Iranian Journal of Reproductive Medicine Vol. 2. No.1 pp 29-33, 2004

Efficacy of Low Dose, Long-acting Gonadotropin Releasing Hormone Analogues (GnRH-a) Compared with Daily Injections of Short-acting GnRH-a in ART Cycles

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Background: The retrieval of good quality oocytes that is accomplished with selection of the best induction ovulation protocol on the basis of patients condition, age and cause of infertility, is one of the most important aspects of ART cycles. The objective was to evaluate the efficacy of low dose, long acting GnRH-a (Decapeptyle) for pituitary desensitization and outcome of ART compared to long protocol of short acting GnRH-a (Busereline).
Materials and Methods: In this randomized clinical trial that was performed at Yazd IVF Center, 60 patients with 61 cycles of ART were included. Patients with endometriosis or age > 40 were excluded in this study. Using COH-ET, patients were randomly divided into two groups. In group one, 30 patients received a single half dose of Decapeptyle (1.87mg) in mid-luteal phase. In the other group, 31 patients received Buserelin daily (0.5mg), starting from previous mid-luteal phase. This was reduced to 0.25mg from gonadotropin administration day and was continued until the day of hCG injection. In these groups, the number of oocytes, the fertilization, cleavage, pregnancy and cancellation rates were compared.
Results: In two groups, there was no case of cancellation due to premature LH surge. In group I, the mean number of gonadotropins was 27.5±4.2 ampoules while in the second group, it was 28.4±2.8 ampoules (P>0.05). 312 oocytes from group I and 294 oocytes from group II were retrieved. Oocyte quality in group II was better than group I (84.3% vs 77.2%, P<0.05). In long-acting GnRH-a group fertilization rate was 81.9% versus 71.1%in group II (P<0.01). However, embryo development in Group I (85.6% vs 94.1%, P<0.05) was lower than group II. Although, pregnancy rate was 20% in Group I which was higher than group II (12.6%) but, there was no significant difference in cancellation, pregnancy rate and gonadotropins dose in two groups.
Conclusion: The low dose long acting GnRH-a is a useful method for pituitary suppression. Low dose GnRH-a combined with gonadotropins permitted the retrieval of good quality oocytes and had no effect on oocytes. The fertilization and pregnancy rates with this method are acceptable and its cost and tolerance is valuable for patients.

Key Words: *Decapeptyle, GnRH agonists, Buserelin, Assisted Reproductive Technology, Infertility, Induction Ovulation*

Introduction

Ovulation induction is the most fundamental and important step in ART cycles. For getting a reasonable number and proper quality of oocytes, patients are selected according to their age, and etiology of infertility. In the past, human gonadotropins were widely used alone, but due to a high prevalence of premature LH surge and its negative effects over oocytes (Gemzell *et al*., 1998), after the availability of Gonadotropin– releasing hormone agonist (GnRH-a) in the market, it has been widely used in IVF (Porter *et al*., 1984). The advantage of the GnRH-a in combination with gonadotropins for controlled ovulation induction in IVF & ICSI cycles have been proved (Daya 1999). Long protocol of GnRH analog improves pregnancy rate and decreases cancellation rate of cycles (Hughes *et al*., 1992). In these days, many types of short and long acting analogues with different methods are being used.

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Albuquerque *et al* (2002) analyzed 552 patients and found that there was no statistically significant differences between the use of depot GnRH-a or daily GnRH-a in the primary and clinical pregnancy outcome. Long protocol method is mostly used for ART. In this method, GnRH analog injection starts at the previous mid luteal phase, but due to the daily injection and complication of applying its different dosages, this method is somewhat strange for patients. In spite of ovulation induction, these patients can not get adequate result because in this method they have to bear the heavy expenditure and its application procedure in such way that many of them make mistakes. For these facts, it is advised to make the therapeutic procedure easier. Scores of clinical studies have been directed toward identification of the optimal doses of GnRH-a. Balasch *et al* (1992) showed that in all cases by using full dose of Decapeptyle, pituitary suppression was achieved successfully. Many researchers have done different types of studies over the usage of long acting GnRH-a such as Decapepetyle (Tasi *et al*., 1995). Some have reported good results, while others reported the negative results (Wiesman and Shohma 1993; Devreker *et al*., 1996). In this study, we have tried to use half dose of Decapeptyle to reduce the cost and its long acting negative effects.

