CLINICAL STUDY

Effect of Combining Recombinant FSH with Recombinant LH on Oocyte and Embryo Quality in the GnRH Agonist Long and Antagonist Cycles

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Abstract

Objective: Luteinizing hormone (LH) requirement in assisted reproductive treatment (ART) cycles is still controversial. The objective of this study was to investigate the impact of providing LH activity with recombinant LH (rLH) on the oocyte and embryo quality in both GnRH agonist long and antagonist ART cycles of normoresponder women <40 years.
Materials and Methods: A retrospective cohort study of 351 normoresponder IVF patients <40 years was carried out. Agonist long protocol was performed in 184 women and the antagonist in 167. Stimulation was performed in both protocols with either recombinant follicle stimulating hormone (rFSH) alone or rFSH plus rLH. Cycle characteristics and outcomes, primarily the proportions of mature oocytes and good-quality embryos were evaluated according to rLH addition in the two most commonly used stimulation protocols.
Results: In the agonist long protocol, a significantly higher number of total oocytes (17.6±5.8 vs 15±6.8; p=0.005) and a significantly higher rate of mature oocytes (75% vs 70%; p=0.02) were obtained, although embryo quality was similar in rFSH alone cycles compared to the rLH supplemented ones. In the antagonist protocol, significantly more oocytes were retrieved in the rFSH alone group (16.3±7.5 vs 12.8±5.1; p=0.001), although this was not reflected to oocyte maturation and embryo development. Fertilization, implantation, clinical pregnancy, miscarriage and ongoing pregnancy rates did not differ when rLH was included in both protocols.
Discussion: Recombinant LH supplementation in agonist long ART cycles was found to have a detrimental effect on the oocyte quality, without a beneficial or adverse effect on the embryo quality in normoresponder women <40 years. Inclusion of rLH during stimulation in antagonist ART cycles, on the other hand, is neither favourable nor deleterious for the oocyte or the embryo quality in normoresponder women <40 years.

Keywords: embryo quality, GnRH agonist, GnRH antagonist, oocyte quality, recombinant LH, IVF, ICSI

Özet

GnRH Agonist Uzun ve GnRH Antagonist Sikluslarında Rekombinant FSH'ye Rekombinant LH Eklenmesinin Oosit ve Embriyo Kalitesi Üzerine Etkisi

Amaç: Üremeye yardımcı tedavi (ÜYT) sikluslarında LH gerekliliği tartışmalıdır. Bu çalışma ile, GnRH agonist uzun protokol ve antagonist protokolü verilen 40 yaş altı normoresponder kadınlarda rFSH ile tedaviye rLH eklenmesinin oosit ve embriyo kalitesine etkisi araştırılmıştır.
Materyal ve Metot: Toplam 351 normoresponder 40 yaş altı hasta incelendi. Bu hastaların 184'ü agonist uzun protokol, kalan 167'si ise antagonist protokolü ile takip edildi. Siklus takibi ya sadece rFSH ile ya da rFSH'ye rLH eklenerek yapıldı. Siklus karakteristikleri ve tedavi sonuçları, primer olarak matür oosit ve iyi kalite embriyo oranları, tedavide çoğunlukla kullanılan iki stimülasyon protokolüne rLH eklenmesine göre değerlendirildi.
Sonuçlar: Agonist uzun protokolde sadece rFSH ile uyarılan hastalarda anlamlı oranda daha fazla sayıda oosit elde edildi (17.6±5.8 vs 15±6.8; p=0.005) ve matür oosit oranı da daha yüksek bulundu (75% vs 70%; p=0.02), ancak embriyo kalitelerinde fark bulunmadı. Antagonist protokolünde ise sadece rFSH verilen hastalarda anlamlı oranda daha fazla sayıda oosit elde edilmesine rağmen (16.3±7.5 vs 12.8±5.1; p=0.001) oosit ve embriyo kaliteleri rLH eklenen hastalardan farklı bulunmadı. Her iki tedavi protokolünde rLH eklendiğinde fertilizasyon, implantasyon, klinik gebelik, düşük ve devam eden gebelik oranlarında farklılık gözlenmemiştir.

