The effects of different gonadotropin releasing hormone analogues in IVF cycles

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ABSTRACT

Objective: To compare Triptorelin, Leuprolide acetate and Nafarelin sodium in women undergoing controlled ovarian hyperstimulation for in vitro fertilization cycles.

Settings: Zeynep Kamil Women and Children's Hospital Reproductive Endocrinology - Infertility - IVF Center.

Material and Methods: Sixty patients were haphazardly distributed for each GnRH-a group. GnRH-a was administrated, starting on the 21st day of menstrual cycles for the long protocol: Triptorelin (Decapeptyl, 0.1 mg) as 0.1 mg/day SC, leuprolide (Lucrine, 5 mg flacon) as 0.5 mg/day SC and nafarelin (Synarel, 2 mg/mi nasal spray) as 200 micrograms to each nostril with a daily total dosage of 800 micrograms. The responses from each group were compared on the basis of duration of stimulation, total dosage of gonadotropin, E2 values on day 5, the down regulation duration, cyst formation, E2 values on the 1st day of stimulation and HCG, progesterone values and endometrial thickness on the HCG day, the number oocytes picked up, fertilization rates, the number of transferred embryos, pregnancy and implantation ratios.

Results: Evaluating Triptorelin (T), Leuprolide (LA) and Nafarelin (NA) groups respectively, E2 values on the 1st day of menstrual cycles, measured to confirm the down regulation for each GnRH-a, were 24.67 pg/ml, 21.23 pg/ml, 29.62 pg/ml (p<0.05); the total gonadotropin usage (ampoules) were 47.15 ± 12.97, 39.45 ± 13.97, 36.72 ± 13.14 (p<0.05); the number of retrieved oocytes were 9.89 ± 5.98, 10.50 ± 3.69 and 9.19 ± 5.31 (p>0.05); the fertilization rates were 89%, 100% and 100% (p>0.05). The implantation and pregnancy rates, the two major parameters of the study were 5% and 15% in T, 16.5% and 40% in LA, 14.8% and 38.8% in NA groups respectively (p>0.05).

Conclusions: Even though the success rates in Triptorelin group were somewhat lower, the results of every GnRH-a group were statistically similar; but to reach the same outcome we had to use more gonadotropins in this former group. Cost-effectivity analysis of the three GnRH-a makes Leuprolide and Nafarelin better choices than Triptorelin. However, we must state that our study has two major limitations to give a concrete conclusion, namely the study was not randomized and the number of patients was very small.

Key words: Assisted reproductive techniques, GnRH-a, controlled ovarian hyperstimulation, Triptorelin, Nafarelin, Leuprolide acetate.

Gonadotropin releasing hormone (GnRH) agonists are used extensively as adjuncts to ovarian stimulation protocols in Assisted Reproductive Technologies (ART). The analogues are used to modulate and control endogenous gonadotropin output, their major role is to prevent LH surge before oocyte retrieval (1) Induction of follicle development and ovulation in patients with anovulatory cycles are secondary usage.

Different GnRH analogues with different biological effectiveness, different plasma half life and different ways of administration have been developed by modifying the 6th and 10th position amino acids. These changes make the analogue more potent and long lasting. Therefore, there may be significant differences between the clinical
effects of these specific GnRH agonists. Leuprolide has a half life of 90 minutes whereas nafarelin's half life is 3-4 hours, triptorelin's half life is 4.2 hours. Leuprolide is 50-80 times, nafarelin 200 times, triptorelin 36-144 times more powerful than the natural GnRH (2,3). Leuprolide and triptorelin are administered as sc injection, nafarelin as intranasal treatment. Both ways have absorption effects, which look like the depot effect. However, the swallowing and the loss of peptides by proteolysis in intranasal treatment do not occur in sc injection.

Our objective is to compare the effects of three different GnRH-a, i.e. Triptorelin, Leuprolide acetate and Nafarelin sodium and their ways of administration in, in vitro fertilization cycles.

