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Impact of Gonadotrophin Releasing Hormone Antagonist Duration on In-Vitro Fertilization Outcome

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ABSTRACT

Objective: To evaluate whether oocyte quality, implantation and pregnancy rates in intra-cytoplasmic sperm injection (ICSI) cycles are related to the duration of gonadotrophin-releasing hormone antagonist (GnRH-ant) use.

Material and Method: A total of 138 patients who were treated with GnRH-ant protocol at the IVF Clinic from March 2010 to January 2012 were enrolled in this study. The patients were classified into three groups according to duration of GnRH-ant use. Group 1: 4 days (n=51); group 2:5 days (n=57); group 3:6 days (n=30) antagonist application. Main outcome measures were implantation rate, pregnancy rate, fertilization rate, number of oocytes retrieved (NOR), number of mature oocytes (NMO).

Results: The NOR and NMO were the lowest in group 1, intermediate in group 2, and the highest in group 3 respectively. There is no statistically significant difference between the groups in regard to total gonadotrophins used, fertilization, implantation or pregnancy rates. The pregnancy rates per ET and cycle were 37.9% and 31.8%, respectively.

Conclusion: We noticed that longer GnRH-ant use was associated with more oocytes retrieved and more mature oocytes, but no difference in fertilization, implantation or pregnancy rates concluding that longer GnRH-ant use does not have a detrimental effect on in vitro fertilization outcome.

Key Words: *GnRH antagonist duration, Fertilization, Implantation, Pregnancy.*

ÖZET

Gonadotropin Salgılatıcı Hormon Antagonisti Uygulama Süresinin In-Vitro Fertilizasyon Sonuçlarına Etkisi

Amaç: Gonadotropin salgılatıcı hormon antagonisti (GnRH-ant) kullanım süresinin, in vitro fertilizasyon (IVF)/ intra-sitoplazmik sperm enjeksiyonu (ICSI) sikluslarında oosit kalitesi, implantasyon ve gebelik oranlarına etkisi olup olmadığını araştırmak.

Gereç ve Yöntem: IVF kliniğimizde Mart 2010- Ocak 2012 tarihleri arasında GnRH-ant protokolü ile tedavi edilen 138 hasta çalışmaya dahil edildi. Hastalar GnRH-ant kullanım sürelerine göre 3 gruba ayrıldı. Grup 1: 4 gün (n=51); grup 2: 5 gün (n=57); grup 3: 6 gün (n=30). İmplantasyon, fertilizasyon ve gebelik oranları, toplanan oosit sayısı (TOS) ve matür oosit sayısı (MOS) bakılan ana parametrelerdir.

Bulgular: Sonuçlara bakıldığında; TOS ve MOS grup1'de en düşük, grup 2'de orta ve grup 3'te en yüksek sayıda gözlendi. Gruplar arasında kullanılan toplam gonadotropin dozu, fertilizasyon oranı, implantasyon ve gebelik oranları açısından istatistiki anlamlı bir fark görülmedi. Siklus ve embriyo transferi başına görülen gebelik oranlarımız sırasıyla %31.8 ve %37.9 idi.

Sonuç: Çalışmamızda GnRH-ant kullanım süresi uzadıkça toplanan oosit ve matür oosit sayısının arttığını, fakat fertilizasyon, implantasyon ve gebelik oranlarının fark etmemesi üzerinden, uzamış antagonist uygulamasının IVF sonucuna negatif etki oluşturmadığını gözlemledik.

Anahtar Kelimeler: *GnRH antagonist süresi, Fertilizasyon, İmplantasyon, Gebelik.*