Materials and Methods

This prospective, controlled, randomized study included a total of 61 IVF/ICSI cycles performed in 60 infertile couples treated at our institution between May 2001 and February 2002. The study was approved by our institution's ethics committee. To be included in this study, patients had to have both ovaries and no ovarian failure on the basis of their basal FSH concentrations of <12 IU/ml. Because of negative effects of endometriosis on the results, patients with endometriosis or age > 40 were excluded from this study.

Age, etiology, and duration of infertility of all patients between two groups were comparatively the same. In our program, ovarian stimulation was routinely accomplished using gonadotropin treatment under pituitary suppression with GnRH agonists with long protocol from the previous cycle. In Group I (no=30) half of one Decapeptyle ampoule (1.87mg) was administered on the 21st day of previous cycle. In the other group (no=31), Leuprolide acetate (Porcrine; Madrid, Spain) suppression was started in the midluteal phase of the previous cycle with an SC daily dose of 0.5mg. This dose was reduced to 0.25mg/d, once ovarian suppression was achieved; then, it was continued until the administration of hCG.

Only one patient was included in both groups who initially was in group I and took single half of Decapeptyle ampoule in previous cycle for ovulation stimulation. There was no good follicular growth, therefore the cycle was canceled, and she entered to the group II in next cycle. The mean age & indication of ART have been evaluated for both groups (Table I).

Gonadotropin stimulation of the ovaries was started when serum E2 concentration declined to < 40pg/ml and a vaginal ultrasonographic scan showed an absence of follicles of >10 mm. From day 2 or 3 of menstruation, 3 ampoules of hMG (Pergonal; serono S.A.) per day were administrated IM to each patient. Sequential transvaginal ultrasonography was performed from day 7 of ovarian stimulation to assess follicular development. From day 8 onward, the dosage of hMG was adjusted on an individual basis according to the ovarian response. Serum LH measurement was performed on the day of hCG administration. Finally, hCG (10,000 IU, Profasi; Serono S.A) was administered IM if the criteria was observed. The criteria for hCG administration included the presence of at least 3 follicles of >17mm. In second group, Gonadotropin and leuprolide acetate administrations, were discontinued in the day of hCG administration. Oocytes retrieval was performed by vaginal ultrasonography under general anesthesia 34-36 hours after hCG administration.

The standard IVF/ICSI procedure was done. Briefly, oocyte-cumulus complexes were evaluated under dissecting microscope and classified. The maturation status of the oocytes was recorded according to the criteria of Veeck (10). Two to 5 embryos per patient were replaced (according to patient's age, number of IVF attempts, and embryo quality) 48-72 hours later. Progesterone 100mg/daily IM was given from the day of embryo transfer to supplement the luteal phase in patients. Cycles were canceled if the ovarian response was poor or excessive.

Serum hCG was measured on 12^{th} and 15^{th} day of transfer. Luteal phase support was continued till 8^{th} weeks of pregnancy. Ultrasonography was done in 6^{th} and 8^{th} week of pregnancy for detection of fetal heart rate. Statistical comparisons were preformed by $\chi 2$ analysis and paired student's t-test, P-value < 0.5 was considered significant.

Results

The results are summarized in tables I and II. As it is shown in table I, the main demographic and baseline characteristics of the patients in groups I and II were almost identical, including age, etiology, FSH level in the early follicular phase, and IVF cycles. The causes of infertility were identical for both groups. This supports the validity of the randomization process.