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Tartışma: Yaşı 40'ın altında olan normoresponder hastalarda agonist uzun protokolde rFSH'ye rLH eklenmesi oosit kalitesini negatif olarak etkilemekte iken, bunun embriyo kalitesi üzerine negatif ya da pozitif etkisi gözlenmemiştir. Antagonist protokolü verilen 40 yaş altı normoresponder kadınlarda ise rFSH'ye rLH eklenmesinin oosit ve embriyo kalitesi üzerine ne faydasının ne de zararının olmadığı gözlenmiştir.

Anahtar sözcükler: embriyo kalitesi, GnRH agonist, GnRH antagonist, oosit kalitesi, rekombinant LH, IVF, ICSI

Introduction

With the introduction of pure FSH produced by using recombinant DNA technology (rFSH) and less availability of the LH-containing preparations, the requirement and the source of exogenous LH supplementation in ART patients became an issue for investigators. The use of recombinant DNA technology has also provided a pure preparation of recombinant LH (rLH).

LH has a well-defined role in both ovarian steroid synthesis and ovulation. However, it is also speculated that some LH activity is essential during ovulation induction to achieve full follicular maturation and obtain fertilizable oocytes (1,2). Although the concept of a 'window' for LH requirement in follicular development, according to which there is not only a threshold requirement for LH but also a ceiling level beyond which LH might be deleterious to follicular development and treatment outcome, is widely accepted, optimal cut-off values still remain to be defined (3). Therefore, addition of exogenous LH activity to ovarian stimulation protocols is still controversial. While investigating the need for LH supplementation in ART cycles, stimulation protocols used were suggested as one of the most important determinants, as they lead to different residual endogenous LH concentrations. According to the type, dose and method of GnRH analogue used, variable suppression of endogenous LH was observed (4).

In patients on GnRH agonist long protocol, the impact of suppressed endogenous LH levels, which may not subsequently be compensated by rFSH, on the outcome is still debatable. It was reported that lower estradiole synthesis (5,6), lower oocyte and embryo yield (5,7), lower fertilization rate and higher biochemical pregnancy rate (8), and higher frequency of early pregnancy wastage (9) were observed in normogonadotropic women down-regulated with a GnRH agonist and stimulated with pure FSH preparations. Conversely, some papers reported LH had a negative effect on oocyte quality (10). Some others failed to detect any relationship between serum LH levels and stimulation outcome (11). Accordingly, some authors recommend (12,13) whereas others are against (14) any exogenous LH supplementation during ovarian stimulation.

When GnRH antagonists are used in controlled ovarian hyperstimulation (COH), LH suppression occurs during the midfollicular phase of ovarian stimulation, since antagonists lead to immediate suppression of LH upon their administration. Therefore, the impact of low residual LH levels which occurs towards the end of follicular phase, is thought to be barely perceptible in antagonist cycles. However, low residual LH concentrations and impaired estradiole secretion with increasing doses of antagonists were suggested to be associated with low implantation rate despite the similar number of oocytes and embryos obtained (15). This was explained by an endometrial impact of low LH levels in antagonist cycles. The beneficial direct action of LH on uterine receptivity has been hypothesized in the model of oocyte donation (16). However, in antagonist cycles a systematic supplementation of LH in an unselected group of patients was not supported (17) and even a higher ongoing pregnancy rate was reported in patients with profound LH suppression after antagonist initiation (18). Nevertheless, it is often preferred to add rLH or partly switch to HMG on the day of antagonist initiation (19).

In the present study, we investigated whether the addition of rLH during stimulation in GnRH agonist long and the antagonist ART cycles of normoresponder women <40 years old had a beneficial or detrimental effect on the oocyte and the embryo quality. Stimulation characteristics and the treatment outcomes were further evaluated.

