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Original Article

Are there any differences between antagonist administration on days <6 and ≥6 of Controlled Ovarian Hyperstimulation on assisted reproductive technique outcomes?

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Abstract

Background: The aim of this study was to investigate the cost-effectiveness of antagonist administration on stimulation on days <6 and ≥6 of COH on assisted reproductive technique (ART) outcomes.
Methods: In this retrospective cohort study, 412 patients who were admitted to the ART Department were evaluated. In group 1 (203 patients), antagonist administration was provided on days <6 of COH. For group 2 (209 patients), antagonist administration was provided on days ≥6 of COH. We preferred a flexible antagonist protocol in clinical practice and added an antagonist treatment regimen when dominant follicles were enlarged to 13 mm or the serum blood E_2 was >300 pg/mL.
Results: There were no differences between antagonist administration on days <6 and days ≥6 of COH in terms of age, BMI, duration and etiology of infertility, AFC, serum FSH, LH, peak E_2 levels, the number of MII oocytes, 2PN, FR, the number of transferred embryos, and CPR per woman. However, there were statistically significant differences between the duration of stimulation, the total gonadotropin dose required, and progesterone levels on day hCG [8.26 ± 1.83 vs 9.56 ± 1.51 ($p = 0.001$); 2173.71 ± 860.00 vs 2749.17 ± 1079.51 ($p = 0.001$); 0.75 ± 0.44 vs 0.92 ± 0.59 ($p = 0.002$), respectively].
Conclusion: Our results have demonstrated that there was no effect of antagonist administration on days <6 and ≥6 of COH on ART outcomes. However, taking cost-effectiveness into consideration, we suggest an antagonist administration on days <6 of COH since the necessary gonadotropin dose is lower.

Keywords: Cost-effectiveness; GnRH antagonist; IVF; Pregnancy outcomes

1. Introduction

Gonadotropins have been commonly used for controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF) procedures, particularly during the last 30 years. In order to obtain a high-quality oocyte yield and embryo by preventing a premature luteinizing hormone (LH) surge, gonadotropin-releasing hormone (GnRH) agonists (GnRHa), which induce gonadotropin release via pituitary desensitization, have started to be employed in IVF treatment.[1] During the past 15 years,

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more potent GnRH antagonists (GnRHant) with fewer side effects have been preferred, because unlike GnRHa, they have the ability to induce a rapid gonadotropin suppression and quickly reverse gonadotropin secretion during IVF treatments.[2−4] GnRHants have certain advantages over GnRHas, which include a shorter duration of stimulation, an absence of vascular symptoms, the requirement for fewer injections, avoidance of ovarian cyst formation and lower gonadotropin doses per cycle.[5] While GnRHa requires longer use, GnRHants competitively block pituitary gland GnRH receptors (fast and reversible suppression) and can be continued for shorter periods.[6] However, GnRHants may have negative effects on the follicles, embryo, and endometrium by reducing growth hormone secretions.[7]

GnRHant therapy in IVF is particularly suitable for women who are undergoing first time COH, have diminished ovarian reserve, are taking oral contraceptives to regulate menstrual cycles, and have been diagnosed with polycystic ovary syndrome (PCOS).[3,6−8]

In COH, GnRHant fixed dosing is started on day 5 or 6 of stimulation, and the flexible dosing begins after the follicles reach 12−14 mm in diameter.[9,10] In a prospective randomized study using a lower gonadotropin dose in the flexible protocol than in the fixed protocol, a higher yield of oocytes was achieved and no difference was observed between the two protocols with regard to the clinical pregnancy rate (CPR).[11,12]

In the literature, there have been several contradicting reports comparing GnRHa and GnRHant use in terms of IVF CPR. GnRHant-treated women manifest lower CPR compared with the GnRHa-treated women.[13,14] On the other hand, systematic reviews and meta-analyses have demonstrated no statistically significant differences between GnRHa and GnRHant treatment with regard to CPR.[5,14]

Recently, some authors have applied several modified treatment methods to reduce IVF treatment costs. These methods have included the alternative use of a short GnRHa,[13] and the administration of recombinant human chorionic gonadotropin (hCG) microdoses in the late stages of COH.[15]

In this retrospective cohort study, we aimed to compare the cost-effectiveness of antagonist administration at <6 days and ≥6 days after stimulation of COH on assisted reproductive technique (ART) outcomes.

