, evidence exists suggesting the presence of extrapituitary receptors which respond to locally produced GnRH (in gonads, placenta, mammary gland etc.). Ligand analogues which interact with the GnRH receptor, and activate or inactivate the intracellular signaling cascade and cellular functions are widely used to treat a variety of diseases, including breast and prostatic cancer, infertility, endometriosis and precocious puberty. These analogues have been designed empirically and represent the outcome of a great number of in-vitro or in-vivo structure function studies with chemically synthesized GnRH analogues or natural GnRHs. (75)

Following their introduction to gynecological practice in the 1980s, the indications and uses have expanded enormously, none more so than in the treatment of aspects of infertility and in particular in their use in assisted reproduction programs. (74)

The amino acid sequencing of gonadotropin releasing hormone (GnRH) was first determined in 1971 (76). Because of its short biological activity, analogues were synthesized by substituting other amino acid bases or complex molecules. (77). These were initially used to treat sex-hormones dependant tumours, particularly cancer of the prostate. However by inducing low levels of pituitary gonadotropins, it was realized that the role of GnRH as could be extended to include the endocrinological manipulation of infertile patients (78).
In 1982, Meldrum et al. (79) first suggested the use of GnRH-a to create a “medical oophorectomy” in the treatment of endometriosis and in the same year Fleming et al. (80) described the use of GnRH-a in combination with gonadotropins for ovulation induction. Shortly after, in 1984, the first report of GnRH-a use in in vitro fertilization (81) was published. Their use in assisted reproduction has resulted in reduced cycle cancellation, convenient timing of treatment, and higher live birth rates (82), as a result most units now use GnRH-a routinely despite the extra costs involved. Following increased use in assisted reproductive treatments, in 1990, Abdalla et al. (83) described the successful use of GnRH-a in women with polycystic ovary syndrome (PCOS) and recurrent miscarriage. The role of GnRH-a in ovarian hyperstimulation syndrome (OHSS) is unclear, but in 1990, Gonen et al. (84) used GnRH-a to induce ovulation in patients at risk of OHSS.

Physiology and Mechanism of Action:

**GnRH is a small peptide of ten amino-acid bases (85):**
The peptide is secreted from the neuron terminals of the hypothalamus in the median eminence and released into the hypothalamic pituitary portal blood system. It reaches the gonadotrophs of the anterior pituitary before dilution and degradation in the peripheral tissues.

The circulating of half-life of the peptide in the general circulation is about 8 minutes (86) and in 1980, Ernst Knobil (87) established the pulsatile pattern of release of 10 minute “bursts” every sixty minutes. Under normal conditions, the pulsatile secretion of GnRH stimulates the release of the gonadotropins - luteinizing hormone (LH) and follicle-stimulating hormone (FSH) - from the gonadotrophs cells. These pituitary hormones go on to establish normal ovulatory menstrual cycles. Whereas GnRH is the major regulator of gonadotropin production, their release is also under the influence of the gonadal steroids and the gonadotropins themselves through the various feedback loops (74).

By making selective amino acid or elhylamide substitutions either at the 6 (Gly) and/or the 10 (Gly) positions (Table 4) GnRH-a were synthesized. These substitutions cause an enhanced affinity for the GnRH receptors and protect against enzyme degradation increasing the half-life from about 8 minutes to as much as 5 hours (88). Initially the GnRHa binds to the receptor on the gonadotroph cell leading to a marked and prolonged release of both LH and FSH. This is called the agonistic phase or the “flare effect” (89).

In the case of continued GnRHa administration the gonadotrophs become insensitive to further stimulation. As shown by Knobil (87) continuous GnRH ultimately leads to the loss of LH and FSH. Secretion by “down regulation” of the receptors. This is caused by a loss of occupied GnRH receptors on the cell surface and an uncoupling of the receptors from the secretory signal. During the “flare effect” there is a concurrent rise in gonadal steroid secretion (90). This declines within tow weeks if the GnRH-a administration is continued with the achievement of pituitary desensitization, secondary to the receptor “down regulation”. Thus, a pharmacological, reversible hypopituitary - hypogonadal state is achieved without affecting other pituitary hormone secretion. There does not appear to be any direct effect on the gonads (91), although this point has been contested (92). Spontaneous pituitary and gonadal activity returns when GnRH-a administration is stopped (93).

In one recent study done by Raga et al. 1998, (94) to study the role of gonadotropin releasing hormone in murine preimplantation embryonic development, they reported that the results of their study demonstrate the presence of GnRH and its receptor in preimplantation embryos at both the mRNA and protein levels and, to the best of our knowledge, this is the first attempt to elucidate the functional significance of GnRH in preimplantation embryo development. Furthermore, on the basis of the observations just described (indicating a specific action on embryo development, rather than a nonspecific or toxic effect), it is tempting to suggest that GnRH plays a positive role in early embryonic development (94).

**Pituitary down-regulation with GnRH agonist in controlled ovarian hyperstimulation**

**Routes of Regulation:**
The usual approach is to desensitise the pituitary, using a GnRH agonist to synchronise follicular growth, before initiating ovarian stimulation with hMG, and to continue GnRH agonist treatment.
during ovarian stimulation in an attempt to reduce the risk of spontaneous luteinizing hormone (LH) surges and premature luteinization.\(^{(10)}\)\(^{(22)}\)

Treatment with the GnRH agonist is usually initiated in the follicular or luteal phase of the preceding cycle (i.e. a long suppression protocol) or in the follicular phase of the treatment cycle (i.e. a short suppression protocol) GnRH agonists are generally administered subcutaneously, intranasally or by single injection of a long-acting GnRH agonist preparation. To desensitize the pituitary subcutaneous administration of a GnRH agonist requires one to three injections daily, while a GnRH agonist administered in the form of an intranasal spray requires two to six doses per day.\(^{(95)}\)\(^{(96)}\)

Intranasal preparations are administered using a spray pump. Initial preparations (e.g. buserelin) needed frequent daily doses (5 times per day), which led to poor compliance despite a high patient acceptability. More recent nasal preparations (e.g. Nafarelin) only require twice daily administration. Nevertheless, drug absorption can be highly variable\(^{(95)}\)\(^{(96)}\)\(^{(97)}\).

Depot injections consist of the GnRH-a microencapsulated in biodegradable matrix (e.g. lactide-glycolide polymer). The drug is released over about 30 - 55 days\(^{(98)}\) and offers increased clinical and patient compliance as well as improved efficacy causing a high degree of pituitary desensitization\(^{(88)}\). This is usually considered too long and the pituitary desensitization too deep for most superovulation programs also requiring continued luteal support. Compared to the shorter-acting GnRH the depot preparations are associated with a requirement for longer treatment and higher doses of gonadotropins\(^{(99)}\)\(^{(100)}\). Some studies also report lower implantation and delivery rates in assisted conception cycles although this remains controversial\(^{(99)}\)\(^{(101)}\).

Subcutaneous injections are more economical and more readily achieve a state of pituitary suppression. A single dose of 500 ug per day is sufficient to induce consistent pituitary receptor down-regulation throughout the day\(^{(102)}\). However, daily subcutaneous injections may limit clinical acceptability and reduce patient compliance.\(^{(74)}\)

**The Use of GnRHa in Superovulation Programmes**

Treatment of infertility with in-vitro fertilization (IVF) or gamete intra-fallopian transfer (GIFT) usually involves ovarian stimulation programmes to achieve multiple follicular development this is because the larger the number of oocytes retrieved the more embryos can be generated and therefore the higher the pregnancy rate.\(^{(103)}\)

The use of GnRHa in superovulation programmes was first described in 1984\(^{(81)}\) in order to prevent the untimely spontaneous LH surge (as a result of the rising serum oestradiol in response to the gonadotropin therapy) and subsequent ovulation GnRHa were shown to prevent this LH surge and avoid the cancellation of the cycle\(^{(104)}\). As a result GnRHa decrease the need for close monitoring to detect the spontaneous LH surge.

