Comparison between cycles of the same patients when using recombinant luteinizing hormone + recombinant follicle stimulating hormone (rFSH), human menopausal gonadotropin + rFSH and rFSH only

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Abstract

Introduction: Recombinant follicle stimulating hormone (rFSH), recombinant luteinizing hormone (rLH), and urinary human menopausal gonadotropin (uHMG) are widely used for controlled ovarian stimulation (COS). This study compares the effects of rFSH only, rLH + rFSH, and HMG + rFSH administration on *in vitro* fertilization (IVF) outcomes for patients in three different yearly follow-up cycles.

Material and methods: This retrospective, single-center cohort study was conducted from January 2001 to June 2016 at Istanbul Memorial Hospital, Artificial Reproductive Technology Center. From a total of 27,024 IVF cycles in women aged 18 to 45 years (17,536 rFSH only; 2147 rLH + rFSH; 7341 HMG + rFSH), the results of 2,147 cycles receiving a treatment of rLH + rFSH over the 3-year evaluation and 2,081 total cycles in which rLH + rFSH was used at least once were evaluated, and different gonadotropin combinations were compared.

Results: The age and body mass index of the patients in the uHMG + rFSH group were found to be significantly higher than those of the patients in the rLH + rFSH and rFSH only groups ($p < 0.001$). The total gonadotropin (GND) dosage of the patients in the rLH + rFSH group was found to be significantly lower than that of the HMG + rFSH group ($p = 0.001$). No statistically significant differences were found between the clinical and ongoing pregnancy rates, while the highest clinical and ongoing pregnancy rate was observed in the rLH + rFSH group at age 35–39 years.

Conclusions: Recombinant luteinizing hormone administration may increase the number of clinical pregnancies for patients aged 35–39 years.

Key words: infertility, luteinizing hormone, recombinant follicle stimulating hormone, recombinant luteinizing hormone, human menopausal gonadotropin, *in vitro* fertilization.

Introduction

The role of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in follicular development is very well known [1]. Consistent with the two-cell two-gonadotropin theory, LH and FSH play an important role

in stimulating the synthesis of steroid hormone and regulation of the ovary micro-environment through stimulating the granulosa and theca follicle cells in the ovary during the menstrual cycle [2]. The most commonly used protocol in Artificial Reproductive Technology (ART) is gonadotropin-releasing hormone (GnRH) agonist or antagonist to preclude a premature LH surge and daily injections of recombinant human FSH to stimulate multiple follicle growth. The importance of FSH for ovarian stimulation is well established and FSH without LH generally leads to successfully developed and mature follicles during *in vitro* fertilization (IVF) cycles [3–5]. Therefore, the part of LH supplementation to the mid-follicular phase in ovarian stimulation cycles during IVF treatment still remains a debated topic and there are conflicting data evaluating the importance of LH supplementation for IVF treatment. Nevertheless, adding LH to recombinant follicle stimulating hormone (rFSH) for ovarian stimulation may be beneficial especially in particular subgroups such as older women and reduced response probably due to the low bioactivity of LH to improve ART results. Moreover, using GnRH antagonists leads to deterioration of serum oestradiol level throughout follicular recruitment because of oversuppression of endogenous LH by the prompt and noteworthy inhibition of pituitary function, which would adversely influence IVF outcomes although the slight quantities of LH after down-regulation are enough to arouse theca and granulosa cells [6–8]. Likewise, during GnRH agonist treatment, selected patients who have a lower LH concentration or a sharper fall in LH from baseline concentrations may have a suboptimal ovarian response and poorer ART outcomes [9, 10]. Supplementation of LH may be beneficial for these patients with LH deficiency during GnRH agonist treatment.

