Comparison of the Recombinant Versus Highly-Purified, Urinary Follicle-Stimulating Hormone (r-FSH vs. HP-uFSH) in the Polycystic Ovary Syndrom Patients who had Antagonist Cycle with In Vitro Fertilization-Embryo Transfer

Antagonist Siklus ile İn Vitro Fertilizasyon-Embriyo Transferi Uygulanan Polikistik Overli Hastalarda Rekombinant ile Üriner Follikül Stimüle Edici Hormonun (r-FSH ve HP-uFSH) Karşılaştırılması

Mustafa KARA, MD,[a]
Tayfun KUTLU, MD,[b]
Kenan SOFUOĞLU, MD[b]

[a]Department of Obstetrics and Gynecology, Bozok University Faculty of Medicine, Yozgat
[b]Reproductive Medicine and IVF Unit, Zeynep Kamil Women's and Children's Hospital, İstanbul

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Yazışma Adresi/*Correspondence:*
Mustafa KARA, MD
Bozok University Faculty of Medicine, Department of Obstetrics and Gynecology, Yozgat
TÜRKİYE/TURKEY
opdrmustafakara@hotmail.com

ABSTRACT Objectives: Although there are a number of studies on the costs and efficacy of the drugs which are used in in vitro fertilization (IVF), the data obtained in antagonist cycles of polycystic ovary syndrome (PCOS) cases are still controversial. The aim of the study was to evaluate follicular development, pregnancy outcome and cost-effectiveness comparing r-FSH and HP-u FSH in antagonist cycles of PCOS cases. **Material and Methods:** A total of 102 patients were included in the study. Patients were treated using the same stimulation protocol. r-FSH (n= 54) or HP-u FSH (n= 48) were given according to the clinician's choice and the patient's preference. **Results:** r-FSH treatment required significantly less amount to induce follicular development and resulted in higher plasma levels of estradiol on the day of human chorionic gonadotropin (hCG) injection. Although pregnancy rate in the r FSH group was higher than HP u FSH group (40.7% vs. 33.3%), the difference was not statistically significant. Total FSH dose was significantly lower in r FSH group comparet to HP-uFSH (1396.3 ± 131.6 iu vs.1820.14 ± 174.6 iu). **Conclusion:** r-FSH is more efficient than HP-u FSH in terms of total FSH dose. The Follicular development and endometrial thickness on the day of hCG were better in the r-FSH group, however these differences were not statistically significant. Although r-FSH is more expensive, the final treatment cost with r-FSH per pregnancy was slightly lower although this difference was not statistically significant.

Key Words: Follicle stimulating hormone; polycystic ovary syndrome; pregnancy

ÖZET Amaç: İn vitro fertilizasyonun (İVF) maliyeti ve kullanılan ilaçların etkinliği hakkında daha önce yayınlanmış birçok çalışmalar olmasına rağmen, antagonist siklus ugulanan polikistik overli (PKO) hastalardan elde edilen bilgiler halen tartışmalıdır. Bu çalışmanın amacı r FSH ve HP-u FSH'nin antagonist siklus yapılan PKO'lu hastalardaki follikül gelişimi, gebelik sonuçları ve maliyete etkisini değerlendirmekti. **Gereç ve Yöntemler:** Toplam 102 hasta çalışmaya dahil edildi. Hastalar aynı stimülasyon yöntemiyle tedavi edildi. r FSH (n= 54) ve HP-uFSH n= 48) klinisyenin seçimi ve hastanın tercihine göre verildi. **Bulgular:** r FSH tedavisi folliküler gelişimi indüklemek için daha az ilaç gerektirdi ve human koriyonik gonadotropin (hCG) injeksiyon gününde daha yüksek plazma estradiol düzeyleriyle sonuçlandı. Gebelik oranı r FSH grubunda HP-uFSH grubuna göre daha yüksek olmasına rağmen (%40.7'ye karşı %33.3), aradaki fark istatistiksel olarak anlamlı değildi. Toplam FSH dozu r FSH grubunda HP-uFSH'tan anlamlı derecede daha düşüktü (1396.3 ± 131.6 iu'ya karşı 1820.14 ± 174.6 iu). **Sonuç:** r FSH total FSH dozu açısından HP-uFSH'tan daha etkilidir. Folliküler gelişim ve hCG günündeki endometrial kalınlık r FSH grubunda daha iyidir ancak, bu farklılıklar istatistiksel olarak anlamlı değildir. r FSH daha pahalı olmasına rağmen gebelik başına maliyet bu grupta daha düşüktür ama, bu farklılık istatistiksel olarak anlamlı değildir.

