

Comparison of ovulation induction and pregnancy outcomes in IVF patients with normal ovarian reserve who underwent long protocol with recombinant-FSH and highly purified-hMG

Normal over rezervi olan ve long protokol uygulanan İVF hastalarında rekombinant fsh ve yüksek derecede saflaştırılmış hmg kullanımının gebelik sonuçlarına etkisinin karşılaştırılması

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Abstract

Objective: Gonadotropins used in controlled ovarian stimulation have been increasing in number. Beside the recombinant preparations such as rec-FSH, rec-LH and h-hMG human-derived preparations have entered the market. We decided to compare the effects of rec-FSH and HP-hMG with GnRHa on embryo quality and pregnancy outcome in women undergoing an IVF cycle.

Material and Methods: In this study, data of 87 patients who had applied to our center from 2007 to 2008 and who had met all inclusion criteria, were analyzed. The patients underwent controlled ovarian hyperstimulation with HP-hMG, rec-FSH following down-regulation with a GnRHa in a long protocol, selected according to determined criteria and acquired embryo via IVF transfer.

Results: Of the 87 patients, 44 were stimulated with rec-FSH and 43 with HP-hMG. Distribution of infertility causes was similar between the groups. Duration of gonadotropin administration ($p=0.677$, Student's t-test) and the total dose of gonadotropin received ($p=0.392$, Student's t-test) were similar between the two groups. The fertilization rate of the rec-FSH group was significantly higher than the HP-hMG group ($p=0.001$, Mann-Whitney U test). No significant differences were observed between the study groups in biochemical, clinical and ongoing pregnancy parameters.

Conclusion: The higher oocyte yield with rec-FSH does not result in higher quality embryos. LH activity in combination with FSH activity positively affected the oocyte and embryo maturation. Therefore, when we consider the clinical and ongoing pregnancy rates there is no inferiority of HP-hMG in controlled ovarian stimulation.

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Key words: Ovarian stimulation, rec-FSH, HP-hMG

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Özet

Amaç: Son dekatta ovulasyon indüksiyonu ve kontrollü over stimülasyonu için kullanılan gonadotropinler hızla çoğalmıştır. Çeşitli rekombinant preparatların yanında; rec-FSH, rec-LH, insan kaynaklı gonadotropinlerde pazara girmiştir. Bu çalışmadaki amaç; in vitro fertilizasyon protokolünde yer alan rec-FSH ve HP-hMG'nin elde edilen embriyoların kalitesi ve gebelik sonuçları üzerindeki etkilerinin karşılaştırılmasıdır.

Gereç ve Yöntemler: Çalışmamıza 2007- 2008 tarihleri arasında Zeynep Kamil kadın ve çocuk hastalıkları eğitim ve araştırma hastanesi tüp bebek merkezine başvuran, çalışmaya dahil edilme kriterlerini sağlayan 87 hastanın verileri kullanıldı. Belirlenen kriterlere göre seçilen, ovulasyon indüksiyonu için rec-FSH ve/veya HP-hMG kullanarak uzun protokol agonist tedavi protokolleri uygulanmış; IVF yöntemi ile ovum fertilizasyonu sağlanmış ve elde edilen embriyolarla transfer yapılmıştır.

Bulgular: Olgular rec-FSH ($n=44$) ve HP-hMG ($n=43$) olmak üzere iki grup altında incelenmiştir. İnfertilite nedenlerin dağılımı her iki grup arasında benzer olarak bulunmuştur. Gruplara göre olgulara uygulanan total gonadotropin dozları ($p=0.677$, Student t-test) ve süresi ($p=0.392$, Student t-test) arasında istatistiksel olarak anlamlı bir farklılık bulunmamaktadır. Rec-FSH grubundaki olguların fertilizasyon sayıları, HP-hMG grubundaki olguların fertilizasyon sayılarından istatistiksel olarak ileri düzeyde anlamlı fazladır ($p<0.01$). rec-FSH ve HP-hMG gruplarında kimyasal gebelik, klinik ve devam eden gebelik görülme oranları arasında istatistiksel olarak anlamlı bir farklılık bulunmamaktadır.

