Hormonal responses and clinical outcome are the same with three doses of gonadotropin-releasing hormone agonist used in the short stimulation protocol

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ABSTRACT

Objective: The aim of this study was to compare 3 different doses of GnRH-a in a short protocol on ICSI outcome.

Methods: 91 ovulatory patients were randomly assigned to three groups; group A (N=34), group B (N=34) and group C (N=23). All started treatment with urinary gonadotropins, 2 ampoules per day and GnRH-a (Triptorelin) on the first day of the menstrual period and continued till the day of (hCG) administration. The daily dose of Triptorelin was 0.1 mg, 50 µg, and 25 µg in groups A, B and C respectively.

Results: Stimulation requirements, days of stimulation and total ampoules of gonadotropins did not differ between the three groups. The mean number of follicles aspirated and the mean number of Metaphase 2 oocytes retrieved were similar in the three groups. Fertilization and cleavage rates were similar in the three groups. The mean number of embryos available for transfer was 6.4 ± 0.5 , 9.4 ± 0.5 and 7 ± 0.7 in groups A, B and C, with a significant difference only between groups A and B. Pregnancy rates per cycle were 35.3%, 35.3% and 34.8 % for groups A, B and C, respectively with no significant difference.

Conclusion: Early and late hormonal changes, stimulation requirements and final outcome were similar with the different doses of the agonist. Other factors should be considered, most importantly: treatment cost and side effects.

Keywords: Gonadotropin-releasing hormone agonist, ICSI clinical outcome, pregnancy rate

INTRODUCTION

Controlled ovarian stimulation for assisted reproduction uses gonadotropin releasing hormone agonist (GnRH-a) routinely either in its short (flare-up) or long protocol (Orvieto *et al.*, 2002). Many studies have been investigating the optimal dose of GnRH-a since the early 1980s (Tolis *et al.*, 1982; Monroe *et al.*, 2006). The routine daily dose of the different agonists used was introduced by trials and is still not uniformly established (Lenton & Mohamed, 2005). Reduced daily dose of GnRH-a has been suggested by several authors (Ben-Rafael *et al.*, 2000; Janssens *et al.*, 2000).

Among the advantages of reducing the dose of the agonist are: decreasing cost and side effects of the medications, decreasing the amount of gonadotropins used for stimulation with its costs and the possible occurrence of ovarian hyperstimulation. We have to weigh these advantages against the possible insufficiency of a smaller dose to maintain pituitary gland suppression and to prevent the occurrence of spontaneous LH surge leading to cycle cancellation. LH surges occur in 20% of IVF patients leading to interruption of the IVF cycles, as shown in placebo-controlled studies involving GnRH agonists (Janssens *et al.*, 2000; Edwards *et al.*, 1996). It is well established that the prevention of spontaneous LH surge is the main function of a GnRH agonist and the main reason for improved IVF outcome associated with its use (Broekmans *et al.*, 1996).

The objective of this prospective randomized controlled study was to characterize the daily changes in the levels of luteinizing hormone (LH) and estradiol (E_2) and to compare intracytoplasmic sperm injection (ICSI) outcomes from 3 different doses of GnRH-a (Triptorelin, 0.1 mg, 50 µg, and 25 µg) used in a short GnRH agonist protocol for normal ovulatory females.

MATERIALS AND METHODS

Ninety-one patients were randomly assigned to one of three groups: group A (34 patients), group B (34 patients) and Group C (23 patients).

All the patients started treatment with the short GnRH agonist stimulation protocol (flare-up) where urinary gonadotropins, and GnRH-a were started on the first day of the menstrual cycle or induced withdrawal bleeding and continued till the day of human chorionic gonadotropins (hCG) administration. The daily dose of Triptorelin (Decapepty, Ferring, Sweden) was 0.1 mg, 50 µg, and 25 µg in groups A, B and C respectively. Stimulation was performed using human menopausal gonadotropin (Merional, IBSA, Italy) or purified urinary FSH (Fostimon, IBSA, Italy) starting at a daily dose of 2 ampoules and adjusted according to ovarian response. Ovarian response was monitored frequently by trans-vaginal ultrasonography. When there were two or more follicles with a mean diameter of >17 mm and serum estradiol (E_2) level of ≥ 200 Pg/ml per mature follicle, we administered 10,000 IU of hCG (Chorion, IBSA, Italy).

