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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Does the quality of an embryo differ between long down-regulated and antagonist cycles among age and cycle day 3 FSH-matched women undergoing ART?

Yaş ve siklusun 3.günü FSH değerleri eşleşmiş kadınlarda uzun ve kısa YÜT'lerinde elde edilen embryo kalitesi farklı mıdır?

Tevfik YOLDEMİR

ABSTRACT

Objective: Lower pregnancy rates have been reported in gonadotropin releasing hormone (GnRH) antagonist cycles in comparison to those with agonist cycles. A non-significant difference of 3.3% in the pregnancy rate per cycle in favour of GnRH agonists was found. The possible difference between these two protocols could be a limiting factor. We aim to determine if the embryos developed in long down-regulated assisted reproductive technology (ART) cycles differ from the ones obtained in antagonist cycles among women matched for age and follicle-stimulating hormone (FSH) administered on cycle day 3.

Patients and Methods: Retrospective cohort analysis was done on a population from a university affiliated hospital. 193 women undergoing standard in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment were included. The age and cycle day 3 FSH matched women undergoing either long or short protocols were compared. Group A consisted of women who had long down-regulation with GnRH analogues, group B consisted of women who had used GnRH antagonists. The clinical and ongoing pregnancy rates were compared. Day 3 embryo scores were compared between the groups.

Results: Although mean number of aspirated and inseminated oocytes were similar for the groups and average embryo scores were comparable, clinical and ongoing pregnancy rates were higher in group A than in group B. The percentage of embryos with zero fragmentation and 8-10 blastomeres on day 3 was similar in antagonist and long agonist cycles.

Conclusion: Long protocol ART cycles will result in comparable percentages of day 3 embryos with symmetric 8-10 blastomeres with zero fragmentation to those in antagonist cycles.

Keywords: Gonadotropin releasing hormone agonist, Gonadotropin releasing hormone antagonist, Pregnancy, Embryo score, Day 3 embryo

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ÖZET

Amaç: Gonadotropin salgılayıcı hormon (GnRH) antagonistlerinin kullanıldığı sikluslarda agonist sikluslarına göre daha düşük gebelik oranları bildirilmiştir. GnRH agonist sikluslarında anlamlı olmayan %3,3' lük bir gebelik oranında artış vardır. Yaş ve üçüncü gün FSH değerleri eşleştirilmiş kadınlarda kısa ve uzun yardımcı üreme teknikleri (YÜT) sikluslarında embriyo gelişiminde farkın olup olmadığının araştırılması amaçlanmıştır.

Hastalar ve Yöntem: Universiteye bağlı hastanenin YÜT merkezine başvuran 193 kadında retrospektif kohort analizi yapıldı. Yaş ve siklusun 3. günü serum folikül stimulan hormon (FSH) değeri benzer olan kısa ve uzun protokol ile klasik in vitro fertilizasyon (IVF) veya intrasitoplazmik sperm enjeksiyonu (ICSI) uygulanan hastalar dahil edildi. Group A, uzun GnRH analog protokolu, group B, ise kısa GnRh antagonist protokolü ile YÜT tedavisi aldı. Klinik ve devam eden gebelik oranları karşılaştırıldı. Üçüncü gün embriyo skorları gruplar arası karşılaştırıldı.

Bulgular: Her iki grupta toplanan ve insemine edilen yumurta sayısı ve ortalama embriyo skorları benzer olmasına rağmen klinik ve devam eden gebelik oranları grup A'da grup B'ye göre daha yüksekti. Sıfır fragmentasyon ve 8-10 blastomer olan 3. gün embriyo yüzdesi gruplar arası benzer idi.

Sonuç: Uzun protokol YÜT tedavilerinde, kısa protokolde elde edilenlere benzer oranda 8-10 blastomerli, simetrik ve sıfır fragmentasyonlu 3. gün embriyoları görülmektedir.