Materials and Methods

Patients and protocols

This study is a retrospective analysis of 351 patients who applied for ART during the period February 2004 to April 2005. Of those 351 patients stimulated at our clinic, GnRH agonist long protocol was administered in 184 and antagonist protocol in 167. Of those 184 patients given agonist protocol, 117 patients were stimulated with rFSH alone and 67 patients with rFSH plus rLH. Of those 167 patients given antagonist protocol, 98 were stimulated with rFSH alone and 69 with rFSH plus rLH. All the patients were stimulated during the same time period and patient characteristics were not taken into consideration while sorting them into either gonadotropin regimes. Stimulation was performed either with rFSH alone or, rFSH plus rLH when available on the market. Recombinant LH was added not from the beginning but on stimulation day 6, when granulosa cells acquired their LH receptors at about the follicular size of 10-12 mm (20,21). Exclusion criteria were high FSH levels (>12 IU/L) and/or ages ≥40 years, previous trials in which ≤4 oocytes were retrieved and polycystic ovarian disease. Our institutional ethics committee agreed that the study did not

need formal ethics review as it was a review of medical records. Informed consent was obtained from all patients.

Patients on the agonist long protocol (n=184) were down-regulated with leuprolide acetate (Lucrin daily; Abbott), 0.1 mg/day, commencing on day 21 of the cycle preceding COH. On day 3 of the subsequent cycle, an ultrasound was performed and serum estradiol (E2) concentration was measured. Gonadotropin treatment was initiated if no follicles were observed larger than 10 mm in diameter and the E2 level was less than 50 pg/ml. The starting gonadotropin dose was individualized according to age, body mass index (BMI), ovarian reserve determined by antral follicle count and experience from previous cycles. Of 184 patients on agonist long protocol, 117 patients (Group 1) received 225-450 IU/day of rFSH only (rFSH-α, Gonal-F, Serono; or rFSH-β, Puregon, Organon), and 67 patients (Group 2) were given 225-450 IU/day of rFSH plus rLH (Luveris, Serono) 75 IU/day starting from the 6th day of stimulation. Follow-up ultrasonography and E2 measurements were performed after 5 days of gonadotropin treatment and the doses were adjusted accordingly.

In patients on the antagonist protocol (n=167), pre-treatment with an oral contraceptive pill was performed during the cycle prior to the IVF/ICSI procedure. On the third day of menses after pill discontinuation, stimulation was carried out starting with a daily injection of 225-450 IU of rFSH alone in 98 patients (Group A), and 225-450 IU/day of rFSH plus rLH 75 IU/day initiated on the 6th day of stimulation in 69 patients (Group B). Doses were modified depending on the hormonal and ultrasound data obtained on day 6 of stimulation. GnRH antagonist 0.25 mg daily injection was started on the 6th day of stimulation and continued up to the day of HCG injection.

An injection of 10 000 IU HCG was administered when the leading follicle reached 20 mm in diameter and oocyte retrieval was performed 36 hours later. Oocytes were assessed according to Veeck (22,23). ICSI was performed as described previously (24). Embryos were evaluated on day 3 after oocyte pick-up according to the size and the shape of blastomeres and degree of fragmentation. Grade I embryos were decided by uniform sized and shaped blastomeres of 6 to 8 in number, ooplasm having no granularity, and a maximum fragmentation of 5%. On day 3 to 5 after oocyte retrieval, determined according to the number of good quality embryos, 3 to 5 embryos with the best morphological grade were selected and transferred into the uterine cavity. Older patient age (≥35 years), poor embryo quality and previous failed cycles caused the number of embryos transferred to increase.

Luteal phase support was commenced on the day after oocyte pick-up and provided with daily i.m. injection of 75 mg progesterone in oil. Pregnancy was assessed 12 days after embryo transfer by measuring serum β-HCG concentration.

Outcome measures

The primary endpoint of this study was the oocyte/embryo quality in rLH supplemented ART cycles in either agonist long or antagonist protocols. Secondary variables evaluated were FSH consumption in IU, stimulation duration, peak E2 levels, number of total oocytes retrieved, fertilization rate, embryo transfer day, implantation rate (number of gestational sacs divided by the number of embryos transferred), clinical pregnancy rate per ET (pregnancy confirmed both by β-HCG measurement on day 12 after ET and by demonstration of intrauterine gestational sac(s) on transvaginal ultrasound 3 weeks after positive β-HCG test result), miscarriage rate (pregnancy confirmed by ultrasonic demonstration of an intrauterine gestational sac but subsequently lost before 12 weeks of gestation) and ongoing pregnancy rate (pregnancies >12 weeks gestational age).