MATERIALS AND METHODS

60 IVF/ICSI patients were haphazardly distributed, in equal numbers, for the 3 GnRH analogue program groups in our study. After physical and gynecologic examinations, basal hormonal analyses were made for each patient on the basis of their blood FSH, LH, and E2 levels through immunoenzymatic methods on the 3rd day of menstruation. Endometrial thickness measurements were performed by transvaginal ultrasonography. Spermiogram, hysterosalpinography, serologic tests, cervical culture, blood type and CBC were carried out for each couple. Any information about their previous treatment modalities was recorded.

Patients who were previously diagnosed with Polycystic Ovary Syndrome and over 38 years old, whose FSH levels were above 10 IU/L, in whom Controlled Ovarian Hyperstimulation were previously tried twice, and for whom TESA or TESE were planned, were not included in this study.

The long protocol was applied to each patient, starting on the 21st day of the menstrual cycle. Three different GnRH analogues were used in each group. Leuprolide (Lucrin, 5 mg/ml, flacon, injections; Abbott) was administered as daily 0.5 mg subcutaneous injections in one group; in another group, 200 micrograms of nafarelin (Synarel, 2mg/ml nasal spray; Ali Raif) was administered to each nostril twice a day, with a total dosage of 800 micrograms per day; the last group received 0.1 mg subcutaneous injection of triptorelin (Decapeptyl, 0.1 mg s.c. injection; Er-Kim) once a day. On the 2nd day of menstruation during the analogue application procedure, gonadotropin treatment, highly purified urofollitrophin (Metrodin HP, 75 IU amp; Serono) with doses between 150-225 IU, was initiated in patients who were assumed to have adequate suppression with E2 levels less than 50 pg/ml, LH levels less than 5 IU/L, progesterone levels less than 1 ng/ml and endometrial thickness below 4 mm. The same day, GnRH analogue dose was halved to lower the gonadotropin requirement and was given until oocyte pick-up. Cysts bigger than 2 centimeters detected by USG were aspirated in the presence of TV-USG.

On 5th day of induction, drug dosage was increased (step-up protocol) in patients whose 9 mm-sized follicles were less than 3 and E2 levels were below 100 pg/ml. On the other hand, step-down protocol was administered in patients whose follicles were bigger than 11-12 mm and E2 levels were above 400 pg/ml.

Gonadotropin treatment was continued as "step-up" or "step-down" on the basis of USG follow-up results and hormonal levels. Treatment cycle was cancelled if developing follicles were less than 3 or E2 levels could not reach 300 pg/ml.

10000 IU hCG (Pregnyl 5000 IU, Organon) was injected intramuscularly when at least 3 follicles bigger than 18 mm were detected through TV-USG and E2 levels were above 150-200 pg/ml per follicle bigger than 14 mm. 34-36 hours after the HCG injection follicular aspiration procedure was carried out.

Mature oocytes were decomposed from the follicular fluid and were placed into IVF-G1 and G-2 culture media. Purification procedure of cumulus cells was completed 4-6 hours after oocyte harvesting followed by microinjection. Evaluation of the fertilization was made 16-18 hours after microinjection and cells with 2 pronuclei were separated. Embryos were transferred in the 48 or 72 hours, using embryo transfer catheter (Wallace 1816 N).

Patients were monitored by serum quantitative hCG levels 12-14 days after embryo transfer.
Ultrasonographic determination of a gestational sac was recognized as a clinical pregnancy. Implantation rate was calculated by dividing the number of gestational sac (single, double, triple) to the number of embryos transferred in patients who became pregnant.

Natural micronised progesterone (Utrogestan, micronised progesterone 100 mg soft capsules, Lab. Besins-Ivresse, Paris) was applied 3x2 vaginally until 12th week of gestation as the luteal phase supply.