Exogenous LH administered in the mid follicular phase can increase serum ovarian steroid production and decrease total doses of FSH, without compromising the total number of mature oocytes retrieved [11]. There are two available sources of exogenous LH activity to use during IVF cycles: recombinant luteinizing hormone (rLH) and human menopausal gonadotropin (HMG). Human menopausal gonadotropin contains both FSH and LH. Recombinant luteinizing hormone has a terminal half-life of 24 h and is structurally and functionally similar to endogenous human LH. Different HMG preparations may show variation in LH quantity and bioactivity because of increased purification and more LH is lost. There may be subtle differences in the clinical outcomes of patients treated with either pure rLH or HMG. Several studies have shown that LH administration in IVF cycles is more efficient for oocyte stimulation and development than HMG + rFSH [12, 13]. However, this effect of LH administration on IVF pregnancy results has not been clearly demonstrated.

Ovarian stimulation is a crucial step to produce the finest follicular response by the most physical method to achieve a maximum success rate in IVF treatment. Determination of the best ovarian stimulation regimens is still important. Strategies to increase the success for IVF treatment are required through the use of new therapeutic options. Luteinizing hormone supplementation to the ovarian stimulus scheme might be one option to improve the IVF success rate. The efficacy of use of rLH supplementation during IVF treatment is still controversial and additional reports of this method are necessary to describe efficacy exclusively.

Considering this gap in the literature, the aim of the present study is to retrospectively compare the effectiveness of rFSH only, rFSH + HMG or rFSH + rLH treatments in agonist and antagonist IVF cycles and also to evaluate the efficacy of adding rLH to r-hFSH.

Material and methods

Participants

A total of 27,074 cycles in the Department of Assisted Reproductive Technologies and Reproductive Genetics, Istanbul Memorial Hospital, Turkey, between 2001 and 2016 were included. Inclusion criteria were patients with primary infertility diagnosed as tubal factor, male factor or unexplained infertility programmed for IVF, body mass index (BMI) $\leq$ 25 kg/m^2, between 18 and 45 years old, with FSH levels of < 12 mIU/ml and estradiol (E2) of < 80 pg/ml on cycle day 3. Exclusion criteria were patients diagnosed with grade 3–4 endometriosis, polycystic ovary syndrome, uterine anomalies, or any lesions in the uterus. Data collected included age, BMI (kg/m^2), number of previous ART attempts, FSH levels on cycle day 3, anti-Mullerian hormone (AMH), total dosage of gonadotropins, duration of stimulation, total number of oocytes retrieved, total number of mature oocytes retrieved, clinical pregnancy rate (PR), ongoing pregnancy rate, and miscarriage.

Assisted reproduction procedures

A controlled ovarian stimulation protocol was used: antagonist protocol (Cetrotide; 0.25 mg; Merck Serono, Istanbul, Turkey) or agonist long protocol (leuprolide acetate; Lucrine; Abbott, Turkey). Patients were divided into three groups according to their add-on treatment protocol: Group I: rFSH only group (follitropin α, Gonal F, Merck-Serono, Istanbul, Turkey) (n = 17,536); Group II: rFSH + HMG group (Menagon, Ferring, Istanbul, Turkey)

(*n* = 7341); Group III: rFSH + rLH group (Luveris, Merck-Serono Istanbul, Turkey) (*n* = 2147). Initial doses ranging from 150 to 450 IU daily were based on age, BMI, and resting antral follicle count detected by early-follicular phase ultrasonography. Controlled ovarian stimulation was initiated on the second day of the cycle with all patients. Doses were adjusted on the basis of serial sonographic measurements of follicular development and serum E2 levels. Recombinant luteinizing hormone was added from the 5th day of the cycle, while HMG was started simultaneously with rFSH. A single dose of 10000 IU of urinary hCG (Pregnyl amp 5000 IU, Organon, Istanbul, Turkey) or 250 µg rec hCG (Ovitrelle amp 250 µg/0.5 ml, Merck-Serono, Istanbul, Turkey) was administered when at least two follicles reached a mean diameter of > 17 mm. Transvaginal US-guided oocyte retrieval was performed between hours 35 and 37 after hCG administration. Fertilization of the oocytes was performed using standard intracytoplasmic sperm injection techniques. According to maternal age, indication for IVF, number of previous attempts and number and quality of embryos available, one or two embryos were transferred on day 3 or 5. Luteal phase support was started on the day of retrieval using vaginal Crinone gel (Crinone 8%, 90 mg; Merck Serono Istanbul, Turkey) daily. Serum quantitative β-hCG levels were obtained at 12 days after embryo transfer (ET). A clinical pregnancy was defined as the presence of a fetal sac visualized by transvaginal US examination at 6–8 weeks of amenorrhea. Pregnancies that continued for longer than 12 weeks were considered ongoing pregnancies.