Statistical methods

Results were expressed as mean ±SD. Between-group differences of continuous variables were analyzed with Student's *t*-test and, χ^2 test was used to assess differences in proportions. $P<0.05$ was considered statistically significant. Data was analysed with the Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc., USA) and MedCalc (MedCalc Software, Ghent, Belgium).

Results

A total of 351 ART cycles were analysed in the present investigation. GnRH agonist long protocol was administered in 184 cycles and antagonist in 167. Four treatment groups based on the combination of GnRH analogue and the gonadotropin preparation were formed; the agonist/rFSH alone group (n=117, Group 1), the agonist/rFSH plus rLH group (n=67, Group 2), the antagonist/rFSH alone group (n=98, Group A), and the antagonist/rFSH plus rLH group (n=69, Group B).

No significant differences were observed among the two gonadotropin regimes in either agonist long or antagonist protocols with regard to age, body mass index (BMI), day 3 FSH levels, infertility duration and infertility diagnosis (Table 1).

Tables 2 and 3 show comparisons among the two gonadotropin regimes in both agonist long and antagonist protocols regarding the oocyte and the embryological data, stimulation characteristics, and the treatment outcomes.

In the agonist long protocol, rFSH alone group revealed better results in terms of gonadotropin consumption, stimulation duration and the number of total oocytes retrieved (Table 2). When the oocyte quality defined by

the proportion of metaphase II oocytes to the total number of oocytes retrieved was evaluated, rFSH alone group yielded statistically higher ratio compared to rFSH plus rLH group (p=0.02). Embryo quality defined by the ratio of grade I embryos to all the embryos available on day 3 was similar in both gonadotropin regimes (Table 2). Therefore, oocyte quality was found to be significantly better, although the embryo quality was similar in rFSH alone group compared to rLH supplemented group in midluteal GnRH agonist long protocol. Implantation, clinical pregnancy, miscarriage and ongoing pregnancy rates were found to be statistically similar in both gonadotropin regimes (Table 2).

In the antagonist protocol, rFSH alone group revealed better results in terms of stimulation duration and the number of total oocytes retrieved (Table 3). With regard to the oocyte and the embryo quality, no significant differences were observed between the two gonadotropin regimes in the antagonist protocol (Table 3). Similarly, no significant differences were found between the two gonadotropin regimes in the antagonist protocol regarding the implantation, clinical pregnancy, miscarriage and the ongoing pregnancy rates (Table 3).

Discussion

This study evaluated the effect on the oocyte and the embryo quality of additional rLH in a normoresponder <40 years old IVF patient population stimulated either with GnRH agonist long or antagonist COH protocol. The results demonstrated that supplementary rLH exerted a negative effect on the oocyte quality, however no effect on the embryo quality in agonist long protocol. In the antagonist protocol, on the other hand, rLH addition exerted neither a beneficial nor an adverse effect on the oocyte and the embryo quality.

Table 1. Demographic characteristics of 351 patients on either midluteal GnRH agonist long or antagonist protocol

	Agonist/rFSH only (Grp 1, n=117)	Agonist/plus rLH (Grp 2, n=67)	Antagonist/rFSH only (Grp A, n=98)	Antagonist/plus rLH (Grp B, n=69)
Age (years)	30±4	30±5	31±5	32±5
Infertility duration (years)	6.9±3.9	7.2±4.8	7.8±5	7.8±4.6
D3 FSH (IU/l)	6.6±2.1	7.0±2.7	7.3±2.4	7.8±2.7
BMI (kg/m^2)	24.6±4.7	24.9±4.6	24.7±4.2	25.4±5.4
Infertility diagnosis, n (%)				
Tubal factor	23 (20)	10 (15)	9 (9)	14 (20)
Male factor	58 (50)	34 (51)	72 (73)	34 (49)
Endometriosis	2 (2)	3 (4)	3 (3)	1 (1.5)
Mixed	-	2 (3)	-	4 (6)
Unexplained	34 (29)	18 (27)	14 (14)	16 (23)

Values are mean ±SD or percentages.
No significant differences between groups in either stimulation protocols (p>0.05).