In each group, duration of stimulation, total dosage of gonadotropin, E2 level on day 5 of the cycle, duration between the first day of GnRH-a and the first day of menstruation, cyst formation during the treatment period, E2 levels at the beginning of the induction date and on hCG day, progesterone level and endometrial thickness on hCG day, number of oocytes retrieved, number of mature oocytes, rate of fertilization, number of embryo transferred, rate of pregnancy and rate of implantation were compared.

To evaluate the data, SSPS for Windows 10.0 program was utilized for statistical evaluation. Following methods were used in the comparison of quantitative data: One way Anova, Tukey Hsd, Kruskall Wallis Variance Analysis, Student T Test, Mann Whitney U Test. Chi-square and Fischer exact tests were used in the comparison of qualitative data. Results were evaluated with 95% confidence interval, and the significance at p<0.05 levels.

RESULTS

Sixty subjects, who were accepted into the ART program, were involved in our study. Each groups (Triptorelin group (T), Leuprolide group (LA), Nafarelin group (NA)) had 20 subjects. The infertility causes of these three groups were "male factor" for 10, 14 and 11 cases, "tubal factor" for 4, 3 and 7 cases, "unexplained infertility" for 4, 3 and 2 cases respectively. The 2 cases of endometriosis were in the Triptorelin group. The distribution of infertility causes between groups was similar (p>0.05). (Figure 1)

Two patients in the NA group were excluded because of inadequate E2 suppression. COH was cancelled in 6 cases out of the remaining 58 because of insufficient ovarian response (2 in each group). Embryo transfer could not be completed due
Table 1. Treatment results in each groups

<table>
<thead>
<tr>
<th></th>
<th>Triptorelin</th>
<th>Leuprolide</th>
<th>Nafarelin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Stimulation duration (day)</td>
<td>10.10 ± 1.30</td>
<td>9.20 ± 1.70</td>
<td>9.00 ± 1.18</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Gonadotropin dosage (ampoules)</td>
<td>47.15 ± 12.97</td>
<td>39.45 ± 13.97</td>
<td>36.72 ± 13.14</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Day 5, E2 (pg/ml)</td>
<td>228.8 ± 160.63</td>
<td>210.67 ± 211.83</td>
<td>228.57 ± 176.53</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Interval between beginning of GNRH-a and 1st day of stimulation</td>
<td>7.10 ± 2.42</td>
<td>8.40 ± 3.15</td>
<td>9.40 ± 2.56</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Cyst formation on GnRHa usage</td>
<td>4 (%20)</td>
<td>1 (%5)</td>
<td>2 (%10.0)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>E2 value on the day first day of stimulation (pg/ml)</td>
<td>24.67 ± 5.91</td>
<td>21.23 ± 2.41</td>
<td>29.62 ± 15.7</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>E2 (pg/ml) on the day HCG</td>
<td>2090.4±1355.7</td>
<td>2117.9 ± 1286.9</td>
<td>1851.3 ± 1405.9</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Endometrial line on the day HCG (mm)</td>
<td>10.36 ± 2.09</td>
<td>11.17 ± 2.31</td>
<td>10.00 ± 1.74</td>
<td>&gt; 0.05</td>
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</tbody>
</table>

Effects of menstrual cycle suppressions on these three GnRH analogues and the results of the stimulation have been compared on the basis of 22 parameters. The period between the first day of the GnRH agonist application and the first day of menstruation was 7.1 days in T group, 8.4 days in LA group and 9.4 days in NA group. This difference was not significant (p>0.05). But the period was observed to be the longest in the NA and the shortest in the T group.

Cyst formation occurred in 7 patients during GnRH treatment: 4 in the T, 1 in the LA and 2 in the NA group. But no significant difference was found (p>0.05). These cysts were aspirated by TV-USG.