Statistical analysis

The IBM SPSS Statistics 22 program was used for the statistical analysis of the research findings. Conformity of the data to normal distribution of the parameters was evaluated with the Shapiro-Wilk test. In the comparison of quantitative data, the one-way ANOVA test was used for the intergroup comparison of parameters with normal distribution. The Tukey HDS test was applied to determine the group causing the difference. The Kruskal-Wallis test was used for the intergroup comparison of the parameters without normal distribution, and the Mann-Whitney *U* test was applied to determine the group causing the difference. The χ^2 test was used for the comparison of qualitative data. A value of $p < 0.05$ was accepted as statistically significant.

Results

A total of 27,074 cycles were retrospectively evaluated. As a result of re-scanning 2147 cycles receiving the treatment of rLH + rFSH in the three-year evaluation, 2081 total cycles in which rLH + rFSH was used at least once were included. After taking into consideration the canceled cycles with no ET, 1711 cycles in total were evaluated.

Separate comparison of agonist and antagonist protocols are shown in Table I. There was no significant difference between groups for clinical and ongoing pregnancies ($p = 0.31$ and $p = 0.19$, respectively) (Table I).

For all groups, patients demographic and endocrine characteristics are shown in Table II. The age, previous IVF attempts and BMI averages of the patients in the uHMG + rFSH group were significantly higher than those of other treatment groups ($p < 0.01$, $p < 0.01$, $p < 0.05$; respectively). There were no significant differences between groups in terms of basal FSH levels. AMH level in the uHMG + rFSH group was determined to be significantly lower than levels in the rLH + rFSH and rFSH groups ($p = 0.04$, $p < 0.01$; respectively).

Table I. Separate comparison of agonist and antagonist protocols

Variable		rFSH (I) (*N* = 179) *n* (%)	HMG + rFSH (II) (*N* = 127) *n* (%)	rLH + rFSH (III) (*N* = 193) *n* (%)	*P*-value
Agonist	Clinical abortus	15 (25.9)	15 (33.3)	22 (30.6)	0.698
	Clinical pregnancy	38 (21.2)	36 (28.3)	62 (32.1)	0.059
	Ongoing pregnancy	23 (12.8)	21 (16.5)	40 (20.7)	0.127
		rFSH (I) (*N* = 418) *n* (%)	**HMG + rFSH (II) (*N* = 267) *n* (%)**	**rLH + rFSH (III) (*N* = 527) *n* (%)**	
Antagonist	Clinical abortus	46 (29.1)	30 (30.6)	59 (28.9)	0.952
	Clinical pregnancy	131 (31.3)	71 (26.6)	166 (31.5)	0.316
	Ongoing pregnancy	85 (20.3)	41 (15.4)	107 (20.3)	0.192

*rFSH – recombinant follicle stimulating hormone, rLH – recombinant luteinizing hormone, HMG – human menopausal gonadotropin, χ^2 test, *p < 0.05.*