Other advantages include better tinning and convenience in regard to oocyte collections insemination and embryo transfer\(^{(105)}\)\(^{(106)}\) avoiding the week-end and making planning for the patient and clinician easier.

Premature follicular luteinization has also been implicated as a cause for poorer embryos leading to lower pregnancy rates and lower live birth rates\(^{(107,108)}\).

Overall the use of GnRHa has increased pregnancy and live birth rates\(^{(83,109,110)}\) mainly by reducing the number of cancelled cycles (as well as allowing the planning of treatment programmes to be made easier by allowing better timing of oocyte retrieval) and possibly by synchronizing follicular maturation.

**Protocols for Multiple Follicular Development**

Multiple follicular development is a prerequisite for a successful in vitro fertilization-embryo transfer (IVF-ET) cycle. Ovulation-inducing agents have been used in a variety of combinations to find an ideal stimulation regimen; however, premature ovulation induced by an endogenous luteinizing hormone (LH) peak is a common reason for interruption of treatment cycle. Pretreatment with a gonadotropin-releasing hormone agonist (GnRHa) before gonadotropin therapy reduces the risk of
an unnecessary, premature, spontaneous LH surge, which reduced the likelihood of premature ovulation and increased the success rate. GnRHa is used in several different protocols for IVF-ET.

**GnRH-a Protocols:**
Three regimens using GnRH-a are currently employed. The “long protocol”, using GnRH-a until pituitary suppression or “down regulation” was the first described \(^{(110,111)}\) and is still the most widely used. The “short protocol” \(^{(112)}\) and the “ultrashort protocol” \(^{(113)}\) utilize the initial increase in gonadotropins, (the flare effect) resulting in shorter treatment times and lower doses of GnRHa and gonadotropins, but can lead to more difficult timing and programming.\(^{(74)}\)

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**Long Protocol (Figure 1) (click image for larger view)**

In the long protocol, it is common to begin GnRHa treatment at the mid-luteal, late-luteal or early follicular phase, and to continue until the day of hCG administration.

Pituitary desensitization is confirmed if the serum oestradiol concentration is less than 150 pmol/l. The time required to achieve pituitary desensitization varies for the different preparations, but is usually between 8 and 17 days. FSH or hMG treatment can start if pelvic ultrasonography shows an absence of follicular activity.

The aim of the "long protocol" is to achieve pituitary desensitization and therefore ovarian quiescence before beginning ovarian stimulation with gonadotropins. The GnRHa can be started in the late luteal phase (day 21, 22, or 23 of the previous cycle) \(^{(111)}\). There is a transient luteotrophic effect resulting in a lower “flare effect” and a more rapid decrease in circulating gonadotropins \(^{(108)}\) \(^{(109)}\). Otherwise GnRHa-a is started early in the follicular phase (day 1 or 2 of the cycle) \(^{(110)}\) until ovarian quiescence is achieved. Some authors have suggested greater follicular recruitment with starting on Day 1 \(^{(114)}\), although there is no evidence to suggest any difference in the outcome of treatment.

The criteria to assess ovarian quiescence and therefore to start ovarian stimulation with gonadotropins are either ultrasonic (regression of follicles to less than 5 mm diameter and thin or no endometrial echo \(^{(115)}\)), biochemical (serum oestradiol < 180 pmol/l) \(^{(110)}\), or both. These criteria are usually met after 14 or 15 days treatment.

As menstruation can occur 9-16 days after initiation of GnRHa-a treatment in the early follicular phase \(^{(109)}\), it would be unwise to start gonadotropin stimulation earlier despite apparent desensitization. Starting in the late luteal phase, the onset of menses can be used as an indicator of ovarian quiescence \(^{(116)}\) and gonadotropin stimulation started as appropriate. However, there is always the risk that GnRHa-a may be administered accidentally during early pregnancy.

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**Short Protocol - (Figure 2) (click image for larger view)**

The short protocol takes advantage of the increase in secretion of gonadotropins produced by the administration of a bolus dose of GnRHa, known as the “flare-up” effect. Administration of the agonist begins in the early follicular phase, after which FSH treatment and ultrasound monitoring start.

The "short protocol" uses the initial "flare up" effect of the GnRHa leading to increased pituitary
gonadotropins presumably initiating follicular recruitment and then continuing exogenous gonadotropin stimulation. GnRH-a should be started on day 1-3 of the cycle (111-117-118) and continued until hCG administration prior to oocyte recovery, in order to avoid the endogenous LH surge. Gnadotropins are usually started 3 days after GnRH-a.

Initial studies (119) showed elevated serum progesterone in the 4 days following initiation of GnRH-a, and lower fertilization rates. Therefore ultrasound scan of the ovaries prior to starting GnRH-a is recommended (117), in order to exclude any cystic structures which may lead to increased progesterone secretion from a corpus luteum. Other authors (120) recommend serum progesterone estimation prior to treatment. Pre-treatment oral norethisterone (10 mg. Daily) has been used to induce ovarian quiescence and plan the timing of IVF/GIFT treatment cycles (121).

Prospective trails (122-123) comparing long and short GnRH-a protocols have consistently shown that in routine use the “long protocol” results in a better ovarian response, more oocytes retrieved, more embryos replaced in IVF-ET, and higher pregnancy rates. However, in previously poor responders (117-121) there may be a role, and in one study the dosage of gonadotropins is reported to be reduced even when compared with protocols without GnRH-a (123)(184).

Ultrashort Protocol (Figure 3) (click image for larger view)

For the ultrashort protocol, GnRHa is administered for 3 days only, followed by injection of FSH. In order to reduce the dosage of GnRHa as well as that of the gonadotropins, the ultrashort or sequential protocol was developed (113). GnRHa, usually subcutaneously, is given only at the start of the cycle (days 3, 4, and 5) and then gonadotropins are continued alone.

Initially it was thought that pituitary depletion of LH may allow follicular development without the LH surge, but unless there is intensive monitoring (thereby losing one of the advantages of GnRH-a treatment) there is a high cancellation rate (124). When compared directly with the long protocol (125) the pregnancy rates are also significantly reduced.

Complications of GnRHa in Superovulation Programmes:

The well documented hypo-oestrogenic effects of GnRHa (126) are usually observed after relatively prolonged treatment and are unusual in superovulation programmes where gonadotropins are also given.

Some transient disturbances have been described (127) consisting of parasthesia or headache. The frequency appears to be less than 6% and the exact mechanism is unknown.

The most common complication is the appearance of ovarian cysts (14-29%) (128) they are more frequent when the short protocol is used, suggesting that they occur as a result of the “flare effect “ although the exact mechanism is unknown. Either continuing the GnRH-a until spontaneous subsidence or draining the cysts transvaginally is the treatment of choice. They do not appear to affect the results of IVF/GIFT treatment (128-129). Multiple pregnancy rates are higher when GnRHa are used (130), and severe OHSS is also more common in cycles with GnRHa (131).