Table 2. Stimulation characteristics and treatment outcomes of 184 patients on midluteal GnRH agonist long protocol

	rFSH alone (Grp 1, n=117)	rFSH plus rLH (Grp 2, n= 67)	*p*
Total FSH used (IU)	2106±719	3094±989	<0.00001
HCG day	12.3±1.0	13.3±1.2	<0.00001
Endometrium on HCG day (mm)	11.5±2.1	11.6±2.3	NS
Serum E2 on HCG day (pg/ml)	3261±1055	3009±1096	NS
Total oocytes retrieved, n	17.6±5.8	15.0±6.8	0.005
MII oocytes/total oocytes retrieved, (%)	75	70	0.02
Fertilization, (%)	81	79	NS
Grade I embryos/all embryos at day 3, (%)	67	73	NS
Grade I embryos transferred, n	3.1±1.2	3.1±1.3	NS
ET day	3.8±0.9	3.5±0.9	NS
Implantation, (%)	16.8	17.4	NS
Clinical pregnancy/ET, (%)	39	37	NS
Miscarriage, (%)	15	12	NS
Ongoing pregnancy/ET, (%)	33	33	NS

Values are mean ±SD or percentages.
FSH: follicle stimulating hormone; HCG: human chorionic gonadotropin; E2: estradiole; ET: embryo transfer; NS: not significant (p>0.05).

Table 3. Stimulation characteristics and treatment outcomes of 167 patients on GnRH antagonist protocol

	rFSH alone (Grp A, n=98)	rFSH plus rLH (Grp B, n=69)	*p*
Total FSH used (IU)	3000±937	2972±1067	NS
HCG day	11.7±1.1	12.2±1.3	0.004
Endometrium on HCG day (mm)	11.2±2.2	11.4±1.7	NS
Serum E2 on HCG day (pg/ml)	2459±1115	2425±1062	NS
Total oocytes retrieved, (n)	16.3±7.5	12.8±5.1	0.001
MII oocytes/total oocytes retrieved, (%)	73	71	NS
Fertilization, (%)	77	79	NS
Grade I embryos/all embryos at day 3, (%)	70	69	NS
Grade I embryos transferred, (n)	3.3±1.3	2.9±1.4	NS
ET day	3.3±0.9	3.1±0.6	NS
Implantation, (%)	16.4	17.7	NS
Clinical pregnancy/ET, (%)	42	43	NS
Miscarriage, (%)	17	23	NS
Ongoing pregnancy/ET, (%)	35	28	NS

Values are mean ±SD or percentages.
FSH: follicle stimulating hormone; HCG: human chorionic gonadotropin; E2: estradiole; ET: embryo transfer; NS: not significant ($p>0.05$).

One possible adverse effect of administering GnRH agonists and also antagonists in stimulation protocols is an excessive reduction in LH levels compared to natural unstimulated cycles. From this observation, arguments came out that this could be avoided by the addition of exogenous LH in COH (1). Some investigators postulated that in GnRH agonist down-regulated cycles, there is sufficient residual endogenous LH for adequate folliculogenesis, steroidogenesis and pregnancy (11,25). Conversely, some others advocated the routine addition of LH in long agonist cycles (26). LH activity of 75 IU daily was suggested to be the appropriate dose providing clinical benefit (27).

Implantation was proposed to be improved by the addition of exogenous rLH in profoundly down-regulated women receiving only rFSH (28). Lisi et al. (29) showed that there was an increase in implantation, clinical pregnancy and delivery rates in patients stimulated with rFSH supplemented with rLH. Conversely, Bjercke et al. (30) showed that even with profoundly suppressed LH serum concentrations (<0.5 IU/L) the clinical outcome was unaffected. Some authors even proposed that LH supplementation could have detrimental effects on ovarian response and IVF outcome in down-regulated women (31).

