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ORIGINAL ARTICLE/ÖZGÜN ARAŞTIRMA

The effect of prolonged time for achieving ovarian suppression before starting stimulation on pregnancy rates in ART cycles

ART sikluslarında stimulasyona başlamadan önce uzamış ovaryen süpresyonun gebelik oranlarına etkisi

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ABSTRACT

Objective: To determine if the prolonged time for achieving ovarian suppression before starting stimulation affected the pregnancy rates.

Patients and Methods: Retrospective cohort analysis was performed on 565 women undergoing first assisted reproductive technologies (ART) treatment in a University-affiliated Hospital. The women were grouped according to the duration of down-regulation by gonadotropin-releasing hormone (GnRH) analogue. Group A consisted of women in the lower 25th percentile, group B consisted of women in the upper 25th percentile, according to the duration of GnRH analogue use. The implantation and clinical pregnancy rates were compared.

Results: Mean number of aspirated and inseminated oocytes were more in group A than in group B. Implantation rates were similar. Clinical pregnancy rates were alike.

Conclusion: Prolonged time for achieving ovarian supression does not compromise clinical pregnancy rates in ART cycles.

Key words: GnRHa, ART, Ovarian suppression, Pregnancy, Extended protocol

ÖZET

Amaç: Stimulasyona başlamadan önce ovaryen süpresyonun uzamasının gebelik oranlarına etkisini araştırmak

Hastalar ve Yöntem: Üniversite affiliye hastanede yardımcı üreme tetkikleri (assisted reproductive technologies (ART)) tedavileri yapılan 565 hasta retrospektif olarak incelendi. Kadınlar gonadotropin salgılayan hormon (gonadotropin releasing hormone (GnRH)) analogu ile ovaryen süpresyon süresine göre gruplandırıldı. GnRH analog kullanım süresine göre grup A alt 25 persentil içinde, grup B de üst 25 persentil içindeki hastalardan oluşmaktaydı. İmplantasyon ve klinik gebelik oranları karşılaştırıldı.

Bulgular: Grup A'da ortalama aspire edilen ve insemine edilen oosit sayısı grup B'dekinden fazla idi. İmplantasyon oranları benzer idi. Klinik gebelik oranları benzer idi.

Sonuçlar: Ovaryen süpresyon için geçen zamanın uzaması ART sikluslarında klinik gebelik oranlarını olumsuz etkilemez.

Anahtar kelimeler: GnRH, ART, Ovaryen süpresyon, Gebelik, Uzun protokol

Introduction

Controlled ovarian stimulation in assisted reproductive technologies (ART) induces multiple follicular recruitment and allows the harvesting of multiple oocytes to obtain embryos. During initial studies with human menopausal gonadotropin (HMG) stimulation of multiple follicle development for in vitro fertilizing (IVF), a premature luteinizing hormone (LH) peak occurred in around 20–25% of the cycles. This advanced exposure to high LH resulted in the premature luteinization of follicles and the induction of oocyte maturation, resulting in either cycle cancellation or compromised IVF outcome. The most widely used protocol for ovarian stimulation in ART cycles is the long down-regulation protocol, for which gonadotropins are administered after pituitary suppression with gonadotropin- releasing hormone (GnRH) agonists. With the clinical development of GnRH agonists in the early 1980s, there was not only the increase in pregnancy and live-birth rates, but also a more flexible timing for oocyte retrieval became possible in IVF treatments [1].

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Because of the intrinsic agonist activity of the GnRH agonists, pituitary down-regulation is preceded by an initial stimulatory phase, which lasts for two weeks. The timing of ovarian quiescence after this initial stimulation phase is highly variable. This flare effect renders the GnRH agonist 'long-protocol' approach more time consuming, because ovarian stimulation can only begin when pituitary quiescence has occurred. Long-acting forms of GnRH analogues were studied in IVF cycles and no difference in cycle outcome was demonstrated [2,3]. Scott et al. [4] did not observe any impact of the duration of GnRH analogue use on ovarian responsiveness to gonadotrophins, nor on IVF success after using leuprolide for 14–15 days prior to ovulation induction. They found no reason to start stimulating patients as soon as suppression was achieved. Moreover, higher implantation rates in amenorrhoeic patients have been reported [5].

