

ASSISTED REPRODUCTIVE TECHNOLOGY

Serum luteinizing hormone, follicle-stimulating hormone and oestradiol pattern in women undergoing pituitary suppression with different gonadotrophin-releasing hormone analogue protocols for assisted reproduction

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Abstract

Gonadotrophin-releasing hormone analogues (GnRH-a) are used widely in controlled ovarian stimulation (COS) cycles for assisted reproduction. At present, there is great debate about the influence of exogenous hormone activity on the hypothalamus-pituitary axis following pituitary desensitization. The objective of this comparative study was to investigate the pattern of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and oestradiol in women undergoing ovarian stimulation with different GnRH-a preparations. We retrospectively analysed 201 women, aged between 27 and 43 years, who were referred consecutively to our infertility clinic between January 2002 and January 2003. All women had no endocrinopathies or occult ovarian failure as assessed by day-3 hormone profile. Women were enrolled in one of the following COS protocols: depot triptorelin long protocol ($n=38$), buserelin long protocol ($n=101$) or buserelin short protocol ($n=62$). Recombinant FSH was used to induce ovulation. Treatment was monitored by transvaginal ultrasound scan and serum measurement of FSH, LH and oestradiol. Among the women initially included, 30 had cancelled cycles due to poor ovarian response. Serum LH levels were significantly higher in the short-protocol group compared with the long-protocol groups ($p < 0.001$). The number of follicles, oocyte yield, number of grade-I embryos and fertilization rate were significantly lower in the short-protocol group than in the long-protocol groups. These findings showed that LH concentrations are significantly higher in women undergoing reversible medical hypophysectomy with a GnRH-a short protocol than in women treated with a long protocol. The hypothesis of an LH ceiling is confirmed.

Keywords: *Controlled ovarian stimulation, gonadotrophin-releasing hormone analogue, long-term and short-term pituitary desensitization, luteinizing hormone*

Introduction

The replacement or deletion of amino acids within the native gonadotrophin-releasing hormone (GnRH) molecule has resulted in the development of GnRH analogues (GnRH-a). These preparations have been used widely for controlled ovarian stimulation (COS) to suppress the pituitary gland in assisted reproduction cycles. Generally, women undergo either long-protocol (i.e., desensitizing) or short-protocol (i.e., flare-up) treatment prior to ovarian stimulation with exogenous gonadotrophins [1,2].

Paracrine signalling regulated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) sustains follicle growth and oestrogen secretion until ovulation [3]. Whether this process is also directly modulated by GnRH-a is still a matter of debate. As a consequence of inadequate evidence and the lack of well-formulated guidelines, COS protocols are often chosen on the basis of the clinician's preference and experience gained from the patient's response to previous treatment cycles. For this purpose, ovarian responsiveness has been employed as a reliable tool for selecting the most appropriate ovarian stimulation protocol. Thus, the assessment of ovarian reserve is

considered mandatory and measurement of serum FSH levels on day 3 of the menstrual cycle is still employed as its main index [4]. On the other hand, the potential direct effect of GnRH-a on steroidogenesis, or its physiological significance, has not been fully established. Attempts to study the exact hormone profile (in particular, of oestradiol and gonadotrophins) of GnRH-a protocols of stimulation are very limited. To this end, only triptorelin long protocol together with recombinant or highly purified gonadotrophins has been studied [5,6].

The aim of the present study was to investigate the pattern of serum concentrations of LH, FSH and oestradiol in women undergoing ovarian stimulation with different GnRH-a protocols for assisted reproduction.

Materials and methods

Study population

Between January 2002 and January 2003, a total of 201 women aged between 27 and 43 years, who were consecutive referrals to the 'Genesis' Centre for Human Reproduction and Therapy of Infertility in Rome, were enrolled in the present study. Informed written consent was obtained from all participants. The protocol regimens and study design were approved by the internal medical ethics committee.