**GnRHa and Ovarian Hyperstimulation Syndrome (OHSS)**

OHSS is the most serious complication affecting medical ovulation induction as well as superovulation programmes of IVF and GIFT (132). In its severe form the condition requires hospitalization, close monitoring, and supportive treatment until the condition resolves spontaneously.

**Incidence of OHSS**

Despite the initial hope that OHSS would be less likely to occur after pituitary suppression with
GnRH agonists, several studies have actually shown that the incidence of OHSS is increased after GnRH-a administration. This may be an unavoidable consequence of the increased pregnancy rate, which in itself is associated with OHSS, the better superovulation control in susceptible patients (i.e. those with severe ) or a tendency to use higher doses of gonadotropins following GnRH-a down-regulation. Alternatively, there may be a direct ovarian effect mediated via the GnRH receptors in granulosa cells.

Interestingly, only mild OHSS has been reported with ovulation induction using pulsatile GnRH.

**Prevention of OHSS with GnRH-as:**
The most commonly used method of preventing OHSS in patients at risk of developing the syndrome is to withhold human chorionic gonadotropin (hCG). In these cases GnRH-a can be continued until menses occur and ovarian stimulation commenced with lower doses of gonadotropins.

The initial “flare effect” of LH and FSH release has been used to achieve ovulation and pregnancies, using both intranasal buserelin and a single subcutaneous injection of leuprorelin, in superovulation and ovulation induction programmes. In all studies so far, no patients have developed OHSS although pregnancy rates (at 16-22%) are lower than with hCG induced maturation and/or ovulation. Unfortunately, this cannot be used in cycles where ovarian stimulation followed pituitary desensitization.

**Extra-Pituitary Side Effects:**
No teratogenic effects of GnRHa have been reported in animal studies and inadvertent use during pregnancy in humans has been reported to cause minimal risk for fetal development. Should conception occur, immediate discontinuation of GnRH-a and routine support with progesterone is recommended. However, ideally non-hormonal contraception should be advised throughout treatment.

Low and high affinity GnRH receptors have been reported in the granulosa cells of the human ovary and administration during the luteal phase in some animal studies has demonstrated increased pregnancy loss, possibly associated with luteal defects. This, and the presence of GnRH in human follicular fluid has led to a consensus of opinion advising the stopping of GnRHa-a treatment before oocyte retrieval and embryo transfer in assisted conception programmes.

Despite stopping GnRH-a treatment before oocyte retrieval and embryo transfer, several authors have reported shortening of the luteal phase and luteal insufficiency as defined as low mid-luteal progesterone levels.

Routine luteal support with progesterone is therefore indicated. Although some authors have suggested a direct endometrial effect to explain better pregnancy rates in frozen embryo treatment cycles, no GnRH receptors have been found in the and there are no abnormalities in endometrium glandular development when GnRHa are used.

**Response Pattern to GnRHa**
It has long been established that patients undergoing ovarian hyperstimulation have different response patterns. In general, patients can be divided into high, intermediate and low responders, depending upon the oestradiol (E2) response prior to oocyte retrieval. Usually the number of mature oocytes retrieved correlates with the E2 response with high responders having the highest number and low responders having the lowest. Success rates for low-responder patients have been generally disappointing and efforts to improve the response and outcome of IVF in these patients are continuing.

Basal gonadotropin follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels on cycle day 3 was found to be useful in predicting the pattern of E2 response and subsequent success rates in IVF. In general, patients can be divided into three groups depending on the basal serum FSH and LH, on cycle day 3: 1) low responders, FSH >15 mIU/ml; 2) intermediate responders, FSH < 15mIU/ml and LH/FSH ratio <1.5; 3) high responders, FSH < 15 mIU/ml and LH/FSH ratio > 1.5.

It should be emphasized that for basal FSH and LH levels to be accurately predictive, the E2 level on
cycle day 3 should be less than 50 pg/ml. Repeatedly, basal FSH levels have been found to be accurately predictive of IVF performance and, as such, can be used to counsel patients and to plan better therapeutic protocols. (154)(155)

During the past 4 years, gonadotropin-releasing hormone (GnRH) agonists have been widely used as adjuncts to gonadotropins for ovarian hyperstimulation. Advantages of Gn-RH agonist use include prevention of a premature LH surge, suppression of endogenous basal LH levels and recruitment of a larger cohort of follicles. GnRH agonists can be used in a long (suppression) or a short (stimulatory, flare-up) protocol. The use of GnRH agonist suppression (starting in the mid-luteal phase) prior to ovarian hyperstimulation was demonstrated to be extremely beneficial in intermediate and high responders, but not in low responding patients. (156)(157) More recently, in low responders the use of a GnRH agonist “flare-up” protocol (GnRH agonist starting on day 2 of the cycle, followed by gonadotropins on day 4) has produced significant improvements in stimulation characteristics and better pregnancy results by taking advantage of the initial agonistic stimulatory effect of GnRH agonist on endogenous FSH and LH secretion. (158)

GnRH analogues (GnRHa-a) are used in the vast majority of IVF cycles all over the world. Long protocol (a period of pituitary desensitization previous to ovarian stimulation) is the most used strategy. Nevertheless, several issues remain unsolved. One of them is the optimal duration of administration of the drug to achieve pituitary suppression when it is started in the early proliferative phase. In these cases, a period of 2 weeks on GnRH-a before assessing pituitary desensitization is favoured by most centers. (95)

Calhaz-Jorge et al. 1998 (159) analyzed the date of IVF programme in order to verify if a shorter period of GnRH-a correlates with:
(i) pituitary down-regulation in a useful proportion of patients;
(ii) a reduction of either duration or number of ampoules of hMG/FSH needed to obtain ovarian stimulation;
(iii) differences in pregnancy rates.

IVF cycles using buserelin (0.3 cc, b.i.d., s.c. long protocol) beginning on day cycle 2 were reviewed. Age of female patients, cycle characteristics (infertility factors and duration, cancellation rate, number of days and ampules of hMG and/or FSH, maximum oestradiol concentrations, oestradiol concentration on day of HCG, retrieved oocytes, fecondation rates, transferred embryos) and results (pregnancy rates) were compared in the following groups of cycles: I, with confirmation of pituitary down regulation after 7-10 days of buserelin; II, with pituitary down-regulation not attained after 7-10 days of buserelin and requiring the prolongation of the drug for a total of more than 14 days; III with first assessment of pituitary down-regulation after more than 14 days of buserelin, usually with scheduling purposes. Cycles of patients with oligo/anovulation were excluded from this study and they concluded that classically accepted levels of pituitary down-regulation were attained in >75% of the cycles after 7-10 days of GnRH-a. Cycles without pituitary suppression after this brief period of buserelin had a higher cancellation rate and gonadotropin consumption, and lower ovarian response. A deliberate duration of buserelin longer than 14 days had no benefit. (159)

The use of GnRH agonist suppression (starting in the mid-luteal phase) prior to ovarian hyperstimulation was demonstrated to be extremely beneficial in intermediate and high responder patients but now in low responders (defined endocrinologically as patient with a basal FSH:LH ratio of 1:1 and a basal LH:FSH ratio of > 1:5 respectively. (95)