It is uncertain whether the ovarian response to exogenous stimulation is affected by GnRH agonist co-treatment [6]. We aimed to determine whether the prolonged time for achieving ovarian suppression before starting stimulation affected the pregnancy rates in IVF/intracytoplasmic sperm injection (ICSI) cycles.

Patients and Methods

Five hundred and sixty five consecutive women undergoing ART cycles in a University- affiliated Hospital, were selected for retrospective evaluation. The women were placed in two groups according to the duration of GnRH analogue use. The mean length of the GnRH analogue use among these women was 27.62± 3.27 days. Group A consisted of women who used GnRH analogue for a duration in the lower 25th percentile, group B consisted of women who used GnRH analogue for a duration in the upper 25th percentile. The period of GnRH analogue was calculated from the time of start on day 21 of the preceeding cycle till the the day of the human chorionic gonadotrophin (hCG) injection. Ethical approval for the study was not required and has not been included as there were no interventions that are not a part of standard care. All practices and protocols conformed to the ethical requirements for assisted reproductive technology programs of the Ethics Commitee of the institution and conform to the provisions of the Declaration of Helsinki.

Hormonal stimulation

All patients underwent ovarian stimulation with gonadotropins and gonadotropin releasing-hormone (GnRH)-agonist for pituitary down-regulation. For long protocols, pituitary desensitization was achieved by s.c. administration of leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL) during the luteal phase of the preceding cycle before the start of gonadotropin stimulation. After down-regulation was confirmed by E2 levels <50 pg/ml, controlled ovarian hyperstimulation was accomplished with s.c. administration of recombinant follicle-stimulating hormone (FSH) (Follitropin alpha; Serono Inc). None of the patients had E2 >50 pg/ml before ovarian stimulation was started. Gonadotropins were initiated the day after the baseline evaluation, or several days later, as required, based on the desired retrieval day. The agonists were sometimes continued longer in order to avoid weekend retrievals. Follicular monitoring was performed by using a Toshiba SAL 77B machine (Toshiba, Tokyo, Japan). Ten thousand IU of human chorionic gonadotropin (hCG, 10,000 IU Profasi, EMD Serono Inc.) was administered when at least one follicle had a mean diameter of 18 mm.

Table I. Women and Infants Hospital (WIH) scoring for day 3 embryos

Cell number	Score	Degree of fragmentation	Score	Symmetry	Score
8-10	4	0	4	Symmetric or slightly asymmetric	1
6-7 or >10	3	<10%	3	Grossly asymmetric	0
4-5	2	10-25%	2		
2-3	1	26-50%	1		
1	0				

Oocyte retrieval, insemination, embryo culture, and grading transvaginal oocyte retrieval was performed 35 or 36 hours after hCG injection. The decision to perform standard IVF, ICSI was based on a diagnosis of infertility. For cleavage-stage embryo transfer, embryos were cultured in P1 medium (Irvine Scientific, Santa Ana, CA). The Women and Infants Hospital (WIH) Scoring system was used on a selection of day 3 embryos (Table I). The individual WIH score of each embryo was calculated as the sum of "development+ fragmentation+symmetry". The average embryo score was recorded for each day 3 embryo transfer. Cleavage-stage embryo transfers were performed by using a Wallace (Cooper Surgical, Shelton, CT) or Embryon catheter (Sage BioPharma, Bedminster, NJ).

Embryo transfer was performed with transabdominal ultrasonographic guidance using a Toshiba SAL 77B machine (Toshiba, Tokyo, Japan).