Table II. Demographic and clinical characteristics of the patients

Parameters	rFSH (I) Total	HMG + rFSH (II) Total	rLH + rFSH (III) Total	*P*-value
Number of cycles	721	491	869	
Number of patients with ET, *n* (%)	597 (82.8)	394 (80.2)	720 (82.9)	0.424[1]
Age, mean ± SD [years]	33.74 ±5.16	36.35 ±5.49	34.68 ±5.32	0.001*[2]
BMI, mean ± SD [kg/m^2]	24.81 ±3.91	26.07 ±4.52	25.32 ±4.43	0.001*[2]
D3 FSH, mean ± SD (median)	8.32 ±3.74 (7.7)	9.19 ±5.71 (8.6)	8.46 ±3.9 (7.7)	0.086[3]
AMH, mean ± SD (median)	1.11 ±1.3 (0.7)	0.7 ±0.75 (0.4)	0.95 ±1.15 (0.6)	0.004*[3]
Number of previous trials, mean ± SD (median)	2.75 ±2.59 (2)	3.42 ±2.99 (3)	2.57 ±2.17 (2)	0.001*[3]
Freeze all, *n* (%)	24 (3.3)	9 (1.8)	39 (4.5)	
Number of induction days, mean ± SD (median)	8.35 ±1.91 (9)	9.16 ±1.84 (9)	9.16 ±1.91 (9)	0.001*[3]
Total GND dosage, mean ± SD	2271.4 ±1024.4	3598.9 ±1386.6	2911.1 ±1245.9	0.001*[2]
Total oocyte count, mean ± SD (median)	10.95 ±7.51 (10)	8.5 ±5.56 (7)	10.34 ±7.1 (9)	0.001*[3]
Total M2 count, mean ± SD (median)	8.2 ±5.74 (7)	6.45 ±4.22 (6)	7.75 ±5.49 (6)	0.001*[3]
Total PN2, mean ± SD (median)	6.68 ±4.9 (5.5)	5.03 ±3.41 (4)	6.24 ±4.52 (5)	0.001*[3]
Total PN2, mean ± SD (median)	6.68 ±4.9 (5.5)	5.03 ±3.41 (4)	6.24 ±4.52 (5)	0.001*[3]

*rFSH – recombinant follicle stimulating hormone, rLH – recombinant luteinizing hormone, HMG – human menopausal gonadotropin, GND – gonadotropin, HCG – human chorionic gonadotrophin, AMH – anti-Mullerian hormone, M – metaphase, PN – pronucleus, ET – embryo transfer, D3 – day 3, [1]χ^2 test, [2]one-way ANOVA test, [3]Kruskal-Wallis test, *p < 0.05.*

Total dosage of gonadotropins in the rLH + rFSH group was found to be significantly lower than in the HMG + rFSH group ($p < 0.01$). Total dosage of gonadotropins in the rLH + rFSH group was significantly higher than that of the rFSH group ($p < 0.01$). Total number of oocytes retrieved, total number of mature oocytes retrieved and number of pronucleus (PN) 2 in the rLH + rFSH and rFSH groups were higher than in the HMG + rFSH group ($p < 0.05$). There was no significant difference between the rLH + rFSH and rFSH groups in terms of total number of oocytes retrieved, total number of mature oocytes retrieved and number of PN 2.

There were no significant differences between clinical pregnancy rates among the rLH + rFSH group, HMG + rFSH group and rFSH group (Table III). There were no significant differences between the groups for ongoing pregnancy rates ($p = 0.14$). Ongoing pregnancies were 20.4%, 15.7%, 18.1% in the rLH + rFSH group, HMG + rFSH group and rFSH group, respectively (Table III). There were no significant differences between the groups for clinical abortion rates ($p = 0.8$).

Among patients in the 35–39 age group, the clinical and ongoing pregnancy rates were higher in the rLH + rFSH group than those of other groups, although not achieving significance ($p = 0.11$ and $p = 0.27$, respectively) (Table IV).

There were no significant differences between the gonadotropin groups for pregnancy, clinical abortion, clinical pregnancy and ongoing pregnancy rates in patients aged 40 years and over who received the agonist and antagonist protocol ($p > 0.05$) (Table V).