Sonuç: rec-FSH grubunda yüksek oosit sayısı olmasına rağmen yüksek kalitede embriyo sayısı elde edilmemiştir. FSH aktivitesi ile birlikte LH aktivitesi oosit ve embriyo gelişimini pozitif yönde etkilemiştir. Bu sebepten dolayı klinik ve devam eden gebelik oranları incelendiğinde, HP-hMG grubunda herhangi bir düşüş rastlanılmamıştır.

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Introduction

Controlled ovarian stimulation in infertile women undergoing treatment for in vitro fertilization (IVF) have usually been provided with highly purified human menopausal gonadotropin (HP-hMG), including both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) activity and recombinant FSH (rec-FSH). Decision as to the most effective preparations between HP-hMG and rec-FSH is a common debatable issue (1). Comparing the outcome of rec-FSH and HP-hMG performed in women undergoing pituitary down-regulation with a gonadotropin-releasing hormone agonist (GnRHa), long protocol has been reported in several studies (2). However, the meta-analysis concluded that large randomized trials were needed to precisely estimate any difference between menotropins and rec-FSH (1).

Results of various studies based on the role of exogenous LH in the process of follicular development, oocyte maturation, and pregnancy rate have raised another argument (3). The concentration of circulating LH that results from the combination of GnRHa and recombinant FSH is much lower than that found in the normal menstrual cycle (4). The lowered basal LH secretion that results from the use of GnRHa, together with the absence of exogenous LH in rec-FSH preparations, may contribute to a decreased PR in IVF (5-8). On the other hand, other studies indicate that very low amounts of LH maintain development of normal follicle and oocyte (9). Moreover, high serum LH during the follicular phase of the menstrual cycle has been associated with lower fertility rates and an increase in the probability of spontaneous abortion (10).

The aim of the present study was to prove the superiority of HP-hMG versus rec-FSH in a relevant clinical setting. We decided to compare the effect of rec-FSH and HP-hMG with GnRHa on embryo quality and pregnancy outcome in women undergoing an IVF cycle. Results were discussed in the light of recent insights on the effects of LH activity on folliculogenesis, embryo quality and endometrial development.

Materials and Methods

The patients underwent controlled ovarian hyperstimulation with HP-hMG, rec-FSH following down-regulation with a GnRHa in a long protocol, selected according to determined criteria and acquired embryo via IVF transfer. Comparing the effectiveness of performed protocols was the retrospective aim. In this study, data of 87 patients who had applied to our center from 2007-2008 and who had met all inclusion criteria, were analyzed.

Inclusion criteria were: primary or secondary infertile women with major indications for IVF, age 20-40 years, body mass index (BMI) 18-29 kg/m², regular menstrual cycles ranging from 25 to 35 days, normal basal serum FSH (<13 IU/l) and estradiol (E₂<75 pg/ml) levels determined on day 3 of the cycle, normal basal serum thyroid stimulating hormone (TSH) and prolactin, no uterine (fibroids, adenomyosis, müllerian malformations), ovarian (endometrioma, polycystic ovaries), or adnexal (hydrosalpinx) abnormalities assessed by transvaginal ultrasonography.

Exclusion criteria were: patients with a history of recurrent pregnancy loss, history of ovarian hyperstimulation syndrome (OHSS) at previous IVF cycles, previous IVF cycles with unsuccessful fertilization polycystic ovary syndrome, stage 4/5 endometriosis, any significant systemic disease, endocrine, or metabolic disorder.

Cycle cancellation criteria

Unsuccessful fertilization, after ovulation induction, all follicles less than 18 mm in diameter decreasing at serum E₂ level of more than 50% between two control days, existence of OHSS risk: on stimulation day 8; 15 or more intermediate follicles (12-16 mm) or on stimulation day 10 or after; 20 or more large follicles (16-20 mm), and/or serum E₂ level ≥3000 pg/ml.

