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Comparison of GnRH antagonist and agonist mini-dose long protocols in infertile cases undergoing controlled ovarian hyperstimulation

H. Akın[1], S. Kumbasar[2], B.A. Şık[3], S. Salman[4], M. Temur[2], F. Çakıroğlu[5], R. Özyurt[6], M. Küçükbaş[7]

[1] Department of Obstetrics and Gynecology, Süleymaniye Research and Education Hospital, Istanbul
[2] Department of Obstetrics and Gynecology, Sakarya University School of Medicine, Sakarya Research and Education Hospital, Sakarya
[3] Faculty of Health Sciences, Aydın University, Istanbul
[4] Department of Obstetrics and Gynecology, Gaziosmanpaşa Taksim Research and Education Hospital, Istanbul
[5] Department of Obstetric and Gynecology, Yeni Ufuk Hospital, Istanbul
[6] Department of Obstetrics and Gynecology, Istanbul Research and Education Hospital, Istanbul
[7] Department of Obstetrics and Gynecology, Zeynep Kamil Research and Education Hospital, Istanbul (Turkey)

Summary

Aim: The purpose of the present study was to determine if there is a difference between multi-dose gonadotropin releasing hormone (GnRH) antagonist protocol and long GnRH agonist protocol. *Materials and Methods:* This retrospective study compared the data pertaining to patients chosen as per predetermined acceptance criteria, 113 of whom were administered multi-dose antagonist protocol for controlled ovarian hyperstimulation (COH) while 133 were administered long agonist protocol for COH at Süleymaniye Teaching Hospital of Obstetrics and Gynecology. *Results:* While cancellation rate was found to be significantly higher in antagonist group (17.7% *vs* 11.28%), the number of follicles > 14 mm and > 16 mm, E2 level, and the number of retrieved oocytes on the day of hCG trigger were significantly lower in the same group. However, there was no difference between fertilization rates and embryonic development rates. The pregnancy rates per transfer and per cycle were found to be 40.9% and 31.7%, respectively; in the antagonist group they were lower, though not significantly, when compared to agonist group (44.1% and 39.1%, respectively). Ongoing pregnancy rates were found to be similar between the groups. *Conclusion:* GnRH antagonist treatment protocol has a level of efficacy similar to agonist treatment protocol in terms of pregnancy results for all groups.

Key Words: Long GnRH agonist; GnRH antagonist; IVF.

Introduction

The first in vitro fertilization (IVF) treatments have been performed in unstimulated, natural cycles. Today, gonadotropins are used in IVF treatment to stimulate multiple follicular development, and gonadotropin releasing hormone (GnRH) analogues are used to prevent premature increases in luteinizing hormone (LH). When stimulation is done without using GnRH analogues in IVF patients, premature LH increases are observed in approximately a 20% rate [1, 2]. The introduction of GnRH upon discovering that endogenous LH increase can be prevented by desensitizing the GnRH receptors localized in the pituitary gland; decreased rate of premature LH peaks down to 2%, thereby increasing pregnancy rates [3, 4]. Today, GnRH agonists are the most commonly prescribed agents along with gonadotropins. Among all agonist treatment protocols, long protocol is the most frequently used. However, this treatment protocol requires two to three weeks of desensitization time which causes increases in treatment time, required gonadotropin dose, and risk of ovarian hyperstimulation syndrome (OHSS). During desensitization period, patients are exposed to some side effects like hot flushes, headache, bleeding, and vaginal dryness. Expectations from GnRH antagonists were high. GnRH antagonists have been created by substitution of amino acids at multiple points with other molecules, they bind to the receptors with high affinity, and inhibit endogenous gonadotropin release; it was thought they would take the place of agonists due to their pharmacokinetic and pharmacodynamic properties. Absence of the initial flare effect not requiring long desensitization time, and thus absence of estrogen deprivation symptoms, having sufficient LH suppression in short time, the dose-dependent effect and reversal of the antagonistic effect by GnRH itself, or its analogue, render antagonists superior to agonists [5, 6]. Additionally, studies suggest treatment duration is shorter and used gonadotropin dose is less in antagonist protocol [6, 7]. However, according to a meta-analysis of first five studies comparing agonist long protocol and GnRH antagonist protocol, there were 5% less clinical pregnancies in antagonist group. Based on these re-

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sults, clinicians have not preferred GnRH antagonists as first choice in treatment. In those studies, GnRH antagonists were essentially used in advanced age and in patients who had unsuccessful previous cycle [8]. However, according to sub-analyses comparing GnRH antagonists and agonists in patients with similar clinical and demographical properties, pregnancy rates were similar in both groups [9].

