Different gonadotropin releasing hormone agonist doses for the final oocyte maturation in high-responder patients undergoing *in vitro* fertilization/intra-cytoplasmic sperm injection

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ABSTRACT

CONTEXT: Efficacy of gonadotropin releasing hormone agonists (GnRH-a) for ovulation in high-responders. **AIMS:** The aim of the current study is to compare the impact of different GnRH-a doses for the final oocyte maturation on cycle outcomes and ovarian hyperstimulation syndrome (OHSS) rates in high-responder patients undergoing ovarian stimulation. **SETTINGS AND DESIGNS:** Electronic medical records of a private in vitro fertilization center, a retrospective analysis. **SUBJECTS AND METHODS:** A total of 77 high-responder cases were detected receiving GnRH-a. Group I consisted of 38 patients who received 1 mg of agonist and Group II consisted of 39 patients who received 2 mg of agonist. **STATISTICAL ANALYSIS:** In order to compare groups, Student's t-test, Mann–Whitney U-test, Pearson's Chi-square test or Fisher's exact test were used where appropriate. A $P < 0.05$ was considered as statistically significant. **RESULTS:** Number of retrieved oocytes (17.5 vs. 15.0, $P = 0.510$), implantation rates (46% vs. 55.1%, $P = 0.419$) and clinical pregnancy rates (42.1% vs. 38.5%, $P = 0.744$) were similar among groups. There were no mild or severe OHSS cases detected in Group I. Only 1 mild OHSS case was detected in Group II. **CONCLUSION:** A volume of 1 or 2 mg leuprolide acetate yields similar outcomes when used for the final oocyte maturation in high-responder patients.

KEY WORDS: Agonist-trigger, antagonist, high-responder, *in vitro* fertilization, leuprolide, ovarian hyperstimulation syndrome

INTRODUCTION

Despite commonly adopted preventive strategies, ovarian hyperstimulation syndrome (OHSS) accounts for significant number of morbidity and even mortality in high-responder patients undergoing ovarian stimulation.[1] Among the strategies that have been proposed for the prevention of OHSS, none has been shown to completely eliminate the risk.[2] Although, a lower incidence of OHSS is observed in antagonist cycles,[3] the risk is not completely eliminated due to the utilization of bolus human chorionic gonadotropin (hCG) injection for the final oocyte maturation. Prolonged luteinizing hormone (LH) activity following hCG during luteal phase not only stimulates the multiple corpora lutea but also promotes the up-regulation of some cytokines including vascular endothelial growth factor, which is a novel promoter in the etiology of OHSS.[1]

For many years, gonadotropin releasing hormone agonist (GnRH-a) induced LH surge which is similar to spontaneous mid-cycle surge has remained largely of theoretical interest. GnRH-a for the final oocyte maturation activates the GnRH receptor with flare-up effect, resulting in a surge of gonadotropins similar to that of the natural cycle. Introduction of GnRH antagonists allowed the replacement of hCG with GnRH-a as for the final oocyte maturation. In many studies, GnRH-a has been shown to be as effective as hCG.[4-6] In agonist-triggered cycles, relatively short half-life and lower amplitude of LH surge (24–36 h vs. 48 h.) interestingly resulted in significant reduction of OHSS as compared with hCG.[7,8]

Different types and doses of agonists have been used for the final oocyte maturation in high-responder patients so far.[9] However, there is no consensus in the literature regarding the optimal agonist type or dose in GnRH antagonist cycles. In the current analysis, impact of different doses of leuprolide acetate (LA) on cycle outcome parameters was analyzed.