Anahtar kelimeler: Gonadotropin salgılatıcı hormon agonisti, Gonadotropin salgılatıcı hormon antagonisti, Gebelik, Embryo skoru, 3.gün embryosu

Introduction

During the luteo-follicular transition of the normal menstrual cycle, follicle-stimulating hormone (FSH) concentrations rise and surpass the threshold for stimulating a cohort of small antral follicles to grow [1]. Around the mid-follicular phase, the most mature follicle gains dominance over other follicles in the cohort [2]. This dominant follicle continues

its growth despite decreasing FSH concentrations [3], whereas the remaining follicles from the recurited cohort become atresic, due to insufficient stimulation by FSH. This decreasing FSH level and mild ovarian stimulation with subsequent closure of the FSH 'gate' or 'window' [4] appears to be essential for the selection of a single dominant follicle. Multifollicular growth is established in current in vitro fertilisation (IVF) protocols by generating FSH serum concentrations far above the threshold for an extended period starting in the early follicular phase.

Conventional ovarian stimulation protocols aim to stimulate growth of many follicles in order to obtain multiple oocytes for IVF and thus multiple embryos allowing for the selection of several for transfer [5]. Preceding the administration of high doses of gonadotropins, pituitary down regulation is normally achieved by prolonged administration of agonists of the gonadotropin releasing hormone (GnRH). This is the "long protocol" . Increasing knowledge about the physiology of ovarian follicle development and selection of the dominant follicle [1,6], together with the clinical introduction of GnRH antagonists in IVF [7, 8] allows ovarian stimulation to be commenced in an undisturbed menstrual cycle. GnRH antagonists have emerged as an alternative approach to prevent surges of luteinizing hormone (LH) [9].

Decreases in serum estradiol (E2) levels and related rates of implantation have been reported for cycles stimulated by a GnRH antagonist [10] suggesting that there is an adverse effect of GnRH antagonists on either oocyte quality, embryo development, or the endometrium [11]. Concerns have been raised regarding the possibility of direct effects of GnRH antagonists on the endometrium [12], and on altered patterns of follicular development [13].

In this study, we determined if day 3 embryo scores and assisted reproductive technology (ART) outcomes of the standard long GnRH agonist protocol for ovarian stimulation differed from those of the GnRH antagonist protocol.

Patients and Methods

One hundred and ninety-three women undergoing IVF cycles at the ART Unit of a University affiliated Hospital, were selected for retrospective cohort analysis. All women were under the age of 38 years. The women were placed in two groups according to the ART protocol. Group A consisted of women for whom the long agonist protocol was used, group B consisted of women for whom the antagonist protocol was used. All practices and protocols conformed to the ethical requirements for assisted reproductive technology programs of the Ethics Commitee of the institution and conformed to the provisions of the Declaration of Helsinki.

Hormonal Stimulation

Two controlled ovarian hyperstimulation protocols were chosen. For the long protocols, pituitary desensitization was achieved by s.c. administration of leuprolide acetate (1 mg/mL; Lucrin flacon , Abbott) during the luteal phase of the cycle preceding before the start of gonadotropin stimulation [14]. When adequate down regulation had been achieved (endometrial thickness <4 mm or serum estradiol <50 pg/ml), usually after at least 10 days of leuprolide acetate administration, controlled ovarian hyperstimulation was accomplished with s.c. administration of recombinant FSH (Gonal-F, Follitropin alpha; Merck-Serono Inc.), at a starting dose of 300 IU per day. For the short protocol, the starting dose of gonadotropin was 400 IU per day of cetrorelix acetate (Cetrotide, Merck-Serono Inc), which was started on cycle day 6. Dose adjustments were individualized after 5 days of ovarian stimulation. 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 Merck-Serono Inc.) was administered when at least one follicle had a mean diameter of 18 mm. Both the analogues and the antagonists were continued till the day of hCG injection.