This study aimed to compare treatment outcomes of antagonist and agonist treatment protocols in a group of normal response women.

Materials and Methods

The study included 211 patients recruited in a controlled ovarian hyperstimulation (COH) program for assisted reproductive techniques (ART) indication, who applied to Süleymaniye Obstetrics and Gynecology Hospital Infertility Polyclinic between October 1, 2003 and April 30, 2005 and met the study inclusion criteria. Of these patients, 118 received mini-dose long protocol and 93 received multi-dose antagonist protocol. The study was approved by local ethics committee.

The study aimed to compare effectiveness of the aforementioned treatment protocols in a retrospective study design investigating patients who were selected according to pre-determined criteria, who received long protocol mini-dose agonist or multi-dose antagonist treatment protocols using r-FSH and/or hMG for COH, who had their ovum fertilized with ICSI method, and in which embryo transfer was performed.

Study inclusion criteria were as follows: patients who suitable for treatment with IVF or ICSI, aged between 20 and 39 years, with a BMI between 18 and 19, with primary or secondary infertility, with regular menstrual cycles (25-32 days), with basal FSH levels less than 13 mIU/ml, and TSH and prolactin levels within normal limits.

Study exclusion criteria were as follows: presence of clinically significant systemic or endocrine disease, history of more than two previous unsuccessful COH+ART, history of previously detected space occupying lesions such as polyps, submucous myoma, and uterine septum after evaluation with hysterosalpingography or office hysteroscopy, and patients who had chlamydia antibody positivity.

The following were determined as criteria for cycle cancellation: premature luteinization (progesterone level ≥ 1.7 ng/ml during COH), premature LH peak: (LH level ≥ 12.1 mIU/ml during COH), drop in estrogen level (more than 50% decrease between two control days), possible development of OHSS (presence of 15 or more intermediate follicle on 8th day of stimulation (12-16 mm) or presence 20 or more large follicles on 10th day or later (16-30 mm) and/or E2 level ≥ 3000 pg/ml), failed fertilization, failed embryo development, and failed follicle development.

COH was performed in all patients using GnRH agonist or antagonist protocol in first instance. In agonist mini-dose long protocol, GnRH agonist leuprolide acetate was initiated on the 21st day of the previous menstrual cycle at a 0.5-mg/day dose, and gonadotropin was added on the third day of menstrual cycle. Ovarian suppression was accepted as serum E2 concentration < 50 pg/ml and absence of >10 mm follicles in the ovary. On the third day of menstrual cycle, stimulation with gonadotropin was initiated and agonist dose was lowered to half on the same day and continued until the day of hCG injection.

In antagonist protocol, gonadotropin was initiated on the third day of menstrual cycle at similar doses, and when dominant follicle reached a size of 11-14 mm, GnRH antagonist was administered at 0.25 mg/day dose until the day of hCG injection.

Gonadotropin dose was adjusted according to serum E2 levels and follicle sizes in ultrasonography. hCG administration criteria for oocyte maturation was the same in both protocols. When the leading follicle reached 18 mm or one of the follicles reached 17 mm, ovulation was triggered with r-hCG 250 µg. Oocyte collection was performed 35-36 hours after hCG administration. During oocyte collection, all follicles at or greater than 14 mm were aspirated. After three days from oocyte collection, one to four embryos preferably with type A quality were transferred into uterine cavity using ICSI standard procedures [10]. Luteal support in all patients were provided with intravaginal micronized progesterone 3×200 mg and/or 1500 IU hCG once in three days intramuscularly (1,500 IU amp. In case of established pregnancy, vaginal progesterone support was continued until 8th-12th gestational week.