Anahtar Kelimeler: Folikül stimüle edici hormon; polikistik over sendromu; gebelik

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Recently, follicle-stimulating hormone (FSH) has gained a central role in ovulation induction and has been shown to be highly effective in achieving superovulation for IVF. Various FSH containing products, both derived from extraction and purification from urine or from recombinant technology have been developed.[1,2] In the early 1980s, production of purified FSH was made possible by the development of affinity purification processes using polyvalent antibodies that yielded a relatively biologically pure FSH extract containing less than 1 IU of LH activity/75 IU FSH but still being contaminated with 95% of urinary proteins. The immunoextraction of urinary FSH using monoclonal antibodies has resulted in the clinical availability of HP-u FSH, containing less than 0.1 IU of LH activity/75 IU FSH and less than 5% of unidentified urinary proteins and could be administered subcutaneously. Recently, biotechnology has made available for the first time a recombinant human FSH preparation which has been and is being marketed in a number of countries. Because FSH needs to be glycosilated for biological activity, r-FSH is produced by genetically engineered mammalian cells, in which genes coding for the FSH subunits have been inserted. Only a mammalian cell can glycosylate the FSH protein correctly thus ensuring full biological activity.

Advances in purification techniques have led to the invention of the HP-u FSH with a spesific activity of >9000 iu FSH /mg protein, which can be characterized and controlled by physico-chemical techniques.[3]

It has become possible to produce FSH for therapeutic use with the application of recombinant DNA technology.[4] r-FSH shows minor differences in the structure of the carbohydrate side chains and contains more basic isohormones than the hormone from natural sources.[5,6]

Several clinical trials were performed to compare the efficacy of r-FSH and HP-u FSH in inducing superovulation in women undergoing assisted reproductive techniques. Clinical results of comparative studies investigating the efficacy of r-FSH vs. HP-u FSH on large patient groups are still controversial.[7]

Polycystic ovary syndrome (PCOS) is a common endocrinopathy and affects 5% to 10% of women in the reproductive age. The syndrome is surrounded by controversies regarding both its diagnosis and treatment. The need to establish universally accepted diagnostic criteria led to the Rotterdam meeting in 2003, during which experts on PCOS from all over the world arrived at a consensus regarding the diagnosis of the syndrome.[8]

Nowadays, several factors are considered in planning the treatment modality and choosing the gonadotropin type in IVF. One of them is the cost effectivity and patient comfort. In this study, the efficacy and cost of r-FSH and HP-u FSH preparations were compared in antagonist cycles of PCOS patients.

MATERIAL AND METHODS

This retrospective study aimed to compare follicular development and cost of therapy with either r-FSH (Puregon® 300 Schering-Plough, USA) or HP-u FSH (Fostimon HP® 75 İBSA, Switzerland) in women undergoing IVF-ICSI (intracytoplasmic sperm injection) treatment.

The patient characteristics (basal hormon levels, duration of infertility and ages of the patients) were analyzed. The groups were homogenous in terms of these parameters. The cases in whom testicular sperm extraction (TESE) procedures were performed were not included the study. The patients whose body mass index (BMI) > 30 were not included in the study. The groups were not compared in relation with BMI.

In all cases, pituitary gland was down-regulated with Cetrorelix (Cetrotide® 0.25 Merck-Serono, Switzerland) in an antagonist protocol starting on the sixth or seventh day of the cycle according to the follicular growth (when the leading follicle reached to 13-14 mm), and E2 level (when the level exceeded 600-800 pg/ml). Cetrorelix was continued until the day of the hCG. Controlled ovarian stimulation (COS) was performed with either r FSH or HP-u FSH starting on cycle day 3. Average FSH starting dose was 225 IU and the dose was individually adjusted according to the previous tre-