Muasher 1992 reported that: there is no any beneficial effects from the use of GnRH agonist suppression in low responder patients (defined endocrinologically as patients with a basal FSH > 15mIU/ml). In such low responder patients, the use of a “flare up” GnRH agonist protocol, (with GnRH agonist starting on day 2 of the cycle followed by gonadotropins on day 4 of the cycle), taking advantage of the initial agonist stimulatory effect of GnRH agonist on endogenous FSH and LH secretion has provided significant improvements in stimulation characterisation and better pregnancy results. (95)

All stimulated cycles from infertile patients undergoing ovarian stimulation for IVF during the years 1989-1990 at the Jones Institute for Reproductive Medicine in Norfolk, Virginia were included in one study. Patients were classified into 3 groups: (95)(160)(161)(162)(163)(164)
A variety of protocols and a number of GnRH agonists have been used for pituitary down-regulation in in vitro fertilisation/embryo transfer (IVF-ET). Although many studies have shown that the use of a GnRH agonist can improve the clinical outcome of IV-ET and gamete intrafallopian transfer (GIFT), this has not been the case in all trials comparing the GnRH agonist stimulation protocol with more traditional approaches, such as clomiphene citrate-gonadotropin or gonadotropin treatment alone. Pituitary down-regulation with a GnRH agonist dose, however, make it more convenient for team members and patients to plan cycle scheduling, and most centres throughout the world apply GnRH agonists in ovarian stimulation for IVF. (165)

Although trials examining the use of GnRH agonist in IVF have produced conflicting results, the combination of GnRH agonist treatment with hMG therapy has been shown to increase the number of oocytes recovered (166)(167) as well as to improve pregnancy rates (168)(169). A recent, carefully performed meta-analysis summarised the results of all randomised and quasi-randomised controlled trials using GnRH agonists for IVF-ET and GIFT. Although this meta-analysis emphasised that more data are needed to address the possible risk of ovarian hyperstimulation syndrome and multiple pregnancies related to GnRH agonist use, the results indicated that the use of GnRH agonist improves the clinical outcome of IVF and GIFT(170).

Goserelin depot for pituitary down-regulation

Goserelin acetate depot (Zoladex*, Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) is a sustained release GnRH agonist preparation, which is about 100 times more potent than the naturally occurring hormone. The depot injection may be given subcutaneously and is designed to release goserelin for about 28 days (171)(172). The mean elimination half-life of goserelin is 4-5 h. Ovarian oestradiol (E2) release increases slightly 3 days after administration of goserelin and then decreases in the 2 weeks to early follicular phase level (172).

Raga et al 1998 (173) reported that:

Our study demonstrates for the first time the presence of GnRH and its receptor in preimplantation human embryos at both the mRNA and protein levels. The administration of GnRHa throughout the luteal phase of the conception cycle and early pregnancy seems to play a positive role in fecundity, with a rise in pregnancy and implantation rates. No deleterious effect was observed among the women or babies exposed to GnRHa. On the basis of the observations just described, it is tempting to suggest that GnRHa is playing a positive role as a regulator in the embryo/endometrial interactions during early pregnancy and that the administration of GnRHa throughout the luteal phase enhances implantation in infertile patients. (173)

The traditional long protocol demanded more human menopausal gonadotropin (hMG) and sometimes causes unnecessary delay in the procedure. A new 7-day GnRHa/hMG protocol required to conserve cost and time is thus evaluated for better outcome. Sixty consecutive IVF candidates less than 40 years of age were recruited for the study. The perspective candidates were divided into two groups, one received the traditional, GnRHa/hMG protocol and the other received the new 7-day regimen. When comparing the results in pregnancy rate (33.3% vs. 30%) cleavage rate (75.7% vs. 75.5%) and the number of oocyte obtained (5.96 +/- 0.91 vs. 6.63 +/- 0.90), the 7-day GnRHa/hMG protocol is as good as those of the traditional regimen. The amount of hMG used nevertheless was significantly less (21.48 +/- 0.78 vs. 50.59 +/- 2.07). The new regimen will surely reduce the cost to relieve patient’s financial burden and to increase patient’s comfort. (174)

GnRH may induce apoptosis in the follicles. In one study done by Waldenstrom and Nilsson 1998, (175) To see if poor responders would respond better if no GnRH-analogue was administered during the ovarian stimulation period and concluded that long down-regulation followed by ovarian stimulation without any GnRH-analogue seemed beneficial for these severe poor responders. A pregnancy rate of 21% per started cycle was promising for the group studied. Much to our surprise, no cycle was
Ovulation Induction in In-Vitro Fertilization
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cancelled because of a premature LH surge and no patient had a spontaneous ovulation.

The improvement of IVF-ET outcome in poor responder is still a matter of concern. In one study done by Kallianidis et al 1998 to investigate the parameters of ovarian stimulation in poor responders after decreasing the dose of GnRH agonists. Patients were classified into three groups - (control group; group A) the dose of GnRH-a was 3.75mg of triptorelin injected intramuscularly in the luteal phase of the previous cycle. Group B consisted of 52 patients that entered IVF-embryo transfer for the second time with the long protocol but half the dose of triptorelin (1.85 mg). Group C consisted of 44 patients that entered IVF-embryo transfer again with the long protocol but with 1.85 mg of leuprolide acetate (half dose).

And concluded that the decrease of the dose of GnRH-a improved most of the parameters of ovarian stimulation but still did not affect pregnancy rate significantly. There was no difference in the two groups that used different GnRH-a.

Cedrin - Durnerin et al 1998 stated that: A short follicular phase is an early clinical feature of an occult ovarian failure. It is usually associated with increased values of plasma FSH and/or oestradiol measured at day 3, attesting that the follicular growth is initiated earlier during the intercycle transition. In addition, the fecundity is usually low in association with both a reduced size of the follicular cohort and a poor oocyte. To test the hypothesis that a short follicular phase worsens conception rate and that this could be improved by increasing the duration of follicular maturation. We started the administration of a GnRH agonist in the late luteal phase to prevent the FSH intercycle rise before stimulating follicular growth by exogenous FSH. These preliminary results show that preventing FSH rise during the intercycle transition by the administration of GnRH agonists is able to increase the duration of follicular maturation and seems to improve the pregnancy rate. Although the miscarriage rate remains high in these women, the results suggest that the oocyte is better with this regimen. Another explanation could be that this protocol improves cervical mucus production and/or endometrial maturation. This needs to be evaluated further in a control study.

In poor responders, a high dosage of gonadotropins may be initially needed to improve follicular recruitment. Nevertheless, once the cohort is recruited, a step-down regimen is as effective as a high-fixed dosage to sustain follicular maturation, and it allows the ovarian stimulation cost to be reduced. Although the overall pregnancy rate remains low, a step-down approach must be considered in this group of patients.

Shalabi et al (1998) concluded that: A long protocol using short-acting GnRHs is preferable to a long protocol with depot GnRH in terms of embryo quality and pregnancy rate. A further multicentre prospective randomized study is needed to confirm these results.

Gonadotropin-releasing hormone (GnRH) analogues have long been used for reduction of premature luteinizing hormone (LH) surges in women undergoing IVF. A variety of different GnRH analogues are being used for this purpose to compare the clinical efficacy of the two commonly used GnRH analogues in the market, leuprolide acetate and triptorelin, in IVF cycles. A study done by Ozgur et al 1998 and they concluded that leuprolide acetate and Triptorelin had similar efficacy in patients undergoing IVF.