Parameters related to effectiveness of protocols compared between the groups

Primary results included: induction time, used gonadotropin dose, mean daily gonadotropin dose, GnRH agonist/antagonist utilization time, folliculometry results on the hCG day (follicle sizes of >10, >14, and >16 mm), E2 level on hCG day, number of collected oocytes, number of MII oocytes, fertilization rate (number of fertilized oocytes/number of ICSI performed oocytes ×100), embryo development rate (number of embryos on the transfer day/fertilized oocyte number ×100), and implantation rate (number of gestational sac/number of transferred embryo ×100).

Statistical analysis

For comparison of parameters such as age, BMI, infertility time, levels of FSH, LH, E2, total FSH dose used, FSH induction time, daily FSH dose, analogue utilization time, treatment time, number of collected oocytes, number of MII oocytes, number of fertilized oocytes, number of developed embryo, and number of embryo transfers between the two treatment groups, "test for equality of means" (independent samples T test)" was used. For comparison of infertility type, history of previous treatment, cause of infertility, overgrade, exogenous LH utilization, pregnancies, and ongoing pregnancies between treatment groups, "Pearson, Yates or Fisher χ^2 independency test" was used. For comparison of MII/total oocyte ratio, implantation ratio, fertilization ratio, and embryo development ratio between treatment groups, "test for significance of difference between percentages in independent samples" was used. Statistical analyses were carried out with SPSS 10.0 statistical software and biostatistical formula [11, 12].

Results

One hundred and forty-five patients from the agonist group, and 119 patients from the antagonist group were included in the study. Since spermatozoa could not be collected by TESE method from male partners of 12 patients in agonist group and of six patients in antagonist group, they were excluded from the study. Records of totally 246 patients, 113 in agonist group and 113 in antagonist group, were examined retrospectively and taken into evaluation. According to criteria for cycle cancellation, cycle or embryo transfer was cancelled in 15 (11.28%) patients in agonist group and in 20 (17.7%) patients in antagonist group. Cycle or embryo transfer cancellation rate due to fertilization failure and inadequate follicular development was significantly

Table 1. — *Cycle or embryo transfer cancellations.*

Reason of cancellation	Agonist mini-dose protocol (n=133) (%)	Antagonist protocol (n=113) (%)	*p* value[a]	T value
Fertilization failure	4 (3)	6 (5.3)	< 0.05	2.53
Absence of embryo development	2 (1.5)	4 (3,\.54)	*p*[b]	1.97
Inadequate follicular development or follicular atresia	5 (3.76)	5 (4.42)	< 0.05	2,53
Failure to obtain MII oocyte		1 (0.88)		
Early luteinization or ovulation	2 (1.5)	4 (3.54)	*p*[b]	1.97
Cycle cancellation due to risk of OHSS	2 (1.5)			
Total	15 (11.28)	20 (17.7)	< 0.05	4.47

a: test for significance of difference between percentages in independent samples, b: α = 0.05 borderline significance.

Table 2. — *Demographical properties and infertility characteristics of patients.*

	Agonist mini-dose protocol (n= 118) (%)	Antagonist protocol (n=93) (%)	*p* value
Age (years)	28.92±4.12	29.87±4.25	0.105[a]
BMI (kg/m^2)	24.30±2.69	24.33±2.61	0.932[a]
Mean infertility time (years)	6.53±3.77	7.42±3.58	0.82[a]
Basal FSH value (IU/ml)	7.06±1.94	7.85±1.82	0.873[a]
Basal E2 value (pg/ml)	53.21±22.81	54.99±22.40	0.572[a]
Basal LH value (IU/ml)	5.28±2.76	6.06±3.36	0.066[a]
Antral follicle (2nd day)	10.48±3.88	9.11±3.18	0.087[a]
Infertility type			0.986[b]
Primary	99 (83.9)	79 (84.9)	
Secondary	19 (16.1)	14 (15.1)	
Number of IVF cycles	1.27±0.56	1.42±0.70	0.059[a]
Cause of infertility			
Female infertility			
- normal	96 (81.4)	73 (78.5)	
- tubal factor	18 (15.3)	18 (19.4)	
- endometriosis	4 (3.4)	2 (2.2)	0.731[c]
Male infertility			
- normal	10 (8.5)	5 (5.4)	
- infertile	108 (91.5)	88 (94.6)	0.549[b]
Total infertility cause			
♀ infertile /♂ infertile	19 (1 6.1)	19 (20.4)	
♀ normal /♂ infertile	89 (75.4)	69 (74.2)	
♀ infertile /♂ normal	3 (2.5)	1 (1.1)	
♀ normal /♂ normal (unexplained cause of infertility)	7 (5.9)	4 (4.3)	0.669[d]