Biochemical pregnancy was determined by serum hCG levels measured 12 days after the embryo transfer. Clinical pregnancy was determined by the presence of a gestational sac during an ultrasound exam. The implantation rate was calculated as the ratio between the number of embryonal sacs diagnosed by sonography and the total number of embryos transferred into the uterus. Clinical pregnancy rates and implantation rates were calculated per embryo transfer. Luteal support consisted of the daily use of transvaginal progesterone gel (Crinone 8%; Serono Inc.) through the 10 weeks of gestation.

Table II. The demographics of the study groups

Groups (n)	Group A (n=163)	Group B (n=146)	P value
Mean age (years)	33.69± 4.77	34.34± 4.38	0.22
Cycle day 3 FSH (mIU/ml)	5.85± 2.17	6.25± 2.34	0.12
Body mass index (kg/m2)	27.84± 8.15	26.22± 7.62	0.12
Duration of GnRH analogue use (days)	24.06± 0.98	32.14± 1.89	0.0001
Ovarian suppression before gonadotropin start (days)	15.33± 1.26	22.38± 2.42	0.0001
Duration of stimulation with gonadotropin (days)	8.72± 1.16	9.75± 1.85	0.0001
Gonadotropin start dose (IU)	294.48± 103.60	317.47± 110.73	0.06
Total gonadotropin dose (IU)	2437.27± 1000.01	3024.83± 1344.34	0.0001
E2 level on HCG day (pg/ml)	1853.64± 955.55	1697.66± 854.03	0.14
Number of aspirated oocytes per retrieval	14.27± 8.47	11.93± 6.17	0.01
Number of inseminated oocytes per retrieval	13.10± 8.30	10.99± 6.04	0.02
Number of fertilized oocytes per retrieval	8.82± 6.82	7.56± 5.29	0.15
Number of embryos with >6 cells by day 3	4.84± 4.97	4.22± 3.74	0.29

All values are shown as mean ± standard deviation (# mean ± SD) ; p< 0.05 is statistically significant

Statistical Evaluation

All analyses used StataSE 10.0 computer software (Statacorp, Texas). Student's t-test was used for the whole group statistical evaluation ; $p<0.05$ was considered significant.

Results

There were 163 women in group A and 146 in group B. The mean age , serum level of FSH on cycle day 3 and body mass index (BMI) were similar between the groups. Diagnosis for infertility in group A were male factor (28.3%), tubal (17.1%), minimal endometriosis (10.5%), polycyctic ovary syndrome (PCOS) (6.6%) or unexplained infertility (27%). The percentages of distribution in group B were 28.3%, 21%, 5.8%, 5.8% and 28.3%, respectively. The mean serum estradiol level on the day of the hCG injection was similar. The ovarian suppression length before the commencement of gonadotropins was longer in group B than in group A (22.38± 2.42 vs 15.33± 1.26, p=0.0001). The duration of gonadotropin stimulation, and the total dose of gonadotropins were higher in group B. The mean number of eggs collected, the mean number of mature oocytes were more in group B than in group A. However the number of fertilized oocytes were similar (Table II).

On day 3, the mean number of embryos with more than 6 cells were similar between the groups. Likewise, the average embryo scores of transfered embryos were comparable between the groups (Table III). The mean number of embryos transferred were similar between groups (3.25± 0.91 vs 3.38± 0.83; p=0.27). The implantation rate and clinical pregnancy rate per cleavage stage transfers was alike between the groups (Table III).