Discussion

To date, there is no established proof as to which gonadotropin is further successful when performing controlled ovarian stimulation (COS) in human IVF. However, patient groups in those studies are highly variable, and, as far as we know, no prior report shows the effects of these treatments compared over long-term follow-ups in a specific patient group (with a higher number of previous trials). The present study aims to assess the IVF outcomes of rFSH, rFSH + HMG or rFSH + rLH replacement therapies administered to the same patients in different cycles and to reveal other factors.

The role of rLH together with improvements in IVF treatment and its use during IVF cycles have become popular research subjects in recent years. The usage of rLH supplementation through ovarian stimulus is a topic of deliberation in the literature and this absence has led to several meta-analyses. Some studies have shown that the use of rLH

Table III. Comparison of pregnancy and abortus results for all ages (18–45)

Variable	rFSH (I) Total *n* (%)	HMG + rFSH (II) Total *n* (%)	rLH + rFSH (III) Total *n* (%)	*P*-value
Number of ET	597 (82.8)	394 (80.2)	720 (82.9)	0.424[1]
Clinic abortus	61 (28.2)	45 (31.5)	81 (29.2)	0.805[1]
Clinic pregnancy	169 (28.3)	107 (27.2)	228 (31.7)	0.215[1]
Ongoing pregnancy	108 (18.1)	62 (15.7)	147 (20.4)	0.149[1]

rFSH – recombinant follicle stimulating hormone, rLH – recombinant luteinizing hormone, HMG – human menopausal gonadotropin, ET – embryo transfer, [1]χ^2 test.

Table IV. Comparison of pregnancy outcomes in protocols applied for the 35–39 age group

Variable		rFSH (I) (*N* = 49) *n* (%)	HMG + rFSH (II) (*N* = 37) *n* (%)	rLH + rFSH (III) (*N* = 71) *n* (%)	*P*-value
Agonist	Clinical abortus	6 (35.3)	5 (33.3)	10 (34.5)	0.993
	Clinical pregnancy	11 (22.4)	13 (35.1)	27 (38)	0.186
	Ongoing pregnancy	5 (10.2)	8 (21.6)	17 (23.9)	0.154
		rFSH (I) (*N* = 147) *n* (%)	**HMG + rFSH (II) (*N* = 89) *n* (%)**	**rLH + rFSH (III) (*N* = 159) *n* (%)**	
Antagonist	Clinical abortus	24 (40.7)	9 (31)	16 (25.4)	0.194
	Clinical pregnancy	48 (32.7)	23 (25.8)	49 (30.8)	0.538
	Ongoing pregnancy	24 (16.3)	14 (15.7)	33 (20.8)	0.495

rFSH – recombinant follicle stimulating hormone, rLH – recombinant luteinizing hormone, HMG – human menopausal gonadotropin, χ^2 test.

Table V. Comparison of pregnancy outcomes for protocols applied in the over-40 age group

Variable		rFSH (I) (*N* = 12) *n* (%)	HMG + rFSH (II) (*N* = 21) *n* (%)	rLH + rFSH (III) (*N* = 24) *n* (%)	*P*-value
Agonist	Clinical abortus	1 (50)	1 (16.7)	1 (25)	0.641
	Clinical pregnancy	2 (16.7)	3 (14.3)	2 (8.3)	0.726
	Ongoing pregnancy	1 (8.3)	2 (9.5)	1 (4.2)	0.766
		rFSH (I) (*N* = 69) *n* (%)	**HMG + rFSH (II) (*N* = 110) *n* (%)**	**rLH + rFSH (III) (*N* = 125) *n* (%)**	
Antagonist	Clinical abortus	3 (21.4)	10 (27)	10 (35.7)	0.586
	Clinical pregnancy	8 (11.6)	25 (22.7)	20 (16)	0.138
	Ongoing pregnancy	5 (7.2)	15 (13.6)	10 (8)	0.249

rFSH – recombinant follicle stimulating hormone, rLH – recombinant luteinizing hormone, HMG – human menopausal gonadotropin, χ^2 test.