a: test for equality of means (independent samples T test);
b: Yates X^2 independency analysis;
c: since values < 5 were more than 20% in the multi-cell table, row 2 and row 3 were combined and Yates χ^2 independency test was performed.
d: Since values that are < 5 were more than 20% in the multi-cell table, row 3 was ignored and Pearson χ^2 independency test was performed on the remaining group including 3 rows.
Mean ± standard deviation. BMI: body mass index.

higher with antagonist multi-dose treatment ($p < 0.05$). According to comparison of cancellation rates due to absence of embryo development, early luteinization or ovulation, the difference between the groups was at borderline significance ($p = 0.05$). In agonist group, two patients (1.5%) had their cycle cancelled due to OHSS, whereas there was no cancellation in antagonist group due to this reason (Table 1).

After exclusion of 15 patients in agonist group and 20 patients in antagonist group according to criteria for cycle cancellation, data belonging in total to 211 patients, 118 patients from agonist group and 93 patients from antagonist group, were taken into evaluation. Demographical properties and infertility characteristics of 211 patients are shown in Table 2. There was no significant difference between two different treatment groups with regards to age, mean BMI, mean infertility time, infertility type, history and number of previous unsuccessful treatments, female-male and total infertility reasons, basal (third day of cycle without treatment) hormone levels, and number of antral follicles ($p > 0.05$). According to infertility reasons, there was male infertility in 91.5% of agonist group and in 94.6% in antagonist group; there was female infertility in 18.7% of agonist group and in 21.6% of antagonist group. Cases with unidentified cause for infertility constituted 5.9% and 4.3%, respectively (Table 2).

Total FSH amounts administered for induction were similar in both groups (2,416 IU in agonist group *vs.* 2,509 IU in antagonist group). Mean induction time with FSH was significantly shorter in antagonist group (9.71 days *vs.* 10.27 days). Mean FSH dose administered in one day was significantly higher in antagonist group (260 IU *vs.* 233 IU). Number of > 10 mm follicles on the seventh day of treatment cycle (at the end of induction with FSH for five days) was less in agonist group (1.28 *vs.* 1.43); on the other hand, it was significantly higher on day of hCG injection (14.75 *vs.* 12.23). There was no difference in the number of 11-14 mm follicles on day of hCG injection (6.7 *vs.* 6.05), and the difference in number of total follicles was due to the difference in the number of >14 and >16 mm follicles. There was linear increase in E2 levels in correlation with follicular development in both groups. In agonist treatment group, E2 levels were significantly lower on the seventh day of the cycle; however, it was significantly higher on the day of hCG injection (468 and 3,179 *vs*. 591 and 2,434 pg/ml). There was no difference in endometrial thickness on the day of hCG injection ($p = 0.003$ and $p = 0$) (Table 3).

In agonist treatment group, average number of collected oocytes, number of MII oocytes, number of fertilized oocytes, and number of embryos on the third day were significantly higher than in antagonist group (14.08, 11.25, 7.69 and 7.58 *vs*. 11.67, 9.27, 6.93 and 6.20, respectively); on the other hand, MII oocyte/total oocyte ratio, fertilization ratio, and embryo development ratio were similar in both groups. In agonist group, 3.76 embryos were transferred on average, whereas in antagonist group, 3.48 embryos were trans-

Table 3. — *Data related to follicular development, hormone levels, and treatment characteristics.*