SUBJECTS AND METHODS

This is a retrospective analysis of 77 high-responder patients undergoing *in vitro* fertilization (IVF)/intra-cytoplasmic sperm injection with GnRH antagonist protocol and with GnRH-a for the final oocyte maturation at two IVF centers between January 2010 and December 2012. After reviewing electronic database of centers, 38 patients in 1 mg of LA (Group I) (Lucrin, Abbott) and 39 patients in 2 mg of LA (Lucrin, Abbott) (Group II) group were detected. In both centers, the criteria for agonist-triggering was defined as: Being at high risk of OHSS by a high number of follicles (>12) measuring ≥12 mm and/or high serum estradiol levels (≥4000 pg/mL) during the late follicular phase of the ovarian stimulation cycle. The decision to apply the GnRH-a trigger as 1 or 2 mg was at the discretion of the treating physician as this study was not designed prospectively.

Patients were recruited for the trial for one cycle only. Ovarian stimulation was carried out with recombinant follicle stimulating hormone (FSH) (Gonal-F, Merck Serono) beginning from the 2nd day of the menstrual cycle depending on the patient's age and body mass index with an average starting dose of 150 IU/daily. Serum estradiol level measurement along with ultrasonography was performed on the 5th day of the stimulation, and gonadotropin dose adjustments were performed individually according to the ovarian response. The GnRH antagonist (Cetrotide, Merck Serono) was introduced (0.25 mg/day) on the 6th day (fix antagonist protocol).

Transvaginal ultrasound–directed oocyte retrieval was performed 35 h after triggering ovulation for all groups. On the day of oocyte retrieval, a single dose of 1500 IU hCG (Pregnyl, Organon) intramuscularly 1 h before the procedure was applied to all participants, as described previously.[10] Embryo transfer was performed 72–76 h after oocyte retrieval. For luteal support, all patients received 8% progesterone gel/daily and 2 mg (p.o.) estradiol hemihydrate 3 times a day, starting with the evening after oocyte retrieval and continuing until a negative pregnancy test or a viable fetus documented by transvaginal sonography. A biochemical pregnancy was defined as β-hCG concentration >10 IU/L on the 12th day after transfer. A clinical pregnancy was defined as an intrauterine gestational sac with a heartbeat 3 weeks after obtaining positive results on the hCG test.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Whether the distributions of continuous and metric discrete variables were normal or not was determined by Kolmogorov–Smirnov test. Continuous and metric discrete variables were shown as mean ± standard deviation (95% confidence interval [CI]) or median (minimum–maximum), where applicable. While the mean differences between groups were compared by Student's *t*-test, otherwise, Mann–Whitney U-test was applied for comparisons of the median values. Nominal data were analyzed by Pearson's Chi-square test or Fisher's exact test, where appropriate. A $P < 0.05$ was considered as statistically significant.

RESULTS

The mean age of participants was 29.9 ± 5.0 (95% CI: 28.2–31.5) in Group I and 30.1 ± 4.5 (95% CI: 28.7–31.6) in Group II. No significant difference was found between two groups regarding the mean age of the cases ($P = 0.811$). Duration of infertility was found as 51.0 (8.0–240.0) months in Group I and 48.0 (12.0–240.0) months in Group II ($P = 0.701$). Antral follicle count on day 3 was similar among groups (13.7 ± 4.5 vs. 13.6 ± 3.1) ($P = 0.920$). Day 3 FSH value was found significantly higher in Group I when compared to Group II (5.5 [3.0–9.0] vs. 5.3 [2.0–8.2]; respectively) ($P = 0.01$). Demographic characteristics of groups are given in Table 1. Number of retrieved oocytes (17.5 [4.0–42.0] vs. 15.0 [9.0–25.0], $P = 0.510$), mature oocytes (13.5 [3.0–40.0] vs. 12.0 [5.0–20.0], $P = 0.503$), implantation rates (46% vs. 55.1%, $P = 0.419$) and clinical pregnancy rates (42.1% [27.9–57.8] vs. 38.5% [24.9-54.1], $P = 0.744$) were similar among groups. The only significant parameter was found as fertilization rate among groups (83.3% [42.8–100.0] in Group I and 75.0% [20.0–100.0] in Group II [$P = 0.006$]). There were no mild or severe OHSS case detected in Group I. Only 1 mild OHSS case was detected in Group II. Cycle outcomes of groups are given in Table 2