Oocyte Retrieval, Insemination, Embryo Culture, and Grading

Oocytes were retrieved transvaginally 35 or 36 hours after hCG injection. The decision to perform standard IVF or ICSI had been based on a diagnosis of infertility. For cleavage-stage embryo transfer, embryos were cultured in P1 medium (Irvine Scientific, Santa Ana, CA). The scoring system of Women and Infants Hospital (WIH) was used for the selection of day 3 embryos [15]. Individual WIH scores for each embryo were calculated as the sum of "development+ fragmentation+symmetry". The average embryo score was recorded for each transferred day 3 embryo. Embryos were transferred at the cleavage-stage by using a Wallace (Cooper Surgical, Shelton, CT) or Embryon catheter (Sage BioPharma, Bedminster, NJ). Embryos were transferred under transabdominal ultrasonographic guidance using a Toshiba SAL 77B machine (Toshiba, Tokyo, Japan).

-HCG titers were drawn 14 days after retrieval and if positive were repeated 2 days later. In patients with a doubling of the -hCG value, transvaginal ultrasonography was used 4 weeks after retrieval to find evidence of a sac to confirm clinical pregnancy. Luteal support consisted of daily use of transvaginal progesterone gel (Crinone 8%; Merck-Serono Inc.) through the 10th week of gestation.

Statistical Evaluation

All analyses used StataSE 10.0 (Statacorp, College Station,

Texas, USA). For the whole group statistical evaluation Student's t-test was used; $p<0.05$ was considered significant. In order to detect the difference between clinical pregnancy rates of 50% and 25%, 145 and 45 patients were needed in two arms for a calculation accepting type I error as 0.05 with the power of 81%. Likewise, 145 and 45 patients were needed in two arms for a calculation accepting type I error as 0.05 with the power of 73% in order to detect the difference between ongoing pregnancy rates of 42% and 20%.

Results

There were 148 women in group A and 45 in group B. The mean age and body mass index (BMI) and serum level of FSH on cycle day 3 were similar between the groups. The duration of gonadotropin stimulation was comparable between groups. Although the starting dose and the total dose of gonadotropin was higher in group B than in group A the mean serum estradiol level on the day of hCG injection was lower in group B than in group A. However, the mean estradiol level per oocyte retrieved was comparable between the groups (Table I).

The mean number of total oocytes and of mature oocytes were comparable between the groups. Likewise, the mean number of fertilized oocytes and of embryos with more than 6 cells on day 3 were comparable between the groups. Similarly, average scores of the transfered embryos were comparable in group A and group B (Table I).

Clinical pregnancy rates per cleavage stage transfer were higher in group A than in group B. Accordingly, there were more ongoing pregnancies per cleavage stage transfer in group A than in group B (Table I).

The percentage of embryos with zero fragmentation on day 3 was higher in group B than in group A so were the percentage of embryos with symmetric blastomeres (Table II).

Discussion

Given the close correlation between the number of embryos from which to select and the chances of pregnancy [5], pregnancy rates may suffer from stimulation protocols where a lower number of good quality embryos are obtained. Tarlatzis et al. [16] and Tan et al. [17] have shown higher implantation and pregnancy rates with the long GnRH analogue protocol. This has been attributed to more effective LH suppression, higher oocyte retrievals per cycle, and more embryos developed. The clinical introduction of GnRH antagonists facilitated the development of new approaches to ovarian (hyper) stimulation in IVF. As the action of GnRH antagonists is characterized by an immediate suppression of pituitary gonadotropin release, treatment can be limited to the days in the mid-to-late follicular phase to prevent a premature LH rise. These compounds offer an opportunity to start the IVF treatment within an undisturbed menstrual cycle.