	Agonist mini-dose protocol (n= 118) (%)	Antagonist protocol (n=93) (%)	*p* value
Total rFSH dose (IU/ml)	2416.91±798.40	2509.82±800.30	0.403[a]
Induction time (days)	10.27±1.53	9.71±1.43	0.007[a]
Average daily FSH dose (IU)	233.25±62.84	260.99±76.76	0.004[a]
7th day folliculometry (average number of > 10 mm follicles)	1.28±1.73	1.43±1.16	0.490[a]
7th day E2 level (pg/ml)	468.29±302.58	591.49±309.44	0.003[a]
Day of hCG injection folliculometry			
- Average number of > 10 mm follicles	14.75±5.38	12.23±5.49	0.01[a]
- Average number of > 14 mm follicles	8.04±3.51	6.17±3.18	0.0[a]
- Average number of > 16 mm follicles	4.53±2.53	3.53±2.119	0.003[a]
- Average number of 11-14 mm follicles	6.70 3±.64	6.05±3.51	0.202[a]
- Average number of 15-16 mm follicles	3.47±2.09	2.65±1.92	0.004[a]
Day of hCG injection mean E2 (pg/ml)	3179.18± 1284.89	2434.231± 102.64	0.0[a]
Day of hCG injection mean endometrial thickness	10.38±2.21	9.92±1.57	0.282[a]

a: Test for equality of means (independent samples T test).
Mean ± standard deviation. ET: endometrial thickness.

Table 4. — *Data related to result parameters in both groups.*

	Agonist mini-dose protocol (n=118) (%)	Antagonist protocol (n=93) (%)	*p* value	T value
Number of collected oocytes	14.08±6.36	11.67±6.5	0.008[a]	
Number of MII oocytes	11.25±5.63	9.27±5.19	0.009[a]	
MII oocyte/total Ratio of oocyte number (%)	79.9	79.5	> 0.05[b]	0.256
Number of fertilized oocytes	7.69±4.04	6.93±4.07	0.012[a]	
Number of embryo on 3rd day	7.58 3.±97	6.20±3.93	0.013[a]	
Number of embryo transfer	3.76±1.01	3.48±1.09	0.056[a]	
Fertilization rate (%)	70.9	70.8	> 0.05[b]	0.05
Ratio of embryo development from fertilized oocytes (%)	95.1	94.6	> 0.05[b]	1.142
Implantation rate (%)	19.6	19.4	> 0.05[b]	0.069
Clinical pregnancies per transfer, number (n), rate (%)	52 (44.1)	38 (40.9)	0.64[c]	
Clinical pregnancies per cycle, number (n), rate (%)	52 (39.1)	38 (33.6)	0.375[c]	
Ongoing pregnancies for > 16 week, number (n), rate (%)	41(34.7)	32 (34.4)	0.959[c]	

a: Test for equality of means (independent samples T test)
b: test for significance of difference between percentages in independent samples
c: Pearson χ^2 independency test.

ferred on average ($p > 0.05$). Implantation rates were similar. Pregnancy rate per transfer and pregnancy rate per cycle were 44.1% and 39.1% in agonist group, and 40.9% and 31.7% in antagonist group, respectively. The lower pregnancy rates in antagonist group were not statistically significant. Ongoing pregnancy rates were similar in both groups (Table 4).

Discussion

Premature endogenous LH increase, which occurs in up to 20% of natural cycles, has fallen to 2% after GnRH agonists have come into use, and pregnancy rates have increased [3, 4]. However, due to long pituitary desensitization period with agonists, treatment duration has increased, patients have been subject to estrogen deprivation symptoms, the amount of gonadotropin used has increased and thus, OHSS risk has increased. Later on, GnRH antagonists have been developed; they did not cause the unwanted effects of agonists, and they could suppress endogenous LH suppression in equal rates.

In the present study, the authors used the data of patients who received COH with antagonist multi-dose protocol and agonist long protocol. In order to provide homogeneity between patient groups, factors that could have an effect on the results were limited. Criteria used when applying this limitation were adopted from other studies in literature, thereby it aimed to make it easier to compare the present results to previous studies [13, 14].