In one prospective study done by Beckers and Fauser (1998) to determine corpus luteum function in IVF cycles in relation to the duration of GnRH agonist medication during the follicular phase. Patients were divided into three different treatment groups and all groups received down-regulation using Decapeptyl® (D) for 3 weeks starting in the early follicular phase. Group A continued Decapeptyl until human chronic gonadotropin (HCG) and luteal support (LS) was given. Groups B and C did not receive LS. In group B, Decapeptyl was stopped after 3 days of hormonal stimulation with human menopausal gonadotropin (hMG:3 ampoules/day). In group C, Decapeptyl was continued until HCG and they concluded that: Early cessation of GnRH agonist medication remained effective in preventing a preovulatory LH rise (group B). HSG 10 000 IU administration per se (35 h before ovum pick-up) did not suppress LH concentrations in the luteal phase (group B). LH concentrations during the luteal phase were higher after early cessation of GnRH agonist (group B versus C). Although luteal phase immunoreactive serum LH concentrations remained extremely low when luteal support was not provided (following extended use of GnRH agonist), adequate quantities of progesterone were produced.
A total of 100 women undergoing ovarian stimulation with gonadotropin-releasing hormone agonist (GnRHa) and a human menopausal gonadotropin (hMG) for in-vitro fertilization (IVF) participated in randomized comparative study. Leuprolide acetate at a dose of 0.5 mg/day s.c. (n=52, group I), or low-dose leuprolide acetate depot at a dose of 1.88 mg. s.c. (n=48, group II) was started on days 21-23 of the cycle. Stimulation with 225 IU/day hMG was started after pituitary desensitization had been achieved. The luteal phase was supported by human chronic gonadotropin (HCG) i.m. injection. No statistical difference existed between these two groups. Thus, a.s.c. low-dose leuprolide acetate depot injection may offer a useful alternative for pituitary suppression in ovarian stimulation for IVF. (182)

Long acting GnRHa in IVF are preferable to daily administration forms, not only for greater acceptance by patients, but for the improved implantation rate they provide, probably in relation to a better hormonal milieu during the implantation window. (183)

In a study done by Filicori et al 1993 (184) 40 normally cycling women with male-related infertility or benign reproductive disorders, were divided into 3 groups, each group of 10 subjects received a different GnRH-a for 3 months: buserelin (group B: 300 ug.sc, every 12 h as a control), goserelin (group G: 3.6 mg, sc, every 28 days), leuprorelin (group L: 3.75 mg, im, every 28 days), and triptorelin (group T:3.75 mg, in, every 28 days). Depot GnRH-a was administered by one of the investigators. GnRH tests (100 ug,iv) were performed before treatment (cycle day 7; test A) and on treatment days 57 (i.e. 1 day after the third depot GnRH-a; test B) and 84 (i.e. 28 days after the third depot GnRH-a; test C). Immunoreactive (i) LH levels were measured with an ultrasensitive immunochemiluminometric assay. Profound suppression of the iLH response to the GnRH test occurred in all subjects during treatment and concluded that in adult women, 1) iLH was profoundly suppressed in the third month of administration of all GnRH-a tested; 2) FSH suppression with depot GnRH-a was less marked than that with high-dose short-acting sc buserelin; and 3) signs of an incomplete block of ovarian function can be present in the third month of depot GnRH-a administration, particularly when goserelin is employed. (184)

Gerris et al (1995) (185) studied the peri-ovulatory and luteal phases in 38 human menopausal gonadotropin (hMG)-stimulated cycles, in which ovulation was triggered with four different i.v. bolus ovulation triggers: 100 micrograms gonadotropin-releasing hormone (GnRH; group A, n=9), 500 micrograms GnRH agonist (GnRHa; group B, n=10), 10,000 IU human chronic gonadotropin (HCG; group C, n=10) and 500 micrograms GnRH (group D, n=9) and these findings show that (i) ovulation occurs and pregnancy can be achieved following an endogenous LH surge induced by GnRH and its agonist, (ii) a high frequency of luteal insufficiency occurs in such cycles even with luteal supplementation and (iii) OHSS cannot be totally prevented by this approach, although cycles with an endogenous LH surge in general result in fewer subclinical signs of ovarian hyperstimulation. (185)

In a study done by Janssens et al 1998 (186) they concluded that: 15 ug triptorelin would be enough to prevent a premature LH surge, but to effect a complete suppression of the pituitary together with a maximum ovarian outcome such a number of oocytes and embryos, a dose of 50 ug triptorelin would be necessary. (186)

Dexamethasone cotreatment with gonadotropins may be useful for patients with both PCO and normal ovaries. Beyond suppression of hyperandrogenism, potential mechanisms of action include direct effects via ovarian glucocorticoid receptors, enhanced growth factor secretion, alterations in cytokine profiles and influences on ovarian cortisol metabolism. Irrespective of the mechanism of action, the clinical benefit observed in this study warrants further investigation. (187)

The use of the GnRH-a flare up regimen results in more cancellations, less follicles, less oocytes, less embryos, but the same pregnancy rate per treatment cycle. The total gonadotropin does is lower and the treatment duration is shorter. The flare-up regimen is clinically safe (no OHSS) and effective. Because of the high cancellation rate, it is questionable whether this regimen is cost-effective. (188)

CONCLUSIONS
GnRH-a are an important arm in the therapeutic arsenal available to treat infertility. Their main role is in the superovulation regimens employed in the IVF or GIFT programmes. Generally the most
effective way is the “long protocol”, although in cases of difficult ovarian stimulation, where there is more intensive monitoring, or where there are concerns about the does and cost of GnRH-a and gonadotropins other regimens or protocols may be more appropriate. Other roles include treatment of moderate or severe endometriosis prior to IVF treatment, and occasionally their use to stimulate ovulation in ovulation induction cycles at risk of OHSS.\(^{(74)}\)

**Antagonistic Analogues of GnRH**

Due to their different pharmacological mode of action, GnRH antagonists are able to suppress serum concentrations of LH within hours of administration. Instead of ‘down-regulation’ and ‘desensitization’, a classic competitive blockage of the GnRH receptors on the cell membrane of the gonadotrophic cells seems to take place. The GnRH antagonists Cetrorelix and Ganirelix have been used in clinical studies to prove that these compounds reliably prevent the premature LH surge within controlled ovarian hyperstimulation. Cetrorelix has been applied in single, dual and multiple dose protocols, while Ganirelix was used until now only according to the multiple dose ‘ Luebecker protocol’.\(^{(249)}\)

Over the past few years the application of GnRH antagonists has been clinically introduced into ovarian stimulation protocols to avoid premature LH surges \(^{(250)}\).

To inspect the efficiency of GnRH antagonist in the case of patients with IVF/ICSI/TESE, a study was done by Schill et al (1998) \(^{(251)}\). During the period of January 1996 to December 1997 where a total of 118 cycles for IVI/ICSI/TESE therapy were stimulated with gonadotropins. Of these, 106 cycles became down-regulated with GnRH agonist, and 12 cycles became suppressed with GnRH antagonists. Stimulation with GnRH agonist occurred according to the so-called ‘Luebecker protocol’. In this protocol, the application of the agonist begins on day 7 and continues until the day of ovulation induction. In three cycles with GnRH agonist neither spermatozoa nor oocytes could be retrieved. The pregnancy rate with GnRH antagonist therapy was 30% and with GnRH agonist therapy 15.6%. The fertilization rate with GnRH antagonist was 33.5%, and with GnRH agonist 35.5%.