2. Methods

A retrospective cohort study was conducted at the Zekai Tahir Burak Women's Health Education and Research Hospital, IVF Unit and the Konya Education and Research Hospital, IVF Unit. We reviewed the outcomes of 412 fresh, non-donor, intracytoplasmic sperm injection (ICSI) cycles which occurred between January and December 2014. Women were included in the study if they were between the ages of 20−39 years. All of the patients had a body mass index (BMI) between 18 and 29 kg/m,[2] regular menstrual cycles, no existence of polycystic ovaries, no presence of endometriosis or uterine abnormalities in the ultrasound, no previous adnexal surgery, and normal basal hormonal levels during the cycle before stimulation. The exclusion criteria were >39 years of age, BMI >30 kg/m^2, a history of recurrent pregnancy loss, any significant systemic diseases or endocrine or metabolic disorders, a low or high response to gonadotropin stimulation in a previous cycle, and any indication for preimplantation genetic diagnosis or screening or concomitant medication interfering with the purposes of the study. This study was approved by the institutional review board of the hospital.

Data were collected for age, BMI (kg/m^2), duration of infertility, infertility factor, baseline at day 3 for follicle stimulating hormone (FSH) and LH levels, antral follicle count, starting day of GnRHant, stimulation parameters (duration of stimulation, total gonadotropin dose, serum estradiol [E_2; pg/ml] and progesterone levels [pg/ml] on day of hCG), laboratory ART outcomes (number of retrieved oocytes, MII and 2 pronucleus [2PN] oocytes, number of transferred embryos, fertilization rate), endometrial thickness (mm), and CPR.

The pituitary down-regulation was achieved and maintained using the flexible GnRHant protocol. Recombinant human FSH (r-hFSH; Gonal-F, Merck-Serono, or Puregon, MSD) or human menopausal gonadotropin (hMG; Menogon or Menopur; Ferring) was used for COH. The initial gonadotropin dose used for ovarian stimulation was individualized according to the patient's age, baseline serum FSH concentrations on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first three days (150−225 IU rec FSH/day), and thereafter, the gonadotropin dose was adjusted according to the individual's ovarian response. Serial estrogen levels and two-dimensional follicle measurements by transvaginal ultrasonography (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South Korea) were performed. A daily dose of 0.25 mg of GnRHant (Cetrotide, Merck-Serono, or Orgalutran, MSD) was initiated when the leading follicle diameter was ≥13 mm or the serum E_2 level reached ≥300 pg/ml. When at least two dominant follicles reached dimensions of 18 mm or greater in diameter, hCG (250 μg, Ovitrell, Merck-Serono) was administered and oocytes were retrieved 36 h after the hCG injection. ICSI was then applied in accordance with our clinical procedures. Embryo transfers were performed on day 3. Luteal phase support was routinely provided as progesterone in the form of Crinone 8% gel (Serono, Istanbul, Turkey) at a dose of 90 mg daily for 14 days until a pregnancy test was performed.

Patients were divided into two groups as follows: group 1 consisted of patients who reached the criteria for GnRHant administration on stimulation day <6, and group 2 consisted of patients who started the GnRHant on stimulation day ≥6.

Clinical pregnancies were defined as those with fetal heart activity documented on an ultrasound examination at 6 weeks after the embryo transfer.

Statistical analysis of data was carried out using SPSS 15 (Statistical Package for Social Sciences, SPSS Inc.) software. The distributions of all of the continuous variables for normal or non-normal distributions were tested using Kolmogorov−Smirnov tests. Variables with normal distributions were compared between groups using independent samples t-tests. The Mann−Whitney U test was applied to the variables that were not distributed normally. The results are expressed as the

mean ± standard deviation (SD). For the categorical variables, Pearson's chi-square analyses and Fisher's exact tests were used. A p value of <0.05 was considered statistically significant.

3. Results

A total of 412 patients admitted to the IVF department were evaluated between January and December 2014. Of the 412 women included in the study, 203 (49.27%) received GnRHant administration on stimulation day <6 (group 1), while 209 women (50.73%) had delayed administration of GnRHant on stimulation day ≥6 (group 2). None of the women had a premature LH surge that led to cycle cancellation.