In the present study, it was observed that there were significantly more cycle cancellations in antagonist group than in agonist group (17.7% *vs.* 11.3%) ($p < 0.05$). According to results of one study, there was no difference in cancellation rates between groups (15.3% *vs.* 17%) [15]. There was no remarkable difference in premature luteinization rates between the groups in the present study (two patients (1.5%) in agonist group, and four patients (3.54%) in antagonist group, $p = 0.05$). There was cancellation due to OHSS risk in two patients (1.5%) in agonist group, whereas there was no cancellation due to this reason in antagonist group. In one study, it was determined that antagonists decreased both OHSS incidence and cycle cancellation rates due to OHSS in high-risk patients [16].

In one non-randomized observational study conducted in Germany with contribution of 116 centers, it was observed that antagonists were used preferentially in patients with poor expectations, and pregnancy rates were almost equal when patients were selected according to restricting criteria like < 35 years of age, tubal infertility, and first treatment cycle (37.8% for agonist *vs.* 36.7% for antagonist) [8]. In the present study, after patient homogeneity was ensured with restriction criteria, there was slightly lower pregnancy rate in antagonist group, and this difference was statistically not significant (pregnancy per transfer ratios were 44.1% *vs.* 40.9%, and pregnancy per cycle ratios were 39.1% *vs*. 33.6%). Similar results were obtained in previous studies. In one meta-

analysis by Ludwig *et al.* that evaluated retrospective and randomized prospective studies, and in one multi-center study published by European Cetrorelix Study Group, it was determined that pregnancy rates were lower in antagonist group, but the difference was not significant and alternative approaches toward multi-dose antagonist utilization were recommended [16, 17]. Ludwig *et al.* also stated that rather than starting antagonist treatment on a certain day, flexible regimes in which early phase of follicular development was less affected and antagonists are started on a patient-specific day, could be more effective [16]. In one study comparing the effects of flexible regime versus starting the antagonist protocol on a certain day, higher clinical pregnancy rates were found, although it was not significant (51.6% *vs*. 44.1%) [8]. In another study by Lautradis *et al.* in 2004 which compared agonist long protocol with antagonist multiple dose flexible regime, pregnancy rates showed a difference in favor of antagonists (24.1% *vs*. 18.5%) [18]. In the present protocols, antagonists were administered as multiple dose flexible regime in order to optimize the treatment. Additionally, in the present treatment protocols, gonadotropins were initiated at different doses depending on the expectation regarding the treatment response of the patient, and dose adjustment was made according to ovarian response. Likewise, the present results regarding pregnancy rates in different treatment protocols were not different than the results of studies comparing agonist long protocol to flexible-regime antagonists that were initiated with a personal gonadotropin dose (ratio of clinical pregnancy per transfer was 20% in agonist group *vs*. 17% in antagonist group) [15, 20].

Total number of collected oocytes and MII oocytes are thought to influence average number of embryo transfer and pregnancy rates indirectly [19]. The possibility to achieve higher quality embryo development is expected to increase with use of greater number of MII oocyte; indeed, this is aimed with COH. In the present study, average number of collected oocytes was 14.08 in agonist group and 11.67 in antagonist group, and the difference was statistically significant ($p < 0.05$). There was no difference between two groups regarding MII/total oocyte ratios, fertilization rates, and embryo development rates on third day. For this reason, it was thought that the significant differences in numbers of MII oocytes, fertilized oocytes, and embryos were due to the limited number of total oocytes. Most of previous studies did not report difference in total number of oocytes between groups [15, 19, 20] whereas Ludwig *et al.* reported 2.6 less number of collected oocytes in antagonist group in their meta-analysis ($p < 0.05$) [16]. Albano *et al.* also reported significantly less number of collected oocytes in antagonist group in their multi-centered study. (4.1 *vs*. 6) [17].