The mean E2 levels to confirm the adequate suppression on the first day of menstruation were 24.67 pg/ml in T group, 21.23 pg/ml in LA group and 29.62 pg/ml in NA group. The difference was statistically significant, but not clinically (p<0.05).

Table 2. Number of oocytes picked up and fertilization rate according to the groups.

<table>
<thead>
<tr>
<th></th>
<th>Triptorelin</th>
<th>Leuprolide</th>
<th>Nafarelin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of retrieved oocytes</td>
<td>9.89 ± 5.98</td>
<td>10.50 ± 3.69</td>
<td>9.19 ± 5.31</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mature oocytes (%)</td>
<td>75.15 ± 3.69</td>
<td>73.70 ± 19.23</td>
<td>85.01 ± 15.33</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Fertilized oocytes (%)</td>
<td>46.37 ± 27.03</td>
<td>55.88 ± 19.57</td>
<td>57.80 ± 20.35</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
<td>3.36 ± 1.180</td>
<td>3.44 ± 1.61</td>
<td>3.68 ± 1.30</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
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DISCUSSION

In ART procedures, GnRH analogues can successfully be used in controlled ovarian hyperstimulation to prevent LH peak and to provide better follicular growth and maturation.

In initial studies on GnRH analogues, it was determined that cycle cancellations were dramatically reduced and rate of pregnancies were clearly increased. Preventing the LH peak, high quality oocytes were retrieved and pregnancy rates increased. However, total gonadotropin dose increased also (2). Yet the expense of increased dosage is compensated by the decrease in cycle cancellation, which results in the rise of the pregnancy ratios (2,3).

In recent studies, the effect of premature luteinization on cyclic consequences is under discussion (1, 4, 5, 6). In addition, it is a fact that ovarian hyperstimulation may cause excessive luteinization even with low LH levels (6). In the study of Dantas, premature LH peak was not observed and premature luteinization did not seem to have a major effect on pregnancies determined by the embryonic quality and endometrial development since pregnancy ratios were 17% in both study groups (1).

Corson et al. in a prospective, randomized, double blind study, used leuprolide (0.5 mg/day sc) in 22 patients and nafarelin (3x200 mcg/day nasal spray) in 17 patients in the IVF long protocol. According to this study, nafarelin as a nasal spray used for pituitary suppression in COH can be an alternative to leuprolide sc injection with a few or no quantitative and qualitative differences in terms of the HMG dosage used, E2 levels, the amount and quality of the collected oocytes and fertilization ratio (2). Even though the difference in our study was not statistically significant, clinical pregnancy and implantation ratios were in favor of LA, and the mature and fertilized oocyte ratios were in favor of NA. In T group, clinical pregnancy and implantation ratios were the lowest.

Tanios et al. compared nafarelin (3x200mcg/day nasal spray) and triptorelin (0.5 mg/day sc.) in a prospective study that consisted of ovulation induction of 40 patients with normal ovulatory functions and bilateral tubal obstruction (7). Triptorelin was administered to 20 patients who took nafarelin in the first cycle and the other 20 patients who took triptorelin in the first cycle were given nafarelin. In this manner, every patient was provided to be a control for herself. Both agonists were found to be effective in suppression, while in triptorelin cycles pituitary and ovarian suppression was more clearly exhibited. This study showed that triptorelin cycles implicate less E2 levels and more gonadotropin usage. Nafarelin was found to be economical because of fewer gonadotropin ampoules used, shorter duration of stimulation and the short period between the first day of GnRH-a interval and OPU day. In our study, we found exactly the same result in point of view of gonadotropin usage; there was no statistically significant difference in terms of the total gonadotropin dosages between the LA and NA groups whereas in T group high total dosages were required. The ovarian suppression was the least in NA group, indicating that not all what is statistically significant should be clinically significant.