The data presented here demonstrate that the results concerning the pregnancy rate as well as the fertilization rate are similar in case of GnRH agonist or GnRH antagonist administration. For the future, the utilization of GnRH antagonist can also be recommended for IVF/ICSI/TESE therapy, because of the equivalent pregnancy rate but far smaller side-effects. \(^{(251)}\)

Another study using Cetrorelix done by Felberbaum and Diedrich (1998) \(^{(249)}\) to study the two ways of its administration. In the multiple dose protocol, COH is started on day 2 or 3 of the cycle with hMG or recombinant (rec.) FSH. Daily administration of the GnRH antagonist at its minimal effective dose (0.25 mg/day) occurs from the sixth day of stimulation onward until ovulation induction. In the single or dual does protocol, 3 or 2 mg of the GnRH antagonist Cetrorelix is injected on day 8 of the stimulation cycle. A second injection is administered 72 h later if ovulation cannot be triggered in the meantime. So far, > 1000 patients have been treated with these protocols.

Both protocols have been proven to be safe and effective. Fertilization rates of >60% in IVF and >70% in ICSI as well as clinical pregnancy rates of ~30% per transfer have been reported. Oestradiol secretion is not compromised by the GnRH antagonist in its minimal effective dose using rec. FSH for COH. The incidence of a premature LH surge is far below 2%, while the pituitary response remains preserved under this regimen in a dose-dependent manner, allowing the induction of ovulation by GnRH or GnRH agonists. However, luteal phase support remains mandatory. The incidence of severe OHSS seems to be lower than in the long agonistic protocol.

The discussion about the advantages and disadvantages of the two possible ways of administration is still going on, although we favour the ‘Lbeck’ multiple dose protocol due to its greater stability; it preserves all the advantages regarding the ‘comfort’ of the therapy of the long agonistic protocol we are accustomed to. In addition, treatment time is shortened and it is easier for the patient to tolerate. The combination of softer stimulation regimens such as clomiphene citrate and low-dose hMG with mid-cycle administration of GnRH antagonists may be the way to cheap, safe and ovarian stimulation. \(^{(248)}\).
To assess the plasma and follicular fluid concentrations of the GnRH antagonist Cetrorelix after daily application of different doses.

A total of 120 patients were treated according to the ‘Lbeck’ protocol for IVF/ICSI cycles in subsequent dose finding studies. Within this protocol, patients started with hMG on day 2 of the menstrual cycle. The GnRH antagonist Cetrorelix was administered daily starting on day 7 of the menstrual cycle: 3 mg (12 patients), 1 mg (12 patients), 0.5 mg (43 patients), 0.25 mg (46 patients) and 0.1 mg (7 patients). Cetrorelix was administered once daily. Cetrorelix plasma concentrations were measured once daily. The follicular fluid concentration was measured on the day of oocyte pick-up (OPU).

The dosage of 0.25 mg daily proved to be the minimal effective dose for prevention of premature LH surges. In the 0.25 mg group, a steady state of the Cetrorelix plasma concentration was reached from 4 days onwards. The Cetrorelix plasma concentration of patients treated with 0.1 mg per day were only rarely above the level of quantification and therefore were not included in the statistical analysis. The mean Cetrorelix plasma concentrations on the day of OPU in the 3 mg (2.03 ± 0.55 ng/ml) and 1 mg group (1.57 ± 0.58 ng/ml) were significantly higher compared to those in the 0.5 mg group (0.80 ± 0.09 ng/ml) (P < 0.05). All concentrations were significantly higher compared to those in the 0.25 mg group on that day, which were in the range of the lower level of quantification (0.07 ± 0.18 ng/ml) (P < 0.05). A significant difference of the 3 mg and 1 mg groups compared to the 0.5 mg group, and a significant difference of the 0.25 mg group, compared to all other groups were also found for the day of embryo transfer (3 mg: 0.95 ± 0.32 ng/ml; 1 mg: 0.58 ± 0.41 ng/ml; 0.5 mg: 0.30 ± 0.36 ng/ml; 0.25 mg: 0.01 ± 0.09 ng/ml). The differences between the day of OPU and embryo transfer were significantly different in all groups. The follicular fluid concentration were significantly lower in the 0.25 mg group (0.11 ± 0.17 ng/ml) compared to all other groups (3 mg: 1.95 ± 0.43 ng/ml; 1 mg: 0.90 ± 0.36 ng/ml; 0.5 mg: 1.18 ± 0.38 ng/ml) (P < 0.05).

This study demonstrated that the plasma and follicular fluid concentration of Cetrorelix were significantly lower in the 0.25 mg group compared to the other groups. The 0.25 mg dose of Cetrorelix is the safest one is relation to possible teratogenic effects. (250)

The GnRH antagonist Cetrorelix is currently used in treatment regimens of controlled ovarian hyperstimulation (COH) in order to prevent premature LH surges. Since it has been shown that human ovaries express GnRH receptors, it might be possible that GnRH antagonists exert actions on ovarian steroidogenesis. To test this hypothesis, Ortmann et al 1998 (252) performed experiments with cultured granulosa lutein (GL) cells that were exposed to different Cetrorelix treatment paradigms and concluded that treatment with Cetrorelix during COH does not impair ovarian steroidogenesis. The data obtained from in-vitro treatments with GnRH analogues which demonstrated no effect of such treatment on steroid secretion do not support the hypothesis of a GnRH-dependent autocrine system but regulate steroidogenesis in the human ovary. (252)

New GnRH antagonists have recently become available for clinical studies. The most promising use of these compounds seems to be in IVF-embryo transfer, where they allow suppression of LH surges. Olivennes et al (1998) (253) compared in their study the results of a single administration of 3 versus 2 mg Cetrorelix in 65 patients undergoing controlled ovarian stimulation and IVF-embryo transfer. The GnRH antagonist Cetrorelix was administered at a dose of 3 mg (34 patients) or 2 mg (33 patients) on day 8 of the stimulation cycle when the LH surge was feared. In some cases of slow follicular development kinetics, the injection was delayed.

No difference was observed in the decrease in LH and in oestradiol secretion between the 3 and the 2 mg groups. However, the LH secretion was suppressed for a shorter time in the 2 mg group. No LH surge was observed in the 3 mg group, while one surge (3%) and one significant rise in LH were observed 4 days after the 2 mg administration. No significant difference was observed in IVF-embryo transfer results in the two groups of patients and concluded that this study demonstrates that a single injection of 3 or 2 mg successfully prevents an LH surge for three days in all the patients treated. The occurrence of one LH surge and one significant LH rise 4 days after the antagonist administration in the 2 mg group led us to choose the 3 mg dose as a safer dose in our single administration protocol. The ‘protection period’ of the 3 mg injection can be estimated at least 4
days, but more patients with a Cetrorelix/HCG interval of 5 or 6 days are needed to see if the period of protection is not longer. This GnRH antagonist single-dose protocol allows us to propose a very simple stimulation regimen with satisfactory IVF results. (253)

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<th>No. of oocyte retrievals</th>
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<th>Embryo</th>
<th>Clinical PR (%)</th>
<th>Implantation Rate (%)</th>
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A case report describes the first attempt to treat imminent ovarian hyperstimulation syndrome (OHSS) by using a gonadotropin-releasing hormone (GnRH) antagonist. A 33 year old, normo-ovulatory woman undergoing in-vitro fertilization received daily subcutaneous injections of 150 IU of recombinant follicle-stimulating hormone (recFSH) from cycle day 2, together with GnRH antagonist (ganirelix) 0.125 mg from cycle day 7 onwards. On cycle day 10 the patient was found to have a serum oestradiol concentration of 16 500 pmol/1 and, on ultrasound examination, four preovulatory (> 16 mm) and nine intermediate sized (10-16 mm) follicles. RecFSH injections were discontinued, human chorionic gonadotropin (HCG) withheld, whereas the ganirelix dose was increased to 2 mg/d. This regimen led to a rapid decrease in serum oestradiol concentrations and the decrease in ovarian size on ultrasound. Since GnRH antagonists will become clinically available for in-vitro fertilisation programmes in the near future this suggested regimen might have a role in preventing severe OHSS. (254)