may increase IVF success in older patients with a poor ovarian response or low serum LH levels. Relevant studies have emphasized that the use of rLH may be beneficial for increasing IVF success, especially in women over 35 years old [14–17]. In recent study Mochtar *et al.* found moderate quality evidence that the use of rLH combined with rFSH may lead to more ongoing pregnancies [18]. Conversely, the conclusions of one systematic review and some individual studies were that accessible proof did not warrant the add-on of rLH both total and in the subgroup of women > 35 years of age [18–20]. In this present study patients of advanced reproductive age (35–39) had higher clinical pregnancy rates in recombinant LH plus recombinant FSH protocols compared with recom-

binant FSH-only and HMG plus recombinant FSH protocols. However, no statistical significance was detected in either agonist or antagonist cycles ($p = 0.18$, $p = 0.53$, respectively).

Possible mechanisms for the improved clinical and ongoing pregnancy rate seen in the present paper are amplified oocyte capability and enhanced endometrial receptivity. Cycles with recombinant LH supplementation have shown lower levels of cumulous cell apoptosis than FSH-only cycles, possibly indicating improved oocyte quality in LH-supplemented cycles [21, 22]. Luteinizing hormone stimulates CYP17 to convert the progesterone (P) into androgens, which can be further aromatized to estrogens (E). The addition of LH may benefit the endometrium by decreasing the risk of a premature P increase and therefore improve the likelihood of implantation and clinical pregnancy [15, 23]. The data from this study indicate a potential benefit to LH administration in patients of advanced reproductive age.

There are two different pharmacological LH preparations that can be used exogenously in IVF cycles. One is urinary uHMG, which also contains FSH, and the other is rLH [24]. Many studies and reviews have compared the effects of rLH and hMG in ART [25]. The findings of the current study are in line with previous literature. Buhler and Fischer compared protocols in 4719 patients and reported more successful results in the FSH + rLH group compared to the FSH + HMG group [26]. In another retrospective study, Dahan *et al.* found that adding rLH to the treatment in patients with serum FSH level > 10 IU/l was more beneficial for clinical pregnancy rates compared to uHMG [27]. According to the German IVF registry results, in which more than 4000 cycles were evaluated, the rLH + FSH protocol resulted in more successful pregnancy and implantation rates than the rFSH + HMG protocol or the rFSH protocol alone. In a study conducted in patients with a poor ovarian response, Revelli *et al.* determined that LH administration with uHMG had a significant effect on clinical pregnancy rates independently of the oocyte count. It was also emphasized that the rates of clinical pregnancy and live births were expected to be higher in patients receiving rLH. The reason for this was emphasized to be the balanced proliferative and controlled effect of rLH on the endometrium that was different from that of uHMG [24]. In addition to the rFSH in the present study, higher pregnancy rates were obtained in comparison to higher uHMG doses, even with the addition of rLH at low doses. Therefore, it was concluded that the use of rFSH + rLH is superior to uHMG, which included LH and FSH.

In conclusion, it can be suggested that rLH may help to reduce the dose of rFSH and the increase in estradiol level, as was seen as an effect of HMG. In addition, a reduced ovarian reserve directly affects the ovarian response during the IVF cycle. This appears to be closely related to age, basal FSH, AMH and basal antral follicle count. Another important effect in the use of exogenous LH replacement is that it facilitates the stimulation of ovarian LH receptors, which decrease with aging. This study observed that despite higher doses of HMG, the addition of low dose rLH to low dose rFSH replacement resulted in more significant clinical and ongoing pregnancy rates. More precise regulation of the rLH dose with higher purity and specific activity (99%) allows for the objective evaluation of the positive effect of LH on the clinical results of IVF treatment.

For patients over 35 years of age, it can be suggested that in addition to rFSH replacement in IVF treatment, rLH administration may increase the number of clinical pregnancies and healthy births. This is attributed to the positive and controlled stimulation effect of rLH on the endometrium, unlike uHMG. In this respect, more randomized, controlled and large-scale studies are needed in similar patient groups.

Conflicts of interest

The authors declare no conflict of interest.

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