Martin et al. (8) in a retrospective analysis of 226 patients and Franco et al. (9) in another study with 238 patients who used nafarelin and leuprolide for the IVF cycle, found no statistically significant difference between the two groups in terms of the maximum E2 level, the gonadotropin ampoules used, the amount of collected oocytes,
ratio of mature and fertilized oocytes, pregnancy miscarriages, implantation rates and cycle cancellation. But take home baby ratio in the study of Martin was 34% in NA and 20% in LA groups respectively (p<0.05). Martin et al. explained the analogues' effect on birth rates with the influence on oocyte quality.

In the study of Parinaud et al. (10) which compared leuprolide, Triptorelin and buserelin, it was considered that the analogues' effect on oocyte quality and implantation rates was not only due to their impact on the pituitary gland but also due to their impact on the ovaries. However, none of these hypotheses have yet been tested. There is no significant difference among the pregnancy ratios in different stimulation cycles. As a conclusion, with this study, Parinaud et al. assumed that in the higher responder group whose ovarian reserves were good, buserelin can be recommended while in the poor responder group with low ovarian reserves triptorelin usage may be appropriate.

Loh et al. (11) in a randomized study which included the pituitary suppression time, total HMG dosage, the number of retrieved and also frozen embryos and clinical pregnancy ratios (for each cycle in LA group 21.4%, in NA group 16.3%), did not determined any statistically significant difference between LA and NA groups. In our study, clinical pregnancy ratios per stimulated cycle were 35% in LA group and 27.7% in NA group. Loh et al. concluded that intranasal nafarelin might be an alternative to and effective as much as Leuprolide for pituitary suppression.

Yuzpe et al. (12) compared nafarelin (400 and 600 mcg/day) and leuprolide (0.5 mg/day) by a retrospective study of IVF. 12 days after usage of GnRH analogue sufficient suppression ratios were 82%, 87%, and 65% respectively. Pregnancy ratios on each embryo transfer were 13/60 (22%), 19/63 (30%) and 18/103 (18%) respectively. Pregnancy ratio in our study for each transfer was better than Yuzpe study and higher in LA group than the NA group (41% and 31.2% in order). In T group, this rate was 17.6%.

Wong et al. (13) formulated a meta-analysis with 1024 patients, 597 of them using nafarelin, 348 buserelin, 14 triptorelin and 55 leuprolide. Pregnancy ratios at every embryo transfer were observed to be equal. The stimulation was shorter and the need for gonadotropin ampoules was less in nafarelin treatment group. Consequently, the effectiveness of nafarelin was identical in comparison to the other GnRH agonists. As our study results are parallel, Nafarelin seems to be more advantageous and comfortable because of the decrease in the number of gonadotropin ampoules and the way of administration.

It is well known that pregnancy ratios are higher in cycles with analogue use (14). Eligendy and Simon et al suggest reducing the dosage of GnRH-a, as we did, after pituitary suppression at the beginning of stimulation in order to lower gonadotropin usage (15, 16). This has no adverse effect on ovarian response and clinical results.

Finally, GnRH-a should be chosen on the basis of the criteria of cost, patient's comfort, availability of the drug, clinical effectiveness and good results. It is apparent that studies have different conclusions, but in general LA and NA exhibited more successful results than T. We must claim that our study has two major limitations to give a concrete conclusion and is not fully powered to answer the question raised: the study was not randomized and the number of patients was very small. However we found out that even though the success rates in Triptorelin group were somewhat lower, the results of every group were statistically similar; but to reach the same outcome we had to use more gonadotropins in this former group. We think that triptorelin achieves more suppression in short terms than leuprolide and nafarelin and requires more gonadotropin ampoules for stimulation. Cost-effectivity analysis of the three GnRH-a makes Leuprolide and Nafarelin better choices than Triptorelin. Clinical effectiveness, patient comfort and its ease of use are the major advantages of Nafarelin. Leuprolide, in the other hand, emerges as a safe and easy handling alternative with a shorter half-life and higher success rates and has the advantages of sc injection pharmacokinetics.

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