**Progesterone therapy:** Vaginal administration of 200 mg of progesterone can produce plasma concentrations of progesterone comparable to those found during the luteal phase of the cycle. They further show that uptake of progesterone even in GnRH-analogue suppressed patients is efficient. In addition, it may be advisable to administer a second suppository after 12 h. (255)

Natural progesterone given as a vaginal tablet is well tolerated, safe and an easily administered treatment. In older women, an enhanced vaginal absorption was observed. Even in nonestrinized vagina the absorption was efficient, and the 100 mg dosage resulted in adequate serum progesterone concentrations. (256)

**Bromocriptine**
To examine whether a new method of ovarian stimulation, bromocriptine-rebound method, improves IVF outcomes compared with the conventional long protocol of GnRH agonist and hMG regimen. A prospective clinical trial. Done by Jinno et al (1998) (257) where patients were assigned to either bromocriptine-rebound method (group 1) or long protocol (group 2). The bromocriptine-rebound method was the same as the long protocol, except that bromocriptine was administered daily from day 4 of the preceding cycle until 7 days before hMG stimulation and concluded that the bromocriptine-rebound method enhanced embryonic development, resulting in an increased pregnancy rate compared with the long protocol. (257)

**Transvaginal Sonography**

**Endometrial thickness and growth during ovarian stimulation**
The development of an adequate receptive endometrium is a crucial factor in the success of IVF-embryo transfer treatment. A few authors have investigated the maturation of the endometrium in different stages of the luteal phase from the day of embryo transfer onwards and demonstrated conflicting results of endometrial dating. (258)

Endometrial thickness is valid screening test for conception outcome in cycles stimulated with hMG. A periovulatory endometrial thickness > or = 10mm defined 91% of conception cycles. No pregnancy occurred when the endometrium measured < 7mm. (259)

Vaginal Sonography has significantly influenced fertility management and greatly extended the role of ultrasound in gynaecology. The transvaginal transducer enables very detailed visualization of the uterine cavity. (260)
As several studies report that transvaginal ultrasound of endometrial thickness may help distinguish fertile from infertile cycles. Beneventi et. al; (1995) assessed endometrial growth and morphology in 124 infertile women. The patients underwent different ovulation induction treatments: clomiphene citrate (CC), human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG), analogous GnRH and hMG (a GnRH+hMG). CC administration is followed by a slackening of endometrial maturation. The US pattern H (typical of the ovulatory phase) appears on day 13 (76.9% of the cases) in spontaneous cycles and on day 16 (75% of the cases) in CC-induced cycles. The H pattern on day 20 in CC-induced cycles persisted in the patients who did not conceive. In a Gn-RH-stimulated cycles the endometrial pattern H appears on days 13 (41.66%) and 16 (83.33%), not preceded by a Hi image. The endometrial pattern Hi was always observed in the patients who did not conceive. Our retrospective study of endometrial US morphology shows that the difference ovulation induction treatments may effect the day of appearance of the various endometrial patterns. The results, which need further confirmation, can allow the changes of conceiving to be investigated during the stimulation protocol of every single stimulated cycle. (261)

Role of 3D ultrasound and Colour Doppler

Satisfactory pituitary down-regulation in the pretreatment phase of IVF cycles has traditionally been measured by thinning of the endometrial lining or suppression of pituitary and ovarian hormones to castration levels. Steroidal hormones play an important role in modulating uterine and ovarian vascularity and colour flow Doppler (CFD) can be used to study these changes. As part of an ongoing trial to explore the role of CFD in assisted conception cycles Dada and Sharma (1998) present data concerning the potential benefits of CFD in monitoring ovarian suppression and concluded that GnRHa-induced suppression of oestradiol can be measured by changes in ovarian artery blood flow with colour Doppler spectral analysis. This has positive implications for aspects of treatment cycles that are currently monitored by invasive investigations which incur both inconvenience for the patient and increased costs from biochemical tests. (262)

3D ultrasound allows precise ovarian volume measurement and may be useful in predicting implantation success. Subendometrial blood flow in combination with the aforementioned volume calculation may give new insights into the complex issues of uterine receptivity. (263)

Blood flow to the individual follicles has been shown to play an instrumental role in the maturation of the follicle, and colour Doppler studies of the growing follicles have demonstrated a good correlation between resistance to flow in the ovaries and follicles and outcome of IVF treatments. Insulin-like growth factor-I (IGF-I) has a regulatory function in folliculogenesis, granulosa cells replication and differentiation and steroidogenesis. Vascular endothelial growth factor (VEGF) induces proliferation and angiogenesis and is involved in the vascularization of the developing corpus luteum to investigate the predictive value of colour Doppler measurements of blood flow in the leading follicle and its correlation with VEGF and IGF-I concentrations in the serum and follicular fluid (FF) and clinical outcome of the treatment. A study was done by Lewin et al (1998) and concluded that: Serum VEGF concentrations and the mean RI of the leading follicles may have a preoperative prognostic value in IVF cycles. (264)

The number of small follicles (3-8mm) present before ovarian stimulation is a good predictor of outcome to gonadotropin stimulation in the IVF estradiol levels obtained on the fourth day of gonadotropin therapy are highly predictive of successful ovulation induction and pregnancy outcome in cycles using luteal phase leuprolide acetate. (266)

Complications

Serour et al 1998 reported complications of medically assisted conception in 3500 cycles (2942 patients) at the Egyptian IVF ET Centre-Cairo, the complications of the procedure encountered were moderate ovarian hyperstimulation syndrome (OHSS) in 5.9%, severe OHSS 1.7%, vaginal bleeding 0.9%, pelvic infection 0.3%, deep vein thrombosis 0.11%, hemiparesis 0.06%, acute abdomen 0.9%, anaesthetic complications 0.06%, testicular infection 0.7% and mortality in one patients (0.03%). Pregnancy complications included 1.9%, heterotopic pregnancy 0.2%, abortion 20.6%, multiple pregnancy 28%, pregnancy-induced hypertension 10%, preterm labour 21.5%, low birth weight 30.5%, intrauterine fetal death 2%, congenital malformation 2.1%. In addition, the rate of abnormal chromosomal karyotyping in 80 babies examined was 2.5%. Other coincidental complications not
proved to be due to the procedure included ovarian malignancy 0.06%, breast carcinoma 0.03%, thyroid adenoma 0.03% and breast adenoma 0.03%. (267)

Table 1. Findings and Outcome at Laparoscopy in Patients Receiving “High Dose” hMG Alone and Clomiphene Alone

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Follicles Aspirated per Patient*</th>
<th>Oocytes Recovered per Follicle Aspirated</th>
<th>Oocytes Recovered per Patient*</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMG</td>
<td>4.7 ± 2.2 (n=18)</td>
<td>82/84 97.6% (n=18)</td>
<td>4.6 ± 2.7 (n=16)</td>
</tr>
<tr>
<td>Clomiphene</td>
<td>3.2 ± 1.2 (n=16)</td>
<td>27/51 52.9% (n=16)</td>
<td>1.7 ± 0.9 (n=15)</td>
</tr>
<tr>
<td>Significance</td>
<td>p&lt;0.05</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Data from Quigley et al. 1984 (5)
*Mean ± standard deviation.