At the first visit, the obstetric and gynecologic history and menstrual cycle regularity were questioned and pelvic examination was performed. In addition, blood pressure, height, weight, and BMI were measured.

Blood sampling for fasting blood glucose, blood urea nitrogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and thyroid function tests were carried out on menstrual cycle day 3.

Blood sampling for endocrine parameters (FSH, LH, hCG, E₂, progesterone, androstenedione, total testosterone, and sex hormone-binding globulin) and basal ultrasonography for assessment of the endometrial thickness, ovarian size, number and diameter of follicles were carried out in the early follicular phase (on menstrual cycle day 2 or 3).

Protocols

All patients underwent controlled ovarian hyperstimulation following down-regulation with a GnRHa in a long protocol. About 44 patients were stimulated with rec-FSH (Puregon; Organon, Holland) and 43 patients with HP-hMG (Menopur, Ferring, Sweden).

All patients received an identical type and dose of concomitant fertility treatment, that is, GnRHa for down-regulation, human chorionic gonadotropin (hCG) for triggering final maturation, and progesterone for luteal support. Pituitary down-regulation using triptorelin acetate, 0.1 mg/day s.c. (DECAPEPTYL; Ferring Pharmaceuticals A/S), was initiated 5-7 days before the estimated start of the next menses and continued until the end of gonadotropin administration. Gonadotropin administration was initiated when down-regulation was confirmed by using transvaginal ultrasonography showing no ovarian cysts, a shredded endometrium with a thickness of <5 mm or serum estradiol <50 pg/ml (0.184 nmol/l). The starting dose of HP-hMG or rec-FSH was 225 IU for the first 5 days, followed by individual adjustments according to the patient's follicular response. The dose could be changed by 75 IU per adjustment and not more frequently than every 4 days. Choriogonadotropin alpha, 250 µg s.c. (OVITRELLE; Serono) was administered to induce final follicular maturation within 1 day of observing three or more follicles of ≥17 mm diameter. Oocyte retrieval took place 36±2 h after hCG administration.

Insemination was done via regular IVF insemination (not ICSI) at 3±1 h after oocyte retrieval. Fertilization was assessed at

20±1 h, and embryo quality was assessed at 28±1 h, 44±1 h, and 68±1 h after oocyte retrieval. A top-quality embryo was defined as four to five cells on day 2 (type A), seven or more cells on day 3, equally sized blastomeres and ≤20% fragmentation on day 3, and no multinucleation. The transfer of one or two embryos of minimum quality, defined as four or more cells with no cleavage arrest (i.e., cleavage must have occurred within the last 24 h and ≤20% fragmentation), was done on day 3 after oocyte retrieval. Vaginal progesterone gel 90 mg/day 8% (CRINONE; Serono) for luteal support was given from the day of embryo transfer until the confirmation of clinical pregnancy (5-6 weeks after embryo transfer) or negative serum β-hCG test (13-15 days after embryo transfer).

Our primary aim was to compare the ongoing pregnancy per started cycle. Positive β-hCG test following embryo transfer was described as chemical pregnancy. Ongoing pregnancy was defined as positive heart beat 10-11 weeks after embryo transfer by ultrasonographic examination. Other outcome parameters assessed were HP-hMG/rec-FSH dose (IU: International Unit), days of gonadotropin stimulation, number of oocytes retrieved, fertilization rate, number of embryos, and ongoing pregnancy.

Statistical analysis

Dates of the study were analyzed by statistical package for social science for window 15.0. Summary statistics were evaluated for all parameters. Differences between groups of normally distributed variables were assessed with the Student’s t-test, while not normally distributed variables were compared with the Mann-Whitney U test. Noncontinuous variables were compared with the Chi square test. P<0.05 was considered statistically significant.