Table II shows the data regarding ovarian response in 2 groups studied. When both protocols in the present study were compared, it was found that group II (Leuprolide acetate) required fewer ampules of gonadotropins (28.4±2.8) for superovulation compared to the Decapeptyle in group

Table I: The characteristics of stimulated cycles.

Variable	Group I (Decapeptyle)	Group II (Buserelin)
Patient age (years)	27.9±4.2	30.6±2.8
No. Tubal factor infertility	3(10%)	5(16%)
No. Ovualtory infertility	25(84.4%)	20(64.5%)
No. Unexplained infertility	0	2(6.4%)
No. .Male factor infertility	2(6.6%)	4(12.9%)
Day 3 FSH level(mIU/ml)	7.1±1.9	6.5±3.0
No. IVF+ET	4(15.4%)	2(6.4%)
No. ICSI+ET	26(84.6%)	29(93.5%)

Table II: Ovarian response in the 2 groups undergoing ART.

Variable	Group I (Decapeptyle)	Group II (Buserelin)	P
Total no. of ampoules of Gonadotropin	27.5±4.2	28.4±2.8	NS
Total no. of oocytes retrieved	10.75±0.7(312)	9.5±0.9(294)	NS
Good quality oocytes retrieved	77.2%(241)	84.35%(248)	<0.05
Fertilized oocytes (Fertilization rate%)	6.27±0.6 (81.93%)	5.48±0.4 (71.12%)	<0.01
Cleaved embryo (Cleavage rate%)	5.75±0.3 (85.6%)	5.71±0.5 (94.11%)	<0.01

Student's t-test and χ^2 were used for statistical analysis
NS: non significant

I (27.5±4.2), although the difference was insignificance. The number of oocytes retrieved (10.75 versus 9.5) were similar for 2 groups of patients. In the Decapeptyle group, a significantly lower percentages of oocytes had good quality compared to 2nd group (77.2% versus 84.35%, P<0.05). A significantly higher fertilization rate was found with Decapeptyle (81.93% versus 71.12%, P<0.01) but cleavage rate was lower in Group I (85.6%) versus 94.11% in group II (P<0.05).

Table III presents outcomes of treatment in patients undergoing ovarian stimulation. No premature LH surge was observed in two groups. In group I, due to low response of ovaries and lack of follicular growth, one cycle was cancelled and she enrolled in the Leuprolide acetate group in the next cycle. Embryo transfer was done in 28 patients of group I and in 31 patients of the other group.

The mean number of embryos transferred was 3.92 and 2.5 in Decapeptyle and Buserelin groups respectively. There was no evidence of significant differences between both groups in terms of ART cycles outcome and pregnancy rate. Pregnancy rate per initiated cycles was higher (20%, n=6) in Decapeptyle group compared to Buserelin group (12.9% n=4, P=0.6). There was no abortion in any patients.

Discussion

The advantage of GnRH-a in combination with gonadotropins for controlled ovulation induction in IVF & ICSI cycles have been proved (Hughes *et al*., 1992). Although different regimens have been advised, but the best regimen must be selected regarding to the type, clinical efficacy, expenditure and usage convenience of the drug. Considering the patient's stress, high expenditure, and intolerance of daily subcutaneous injection of GnRH-a, is a very important factor. Therefore, some authorities have advised to use nasal sprays. Sometimes for reducing stress, long acting (Depot) GnRH agonists was used for pituitary suppression instead of daily injection of short acting GnRH-a (Tasi *et al*., 1995). Although, multitude of studies have appeared on the advantages of using the different GnRH agonists in ovulation stimulation regimen for IVF-ET in the last decade, but the optimal dosage for pituitary suppression in ovulation stimulation has not been pecified yet (Fleming *et al*., 1982; Porter *et al*., 1984).