Trans-vaginal oocyte retrieval was performed 35h after hCG injection. Intra-cytoplasmic Sperm injection (ICSI) was performed on metaphase II oocytes according to the conventional protocol. Embryo transfer was performed vaginally under abdominal ultrasound guidance on the second or third day after retrieval. Up to four embryos were transferred. The luteal phase support was started on the day of embryo transfer by either vaginal progesterone 400 mg (Cyclogest, Actavis Barnstaple EX32, UK) or intramuscular progesterone 100 mg (Prontogest, EIPCO, Egypt) according to patient preference.

Clinical pregnancy was defined by the presence of intrauterine gestational sac(s) with pulsating heart beats on trans-vaginal ultrasound scan at 5–6 weeks' gestation with increasing serum B-hCG levels.

Serum levels of LH and E_2 were measured daily with commercially available enzyme immunoassay kits (Cobas, Elecsys, Enzymun, Roche diagnostics GmbH, Sandhofer Strasse116, Germany).

Statistical analysis:

The demographic data of the patients, hormonal levels, stimulation requirements and outcome variables were compared for the three groups. Results are presented as mean $\pm$ standard deviation. Where appropriate, x2 test and t-test were used for analysis, using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL USA). $P < 0.05$ was considered statistically significant.

RESULTS

The mean age of the patients (34.6 ± 3.2 , 34.6 ± 3.8 and 35 ± 4.1 years), and the mean duration of infertility (7.4 ± 0.8 , 6.6 ± 0.6 and 7.7 ± 0.7 years), did not differ between the three groups. The distribution of the different causes of infertility was similar in the three groups. The basal levels of LH (3.8 ± 0.4 mIU/ml, 3.6 ± 0.3 mIU/ml and 3.7 ± 0.4 mIU/ml) and that of E_2 (49 ± 3.1 Pg/ml, 49 ± 3 Pg/ml and 55 ± 6 Pg/ml) were similar in the 3 groups (table 1).

Early changes in LH levels:

The basal levels of LH (3.8 ± 0.4 mIU/ml, 3.6 ± 0.3 mIU/

ml and 3.7 ± 0.4 mIU/ml) were similar in the 3 groups. 24 hours after treatment onset, the level of LH rose markedly to 4-5 times the basal levels in the three study groups with a mean of 22 ± 2.3 mIU/ml, 19 ± 1.5 mIU/ml and 21 ± 2.5 mIU/ml in groups A, B and C respectively. The level of LH dropped on the third day to a mean value of 13.3 ± 2.6 mIU/ml, 9 ± 0.8 mIU/ml and 9 ± 1 mIU/ml and continued to decrease on the fourth and fifth days. The level of LH on days 3 and 4 were significantly higher in group A than the other 2 groups (Table 2, Figure 1)

Early Changes in E_2 level:

The basal levels of E_2 (49 ± 3.1 Pg/ml, 49 ± 3 Pg/ml and

Table 1. demographic data of study groups

Group	Group A (N=34)	Group B (N=34)	Group C (N=23)	P Value
Demographic Data				
Age (years)	34.6 ± 3.2	34.6 ± 3.8	35 ± 4.1	NS
Duration of infertility (years)	7.4 ± 0.8	6.6 ± 0.6	7.7 ± 0.7	NS
Tubal factor infertility	14 (41.2%)	13 (38.2%)	9 (39%)	NS
Male factor Infertility	15 (44.1%)	17 (50%)	11 (48%)	NS
Unexplained Infertility	5 (14.7%)	4 (11.8%)	3 (13%)	NS
Basal LH (mIU/ml)	3.8 ± 0.4	3.6 ± 0.3	3.7 ± 0.4	NS
Basal E_2 (Pg/ml)	49 ± 3.1	49 ± 3	55 ± 6	NS