Results

Demographics and baseline characteristics of the participants are shown in the Table 1. No difference was observed between the two treatment groups. Distribution of infertility causes was similar between the groups (Figure 1). About 87 cases were reported in this study. Of the 87 patients, 44 were stimulated with rec-FSH and 43 with HP-hMG. Data of 87 patients were analyzed by dividing them into two groups as rec-FSH (n=44) and HP-hMG (n=43) treatments.

Duration of gonadotropin administration (p=0.677, Table 1) and the total dose of gonadotropin received (p=0.392, Table 2) were similar between the two groups. Serum E₂ levels on the day of rec-hCG administration were different between the HP-hMG and rec-FSH group, which was statistically significant. Serum E₂ levels were higher in the HP-hMG group (p=0.041, Table 2). On the day of hCG injection, there was no significant difference in endometrial thickness (p=0.282, Table 2). Oocyte retrieval was performed in 41 (95.3%) patients stimulated with HP-hMG and in 43 (97.7%) patients stimulated with rec-FSH. In the HP-hMG group, 2 patients did not attend the oocyte retrieval visit because of insufficient endometrial thickness (1 patient) and degenerated oocyte (1 patient). In the rec-FSH group, oocyte retrieval was not performed in 1 patient due to endometrial thickness. The mean

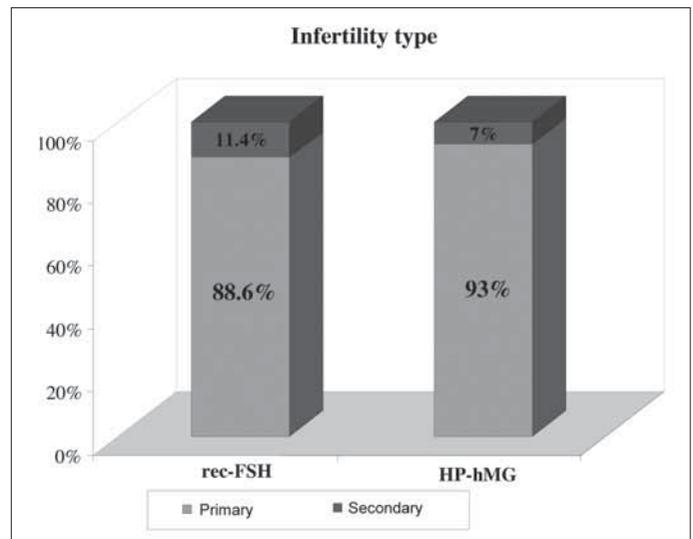


Figure 1. Distribution of infertility type in the groups

Table 1. Demographics and baseline characteristics of patients in the groups

		rec-FSH n (%)	HP-hMG n (%)	p
Age	<35	26 (59.1)	20 (46.5)	0.240
	≥35	18 (40.9)	23 (53.5)	
		mean±sd	mean±sd	
Weight (kg)		63.54±9.54	65.44±9.38	0.353
BMI (kg/m ²)		24.24±4.18	25.17±3.40	0.257
Antral follicles at day 1		10.58±3.98	9.21±3.28	0.087
Duration of infertility (years)		10.53±5.23	10.14±5.00	0.720
FSH (IU/l) at day 3		7.76±2.50	8.09±2.24	0.512
E ₂ (IU/l) at day 3		59.97±22.37	59.74±34.04	0.970
Duration of GnRH agonist (days)		22.35±3.60	21.63±3.58	0.354
Duration of Gonadotropin (days)		9.37±1.46	9.51±1.62	0.677
Chi square test was used for age (p<0.05). Student's t-test was used for other parameters (p<0.05)				