The mean ages of the women, BMI, duration of infertility, causes of infertility, baseline FSH and LH levels, antral follicle count, serum E_2 levels on the day of hCG, and the endometrial thickness were not different between the two groups ($p > 0.05$). The starting day of GnRHant administration was 4.74 ± 0.50 vs 6.52 ± 0.74 in groups 1 and 2, respectively ($p = 0.001$). Women in group 2 required a significantly longer ovarian stimulation period, significantly higher progesterone levels on day hCG, and a significantly higher extent of gonadotropin use ($p = 0.001$). Patient demographic and stimulation characteristics are shown in Table 1.

There were no differences between groups 1 and 2 regarding the number of retrieved oocytes, the number of MII oocytes, the number of 2PN, the fertilization rates, the number of transferred embryos, and CPRs ($p > 0.05$). Laboratory and reproductive outcome parameters are shown in Table 2.

Table 2
Laboratory and reproductive outcome parameters of the patients.

The day of antagonist administration	<6 (n = 203)	≥6 (n = 209)	p
Number of oocytes retrieved	6.42 ± 5.72	6.88 ± 5.10	0.425
Number of MII oocytes	4.72 ± 3.68	5.23 ± 3.51	0.195
2 PN	3.31 ± 2.43	3.59 ± 2.74	0.335
Fertilization rate	73.58 ± 26.05	68.24 ± 29.91	0.085
Number of transferred embryos	1.55 ± 0.49	1.60 ± 0.49	0.294
Clinical pregnancy (%)	68 (33.5)	74 (35.4)	0.684

PN: pronucleus.
$p < 0.05$ is significant.

4. Discussion

In this retrospective cohort study, which was based on the starting day of GnRHant administration in a multiple-dose protocol (0.25 mg/day), no differences were found between the reproductive outcome parameters of the IVF cycle. Although CPR was higher in the ≥6 days group, this difference was not statistically significant. In our study, 50.73% of patients belonged to the general IVF population treated with the flexible GnRHant protocol.

There are three positive contributions of an earlier initiation of GnRHant treatment in the overall IVF-ICSI treatment; 1) moderation of ovarian stimulation by reducing E_2 levels via the suppression of endogenous FSH during the early period; 2) prevention of premature ovulation by inhibiting an untimely LH surge; and 3) reduction of the negative effects of progesterone on the endometrium by controlling progesterone levels during the early and late follicular phases.

Intervals >3 days between endometrial advancement and the oocyte retrieval day in IVF treatments lead to high LH levels during the initiation of stimulation, a prolonged stimulation before the adjustment of the GnRHant treatment, and a decreased chance of pregnancy.[5] Similarly, untimely elevations of E_2 and progesterone may cause a reduced chance of pregnancy by negatively influencing endometrial receptivity.[16] LH elevations may occur in 1.4–35% of patients during GnRHant stimulation cycles.[17] While a possible negative impact of GnRHant on the oocyte and embryo has been asserted in the past, authors currently do not recognize such an effect.[18] High-dose gonadotropin in the IVF cycles may have detrimental effects on oogenesis, embryo quality, endometrial receptivity, and perhaps also perinatal outcomes.

Table 1
Demographic and stimulation characteristics of the patients.

The day of antagonist administration	<6 (n = 203)	≥6 (n = 209)	p
Age (years)	33.66 ± 5.25	32.79 ± 4.87	0.085
BMI (kg/m^2)	24.75 ± 3.97	25.09 ± 4.46	0.404
Duration of infertility (years)	6.14 ± 5.27	5.56 ± 3.97	0.205
Etiology of infertility (%)			
Male factor	84 (42%)	91 (43.5%)	0.946
Unexplained	49 (24.5%)	49 (23.4%)	
Poor responder	67 (33.5%)	69 (33.0%)	
Bazal-FSH (IU/mL)	10.54 ± 2.55	10.21 ± 2.48	0.184
Bazal-LH (IU/mL)	6.34 ± 3.10	6.09 ± 3.21	0.424
AF	4.71 ± 2.96	5.10 ± 3.16	0.240
The starting day of antagonist administration	4.74 ± 0.50	6.52 ± 0.74	0.001*
Duration of stimulation (days)	8.26 ± 1.83	9.56 ± 1.51	0.001*
Gonadotropin dose (IU)	2173.71 ± 860.00	2749.17 ± 1079.51	0.001*
Estradiol levels on day hCG (pg/mL)	1155.89 ± 754.21	1248.65 ± 751.23	0.241
Progesterone levels on day hCG (pg/mL)	0.75 ± 0.44	0.92 ± 0.59	0.002*
Endometrial thickness (mm)	9.12 ± 1.30	9.27 ± 1.32	0.283

BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; AF: antral follicle.
* $p < 0.05$ is significant.