All women underwent routine infertility work-up, including laparoscopy, pelvic ultrasonography and partner's semen analyses, prior to ovarian stimulation. Women were menstruating regularly (cycles of 26–34 days) and had a body mass index (BMI) of 17–33 kg/m². Pelvic ultrasound scanning showed uterus and ovaries of normal size and structure. Haematology and serum biochemical indices were well within the normal range. There was neither endocrinopathy nor occult ovarian failure as assessed by baseline hormone profile (FSH < 14 mIU/ml). Infertility causes were reported as follows: male-factor infertility (*n* = 101), Fallopian tube disease (*n* = 45), unexplained infertility (*n* = 42) and pelvic endometriosis (*n* = 13).

Sample size and ovarian stimulation protocols

Participants were assigned to one of three pituitary desensitization protocols for assisted reproduction as follows: triptorelin long protocol during the first 3 months of the study, buserelin long protocol during the following 5 months and buserelin short protocol during the last 4 months of the study. No woman underwent more than one cycle of stimulation with the protocols considered.

- Group A (*n* = 38): a single intramuscular injection of 3.75 mg depot triptorelin (Decapeptyl 3.75[®]; IPSEN SpA, Milan, Italy) was administered on day 21 of the previous spontaneous menstrual cycle.

- Group B (*n* = 101): subcutaneous injection of 0.3 ml buserelin acetate (Suprefact[®]; Hoechst, Rome, Italy) was given daily from day 21 of the previous spontaneous menstrual cycle for an average of 13 consecutive days.
- Group C (i.e., flare-up group) (*n* = 62): subcutaneous injection of 0.5 ml buserelin acetate was given from day 2 to day 4, subsequently reduced to 0.2 ml from day 5 onwards for ten consecutive days.

Ovarian stimulation with exogenous gonadotrophins was commenced 12–16 days after pituitary desensitization in groups A and B, and on cycle day 3 in group C. All women received a subcutaneous injection of recombinant FSH (rFSH) (Gonal-F[®]; Serono, Rome, Italy) daily at 18.00–20.00 hours, with a starting dose of 300 IU. The stimulation was then continued in a step-down fashion according to the individual ovarian response as assessed by serum oestradiol concentration and serial follicle growth. In practice, rFSH supplementation was reduced to 225 IU or even less when oestradiol levels doubled. When at least three follicles reached a diameter > 15 mm and serum oestradiol levels were > 1.500 pg/ml, 5.000 IU of human chorionic gonadotrophin (hCG) (Profasi[®]; Serono) was administered intramuscularly to trigger final follicular maturation. Ultrasound-guided transvaginal retrieval of oocytes was performed 35 h after hCG administration. Luteal phase support was given by daily intramuscular administration of 50 mg progesterone in oil (Prontogest[®]; AMSA, Rome, Italy) starting on the day of embryo transfer and continuing until the day of the pregnancy test (i.e., day 12 after embryo transfer).

There were no statistically significant differences between the study groups with regard to age, baseline serum FSH, weight, height, BMI, menstrual cycle length (Table I) and causes of infertility. Assisted reproduction techniques (*in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI)) were distributed equally between the study population (*n* = 171).

Laboratory procedures

Fresh spermatozoa were prepared early in the morning of oocyte retrieval with the mini swim-up method [7]. In the IVF procedure, the oocytes were placed in IVF-20 medium under mineral oil (Vitrolife, Gothenburg, Sweden) and incubated at 37°C in a humidified atmosphere with 5% CO₂. In cases where ICSI was performed, oocytes were cultured for up to 3 h prior to microinjection. The oocytes were then checked for signs of degeneration and cultured in 20 µl of IVF-50 medium under mineral oil (Vitrolife). Pronucleated oocytes were assessed and embryo grading was performed according to published criteria [8]. Two embryos per woman were replaced 44–50 h later.

Table I. Descriptive and clinical characteristics for the women included in the study ($n = 201$).

	Long protocol		Short protocol
	Triptorelin ($n = 38$)	Buserelin ($n = 101$)	Buserelin ($n = 62$)
Age (years)	32.80 \pm 0.61	33.27 \pm 0.41	34.22 \pm 0.53
Weight (kg)	60.83 \pm 2.87	60.61 \pm 0.84	60.05 \pm 1.78
Height (m)	1.62 \pm 0.01	1.65 \pm 0.01	1.62 \pm 0.01
Body mass index (kg/m ²)	23.13 \pm 1.02	22.31 \pm 0.29	22.72 \pm 0.64
Menstrual cycle duration (day)	28.00 \pm 1.38	28.42 \pm 1.22	28.23 \pm 1.18
Basal follicle-stimulating hormone level (mIU/ml)	6.43 \pm 0.42	6.61 \pm 0.19	7.10 \pm 0.39

All values are expressed as mean \pm standard error.

