Ovulation Induction in In-Vitro Fertilization

July 05, 2011 | Ovulation Induction in In-Vitro Fertilization

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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.\(^\text{(1)}\)

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.\(^\text{(2)}\)

During their initial effort human menopausal gonadotropins (hMG) were utilized for ovarian stimulation to produce multiple follicles for ovum retrieval.\(^\text{(3)}\)

Their technique using the spontaneous cycle was based on endocrine abnormalities and luteal phase defects (LPD) associated with stimulation.\(^\text{(4)}\)
However, in view of the relatively low pregnancy rate, due not only to the presence of a single preovulatory follicle in a spontaneous cycle but also to the difficulty of monitoring a spontaneous cycle and performing the oocyte retrieval 24 hours a day. (5)

Several groups of investigators again adopted the use of ovulation inducing agents. (6) (7) 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. (7)

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. (5) (8) (9) (10) (11)

**Ovarian simulation**
The first pregnancy obtained by IVF and embryo transfer was obtained using ovarian stimulation but it proved to be an ectopic pregnancy (3). The first full-term pregnancies were achieved with oocytes from unstimulated cycles (2). Subsequent studies, however, have shown that ovarian stimulation is associated with better results (5). Hence, most centres are now performing IVF and other assisted reproduction techniques in stimulated cycles (12). 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 (13) (14) and
(v) recombinant FSH (15).

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 (16) (17).

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 (12). Oocyte aspiration is performed 34-38 h after HCG injection or 26-28 h after the detection of an endogenous LH surge (18).

Follicular development can be monitored by serial hormonal measurements (oestradiol, LH, progesterone) and by ultrasonography (12) (18). 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. (12) (18)

**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 (10) (19) (20).

In a spontaneous cycle the levels of FSH are rising immediately prior to and during menses (10) (21).

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.** (5) (22).

**Clomiphene Citrate (CC)**
The single agent most commonly used for enhanced follicular recruitment for in-vitro fertilization has
been clomiphene citrate \(^{(5)}\).

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 \(^{(10)}\).

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 \(^{(23)}\).

There are problems associated with CC use:

- Its effects are long-lasting \(^{(24)}\). 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 \(^{(25)}\) \(^{(26)}\).
- The En isomer is the active component in initiating follicular development \(^{(27)}\). 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 \(^{(28)}\).
- The induced increase in luteinsing hormone (LH) secretion can far exceed that for follicle stimulating hormone (FSH) \(^{(29)}\), which is further exaggerated in a polycystic ovaries (PCO) patient. High LH has been associated with miscarriage \(^{(30)}\), which in a PCO patient can be best corrected by the use of a gonadotropin-releasing hormone (GnRH) agonist \(^{(31)}\) \(^{(32)}\).
- There is a high reported incidence of luteinised unruptured follicle (LUF) syndrome in patients with unexplained infertility \(^{(33)}\). The anti-oestrogenic effects are at the level of the cervix and endometrium \(^{(34)}\).
- There is an increased incidence of ectopic pregnancies in in vitro fertilisation (IVF) \(^{(35)}\) \(^{(36)}\).
- There is substantial literature support for a possible direct adverse effect at the level of the rat, rabbit and human oocyte \(^{(37)}\).

**Clomiphene citrate in IVF**

Saunders et al (1992) \(^{(38)}\) have associated the use of clomiphene citrate in superovulation cycles with a higher miscarriage rate than GnRH agonist \(^{(38)}\).

Corson and Batzer (1986) \(^{(39)}\) and Cohen et al (1986) \(^{(35)}\) have suggested a relationship between clomiphene citrate use and ectopic pregnancy. However, Grab et al (1992) \(^{(40)}\) 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 \(^{(40)}\). Harrison et al (1993) \(^{(36)}\) have reported a trend towards an increasing ectopic pregnancy rate with increasing daily doses of clomiphene citrate \(^{(36)}\). (This could be related to clomiphene citrate effects on tubal transport \(^{(42)}\). 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” \(^{(42)}\).

Gonen and Casper (1990) \(^{(41)}\) 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 \(^{(41)}\). This may be due to the anti-oestrogenic effect of clomiphene citrate on the endometrium.

*Most published series on the use of clomiphene citrate alone for enhanced follicular recruitment report a mean of less than 2 oocytes recovered per patient undergoing follicular aspiration* \(^{(43)}\) \(^{(44)}\).

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 \(^{(43)}\).
In 1995 Benadiva et al (1995) \(^{45}\) 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 \(^{47}\). 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 refute 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 \(^{61}\).
The principal experience with the “physiologic” use of hMG for follicular recruitment comes from the Eastern Virginia Medical School. (62). 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 (63) and 2.4 (64) 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 (65). 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) (66). 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) (2) 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 (2). 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 (67). 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. (67)

Other groups have also reported the use of differing combinations of clomiphene and hMG for enhanced follicular recruitment. Lopata (1983) (68) 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) (69) 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 (69). Mandelbaum et al. (1983) (70) 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. (70)

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 \(^{(71)}\).