DISCUSSION

In an effort to achieve favorable clinical outcomes with less OHSS, GnRH-a has been widely adapted to ovarian stimulation protocols as for the final oocyte maturation. Once the decision to use GnRH-a trigger has been made, there are different options regarding the kind and the dose. There is no report available in the literature evaluating the impact of different agonists for the final oocyte maturation on cycle outcomes. In addition, no universal consensus has

Table 1: Demographic characteristics of groups

	Leuprolide 1 mg group (*n*=38)	Leuprolide 2 mg group (*n*=39)	*P*
Age (years)			
Mean±SD (95% CI)	29.9±5.0 (28.2-31.5)	30.1±4.5 (28.7-31.6)	0.811
Duration of infertility (months)			
Median (minimum-maximum)	51.0 (8.0-240.0)	48.0 (12.0-240.0)	0.701
Cause of infertility (%)			
Anovulatory	18 (47.3)	20 (51.2)	
Male factor	10 (26.3)	9 (23)	
Endometriozis	5 (13.1)	2 (5.1)	
Tubal	3 (7.8)	4 (10.2)	
Unexplained	2 (5.2)	4 (10.2)	
Day 3 AFC (numbers in both ovaries)			
Mean±SD (95% CI)	13.7±4.5	13.6±3.1	0.920
Day 3 FSH (IU/L)			
Median (minimum-maximum)	5.5 (3.0-9.0)	5.3 (2.0-8.2)	0.011
Day 3 estradiol			
Median (minimum-maximum) (pg/mL)	41.5 (22.0-66.0)	39.0 (24.0-84.0)	0.729

SD=Standard deviation, CI: Confidence interval, AFC= Antral follicle count, FSH= Follicle stimulating hormone

Table 2: Cycle outcomes of groups

	Leuprolide 1 mg group (*n*=38)	Leuprolide 2 mg group (*n*=39)	*P*
Duration of stimulation (days)			
Median (minimum-maximum)	10.0 (6.0-20.0)	9.0 (8.0-12.0)	0.013
Total dose of gonadotropins (IU)			
Median (minimum-maximum)	1475.0 (635.0-2550.0)	1525.0 (1025.0-3500.0)	0.695
Number of retrieved oocytes			
Median (minimum-maximum)	17.5 (4.0-42.0)	15.0 (9.0-25.0)	0.510
Number of M2 oocytes			
Median (minimum-maximum)	13.5 (3.0-40.0)	12.0 (5.0-20.0)	0.503
Number of fertilized oocytes (2PN)			
Median (minimum-maximum)	10.0 (3.0-32.0)	10.0 (1.0-16.0)	0.128
Fertilization rate (%)			
Median (minimum-maximum)	83.3 (42.8-100.0)	75.0 (20.0-100.0)	0.006
Number of embryos transferred	1.23±0.43	1.17±0.38	0.541
Number of good quality embryos transferred	0.76±0.67	0.61±0.54	0.227
İmplantation rate (%)	46	55.1	0.419
Clinical pregnancy rate			
n (%) (95% CI)	16 (42.1) (27.9-57.8)	15 (38.5) (24.9-54.1)	0.744
Mild/severe OHSS			
n (%) (95% CI)	-	1 (2.6) (0.004-13.2)	1.000

CI= Confidence interval, OHSS= Ovarian hyperstimulation syndrome

been defined regarding the kind and the dose. The results of this study indicate that 1 and 2 mg LA yields similar outcomes when used for triggering final oocyte maturation.