atment cycles, BMI, and age. Follicular development was monitored and dose adjusment was done according to E2 level and ultrasonographic measurements. When at least three follicles reached 17 mm size, hCG (Pregnyl® 5000 IU × 2, Schering-Plough, USA) was administered for final maturation. Transvaginal ultrasound-guided needle aspiration of follicular fluid was carried out 35 to 36 hours after hCG administration. Immediately after follicle puncture, the oocytes were incubated for 2-4 hours in the incubators, and then hyaluranidase (Vitrolife, Sweden AB, Kungsbacka, Sweden) was applied for denudation procedure. In all cases, ICSI was performed. Semen samples were washed by using gradient method. Isolate sperm seperation medium (Irvine Scientific, Santa Ana, California) and Quinn's sperm washing medium (Sage, Trumbull, CT, USA) were used for sperm preparation. G-MOPS plus, G-IVFplus, G1-plus, G2-plus (Vitrolife, Sweden AB, Kungsbacka, Sweden) were the media which were used for embryo culturing. Embryos were classified according to the number of blastomeres, percentage of fragmentation and blastomere appearences as type I, II, III or IV on 1st, 3rd and 5th days. Up to three embryos were transferred into the uterine cavity on day 2, 3 or 5, after oocyte retrieval. All transfers were made by using Rocket Thin wall Transfer set (Rocket Medical, Hingham, MA,USA).

Luteal phase support was given by transvaginal progesterone administration (Crinone %8 vaginal gel® Merck-Serono, Switzerland). Progesterone administration was initiated on the oocyte pick-up day and continued for 12 days (until the serum beta hCG measurement day). In case of pregnancy, progesteron was given until the 12th gestational week. The cycle cancellation rate was not calculated since such patients were not included in the study.

The primary end point of the study was the clinical pregnancy rate after IVF-ICSI treatment. Clinical pregnancy was defined by the presence of a fetal sac on ultrasound examination 6 weeks after embryo transfer. Secondary variables included the number of treatment days, the total dose of gonadotropin administered, the number of oocytes retrieved, the number of mature oocytes in case of ICSI and plasma levels of estradiol on the day of human chorionic gonadotropin injection.

Student's t-test, one-way analysis of variance (ANOVA) and χ^2 tests were performed. Differences were considered significant at $p< 0.05$. Results are expressed as mean ± SD.

RESULTS

A total of 102 patients were included in the study and only one cycle was used for each patient. Fifty four patients were treated with r FSH while HP-u FSH was used for 48 patients. The patient characteristics of both groups were comparable (Table 1).

The characteristics of the patients were similar. The patients' age, duration of infertility, basal FSH levels, basal E2 levels and prolactin levels were evaluated, however but there was no statistical differences.

The follicular development was better in r-FSH group but the difference was not statistically significant. The number of the follicles larger than 12 mm in diameter were higher in number on the day of the HCG administration in the r FSH group, but it was not statistically significant. Number of follicles >18 mm and size of dominant follicle on the hCG day were compared but, there was no statistical difference. Total doses of FSH, E2 levels and endometrial thickness on the day of hCG administration, and duration of stimulation are presented in Table 2. Mean total FSH dose was 1396.3 ± 131.6 İU for the r FSH group, and it was 1820.14 ± 174.6 İU in the HP-u FSH group, and this difference was

TABLE 1: Characteristics of the patients. Values are means ± SD.All P values were not significant statistically.

Characteristic	r- FSH (n= 54)	HP-u FSH (n= 48)	p
Age	26 ± 2.33	30.4 ± 2.6	ns
Duration of infertility (year)	5.3 ± 0.51	5.6 ± 1.09	ns
Basal FSH level (iu/l)	6.11 ± 0.51	6.53 ± 0.62	ns
Basal E2 level (pg/ml)	50.14 ± 10.2	47.88 ± 9.13	ns
Prolactin level (ng/ml)	13.08 ± 1.31	14.22 ± 3.01	ns

ns: not significant.

statistically significant (p= 0.0001). The pregnancy rate was slightly higher in r FSH group, however pregnancy outcomes (e.g abortion rate, multiple pregnancies) were not assessed.

Both HP-uFSH and r-FSH were very well-tolerated by the patients. No systemic adverse effects were observed and no severe ovarian hyperstimulation syndrome (OHSS) occurred.

Total dose of FSH per pregnancy was lower in r-FSH group. In spite of the fact that the mean expense per 75 IU gonadotropin for r-FSH were more expensive, the final treatment cost for pregnancy was not statistically significantly different between two groups. Expense per pregnancy was 2392 TL and 2419 TL for r-FSH and HP-u FSH, respectively (Table 3).