Gonadotrophin-releasing hormone antagonist (GnRH-ant) protocols are recently preferred to prevent luteinizing hormone (LH) surge during controlled ovarian hyperstimulation (COH) of in vitro fertilization (IVF) procedures (1). Absence of hypo-estrogenic side-effects, no flare-up effect, short cycle duration, significant reduction in severe ovarian hyperstimulation syndrome (OHSS) incidence and gonadotropin amounts are the advantages (2, 3). Immediate and rapid inhibition of LH release within several hours through competitive binding of pituitary GnRH receptors allows GnRH-ant use at any time during the follicular phase (2, 3). However, there is still no consensus on the effect of GnRH-ant on pregnancy and live birth rates. Possible deteriorating effects of GnRH-ant on the endometrium might reduce embryo implantation and pregnancy rates (4-6). GnRH antagonists would decrease the effect of endometrial growth factors and thus alter oocyte development and decrease endometrial receptivity. The half-life of GnRH-ant is about 30 hours, and embryo transfer is usually performed on day 3 after retrieval (7). Therefore, the hypothesized effect on the endometrium might still persist at the time of embryo implantation and development (8). In the present study, we investigated whether longer duration of GnRH-ant use might have an impact on IVF outcome.

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MATERIAL AND METHOD

A total of 138 patients who were treated with GnRH-ant protocol at the IVF Clinic from March 2010 to January 2012 were enrolled in this retrospective study. The age of women was ranged between 20 and 44 years old. The 67% of women were suffering from primary infertility. The patients were evaluated with following investigations: gynecological examination, transvaginal sonographic imaging of uterus and ovaries, day 3 serum FSH-LH-E2-TSH-PRL levels, semen analysis, hysterosalpingography or office hysteroscopy. 'Decreased ovarian reserve' defined as total antral follicle count under 10, FSH level higher than 12mIU/mL and total retrieved oocyte number under 6 in previous IVF cycle. The patients with gynecological cancer, Mullerian anomaly and thyroid dysfunction were excluded from the study. The patients were classified into three groups according to the duration of GnRH-ant use. Group 1, 4 days (n=51); group 2, 5 days (n=57); group 3, 6 days (n=30) antagonist application. Main outcome measures were number of total oocytes retrieved (NOR), number of mature oocytes (NMO), fertilization rate, implantation rate and, clinical pregnancy rate.

All the patients underwent ovarian stimulation with gonadotrophins (Gonal-F®, Merck Serono; Puregon®, Organon) and GnRH-ant for pituitary down-regulation (Cetrotide®, Merck Serono; Orgalutran®, Organon). Gonadotrophins were initiated on the second day of menstruation and continued until the day of the hCG administration. GnRH-ant (0.25 mg daily) was added when the leading follicle reached 12-14 mm in diameter or E_2 level exceeding 300 pg/mL and continued until and including the day of hCG administration. Gonadotrophin stimulation was started as 150-225 IU for normoresponders and 375-450 IU for poor responders according to each patient's basal antral follicle count, day-3 hormonal status, body mass index (BMI), and prior response. When at least three follicles with a mean diameter exceeding 17 mm were measured, 250 mcg recombinant hCG (Ovitrelle®, Merck Serono) was administered. Estradiol level and endometrial thickness were both measured on the day of hCG administration. Oocyte pick-up was scheduled 35 hours after hCG administration. Embryo transfer (ET) was performed on day 3 or 5 after retrieval under guidance of transabdominal ultrasound. The ETs performed on day 2 were not included in the study. Luteal phase supplementation was done with vaginal progesterone gel twice a day (Crinone 8% gel, 90 mg; Merck Serono) starting the oocyte retrieval day. βhCG levels were measured 12 days after ET.

The statistical analyses were performed using Statistical Package for Social Sciences version 12.0 (SPSS Inc., USA). Continous variables were expressed as mean ± SD and analyzed with One Way ANOVA test. The differences between groups were analysed with post-hoc Tukey test. Pearson correlations were used to predict whether the duration of GnRH-ant use would influence oocyte number and quality (NOR, NMO, fertilized oocytes), fertilization and implantation rates. We then conducted stepwise forward logistic regressions with the variables indicative of oocyte quality. 'Implantation rate' (defined as the number of gestational sac(s) per total number of embryos transferred), as the dependent variable, was also evaluated. 'Fertilization rate' defined as the number of fertilized oocyte(s) per total number of mature oocytes. 'Clinical pregnancy' defined as the pregnancy with positive fetal cardiac activity detected on ultrasonographic evaluation. $P<0.05$ was considered as statistically significant.