Cycle monitoring and serum hormone assays

All women were followed up and hormone levels were monitored throughout the stimulation phase. A blood sample was drawn daily from each woman at 08.00–09.00 hours, and serum LH, FSH and oestradiol levels were measured for clinical monitoring. Also, follicular growth was monitored using transvaginal pelvic ultrasonography (Echo Camera SSD-500; Aloka Co. Ltd, Tokyo, Japan) on gonadotrophin stimulation day 1 and day 5, and on alternate days thereafter until hCG administration. LH, FSH and oestradiol concentrations were measured using commercial microparticle immunoassays (AXSYM[®]; Abbott SpA, Rome, Italy). The minimum detectable assay levels for the three hormones were 0.50 mIU/ml, 0.37 mIU/ml and 20 pg/ml, respectively. The inter- and intra-assay coefficients of variation (CV) for FSH, LH and oestradiol were 3.5% and 3.1%, 4.8% and 4.5%, and 4.2% and 4.5%, respectively.

Statistical analysis

Power statistical analysis was calculated with a view to improving pregnancy rate from 25 to 35%, taking 5% as the significance level and accepting 80% probability of finding a true difference.

To compare the pattern of serum hormone levels during COS cycles, the area under the curve (AUC) was calculated individually for each of the three hormones and the mean value obtained by relating it to the number of days of measurement. One-way analysis of variance and Scheffe's *post hoc* test of significance were used to compare different parameters among study groups. Data analysis was carried out using the StatView statistical package (SAS Institute Inc., Cary, NC, USA). Differences were considered statistically significant if $p < 0.05$.

Results

The mean duration of infertility in the study population was 4.2 years (range 3.0–5.5 years). Among the 201 women initially considered, 30

(14.9%) who had cancelled COS cycles due to poor response at stimulation were excluded from the final analysis. The cancellation rate was 7.9% (3/38 women), 11.8% (12/102 women) and 24.2% (15/62 women) in the triptorelin group, buserelin long-protocol group and buserelin short-protocol group, respectively ($p < 0.01$).

The overall dose of gonadotrophins administered was not significantly different between the three groups, whereas the duration of gonadotrophin stimulation was significantly longer in the triptorelin group than in the buserelin long- and short-protocol groups ($p < 0.001$) (Table II).

Serum FSH, LH and oestradiol pattern, expressed as AUC during ovarian stimulation, is also shown in Table II. During COS cycles, women in the triptorelin and buserelin long-protocol groups had significantly lower serum FSH levels than women in the buserelin short-protocol (flare-up) group ($p < 0.05$). Serum concentrations of LH calculated as AUC were significantly higher in the flare-up group with respect to the other two groups ($p < 0.001$). The AUC for oestradiol was not significantly different among the three groups. LH/FSH ratio followed the LH pattern, being higher in the flare-up group ($p < 0.001$). The oestradiol/FSH ratio was significantly lower in the short-term protocol compared with the long-term ones ($p < 0.05$) whereas the oestradiol/LH ratio was significantly higher in the triptorelin group compared with the buserelin groups ($p < 0.01$).

The total numbers of follicles and leading follicles, the oocyte yield and the numbers of mature oocytes, grade-I embryos and embryos transferred were significantly lower in the buserelin short-protocol group compared with the long-protocol groups (Table III). Conversely, the number of oocytes fertilized and fertilization rate were significantly higher in the triptorelin group than in the buserelin long- and short-protocol groups ($p < 0.02$).

The pattern of serum LH, FSH and oestradiol during the ovarian stimulation cycle, as well as the ratios LH/FSH, oestradiol/FSH and oestradiol/LH, are shown in Figures 1 and 2.

Table II. Hormone levels before and during controlled ovarian stimulation cycles for all women undergoing oocyte recovery ($n = 171$), by protocol of stimulation.