Table 2. Early changes in LH levels

Group	Group A (N=34)	Group B (N=34)	Group C (N=23)	P Value
LH level (mIU/ml)				
Basal LH	3.8 ± 0.4	3.6 ± 0.3	3.7 ± 0.4	NS
Day 2 LH	22 ± 2.3	19 ± 1.5	21 ± 2.5	NS
Day 3 LH	$13.3 \pm 2.6^{**}$	$9 \pm 0.8^*$	$9 \pm 1^*$	<0.05
Day 4 LH	$9.9 \pm 1.6^{**}$	$6.8 \pm 0.6^*$	$6.2 \pm 0.7^*$	<0.05
Day 5 LH	7.2 ± 1.3	5.4 ± 0.4	4.8 ± 0.8	NS

Figure 1. Early changes in KH level

Table 3. Early changes in E₂ level

Group	Group A (N=34)	Group B (N=34)	Group C (N=23)	P Value
E ₂ level (Pg/ml)				
Basal E ₂	49±3.1	49±3	55±6	NS
Day 2 E ₂	139±12,	111±10	115±6	NS
Day 3 E ₂	163±16	130±14	141±17	NS
Day 4 E ₂	289±34	212±29	227±31	NS
Day 5 E ₂	441±55	332±44	324±50	NS

Figure 2. Early changes in E₂ level **Figure 3.** Late changes in LH levels **Figure 4.** Late changes in E₂ levels

Table 4. Late hormonal changes

Group	Group A (N=34)	Group B (N=34)	Group C (N=23)	P Value
Hormonal level				
Day -2 LH level (mIU/ml)	6.2±0.6	4.7±0.4	5±0.7	NS
Day -1 LH level (mIU/ml)	5.5±0.3	4.4±0.3	4.2±0.5	NS
Day (hCG) LH level (mIU/ml)	5±0.3	4.4±0.3	3.7±0.4	NS
Day -2 E ₂ level (Pg/ml)	593±36	577±38	581±102	NS
Day -1 E ₂ level (Pg/ml)	882±61	851±66	865±150	NS
Day (hCG) E ₂ level (Pg/ml)	1200±89	1253±94	1317±285	

Table 5. Stimulation requirements and outcome

Group	Group A (N=34)	Group B (N=34)	Group C (N=23)	P Value
Retrieval cycles	31	31	21	NS
Cancellation	3	3	2	NS
Days of stimulation	7.25± 0.3	7.7±0.3	7.5±0.5	NS
Number of ampules	14.5±0.6	15.4±0.6	15±1	NS
Metaphase 2 oocytes	10±0.8	11±0.8	9.5±1.17	NS
Immature oocytes	1.3±0.2	1.2±0.25	1±0.2	NS
Metaphase 2 oocytes	6.7±0.7	8±0.7	7.4±1.1	NS
Embryos	3.2± 0.5*	4.6± 0.5*	3.5 ±0.6	NS
Pregnancy	12	12	8	
Pregnancy Per cycle (%)	35.3	35.3	34.8	NS

55±6 Pg/ml) were similar in the 3 groups. The level of E₂ rose markedly on the second day of stimulation to a mean of 139±12 Pg/ml, 111±10 Pg/ml and 115±12 Pg/ml in groups A, B and C respectively and kept rising on the days after. These values did not differ significantly between the three groups. (Table 3, Figure 2)

Late hormonal changes:

All the three doses of the agonist were sufficient to suppress LH and prevent its spontaneous surge. The mean level of LH on the day of hCG administration was 5±0.3 mIU/ml, 4.4±0.3 mIU/ml and 3.7±0.4 mIU/ml in groups A, B and C respectively. These values did not differ significantly from those of the preceding 2 days and between the three groups (Table 4, Figure 3,4)

Stimulation requirements and outcome:

The stimulation requirements in the form of days of stimulation (7.25±0.3, 7.7±0.3 and 7.5±0.50) or the total number of ampoules of gonadotropins (14.5±0.6, 15.4±0.6 and 15±1) did not differ between the three groups. The mean number of follicles aspirated (10±0.8, 11±0.8 and 9.5±1.1) and the mean number of Metaphase 2 oocytes retrieved (6.7±0.7, 8±0.7 and 7.4±1.1) were similar in the three groups. The rates of fertilization and cleavage were similar in the three groups. The mean number of embryos available for transfer was 6.4±0.5, 9.4±0.5 and 7±0.7 in groups A, B and C, respectively; with a significant difference only between groups A and B. Pregnancy rates per cycle were 35.3%, 35.3% and 34.8 % for groups A, B and C, respectively; with no significant difference. (Table 5)

DISCUSSION

Controlled ovarian stimulation for assisted reproduction

uses gonadotropin releasing hormone agonist (GnRH-a) routinely, either in its short (flare-up) or long protocol. The main benefit achieved by GnRH agonist use is the prevention of spontaneous LH surge leading to cycle cancellation (Broekmans *et al.*, 1996). Such a benefit explains why a marked improvement of IVF results was associated with the introduction of the agonist use. LH surges occur in 20% of IVF patients leading to cancellation of the IVF cycles as shown in placebo-controlled studies of GnRH agonists (Janssens *et al.*, 2000; Edwards *et al.*, 1996).

Many studies have investigated the optimal dose of GnRH-a since the early 1980s (Tolis *et al.*, 1982; Monroe *et al.*, 2006). The routine daily dose of the different agonists was introduced by trials and is still not uniformly established. The adopted daily dose of triptorelin, for multifollicular ovarian stimulation (MFOS) has been 100 mg. Due to the slower and lower ovarian response after complete desensitization of the pituitary (especially in poor responders), several authors have suggested that the GnRH-a dose reduction of should be possible (Ben-Rafael *et al.*, 2000; Janssens *et al.*, 1998; 2000; Broekmans *et al.*, 1996).

It has been shown that once the pituitary gland has been suppressed, the GnRH-a dose required to prevent the LH surge decreases with the length of treatment (Sandow & Donnez, 1990). Reducing the daily administered dose of GnRH-a for MFOS has been successful in poor responders, without having a detrimental effect on the ovarian response to stimulation (Takeuchi *et al.*, 2001; Yakin *et al.*, 2001). However, a comparative study has reported the cancellation rate to be significantly higher without a favourable pregnancy rate in those receiving a microdose in the GnRH-a protocol (Leondires *et al.*, 1999).

Among the advantages of reducing the dose of the agonist are decreasing the cost and side effects of the medications, decreasing the amount of gonadotropins used for

stimulation with its costs and the possible occurrence of ovarian hyperstimulation. We have to weigh these advantages against the possible insufficiency of the smaller dose to maintain suppression of the pituitary gland and to prevent the occurrence of the spontaneous LH surge leading to cycle cancellation.

Simon *et al.* (1994) compared a continuous fixed dose of 0.5mg triptorelin to tapering the dose; from a starting dose of 0.5mg to a dose of 0.1 mg after achieving down regulation. They reported this method manages to reduce the total dose of GnRH-a without any adverse effects on ovarian response or clinical results. In another trial, Elgendy and co-workers reported that more oocytes were retrieved and more embryos were obtained with intranasal nafarelin 200 mg three times daily followed by a reduction to twice the daily dose after down regulation, versus continuous 200 mg three times daily until the hCG day (Elgendy *et al.*, 1998).

In a study by Dal Prato and associates, partial desensitization with reduced GnRH-a dose was superior to the long-acting depot form (Dal Prato *et al.*, 2001). Desensitization using 100 mg triptorelin followed by 50 mg significantly reduced the duration of MFOS and gonadotropin doses compared with the group on 3.75 mg depot triptorelin. In another more recent study by Ellenbogen, *et al.* (2006), 106 IVF patients were divided into 2 groups comparing single half dose of triptorelin to its full dose. The authors concluded that half the dose offers a useful alternative for pituitary gland suppression in IVF patients, improving treatment outcome. It also reduces cost and undesirable side effects of the GnRH-a (Ellenbogen *et al.*, 2006).