In the literature, various agonists in different doses have been used for triggering final oocyte maturation. Buserelin 0.5 mg (subcutaneous [sc]),[11] triptorelin 0.2 mg (sc)[12-15] and LA with different doses (0.5–4 mg) (sc)[14,16-19] were reported for the final oocyte maturation and almost all studies have compared the outcomes of GnRH-a triggered cycles with cycles which were triggered with hCG. Nevertheless, there is no report available comparing the outcomes of different type of agonists along with different doses except one.[14] In that study, triptorelin 0.2 mg and LA 0.5 mg were compared with that of hCG for triggering final stages of oocyte maturation in normo-responders, and the cycle outcomes were similar.

The doses of LA used for final oocyte maturation in the literature ranges from 0.5 mg[14] to 4 mg,[18] where the difference is nearly eight-fold. Although, increasing the dose of agonist for triggering final oocyte maturation may have a potential to improve the oocyte quantity, study populations are slightly different in these studies. In high-responders when LA is used for final oocyte maturation, the mean number of retrieved oocytes varies in

the current literature.[20-22] One study reported 27 retrieved oocytes using 4 mg LA,[20] while another study revealed 16 retrieved oocytes with 2 mg LA.[21] And finally Kummer *et al*. reported 25 retrieved oocytes with 1 mg LA for final oocyte maturation.[22] In our study, the mean number of retrieved oocytes was 17 and 15 in 1 mg and 2 mg LA groups, respectively (P = 0.510). Higher doses of agonists for the final oocyte maturation may result in higher gonadotropin surge amplitude, where one may think that better oocyte yield could be possible with incremental doses. However, previously mentioned reports and the current analysis have failed to demonstrate any clear benefit of this approach.

Undoubtedly, in the above-mentioned studies, the decision to apply the GnRH-a trigger was based on OHSS prevention, as the method is associated with a lower incidence of OHSS. However, there is no previously defined standard criterion for agonist-triggering. Therefore, some authors used increased estradiol levels as a criterion,[20,23] whereas others did not include this parameter and assessed only excess number of available follicles during late follicular phase of ovarian stimulation.[21,24] The diversity among the study populations, GnRH-a trigger criteria, and different doses obviously limit us to draw strong conclusions however further studies will help to determine a possible standardization for GnRH-a trigger criteria that will yield optimal outcomes.

Despite acceptable cycle parameters following agonist-triggered cycles, initial studies revealed poor on-going pregnancy rates.[25] A recent Cochrane Systematic review recommended that agonists should not be used routinely as final oocyte maturation in fresh autologous IVF cycles because of a very significant reduction in live birth rates compared with the use of a traditional hCG trigger (odd ratio 0.44, 95% CI 0.29–0.68).[25] The method has been criticized for the altered luteal dynamics rather than the adverse impact on oocyte quality and quantity. Excellent conception rates in oocyte recipients receiving embryos originating from donor cycles triggered with agonist further support this hypothesis.[26-30] Subsequent analysis reported improved clinical and on-going pregnancies either with intensive steroidal support[16] or with low dose (1500 IU) luteal hCG rescue protocol.[10,21,24] In our study, 1500 IU of bolus hCG was administered as luteal rescue additional to steroid supplementation.

Evidence from observational trials and randomized studies in the last decade suggests that the GnRH-a for final oocyte maturation significantly reduces or even eliminates the OHSS in high-risk populations.[12,16,20,24] In fact, no other prevention strategy even comes close to this result. Interestingly, a recent retrospective report underscored high incidence of early-onset OHSS (22%) after agonist-trigger, in a population of high-responder patients.[29] Very recently, two cases of severe OHSS has been reported after GnRH-a triggering with freeze-all attitude without the administration of any hCG for luteal support.[30] In our study, one mild early OHSS case in 2 mg LA arm was noted.

Currently, there is no protocol available which fully imitates the natural mid-cycle surge of gonadotropins. Before dose finding studies conclude on this issue, a successful oocyte recovery cycle can be achieved by triggering an endogenous gonadotropin surge using either 1 or 2 mg LA in an antagonist cycle.

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