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/1, 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 Ca\(^{2+}\) 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 endocrinoiological 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 gonadotroph 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 ethylamide 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 desensitise 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.\(^{(5)}\)\(^{(6)}\)\(^{(7)}\)\(^{(8)}\)

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 Table 9\(^{(178)}\) Shalabi et al (1998)\(^{(179)}\) concluded that: A long protocol using short-acting GnRHa is preferable to a long protocol with depot GnRHa in terms of embryo quality and pregnancy rate. A further multicentre prospective randomized study is needed to confirm these results.\(^{(179)}\)

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\(^{(180)}\) and they concluded that leuprolide acetate and Triptorelin had similar efficacy in patients undergoing IVF.\(^{(180)}\)
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 L.S. 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.

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.

A study done by Filicori et al 1993 40 normally cycling women with male-related infertility Table 1. Findings and Outcome at Laparoscopy in Patients Receiving “High Dose” hMG Alone and Clomiphene Alone

**Treatment Group**

<table>
<thead>
<tr>
<th>hMG</th>
<th>Clomiphene</th>
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<tbody>
<tr>
<td><strong>Significance</strong></td>
<td></td>
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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>
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<tbody>
<tr>
<td>hMG</td>
<td>21.1 ± 1.5 days</td>
<td>11.6 ± 1.5 days</td>
</tr>
</tbody>
</table>
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></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-αs

<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>Goserein</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>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></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>1. Stimulation cycles</td>
<td>524</td>
<td>299</td>
<td>332</td>
</tr>
<tr>
<td>2. Patients</td>
<td>429</td>
<td>243</td>
<td>278</td>
</tr>
<tr>
<td>3. Retrieval cycles</td>
<td>507</td>
<td>268</td>
<td>275</td>
</tr>
<tr>
<td>4. Cancellation rate (%)</td>
<td>3*</td>
<td>10</td>
<td>... ..</td>
</tr>
<tr>
<td>5. Transfer cycles</td>
<td>492</td>
<td>244</td>
<td>244</td>
</tr>
<tr>
<td>(94% of 1.)</td>
<td>(82% of 1.)</td>
<td>(73% of 1.)</td>
<td></td>
</tr>
</tbody>
</table>

A<B<C, P<0.05
(Muasher 1992) (95)

Table 6. Causes of infertility in the three stimulation protocols, 1989-1990

<table>
<thead>
<tr>
<th>Cause</th>
<th>Luteal Leuprorelin Acetate (A) (n=507) (%)</th>
<th>Follicular Leuprorelin Acetate (B) (n=268) (%)</th>
<th>FSH/hMG (C) (n=275) (%)</th>
</tr>
</thead>
<tbody>
<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>
</tbody>
</table>
Table 7. Patient and stimulation characteristics in the three stimulation protocols, 1989-1990

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Luteal Leuprorelin Acetate (A) (n=52)</th>
<th>Follicular Leuprorelin Acetate (B) (n=29)</th>
<th>FSH/hMG (C) (n=32)</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</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>Total oocytes/cycle</th>
<th>Luteal Leuprorelin Acetate (A) (n=507)</th>
<th>Follicular Leuprorelin Acetate (B) (n=268)</th>
<th>FSH/hMG (C) (n=275)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preov. oocytes/cycle</td>
<td>12.9±5.2*</td>
<td>6.6±3.9</td>
<td>6.4±4.1</td>
</tr>
<tr>
<td>Preov. oocytes</td>
<td>9.3±2.0*</td>
<td>4.6±1.6</td>
<td>3.9±1.4</td>
</tr>
<tr>
<td>transfer/cycle</td>
<td>3.8±1.0*</td>
<td>2.9±0.8</td>
<td>2.6±0.7</td>
</tr>
<tr>
<td>Cycles with cryo %</td>
<td>54(274/507)*</td>
<td>20(53/268)</td>
<td>19(52/275)</td>
</tr>
<tr>
<td>Embryos cryo/cycle</td>
<td>5.5±1.8**</td>
<td>3.6±2.0</td>
<td>2.5±2.0</td>
</tr>
</tbody>
</table>

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 &gt;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>
</tbody>
</table>
References:


<table>
<thead>
<tr>
<th>Replaced oocytes</th>
<th>2.3±0.9</th>
<th>1.9±0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pregnancies</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>


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Links:
[8] http://www.obgyn.net/authors/m-nabil-el-tabbakh-md