In the long agonist cycles, as ovarian stimulation with rFSH progresses, the suppression of pituitary LH secretion becomes more effective and the concentrations of endogenous LH decrease (8). Two investigators evaluated rLH addition in agonist long protocol from day 6-8 onwards and found no detrimental effect on ovarian response and pregnancy outcome and even found better implantation rate in patients >35 years (32,33). De Placido et al. (5,34) suggested that ART cycles could be rescued by the substition of HMG or rLH from day 8 onward in pituitary down-regulated normogonadotropic young women with an initial poor response to rFSH and they demonstrated the beneficial effect of using HMG or rLH from the mid-follicular phase of the cycle on IVF outcome. Ferraretti et al. (35) also agreed with them. Tarlatzis et al. (36), on the other hand, found no benefit of exogenous rLH, added when the follicles reached 14 mm, on stimulation outcome in <37 years old women. Similarly, in the present study of normoresponder IVF patients <40 years old given long agonist protocol, both rLH supplemented group and rFSH alone group gave comparable results regarding the treatment outcome, however oocyte quality was found to be significantly lower in rLH supplemented one.

Balasch et al. (14) concluded that the addition of rLH to rFSH in pituitary-suppressed women did not improve the ovarian response and even might have a negative impact on oocyte maturation and fertilization. Our results support these partially since a higher percentage of MII oocytes was found in rFSH alone group compared to the rFSH plus rLH group in the long agonist protocol although the fertilization rates were similar. In the study by Balasch et al. (14), rLH was initiated from the beginning of stimulation, whereas we added rLH from the 6th day of stimulation onwards.

According to various studies, profoundly suppressed women having reduced follicular phase serum LH levels were identified in ART population as ranging between 12% to 70% (37). In another study, it was reported that in about 15% of patients down-regulated with GnRH agonists, suboptimal ovarian response to rFSH was observed which might be due to excessive pituitary LH suppression (38). Therefore, if LH supplementation is safe during COH, it might be encouraged in the long agonist cycles to prevent possible unexpected suboptimal response to stimulation with rFSH alone.

LH supplementation has also been evaluated in antagonist cycles. In a study with donor cycles and rLH supplementation, the antagonist initiated improved oocyte and embryo quality and also fertilization and implantation rates (39). Conversely, Cedrin-Durnerin et al. (17) suggested no benefit of rLH addition to the antagonist. Griesinger et al. (40) reported no benefit of additional rLH except for higher peak E2 levels when rLH was combined with rFSH from the beginning of stimulation. Even profound LH suppression after antagonist administration was claimed to be associated with a significantly higher ongoing pregnancy rate (18). Nonetheless, some clinicians have suggested that the decrease or plateau in E2 concentrations after antagonist administration in cycles stimulated with rFSH, which are most likely the result of oversuppression of LH, might compromise the pregnancy outcome (41). Therefore, it is worth considering LH supplementation in antagonist cycles as no adverse effect on oocyte and embryo quality and also pregnancy outcome was observed in the present study.

Miscarriage rate according to rLH supplementation was also investigated in our study. It was proposed that while the use of GnRH agonist led to a fall in LH levels from the beginning of the follicular phase and, as a result, a significant increase in the rate of abortion in profoundly suppressed LH group (9). Merviel et al. (42) showed that antagonist-rFSH protocols maintained reasonable LH levels at the beginning of the follicular phase and did not therefore give harm to the course of pregnancies thus obtained, suggesting that the LH concentrations <0.5 IU/L in the late follicular phase, after antagonist administration, did not interfere with the pregnancy rate or outcome. We did not observe any significant difference between rFSH alone and rFSH plus rLH groups in either agonist long or antagonist COH protocols with respect to miscarriage and ongoing pregnancy rates.

Serum LH levels were monitored in most of the studies at some points during COH. However, existing data are controversial for recommending rLH addition in IVF based on LH measurements (9,11,43,44). Moreover, not the LH concentration itself but the direction and rate of change in LH concentrations was suggested to be effective in follicular growth (19). In our center we are not monitoring LH levels routinely during stimulation.

In conclusion, this study is one of the largest series investigating the impact of rLH addition in agoinst long and the antagonist COH cycles, mainly on the oocyte and the embryo quality. Our results have demonstrated that supplemental rLH during stimulation in the long agonist protocol has a significant negative effect on the oocyte quality, without a similar effect on the embryo quality and the treatment outcome in normoresponder women <40 years old. In the antagonist protocol, on the other hand, rLH addition had no beneficial or detrimental effect on the oocyte and the embryo quality and also the treatment outcome in normoresponder women <40 years old.

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