RESULTS

Table 1 describes the characteristics of all the patients included (138 ICSI cycles). There was no difference between groups according to etiology. The 22 ET was not performed according to following reasons: total fertilization failure (n=5), absence of mature oocyte (n=2), empty follicle (n=2), absence of sperm on TESE (n=13). The coasting was applied on 7 patients. The pregnancy rates per ET and cycle were 37.9% (44/116) and 31.8% (44/138) respectively. The pregnancy outcomes were as follows: 7 biochemical abortions (16%), 5 abortions (11%), 18/14 ongoing pregnancies/live births.

Table 1. *Demographic characteristics of the women included in the study*

Characteristics	**Mean±SD**
Female age (years)	31.8±5.9
Male age (years)	35.3±6.4
Infertility duration (years)	6.2±4.6
BMI[a] (kg/m^2)	25.6±4.6
Day3 FSH[b] (mIU/mL)	6.9±3.6
Previous ART[c] cycle (n)	1.6±0.9
Etiology of infertility	**n (%)**
Male factor	54 (39)
Decreased ovarian reserve	33 (24)
Anovulation	20 (15)
Tubal factor	22 (16)
Unexplained	9 (6)
Type of infertility	**n (%)**
Primary infertility	93 (67)
Secondary infertility	45 (33)

[a]: Body mass index, [b]: Follicle stimulating hormone, [c]:Assisted reproductive technology

The estradiol level on the day of hCG administration, duration of stimulation, NOR, NMO and fertilized oocytes were the lowest in group 1, intermediate in group 2, and the highest in group 3, respectively. The NOR, NMO and fertilized oocytes of group 1 were significantly lower than of group 2 and 3. Only the number of germinal vesicle oocytes of group 2 was significantly higher than of group 1 (p=0.006). There was no statistically significant difference between the

numbers of metaphase1 oocytes, total amounts of gonadotrophins used, endometrial thickness on the day of hCG administration, fertilization rate and implantation rate of all groups (Table-2). The percentage of blastocyst stage transfer was the highest in group2, intermediate in group3 and the lowest in group1 and the difference for this parameter between groups was near significant (p=0.05); and there was no significant difference between groups for the percentage of grade 1 embryo on the day of ET (Table-2). The longer stimulation duration pointed out the higher E2 levels on the day of hCG administration. The comparison of main outcomes according to etiologic classification of the patients revealed out no difference from the main results.

With Pearson correlations, 'duration of GnRH-ant use' did not show a significant correlation with implantation or with the oocyte quality parameters (NOR, NMO, number of fertilized oocytes). Neither the duration of GnRH-ant nor the length of ovarian stimulation were predictors of implantation in regression analysis.

Table 2. *Cycle characteristics and the treatment outcomes of the groups*

Parameters	Group 1(n=51)	Group 2(n=57)	Group 3(n=30)	P value
Age (years)	32.6±5.6	31.4±6.0	30.5±5.5	0.27
BMI[a] (kg/m2)	25.7±4.3	24.9±4.4	24.4±4.3	0.06
Day 3 FSH (mIU/mL)	7.3±4.1	6.9±3.4	6.1±2.6	0.34
Total FSH[b] dose (IU)	1743±1042	2130±1125	2135±1019	0.12
Duration of stimulation (days)	8.5±0.8	9.2±1.2	10.8±0.8	<0.01
Endometrial thickness on hCG[c] day (mm)	10.4±2.4	10.7±1.9	11.0±2.7	0.45
Serum E_2 on hCG day (pg/mL)	1535±862	2163±1025	3087±1035	<0.01
Number of oocytes retrieved	9.6±6.5	14.4±8.8	17.9±9.4	<0.01
Number of mature oocytes	6.6±3.9	10.7±7.7	14.4±8.4	<0.01
Number of metaphase 1 oocytes	1.1±1.4	1.4±2.1	2.1±2.1	0.07
Number of GV[d] oocytes	1±1.4	2.4±2.8	1.4±1.6	<0.01
Number of fertilized oocytes	4.6±3.1	8.3±6.3	10.7±7.9	<0.01
Fertilization rate (%)	62	66	63	0.84
Grade1 embryos transferred	1.3±0.8	1.3±0.8	1.5±0.9	0.55
Grade1 embryo % on the day of ET[e]	23±3	20±2	16±3	0.12
Implantation rate (%)	15	30	25	0.16
ET on day 5 (%)	24.4	40	30.8	0.05
Pregnancy rate / ET (%)	12/40 (30%)	25/48 (52%)	14/25 (56%)	0.054
Pregnancy rate / cycle (%)	12/51 (23%)	25/57 (44%)	14/30 (46%)	0.052