In two randomized and controlled studies,[5,10] GnRHant administration after stimulation day 6 was found to have the potential to adversely affect pregnancy outcomes. However, several studies and meta-analyses have shown that GnRHant administration either before stimulation day 6 or after stimulation day 6 does not affect pregnancy outcomes.[5,12,18,19] In the current study, we found no difference between the GnRHant administration before or after stimulation day 6 with regard to pregnancy outcomes. Tannus et al.[19] reviewed 442 IVF cycles retrospectively, and the GnRHant administration after stimulation day 6 was associated with a higher number of PCOS patients, a higher level of gonadotropin use, fewer instances of oocyte achievement, fewer 2PN oocytes, and 10% higher CPRs. However, higher CPRs were not statistically significant. Since there was no difference between the groups with regard to the number of transferred embryos, there was also no difference in relation to pregnancy outcomes. This group claimed that embryo quality and endometrial receptivity are not adversely affected by the delayed administration of GnRHant. In our study, we found that there was no difference for CPR between the two groups. The aim of GnRHant administration in IVF cycles is to prevent an untimely LH surge and premature luteinization.[16,17] Therefore, if there is no progesterone increase on hCG day, it is expected that there will be no difference in pregnancy rates between the groups. The low progesterone levels (<1 pg/mL) in both groups in our study indicate that there is no early luteinization.

Because we included patients from the general IVF population who presented to our daily clinical practice, our study population could be generalized to all patients undergoing IVF treatments.

In this study, our purpose was not to compare fixed and flexible GnRHant protocols. Instead, we aimed to compare patients receiving GnRHant administration before or after stimulation day 6. Our results indicated that early GnRHant administration is appropriate for patients with a follicle diameter of ≥13 mm and serum E_2 levels ≥300 pg/ml. These findings are compatible with those in the literature.[5,10]

We used an individualized starting dose of r-FSH to induce an optimal ovarian response. We began monitoring the cycles on stimulation day 5 and initiated GnRHant administration when the follicle size reached a diameter of ≥13 mm, while also taking into consideration that serum E_2 levels should be ≥300 pg/ml. The luteal phase was supported by routine administration of progesterone in the form of Crinone 8% gel (Serono, Istanbul) at a dose of 90 mg daily for 14 days.

Since the advent of GnRHants use in IVF treatment, many studies have been performed to determine the optimal starting day. The fixed protocol, which starts on day 5 of stimulation, was first developed.[20] A flexible protocol was then created to reduce the GnRHant injections and duration of stimulation. Since the majority of an LH surge occurs before stimulation day 6, there has been a recent tendency to start GnRHant administration before stimulation day 6.[21−23] In order to predict a premature LH surge, strict criteria have been developed for antagonist initiation based on ultrasound and hormonal parameters.[15,24]

While the first monitoring visit is scheduled for stimulation day 6 in the fixed protocol, it is performed on stimulation day 3 or 4 according to the strict criteria of the flexible protocol. Lainas et al.[15] showed that the initiation of GnRHant therapy on stimulation days 4 or 5 was associated with higher CPRs than when initiated on day 6. Moreover, they also observed that GnRHant administration should be initiated earlier than stimulation day 6 to prevent premature LH surges.

Recently, Kolliniakis et al.[24] found that when GnRHant was started at a time point where the leading follicle diameter was >12 mm or the serum E_2 level was >150 pg/ml, there was no difference between the flexible and fixed protocol groups regarding the incidence of LH surge or pregnancy outcomes.

This study did have certain limitations. Namely, one limitation may be related to the investigation's retrospective study design. However, we do not consider this a limitation of considerable relevance.

In conclusion, among a considerable proportion of patients in whom GnRHant administration was started after stimulation day 6, a longer duration of stimulation and higher total gonadotropin consumption and progesterone levels were observed on day hCG. When the cost-effectiveness of IVF treatment is considered, we suggest that GnRHant administration should start before stimulation day 6. However, it remains apparent that there is a need for further studies to optimize GnRHant administration time.

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