As result of multifollicular growth, hormone levels of estradiol are increased in the late follicular phase. The effects of these supraphysiological hormone concentrations on follicle- and oocyte quality remain unclear [6]. The potential detrimental effect of ovarian hyperstimulation on endometrial receptivity and the negative influence of endometrial changes on embryo implantation have all been investigated [6, 18,19]. In our study, both groups had maximum E2 levels below 2000 pg/ml; this was lower than the levels mentioned in the literature [20]. Mean E2 levels at the late follicular phase between 2,800 and 4,500 pmol/l (conversion factor 3.671) do not represent clinically important differences in the ovarian response [21,22]. In our study, the mean E2 levels on the day of hCG administration were higher than these ranges. Hence, the ovarian response in our groups were neither high nor poor. These (physiologic) hormone concentrations at the follicular phase probably resulted in a similar number of mature oocytes. Good quality oocytes lead to better embryos, which is a predictive factor for pregnancy [23]. In our study, the chance of subsequently producing good quality embryos was similar for the protocol using the long stimulation with GnRH agonist and the protocol using the GnRH antagonist. Both protocols resulted in comparable number of mature oocytes, which are likely to result in good quality of embryos.

Women who respond poorly or not at all to standard controlled ovarian hyperstimulation treatments are referred to as 'low, or poor responders'. Numerous criteria have been proposed to characterize a poor response. The number of developed follicles and/or the number of oocytes are two of the most important criteria for defining a poor ovarian response [24]. The proposed number ranges from less than three to less than five dominant follicles on the day of hCG administration [5-8], and/or less than three to less than five retrieved oocytes [25]. Both the number of follicles and a peak estradiol value of <300 pg/ml during ovarian stimulation are also used in defining a poor response. None of the patients in our study groups fulfilled these latter criteria. Hence, the women in our study did not have a low ovarian reserve.

Although, embryo quality is not the only factor determining implantation rate, embryo score is predictive of pregnancy [26]. Since day 3 embryos with more than 6 cells and the average scores of embryos transfered at cleavage stage were comparable in both groups, similar pregnancy rates would have been expected. Comparison of implantation rates between different protocols have led to the belief that the beneficial effects of GnRH analogues were related to an improved endometrial receptivity [14]. However, a direct action of GnRH analogue on the endometrium remains

speculative. Since, LH/hCG receptors have been recognized in the endometrium level [27,28], another way for an GnRH analogue to influence uterine receptivity might be through the reduction of gonadotropin synthesis, with subsequent consequences on endometrial LH receptor function. Lower pregnancy rates associated with GnRH antagonist use [7, 29] have been reported. Even though a non-significant difference of 3.3% in the pregnancy rate per cycle in favour of GnRH agonists was given, our results showed a significant difference both in clinical and ongoing pregnany rates.

Ovarian sensitivity to FSH is regulated by intra-ovarian factors, like FSH receptor inhibitors [30] and growth factors [31]. Growth factors act in an auto- or paracrine fashion through their specific receptors. Within cells, their signal transduction pathways merge with the FSH-activated pathways and subsequently modulate FSH stimulated responses within the cell. Numerous growth factors have been identified which contribute to this regulation of normal ovarian function. In our study, the morphology of the embryo on the third day in each group was similar. There were more embryos with zero fragmentation in the antagonist group. However, this non-significant difference together with the non-significant increased percentage of day 3 embryos with symmetric blastomeres did not conclude to favourable pregnancy rates.

One of the limitations of our study could be the difference in both the starting and the total doses of gonadotropins in the two groups. However, the level of serum estradiol per oocyte collected was similar for the two groups. Hence, the effect of gonadotropins on the ovaries could be accepted as identical. Moreover, the number of retrieved, fertilized oocytes were almost the same. The embryo gradings of the transferred embryos were also similar. Our primary outcome measure was to determine whether the embryos developed in long down-regulated ART cycles differed from the ones obtained in antagonist cycles for women matched for age and FSH levels on day 3. Thus, other confounding factors which would affect implantation have not been investigated. These might be due to more suppression of GnRH antagonists on the endometrium than that expected with GnRH agonists.

We have shown that women younger than 38 years of age are very likely to obtain higher pregnancy rates with long GnRH analogue cycles than with antagonist cycles if the duration of stimulation is shorter than 12 days. The women seem to have one more frozen embryo on average after long GnRH analogue protocol than the women with antagonist protocol. This will probably increase the chance for subsequent pregnancies obtained from frozen-thawed embryo transfers. Even though cycle characteristics closely resemble one another and the day 3 embryo characteristics are almost similar, more pregnancies are achieved with long GnRH analogues. The endometrial receptivity should be well-evaluated before embryo transfer and if needed, then embryos should be frozen for future natural cycle with thawed embryo transfer treatments.