number of oocytes retrieved was lower in the HP-hMG group compared with the rec-FSH group (p=0.029, Table 3). The mean number of oocytes fertilized was also lower in the HP-hMG group compared with the rec-FSH group (p=0.001, Table 3). Between the study groups, there were no significant differences in the number of embryos on day 2 and 3. The number of top-quality embryos did not differ between the patients of the two groups, but the rate of top-quality embryos to total embryos were significantly higher in the HP-hMG group (p=0.006, Table 3). The fertilization rate of rec-FSH group was significantly higher than HP-hMG group (p=0.001, Table 3). Embryo transfer was performed in 40 patients in the rec-FSH group and 28 patients in HP-hMG group. 3 patients in the rec-FSH group and 12 patients

that the fertilization rate was 51.6% in the HP-hMG group and 52.6% in the rec-FSH group ($p=0.065$) (13). In the same study, the mean numbers of cells with 2 pronucleus at the 20th hour were 5.1 ± 4.0 in the HP-hMG group, 6.0 ± 4.3 in the rec-FSH group (13). In our study, the proportion of fertilization rate was 90.9% in the rec-FSH group, 58.1% in the HP-hMG group. There were significant statistical differences ($p=0.001$, Table 3). The mean numbers of cell with 2 pronucleus at the 20th hour were 1.87 ± 1.96 in the HP-hMG group and 4.22 ± 3.57 in the rec-FSH group. Between the two groups, patients stimulated with rec-FSH have a statistically higher fertilization number ($p<0.01$). The mean number of oocytes retrieved was significantly lower in the HP-hMG group compared with the rec-FSH group ($p<0.05$, Table 3). The numbers of mature oocytes differ between the treatment groups, and was found higher in the HP-hMG group than the rec-FSH group ($p<0.005$), but the proportion of mature oocytes to total oocytes retrieved was similar in the two groups ($p=0.05$, Table 3). The mechanisms whereby the LH activity could mediate improvements in some oocyte/embryo quality parameters in IVF cycles are not yet fully understood. Cumulus cells are considered an ideal surrogate for assessment of oocyte development potential. It is speculated that a set of cumulus genes may determine oocyte maturation, fertilization potential, and embryo quality (15). Magier et al. detected that cumulus cells have a positive effect on embryo development (16). Furthermore, cumulus cells and cytoplasmic maturation of oocytes that may be directed with LH activity was considered in some studies (5). Ziebe et al. obtained a higher percentage of top-quality embryos per oocyte retrieved from stimulation with HP-hMG. The subgroup of top-quality embryos obtained from HP-hMG-treated women was associated with increased ongoing implantation and pregnancy rates in comparison with rec-FSH-treated women (13). Formerly, articles had declared that LH activity with the effect of Rec-FSH increased the rates of implantation and pregnancy (17). In our study, the proportion of top-quality embryos per obtained oocyte in patients treated with HP-hMG and rec-FSH was 28.4% and 20.37%, respectively. We did not observe a statistical difference between the two groups ($p>0.05$). The rate of top-quality embryo to total embryo was 45.8% in rec-FSH and this rate was 71.31% in HP-hMG group. The higher proportion of HP-hMG group was significant statistically ($p<0.001$, Table 3). Information on ongoing pregnancy rate was not relevant between the rec-FSH and HP-hMG groups. In their study, Andersen and Coworkers conducted a research in which the ongoing pregnancy rate was 27% in the HP-hMG group and 22% in the rec-hMG group (14). In our study, the clinical pregnancy rates were 25.8 in the rec-FSH and 23.1 in the HP-hMG groups and the ongoing pregnancy rates were 38.9 and 35.3, respectively. However, the superiority of rec-FSH on the clinical and ongoing pregnancy rates are not shown statistically ($p>0.05$). In conclusion, the higher oocyte yield with rec-FSH does not result in more high quality embryos. LH activity with the combination of FSH activity positively affected the oocyte and embryo maturation. Therefore, when we compare the clinical and ongoing pregnancy rates, there is no inferiority of HP-hMG in controlled ovarian stimulation.

Conflict of interest

None declared.

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