[a]: Body mass index, [b]: Follicle stimulating hormone, [c]: Human chorionic gonadotrophin, [d]: Germinal vesicle, [e]: Embryo transfer

DISCUSSION

In the present study, it was observed that the longer duration of GnRH-ant use did not have a detrimental effect on oocyte quality, fertilization rate, implantation rate or pregnancy rate. It is still controversial whether pregnancy rate is lower with GnRH-ant protocols compared to the well established GnRH-agonist regimens (9). Gonadotrophin releasing hormone and its receptors were found in extrapituitary tissues such as ovary, myometrium, endometrium, mammary gland, placenta, and embryo (10). Thus, the extrapituitary actions of GnRH-ant were thought to affect ovarian stimulation outcomes and could be one of the causes of lower pregnancy rates (6). It is still unclear whether lower pregnancy rates are the result of detrimental effects of GnRH-ant on oocyte quality, embryo development, or endometrial maturation. Kinay et al (11), reported no relation between endometrial thickness and pregnancy rates in GnRH-ant cycles. In our study there was no difference between groups for endometrial thickness on the day of hCG administration and implantation rate.

The quality of oocytes (12-15) and embryos (16) are among the most significant factors determining the success of an IVF treatment. Increased cytoplasmic abnormalities in the retrieved oocytes, lower rate of zygotes showing normal pronuclear morphology and, higher rate of embryos on day 2 with an increased number of blastomeres were reported in GnRH-ant cycles (17). In our study we did not observe any relation between antagonist duration and oocyte and embryo quality. Blastocyst development did not show significant difference according to antagonist duration, but in group2 blastocyst transfer percentage was higher than group 1 and 3. This difference could not be attributed to only antagonist duration, because embryo development is a multifactorial process.

GnRH-ant did not show any difference in terms of follicular growth, the maturity of the oocytes, embryo quality, implantation, clinical pregnancy, ongoing pregnancy and miscarriage rates when compared to GnRH agonists (18). Ovarian stimulation response of GnRH-ant cycles were not inferior to agonist cycles, thus the reduced embryo implantation and pregnancy rates could be the result of possible deteriorating effects on the endometrium (19). The endometrial thickness on the day of hCG administration < 9.8 mm in GnRH-ant cycles was reported to be inversely related

to the early pregnancy loss (20). In our study we did not observe any detrimental effect of antagonist duration on endometrial thickness. Early pregnancy loss was reported to be significantly higher after day 3 single embryo transfer than after day 5 single blastocyst transfer in GnRH-ant stimulated IVF cycles and this result might be explained with asynchronization between endometrium and cleavage-stage embryos (21). In our study we also observed increased abortion rate for day 3 embryo transfer (29%) compared to day 5 embryo transfer (23%). It was also reported that the achievement of ongoing pregnancy in frozen embryo transfer cycles was not affected from the duration of GnRH-ant administration in the fresh cycle (22).

The hyper-responder patient's duration of stimulation was longer than of normo- and hypo-responders. This duration was correlated with antagonist administration. It could not be thought that longer antagonist administration improved the ovarian response. Higher estradiol levels and more retrieved oocytes were the result of ovarian reserve. In this study, we used two different kind of gonadotrophins and GnRH antagonists. This difference may have influence on our results. We demonstrated that longer GnRH-ant use was associated with higher estradiol levels, increased numbers of total and mature oocytes retrieved, but had no effect on fertilization rate, implantation rate or pregnancy results. In conclusion we thought that longer GnRH-ant use did not have a detrimental effect on IVF outcome.

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