Table III. Pregnancy outcome in groups

	Group A (n=163)	Group B (n=146)	P value
Average score of transferred embryos on day 3 *	6.51±1.34	6.46±1.20	0.78
Number of embryos transfered on day 3	3.25± 0.91	3.38± 0.83	0.27
Implantation rate per transfer (%)	21.90± 0.30	20.79± 0.29	0.77
Biochemical pregnancy rate per cleavage stage transfer (%)	47.26±0.50	54.48±0.50	0.23
Clinical pregnancy per cleavage stage transfer (%)	41.78±0.49	45.19±0.50	0.56

Mean ± standard deviation.
P < 0.05 is considered as statistically insignificant
* WIH Score = development+ fragmentation+symmetry

Discussion

Because of the intrinsic agonist activity of the GnRH agonists, pituitary down-regulation is preceded by an initial stimulatory phase which lasts for two weeks. A progressive decrease in LH β subunits within the 7 days of the agonist administration, regardless of the duration of GnRH agonist administration is observed. By contrast, LH α subunit secretion seemed to be dependent on the duration of the treatment [7]. Unlike LH [8], FSH bioactivity does not decrease during GnRH agonist administration [9]. Plasma FSH concentrations are not influenced by the duration of the treatment. It may therefore be speculated that the apparent beneficial effect of maintaining GnRH agonist administration for 14 days is related to the persistence of bio-FSH secretion. With a progressive decrease in plasma LH concentrations, a second inhibition phase is observed. During this period and as long as GnRHa administration is maintained, the pituitary seems completely refractory to GnRH action [10]. The intensity and duration of hypophyseal desensitization for LH are dose dependent [11]. After 21 days of desensitization, LH bioactivity is completely suppressed [9].

The present study investigated whether the duration of ovarian suppression by GnRH agonist affects pregnancy rates in long-down regulated IVF/ICSI cycles. ART cycles pre-treated with GnRH agonists have resulted in superior pregnancy rates when compared with cycles not utilizing analogues [12- 14]. There was not only an increase in the number of developing follicles [15, 16], but also premature luteinizing hormone (LH) surges were prevented [17, 18]. A long protocol of down-regulation allows for the complete suppression of estradiol before ovarian stimulation is begun. This suppression process may take at least 14 days [19, 20]. Tarlatzis et al. [21] and Tan et al. [22] have shown higher implantation and pregnancy rates using the long protocol. This has been attributed to more effective LH suppression, higher oocyte retrieval per cycle and more developed embryos. In our study, although higher doses of gonadotropins were used in group B than in group A, more oocytes were retrieved and inseminated in group A. However, mean number of fertilized oocytes were comparable in both groups. Similarly, the average embryo score of transferred embryos and the number of transferred embryos on day 3 were comparable. Similar clinical pregnancy rates were detected in both groups.

A comparison of implantation rates between different protocols has led us to believe that the beneficial effects of GnRH agonists were related to an improved endometrial receptivity [23]. However, a direct action of GnRH agonist on the endometrium remains speculative. In our study, the implantation rates between the groups were compatible. Since LH/hCG receptors have been recognized at the endometrial level [24, 25], another way for GnRH analogue to influence uterine receptivity might be through the reduction of gonadotrophin synthesis, with subsequent consequences on endometrial LH receptor function. In our study, even though a higher total dose of FSH was used per cycle in group B, the mean serum estradiol level on the day of the hCG was similar between the groups and thus the effect of rising estradiol levels on the endometrium was comparable. Hence, the prolonged use of GnRH analogue did not influence uterine receptivity, as shown by the similar implantation rates.

Patients with hypogonadotropic hypogonadism showed a significantly higher pregnancy rate after ovarian stimulation using exogenous gonadotropins, than in normogonadotropic women [26]. Even though a significantly higher rate of implantation has been reported, the reasons for this enhanced fertility in amenorrhoeic women are still partially unknown. Our findings could not support this increased rate of implantation. With comparable numbers of embryos transferred in both groups, clinical pregnancy rates were similar between the groups.

Conclusion

It appears that prolonged GnRH analogue use does not compromise IVF/ICSI results. Hence, IVF laboratory schedules might be adjusted for normo-responders in order to omit weekend egg retrievals and embryo transfers. However, prospective cohort studies are necessary to support our results.

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