	Long protocol		Short protocol
	Triptorelin ($n = 35$)	Buserelin ($n = 89$)	Buserelin ($n = 47$)
Duration of gonadotrophin stimulation (days)	11.44 \pm 0.27*	9.93 \pm 0.21	10.09 \pm 0.36
Total amount of gonadotrophin administered (IU)	3396.40 \pm 342.78	3633.39 \pm 206.48	3659.22 \pm 328.45
FSH (AUC) (mIU/ml \times day)	147.0 \pm 12.8	176.9 \pm 10.6	234.8 \pm 14.1 [†]
LH (AUC) (mIU/ml \times day)	13.0 \pm 1.23	19.9 \pm 1.2	117.7 \pm 7.6 ^{††}
E ₂ (AUC) (pg/ml \times day)	8973 \pm 574	8750 \pm 427	8107 \pm 548
LH/FSH	0.09 \pm 0.02	0.11 \pm 0.01	0.50 \pm 0.08 ^{††}
E ₂ /FSH	61.04 \pm 6.50	55.37 \pm 5.21	34.64 \pm 6.84 [†]
E ₂ /LH $\times 10^3$	690.06 \pm 89.55**	439.21 \pm 42.51	69.00 \pm 10.32

FSH, follicle-stimulating hormone; AUC, area under curve; LH, luteinizing hormone; E₂, oestradiol; all values are expressed as mean \pm standard error; significant difference vs. long- and short-protocol buserelin: * $p < 0.001$; ** $p < 0.01$; significant difference vs. long-protocol buserelin and long-protocol triptorelin: [†] $p < 0.05$, ^{††} $p < 0.001$.

Table III. Outcome of ovarian stimulation by protocol group.

	Long protocol		Short protocol
	Triptorelin ($n = 35$)	Buserelin ($n = 89$)	Buserelin ($n = 47$)
Total number of follicles	12.44 \pm 80	11.11 \pm 0.49	8.53 \pm 0.59**
Number of follicles with diameter ≥ 15 mm	9.32 \pm 0.78	8.40 \pm 0.47	6.70 \pm 0.60
Number of retrieved oocytes	12.55 \pm 1.30	10.22 \pm 0.59	6.73 \pm 0.85*
Number of mature oocytes (% mature)	9.52 \pm 1.06 (80.54)	7.55 \pm 0.49 (78.11)	5.62 \pm 0.81** (84.32)
Number of grade-I embryos	2.33 \pm 0.19	2.22 \pm 0.11	1.58 \pm 0.14**
Number of grade-II embryos	2.07 \pm 0.23	1.75 \pm 0.11	1.69 \pm 0.21
Number of fertilized oocytes (fertilization rate, %)	8.10 \pm 0.75 [†] (66.89)	6.20 \pm 0.40 (61.11)	4.95 \pm 0.65 (70.58)

All values are expressed as mean \pm standard error; significant difference vs. long-protocol buserelin and long-protocol triptorelin: * $p < 0.05$, ** $p < 0.001$; significant difference vs. long- and short-protocol buserelin: [†] $p < 0.02$.

Discussion

It is well established that each GnRH-a preparation has potential advantages and disadvantages as a result of its intrinsic activity. For instance, long-acting depot preparations produce more profound pituitary suppression than short-acting preparations [9]. This activity induces a functional and transitory hypogonadotrophic condition and, albeit rarely, may decrease ovarian response to exogenous gonadotrophins [10]. On the other hand, short and ultra-short COS protocols [2,11] as well as lower GnRH-a dosages [12] have been used extensively to modulate treatment duration, gonadotrophin consumption and ovarian response, but with different degrees of success.

The present study confirms that GnRH-a short protocol used in ovarian stimulation for assisted reproduction carries a lower reproductive outcome compared with GnRH-a long protocol [13]. In accordance with previous studies [14,15], our data demonstrate that the number of leading follicles,

oocyte yield, oocytes fertilized and high-quality embryos were significantly higher in the GnRH-a long-protocol group.