Table 2. Length of the Menstrual Cycle and Luteal Phase in Patients Receiving “High Dose” hMG Alone and Clomiphene Alone

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cycle Length*</th>
<th>Length of Luteal Phase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMG</td>
<td>21.1 ± 1.5 days</td>
<td>11.6 ± 1.5 days</td>
</tr>
<tr>
<td>Clomiphene</td>
<td>26.8 ± 2.3 days</td>
<td>14.3 ± 2.3 days</td>
</tr>
<tr>
<td>Significance</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Data from Quigley et al. 1984 (5)
*Mean ± standard deviation.

Table 3. Follicular Development at hCG Administration, Oocytes Recovered at Laparoscopy, and Embryos Transferred in Patients Receiving Clomiphene Alone and a Clomiphene hMG Combination

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Number of Follicles (hCG day)</th>
<th>Oocytes Recovered per Patient*</th>
<th>Embryos Transferred per Patient*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clomiphene</td>
<td>3.7 ± 1.2 (n=17)</td>
<td>1.9 ± 0.9 (n=15)</td>
<td>2.0 ± 0.4 (n=12)</td>
</tr>
<tr>
<td>Combination</td>
<td>5.1 ± 2.0 (n=17)</td>
<td>2.8 ± 1.9 (n=13)</td>
<td>2.5 ± 1.2 (n=13)</td>
</tr>
<tr>
<td>Significance</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Modified from Quigley et al. 1984 (5)
NS, not significant,
*Mean ± standard deviation.

Table 4 - Structure and relative potencies of current GnRH-as

<table>
<thead>
<tr>
<th>GnRH Analogue</th>
<th>Substitution</th>
<th>Relative Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buserelin</td>
<td>D-Ser (TBU) for Gly-6</td>
<td>100</td>
</tr>
<tr>
<td>Nafarelin</td>
<td>D-(2Nal) for Gly-6</td>
<td>100</td>
</tr>
<tr>
<td>Leuprorelin</td>
<td>D-Leu for Gly-6</td>
<td>50</td>
</tr>
<tr>
<td>acetate</td>
<td>Ethylamide for Gly-10</td>
<td></td>
</tr>
<tr>
<td>Goserelin</td>
<td>D-Ser (TBU) for Gly-6</td>
<td>50</td>
</tr>
<tr>
<td>Triptorelin</td>
<td>D-Trp for Gly-6</td>
<td>100</td>
</tr>
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</table>

Table 5. Classifications of patients in the three different stimulation protocols, 1989-1990

<table>
<thead>
<tr>
<th>Stimulation cycles</th>
<th>Luteal Leuprorelin Acetate (A) (45%)</th>
<th>Follicular Leuprorelin Acetate (B) (26%)</th>
<th>FSH/hMG (C) (29%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>524</td>
<td>299</td>
<td>332</td>
</tr>
</tbody>
</table>
2. Patients

<table>
<thead>
<tr>
<th></th>
<th>Luteal Leuprorelin Acetate (A)</th>
<th>Follicular Leuprorelin Acetate (B)</th>
<th>FSH/hMG (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=507)</td>
<td>(n=268)</td>
<td>(n=275)</td>
</tr>
<tr>
<td>Tubal</td>
<td>269 (53)</td>
<td>157 (59)</td>
<td>152 (55)</td>
</tr>
<tr>
<td>Male</td>
<td>102 (20)</td>
<td>49 (18)</td>
<td>62 (23)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>57 (11)</td>
<td>38 (14)</td>
<td>38 (14)</td>
</tr>
<tr>
<td>Unexplained &amp; Other</td>
<td>79 (16)</td>
<td>24 (9)</td>
<td>23 (8)</td>
</tr>
</tbody>
</table>

(Muasher 1992) (95)

Table 7. Patient and stimulation characteristics in the three stimulation protocols, 1989-1990

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Luteal Leuprorelin Acetate (A)</th>
<th>Follicular Leuprorelin Acetate (B)</th>
<th>FSH/hMG (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33.6±3.6*</td>
<td>33.5±3.6</td>
<td>36.1±3.7</td>
</tr>
<tr>
<td>Basal FSH (ml U/ml)</td>
<td>9.8±3.3*</td>
<td>16 ±.5</td>
<td>14.5±7.1</td>
</tr>
<tr>
<td>Basal LH (ml U/ml)</td>
<td>16.5±6.3</td>
<td>14.2±6.0</td>
<td>13.5±6.7</td>
</tr>
<tr>
<td>Ampoules of FSH/hMG</td>
<td>22±6</td>
<td>19±5</td>
<td>19±4.5</td>
</tr>
<tr>
<td>E₂ day of hCG (pg/ml)</td>
<td>1069±684**</td>
<td>768±497</td>
<td>587±389</td>
</tr>
<tr>
<td>Peak E₂ (pg/ml)</td>
<td>1430±897**</td>
<td>992±597</td>
<td>764±510</td>
</tr>
</tbody>
</table>

All values means ± SD. *A<B,C, P<0.05: **A>B,C, P<0.05

Table 8. Classification of oocytes retrieved, transferred and cryopreserved in the three stimulation protocols, 1989-1990

<table>
<thead>
<tr>
<th></th>
<th>Luteal Leuprorelin Acetate (A)</th>
<th>Follicular Leuprorelin Acetate (B)</th>
<th>FSH/hMG (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=507)</td>
<td>(n=268)</td>
<td>(n=275)</td>
</tr>
<tr>
<td>Total oocytes/cycle</td>
<td>12.9±5.2*</td>
<td>6.6±3.9</td>
<td>6.4±4.1</td>
</tr>
</tbody>
</table>
Preov. oocytes/cycle | 9.3±2.0* | 4.6±1.6 | 3.9±1.4
Preov. oocytes transfer/cycle | 3.8±1.0* | 2.9±0.8 | 2.6±0.7
Cycles with cryo % | 54(274/507)* | 20(53/268) | 19(52/275)
Embryos cryo/cycle with cryo | 5.5±1.8** | 3.6±2.0 | 2.5±2.0

Preov. = preovulatory; cryo = cryopreserved, cryopreservation
All values means ± SD. *A>B,C P<0.05; **A>B>C, P<0.05 (Muasher 1992) (95)

Table 9 - Final IVF parameters (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>High-fixed dose (n=23)</th>
<th>Step-down (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of FSH vials</td>
<td>61.2±8</td>
<td>38.2±9.6</td>
</tr>
<tr>
<td>Maximum oestradiol (pg/ml)</td>
<td>1459±844</td>
<td>1223±696</td>
</tr>
<tr>
<td>Follicles ≥³ 12mm</td>
<td>6.3±3.1</td>
<td>5.1±2.3</td>
</tr>
<tr>
<td>Total oocytes</td>
<td>5.4±2.4</td>
<td>5.6±3.4</td>
</tr>
<tr>
<td>Fertilized oocytes</td>
<td>4.1±2.2</td>
<td>3.8±1.9</td>
</tr>
<tr>
<td>Replaced oocytes</td>
<td>2.3±0.9</td>
<td>1.9±0.8</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>


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