DISCUSSION

Recommended third-line treatment in PCOS is IVF because this treatment is effective in women with this disease. Data concerning the use of single-embryo transfer in (young) women with PCOS undergoing IVF, which significantly reduces the chance of multiple pregnancies, are awaited. Several stimulation protocols have been published for the treatment of patients with PCOS undergoing IVF: GnRH-agonist associated with hMG or recombinant FSH and GnRH-antagonist associated with hMG, HP-u FSH or recombinant FSH. Currently, the most widely accepted standard protocol is the long desensitization protocol associated with FSH. However, long protocol requires more time, vials and injections.[9-11]

In principle, anovulation is not an indication for IVF. During the past decade, an important increase has occurred in the use of ovulation induction regimens, mainly those using gonadotropins. The logical therapy for women with PCOS is induction of ovulation, especially by clomiphene citrate administration, and in case of failure, by using exogenous gonadotropin therapy. The major complication of ovulation induction is the 10% multiple pregnancy rate, especially after the use of gonadotropin therapy. For this reason, use of gonadotropins may be questioned.[9,10]

TABLE 2: Outcomes of antagonist protocol used in patients with PCO and comparison between r FSH and HP-u FSH.

	r FSH	HP-u FSH	P
Total FSH dose (iu)	1396.3 ± 131.6	1820.14 ± 174.6	0.0001
Duration of follicular phase (day)	9.61 ± 0.28	10.12 ± 0.31	ns
Endometrial thickness on HCG day (mm)	10.3 ± 0.39	10.58 ± 0.48	ns
No.of follicles 12-17 mm on HCG day	9.91 ± 1.41	9.11 ± 1.71	ns
No.of follicles >18 mm on HCG day	3.12 ± 1.05	2.95 ± 0.86	ns
Size of dominant follicle on HCG day (mm)	19.18 ± 0.68	17.55 ± 0.54	ns
E2 level before HCG injection day	1885.3 ± 302	1696.17 ± 251.42	ns

ns: not significant.

TABLE 3: Expenses of the two treatment groups.

	r-FSH	HP-u FSH	P
Mean expense per 75 gonadotropin unit (TL)	58.06	30.89	
Mean expense per cycle (TL)	975.4 ± 71.32	806.84 ± 64.2	ns
Percent of pregnancies (%)	40.7 (22/54)	33.3 (16/48)	ns
Expense per pregnancy (TL)	2392	2419	ns

TL: Turkish liras.

Higher number of oocytes were retrieved and higher clinical pregnancy rates were observed in the r-FSH group and cost of the treatment was lower, but none the differences between the groups reached statistical significance (Table 2). Treatment with r-FSH resulted in a significantly higher number of recovered oocytes compared to the HP-u FSH group, and this advantage of FSH was associated with a lower smaller of vials needed to reach the criterion for hCG administration. This fact was mentioned before in a number of studies.[11,12]

Strehler et al. assessed 578 patients.[7] Treatment with human menoposal gonadothropin (hMG) was used in 282 patients, whereas 296 patients were treated with r FSH. In terms of the clinical pregnancy rate, no significant differences between the two stimulation regimens were detected.

Bergh et al. carried out a prospective, randomized study in two centers to compare the efficacy and safety of r-FSH and HP-u FSH in women undergoing ovarian stimulation for IVF including ICSI.[13] A total of 235 patients started a long gonadotrophin-releasing hormone agonist protocol: 119 received r-hFSH and 114 received u-hFSH HP (150 IU/day) for the first six days. Total FSH dose was significantly less ($p< 0.0001$) in the r-hFSH group compared to the HP-u FSH group. The clinical pregnancy rates per started cycle and per embryo transfer were 45 and 36%, and 48 and 47%, respectively in the r-FSH and HP-u FSH groups (not significant). In conclusion, it was found that r-FSH was more effective than HP-u FSH for inducing multiple follicular development.[13]

Finally, these comparative studies demonstrated that r-FSH has statistically significant advantages in terms of efficacy, resulting in greater numbers of follicles and oocytes retrieved, although the amount of gonadotropins administered was lower and the treatment period was shorter.[14] Balasch et al. found the smilar results.[15] OHSS did not develop in our patients because of close monitoring of follicular development and estradiol measurement.

The optimal stimulation protocol is still under debate in PCOS patients. There is a need to perform further randomized controlled trials comparing FSH stimulation protocols with use of GnRH agonists versus GnRH antagonists.[16]

In conclusion, the present study shows that both HP-u FSH and r-FSH can be safely and effectively used in antagonist cycles of PCOS cases. Although slightly more effective than HP-u FSH, r-FSH seems to be efficient to decrease the cost of the treatment.

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