Table I. The patient demographics of the study groups

	Group A (n=148)	Group B (n=45)	p value
Mean age (years)	34.62 (33.90 - 35.34)	36.09 (34.79 –37.39)	0.05
Body mass index (kg/m2)	26.84 (25.29 – 28.38)	25.29 (23.11 –27.47)	0.33
Cycle day 3 FSH (mIU/ml)	7.00 (6.56 – 7.44)	7.20 (6.38 - 8.02)	0.67
Duration of stimulation with gonadotropin (days)	11.52 (10.40 - 11.64)	11.71 (11.47 - 11.96)	0.14
Gonadotropin start dose (IU)	322.80 (304.02 – 341.59)	443.33 (402.34 – 484.33)	0.0001
Total gonadotropin dose (IU)	3662.76 (3421.80-3903.71)	5355.68 (4844.48 –5866.89)	0.0001
Mean estradiol level on hCG day (pg/ml)	1666.33 (1508.48-1824.18)	1227.05 (957.52-1496.57)	0.01
Mean estradiol level per oocyte collected (pg/ml)	172.63 (157.79-187.48)	170.02 (128.61-211.43)	0.94
Mean number of aspirated oocytes per retrieval	11.01 (9.93-12.10)	9.26 (7.05- 11.46)	0.13
Mean number of inseminated oocytes per retrieval	10.24 (9.22 - 11.27)	8.67 (6.74 – 10.60)	0.15
Mean number of fertilized oocytes per retrieval	7.63 (6.77- 8.50)	6.26 (4.58 – 7.95)	0.14
Mean number of embryos with >6 cells by day 3	4.44 (3.80 - 5.08)	3.89 (2.42 - 5.36)	0.46
Number of embryos frozen on day 3	2.32 (1.83 - 2.81)	1.23 (0.49 -1.96)	0.03
Average WIH score of embryos transferred on day 3 *	6.61 (6.41 - 6.83)	6.18 (5.77 - 6.60)	0.06
Mean number of embryos transferred on day 3	3.50 (3.34- 3.65)	3.81 (3.48- 4.14)	0.07
Implantation rate	21.11 (15.03 – 25.20)	5.83 (0.4- 12.08)	0.01
Clinical pregnancy per day 3 transfer (%)	50.00 (41.85 – 58.15)	22.22 (9.59 – 34.85)	0.001
Ongoing pregnancy per day 3 transfer (%)	40.30 (31.89 – 48.71)	20.75 (1.74 – 22.65)	0.001

All values are given as mean and 95% confidence intervals (CI) in parentheses, $p < 0.05$ is statistically significant, * The sum of "development+ fragmentation+symmetry"of each embryo transfered divided by number

Table II. Day 3 embryo morphology in groups

		Group A (n=714)	Group B (n=143)	p value
Cell number				
	8-10 cells	40.47 (36.87 – 44.09)	41.96 (33.77 - 50.14)	0.74
	6-7 or >10 cells	36.83 (33.29 – 40.38)	39.16 (31.06 – 47.26)	0.60
	4-5 cells	19.75 (16.82 – 22.67)	15.38 (9.40 – 21.37)	0.23
Fragmentation	0%	13.11 (10.59 – 15.63)	16.43 (10.21 – 22.64)	0.30
	<10%	35.88 (32.30 – 39.46)	32.14 (24.31 – 39.97)	0.40
	10-25%	33.72 (30.19 – 37.24)	32.14 (24.31 – 39.97)	0.72
	26-50%	15.71 (12.99 – 18.42)	15.71 (9.61 - 21.82)	0.99
Symmetry	symmetric	55.64 (51.93 – 59.35)	57.25 (48.89 – 65.60)	0.73

Values are expressed as percentage (%) and 95% confidence intervals (CI) in parentheses, p <0.05 is statistically significant.

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