The clinical efficacy of the 100 mg dose of triptorelin was suggested by Janssens and his team in their two dose-finding studies. They found that 15, 50 and 100 mg were effective doses that induced adequate desensitization (Janssens *et al.*, 2000), and that more oocytes and embryos were obtained with 50 and 100 mg compared with 15 mg or placebo, in women with idiopathic, tubal, or male factor infertility with basal FSH levels of ≤ 10 mIU/ml.

If the reduced dose of the agonist used in long protocols were successful in avoiding spontaneous LH surge, as shown in most of the above mentioned studies, this advantage will apply also to the short protocol.

An earlier report addressed another aspect of GnRH-a dose reduction and warned of the possible occurrence of ovarian hyperstimulation, even when using sub therapeutic doses of GnRH-a (Navot *et al.*, 1991). This possibility however, may not apply to those who are poor or low responders, where a reduced GnRH-a dose may avoid profound pituitary suppression and improve ovarian responsiveness to gonadotropin.

Based on these experiences, the objective of this prospective randomized controlled study was to characterize the daily changes in the levels of luteinizing hormone (LH) and estradiol (E_2) and to compare the intracytoplasmic sperm injection (ICSI) outcome with 3 different doses of GnRH-a (Triptorelin, 0.1 mg, 50 μ g, and 25 μ g) used in a short protocol for normal ovulatory females. The three study groups were similar regarding their age and contributing factors of infertility and its duration. They also started with the same dose of gonadotropins used for stimulation to avoid any bias that may result from variation in the characteristics of patients or treatment regimens. Such variations are well known to affect the results of ART cycles.

It was expected that the use of smaller dose of the agonist would decrease the dose of gonadotropins used, as suggested by some studies (Dal Prato *et al.*, 2001). But this was not shown in this study, as stimulation requirements in the form of the number of ampoules of gonadotropins

and the days of stimulation required to reach the criteria of hCG administration were comparable in the three groups. The difference between the three doses may be less than required to produce this effect.

The administration of GnRH agonist during the early follicular phase results in a flare of both FSH and LH for the following 3 to 5 days before the down regulation phenomenon takes place. This study showed that the three doses of the agonist used were capable of producing such a flare in LH, which reached a peak 24 hours after initiation of therapy and showed a sharp drop on the second day, fading away after 4-5 days. Similar duration of this flare up was recorded in other studies (Navot *et al.*, 1991; Lemay *et al.*, 1984). The significance of such an occurrence in gonadotropins induced by the agonist is still controversial, and whether it is detrimental or beneficial to the follicular development is not settled. Some studies reported it as being useful and stimulating to the recruitment of a higher number of follicles (Surrey *et al.*, 1998), while others stressed on the possible association between high LH level and follicular atresia (Polan *et al.*, 1986; Shima *et al.*, 1987). In the current study, the three doses of the agonist were sufficiently potent to produce a flare of LH and E_2 level. Although the magnitude and duration of this flare seems to be related to the dose of the agonist used, we did not find any significant effect of this variation on the final IVF outcome.

The most meaningful statistics of IVF is the number of live birth per number of initiated cycles. It was recognized before commencing this study that the sample size would not provide sufficient power to test the possible difference, if any, in the pregnancy rate between the three groups.

CONCLUSION

- The three doses of the agonist were sufficiently potent to produce a flare of LH and E_2 , although the magnitude and duration of this flare seems to be related to the dose of the agonist used. We did not find any significant effect of this variation on the final IVF outcome. The three doses of the agonist were sufficiently potent to avoid spontaneous LH surge and to block the pituitary response to the rising estradiol level.

- Stimulation requirements, such as the number of ampoules of gonadotropins and the days of stimulation required to reach the criteria of hCG administration were comparable in the three groups.

- The dose of 50 μ g Triptorelin per day yielded a higher number of follicles and embryos, but this difference did not reach statistical significance and did not reflect on the final outcome in terms of pregnancy rates.

- Based on the similarity in the stimulation requirements and final outcome of the three doses of the agonist used, treatment cost would be the most important factor to consider.

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CONFLICT OF INTERESTS

No conflict of interest have been declared.

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