All women included in the present study had similar demographic features, causes of infertility and ovarian reserve characteristics. The increase in serum oestradiol levels was similar in all groups until the day of hCG administration, although the overall levels were significantly lower in the flare-up group. Similar results were recently reported by other authors [6].

Serum LH levels on day 3 of ovarian stimulation were found to be significantly higher in the buserelin short-protocol group than in the long-protocol groups. These findings confirm previous studies showing a positive correlation between increased concentrations of serum LH [16] and urinary LH [17] and lower oocyte and embryo quality and poor reproductive outcome. Similarly, Loumaye and colleagues found that GnRH-a short and ultra-short protocols were associated with raised LH levels, hence exposing the developing ovarian follicles to

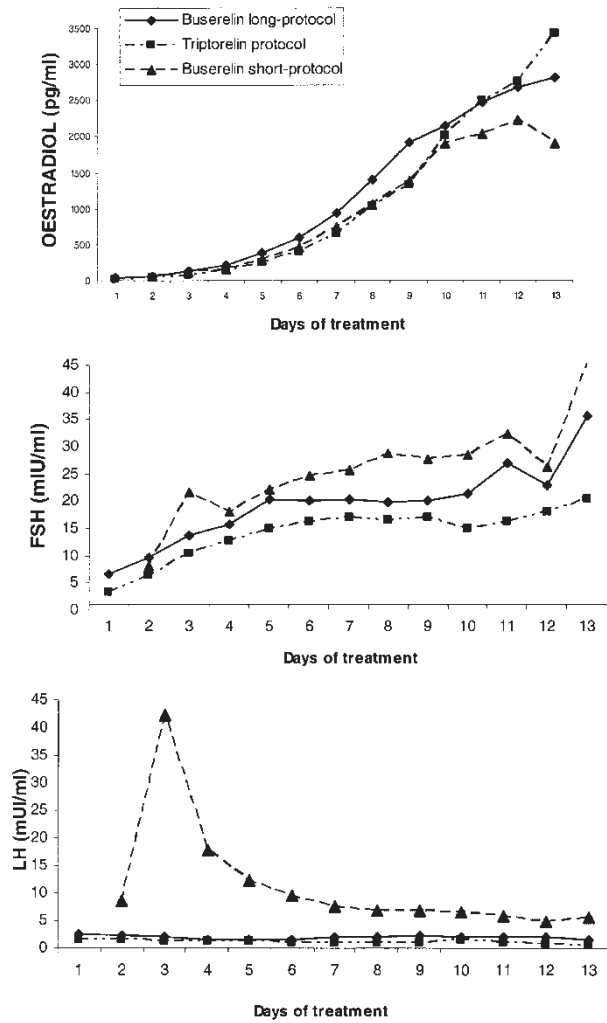


Figure 1. Mean daily concentrations of oestradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) during ovarian stimulation using the three protocols.