In the present study, similar with the total number of oocytes, there was also significant difference in the total number of >10 mm follicles between the groups ($p < 0.05$). This difference in the number of follicles may be related to the dose and duration of gonadotropin. It is proposed that increased dose of gonadotropin yields greater number of developed follicles and increases the number of collected oocytes [20]; in the present study, total amount of gonadotropin stimulation was similar in both groups. In one study reporting significantly less number of total oocytes, the number of 11-14 mm follicles was less in antagonist group (3.2 *vs*. 4.3, $p < 0.05$), but the decreased number of follicles in other sizes was not statistically significant [17]. Lee *et al.* found similar number of all follicles larger than 10 mm and total number of collected oocytes in antagonist group, but showed that small and medium sized follicles were less in number ($p = 0.003$ and $p = 0.007$, respectively [12]. Similar results were reported in Ludwig *et al.*'s meta-analytic study (16). On the other hand, in the present study, the number of 15-16 mm and >16 mm follicles and the number of follicles larger than 10 mm were significantly less in antagonist group; however on the contrary to aforementioned studies, the numbers of 11-14 mm follicles were similar in both groups ($p = 0.202$). One reason for the difference in follicular development may be related to gonadotropin dose and duration. In the study by Albano, lower dose of gonadotropin was administered in shorter time in antagonist group [17], whereas in the present study, similar gonadotropin dose was administered in shorter time. That is, follicles in antagonist group were exposed to higher dose of daily gonadotropin compared to agonist group in the present study (260 IU/day in antagonist group *vs*. 233 IU/day in agonist group).

In summary, antagonist group appears to be non-favorable regarding total number of oocytes and follicles which are parameters that could affect pregnancy rates. This condition has an influence on number of developed embryos and embryo transfer. There were 3.48 embryo transfers in antagonist group and 3.76 embryo transfers in agonist group on average ($p = 0.056$). However, similar pregnancy rates suggest adequate number and quality of oocyte development were achieved in antagonist group.

In the present study, E2 levels on seventh day of stimulation were higher in antagonist group and with the initiation of antagonist, they showed an increase with less acceleration compared to the agonist group. There was a significant difference in E2 levels on the day of hCG injection in favor of antagonist group, as in total number of follicles ($p < 0.05$). This difference was found significant in some of the studies in literature [4, 17, 20]. On the contrary, in the study by Xavier *et al.*, treatment was initiated with personal gonadotropin dose, antagonist flexible regime was used, and equal doses of gonadotropin was administered in both groups; however, there was no significant difference in E2 levels between the two groups on the day of hCG injection [14]. One reason for lower E2 level on the day of hCG injection was proposed to be less number of small follicles [18]. Although number of small follicles were similar in the present study, significantly lower levels of E2 suggest that

number of follicles of other sizes are important. In one study comparing single dose three-mg cetrorelix with long protocol, it was stated that E2 concentration in follicular fluid was lower with antagonist treatment (572 *vs*. 873 pg/ml), and this could be due to the direct effect of antagonists on ovarian cells and reduced aromatase activity in granulosa cells [4]. It was proposed that this condition could have a favorable effect on endometrial receptivity, and absence of difference between the groups with regards to other parameters (fertilization, implantation, and pregnancy rates) may not be related to E2 concentration, which is a classical index for follicular health [4]. In contrast, in one study involving cases who received long protocol with buserelin, it was reported that more successful fertilization and pregnancy could be achieved with oocytes obtained from estrogen-rich follicles, and that estrogen plays an important role in oocyte maturation and successful embryogenesis [21]. In the present study, there were equal rates of MII/total oocyte, fertilization, embryo development and implantation, which is more consistent with the first proposal.

Some studies state agonists can directly act on ovarian GnRH receptors, therefore directly effecting GnRH and similar peptides produced in ovaries, which in turn influence serum E2 levels; however another study did not support this idea of direct effect on ovary [22].

In conclusion, follicular development and number of obtained oocytes were significantly less in GnRH antagonist multi-dose protocol compared to GnRH agonist long protocol in COH program for ART; however, fertilization , embryo development, implantation, and pregnancy and ongoing pregnancy rates, which are primary parameters, were at comparative levels. In conclusion, it can be stated that GnRH antagonists have a similar effectiveness to GnRH agonists in all patient groups.

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Corresponding Author:
S. KUMBASAR, M.D.
Department of Obstetrics and Gynecology
Sakarya University School of Medicine
Sakarya Research and Education Hospital
Sakarya (Turkey)
e-mail: doktor1977@hotmail.com