Geber *et al* (2002) compared single dose of Gosereline with daily injection of Leuprolide and concluded causes of infertility were identical for both groups. This supports the validity of the randomization process. Table II shows the data regarding ovarian response in both studied groups. When both protocols in the present study were compared, it was found that group II (Leuprolide acetate) required fewer ampules. In another study, Ben Rafael (2001) indicated that by using the dosages of 500µg and 600µg of GnRH-a, there are not significant differences between duration of pituitary suppression, number of oocytes, embryos, fertilization and pregnancy rates. But, those patients who have received 200µg of GnRH-a, had lower fertilization and pregnancy rates. Therefore, GnRH-a less than 200µg should not be used.

Short acting GnRH-a is being used, because it is

Table III: Outcome of treatment in the 2 groups of stimulated cycles

Variable	GroupI (Decapeptyle)	Group II (Buserelin)	P
No. of cancellation due to low response	1(3.3%)	0	NS
No. of punctured cycles	29	31	NS
No. of transferred cycles	28	29	NS
No. of embryo transferred	3.92±0.5	2.5±0.6	NS
Pregnancy/initiated cycle(%)	6/30(20)	4/31(12.9)	NS(0.6)
Pregnancy/transfer cycle(%)	6/28(21.4)	4/29(13.7)	

Note: P value<0.05 was significant

thought that the long acting products inhibit gonadotropins, cause disturbance and insufficiency in luteal phase. These products have negative effects over pregnancy and abortion (El-Nemr *et al*., 2002). It seems, that drug effect remains for 4 to 7 weeks, this issue may cause insufficiency for corpus luteum follicle. However, we can overcome this problem by using progesterone until 7th week of pregnancy (Wiesman and Shohma 1993). Because daily use of GnRH-a is difficult for patients and it causes mistakes for patients in continuing cycle and starting ovulation drugs simultaneously. As psychiatric stress is one of the etiologies of infertility and has strong effect on success rate, it is better to select simple therapeutic approach (Samuel *et al*., 1993).

Based on this fact, we prefer to use long acting products (Dada *et al*., 1999). Researches on Busereline, Triptroline and Leuprolide have indicated that the number of oocytes and clinical pregnancies were all the same (Tarlatitzis *et al*., 1994). According to the results full and half dose of Triptroline had the same outcome (Juan *et al*., 1992; Hazout *et al*., 1993). Regarding ART cycles, our have proved that half dose of long acting products on 21st day of menstrual cycle are as effective as short acting ones. Also, it does not have negative effects on the outcome. So, we recognized that by using half dose of Decapeptyle no cycle due to premature LH surge was cancelled.

The effects of long acting GnRHa on the specific receptors of oocyte are suppressing (Dekel and Granot, 1996), increasing the number of immature oocytes (Ron-EI *et al*., 1990), increasing the number of diploid oocytes and early chromosal condensation of sperms after fertilization (Racoowsky *et al*., 1997). GnRH-a causes occlusion of LH dependent gap junctions on cumulus, so the final stages of oocyte maturation needs lower amount of hCG (Dekel *et al*., 1988). In our study, in comparison with control group it has been proven that by using Tryptroline the number of immature oocytes are significantly less than the Buserelin. But on the other hand, the fertilization and pregnancy rates is higher (Racoowsky *et al*., 1997). Although , Depot GnRH-a disturb developmental growth of embryo and implantation (Devreker *et al*., 1996), but we demonstrated that not only there is no significant difference in the number of oocytes, but also the fertilization rate and subsequent divisions are higher.

Also, we showed that injecting half dose of Triptoreline ampoule for pituitary suppression is sufficient. In the same way, we showed that by reducing the dosage of long acting GnRH–a the optimal dose of gonadotropins is also reduced. In contrast to other studies (Gianaroli *et al.,* 1994; Porcu *et al.,* 1994) there is no need for higher dose of hMG. However, more investigations with the help of Doppler ultrasound and measuring the uterine blood supply are needed.

Cost, side-effects, and efficacy simplification of application and lower rate of injections mistakes, make the GnRH-a as the first choice. Also, it is advised to do more researches on its appropriate dosage. However, we should share these data to pharmaceutical companies to make the required dose injections.

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