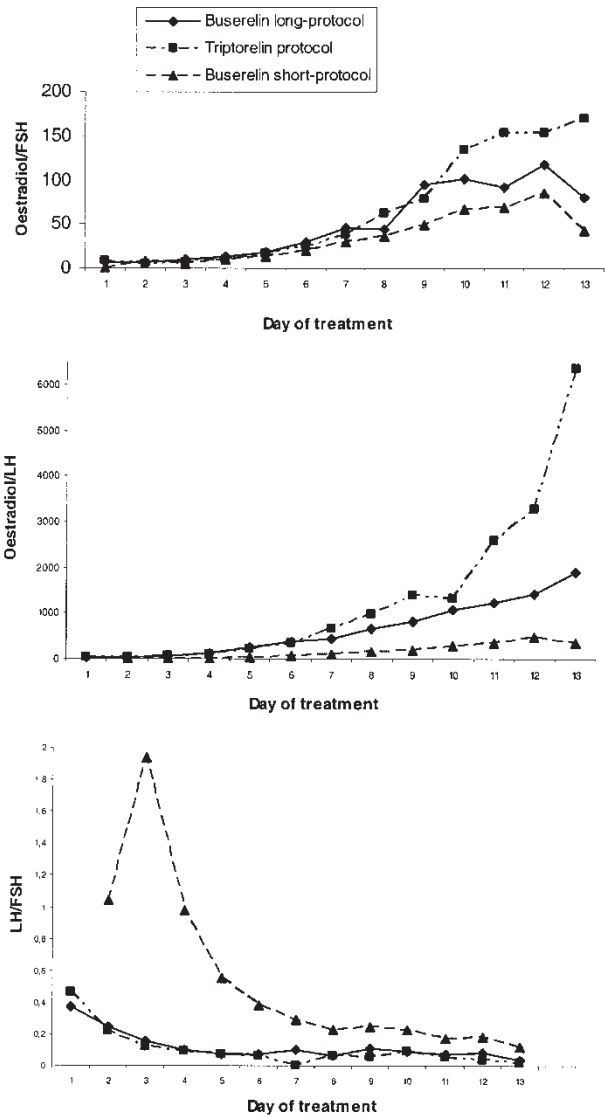


Figure 2. Mean daily concentration ratios of oestradiol/follicle-stimulating hormone (FSH), oestradiol/luteinizing hormone (LH) and LH/FSH during ovarian stimulation using the three protocols.

abnormal LH concentrations [14]. However, these studies were performed using LH-containing preparations (i.e., human menopausal gonadotrophin, hMG), whereas in our work only rFSH was used. More recently, Chappel and Howles [18] argued that such follicles were more prone to entering atresia or becoming prematurely luteinized to the extent of compromising oocyte development. Apart from effects on the follicles, a large body of evidence indicates that high LH concentrations may impair fertility through a direct effect on the oocytes [16,19,20] and uterine receptivity [21]. The effect of LH on steroidogenesis and cell growth has also been well documented. Low doses of LH enhance steroidogenesis without inhibiting DNA synthesis, while high doses of LH enhance the synthesis of progesterone, the suppression of aromatase activity and the inhibition of cell growth [22,23]. Our findings therefore agree with the theory of Hillier, who postulated the existence of an LH ceiling above which LH becomes detrimental to oocyte development [24].

In the present study, oestradiol levels were similar in the three groups whereas FSH levels were significantly higher in the flare-up group (Table II). These findings let us assume that, in the case of raised serum LH levels, rFSH is unable to influence oestradiol production if using a GnRH-a short protocol for pituitary suppression. It is likely that high LH levels increase androgen production to such an extent that it cannot be transformed into oestrogens owing to lack of aromatase. Previous studies have in fact demonstrated that increased intrafollicular concentration of androgens yields a deleterious hormonal milieu for oocytes [25]. As previously reported by Richards and associates [26], an ordinate chain of events (in particular LH receptor formation) is mandatory for a proper follicular development.

As expected, we found that the pattern of LH/FSH follows a similar pattern to that observed for LH alone (Table II). Gordon and co-workers reported

that a physiological LH range has to be set between 0.5 and < 10 mIU/ml in ovarian stimulation protocols [27]. Other studies demonstrated that very low LH levels (< 0.5 mIU/ml) were sub-optimal for both follicular development [10] and reproductive outcome [28,29].

Whether the administration of additional LH or hCG should be considered when serum LH levels are less than 0.5 mIU/ml is still a matter of debate [28]. Recently, Tesarik and Mendoza [30] found that exogenous LH supplementation negatively affects embryo morphology and implantation rate in women who had serum LH levels > 1 mIU/ml. In contrast, Kilani and colleagues [6] could not demonstrate any adverse effect on reproductive outcome when comparing ovarian stimulation with and without supplemental hMG, regardless of serum LH concentrations.

In conclusion, these findings confirm that, compared with long-stimulation protocols, the GnRH-a short protocol is associated with higher cancellation rate, lower numbers of growing follicles, retrieved oocytes and grade-I embryos, and lower fertilization rate. We believe that higher associated levels of serum LH following GnRH-a short protocol may play a certain role. These data support the 'LH ceiling hypothesis' first proposed by Hillier [31]. Whether the recently introduced GnRH antagonists may be useful in shortening the stimulation period, preventing the LH surge and ovarian hyperstimulation syndrome, and controlling detrimental rises in LH during COS is awaited. Further large, prospective, randomized studies are needed to confirm these data and also to improve our understanding of the treatment strategy for assisted reproduction.

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