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The effect on clinical results of adding recombinant LH in late phase of ovarian stimulation of patients with repeated implantation failure: a pilot study

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Abstract. – OBJECTIVE: To evaluate the efficacy of recombinant LH (r-LH) addition in the late phase of ovarian stimulation in patients with repeated implantation failure (RIF).

PATIENTS AND METHODS: 66 infertile couples undergoing ICSI treatment due to male factor were allocated to group A (33) and to group B (33). Group A (29 subjects) received recombinant FSH (r-FSH) supplemented by r-LH in the late follicular phase starting the same day of GnRH-antagonist (GnRH-ant) administration, and group B (32 subjects) received r-FSH alone. All patients were stimulated with a GnRH-ant flexible protocol starting r-FSH on day 2 of a spontaneous or induced cycle. hCG (10000 IU) was administered by intramuscular route when at least 2 follicles reached 18 mm in diameter.

RESULTS: Metaphase II (MII) oocytes with cytoplasmic maturation showed a significant difference in the r-LH group (89.02%) compared to the one with FSH alone (81.15%) ($p < 0.01$). The number of positive pregnancy test, 14 (48.3%) and 8 (25%), was significantly greater in the r-LH group compared to the group treated with r-FSH alone ($p < 0.03$). The number of gestational sacs was 20 in the r-LH group vs. 9 in the r-FSH group ($p < 0.001$). The implantation rate was significantly higher in the r-LH group compared to the r-FSH only group (19% *vs.* 7% respectively; $p < 0.01$). Also, a lower abortion rate was found in the r-LH group (21% *vs.* 37.5% respectively – $p < 0.01$).

CONCLUSIONS: Ovarian stimulation should be personalized because it seems that some subgroups of patients, like those with RIF, reach a better clinical outcome with the addition of r-LH in the advanced follicular phase stimulation.

Key Words:
rLH, Repeated implantation failure, IVF.

Introduction

During the last decades, GnRH-agonist has been widely used as a standard protocol for assisted reproductive technologies. More recently GnRH-antagonist was introduced to control the endogenous LH surge[1]. GnRH-ant acts by fast suppression of gonadotropin release, without the flare-up effect. While the GnRH agonists act via downregulation, the GnRH-ant specifically block GnRH receptors and induce a decrease in both LH and FSH secretion[2].

For these reasons, LH supplementation has been considered useful in GnRH-antagonist cycles[3]. However, the use of LH supplementation in addition to GnRH-ant in women undergoing *in vitro* fertilization (IVF) raised a debate in the scientific community and is still under full evaluation[4].

LH plays a key role in the intermediate-late phases of folliculogenesis[5,6]. The presence of receptors for LH in cumulus granulosa cells and its correlation with oocyte maturation has been demonstrated[7]. Ovarian stimulation is achieved by the administration of r-FSH alone, although some subgroups of women may also benefit from r-LH supplementation[8]. For example, it was demonstrated that supplementation with LH activity improves the clinical outcome in advanced age women[9]. In particular, the combined use of r-LH and r-FSH versus r-FSH alone results in a similar number of metaphase II oocytes but displays a better fertilization rate, suggesting that the oocytes retrieved were of better quality, leading to higher implantation rates[9].

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A subgroup of women who might well benefit from r-LH supplementation is that at high risk of poor ovarian response (selected in accordance with the Bologna criteria) when treated with GnRH-agonist. In these patients, LH pretreatment was shown to improve oocyte quantity and quality[4]. Some studies by Sauer et al[11], Griesinger et al[12], and Levi-Setti et al[13] did not demonstrate beneficial effect to the oocytes quality and pregnancy outcomes after the supplementation of r-LH.

Recent data demonstrate that in a flexible GnRH-ant administration protocol, the addition of r-LH in the ovarian stimulation improves the number of mature oocytes retrieved, when compared to the standard GnRH-agonist flare-up protocol. The r-LH calibrated administration seems to improve the ovarian outcome, especially in patients older than 35 years, in those with an initial abnormal ovarian response to r-FSH monotherapy, and in the 'low prognosis' women treated with GnRH-ant[2].

Furthermore, the recent meta-analyses by Xiong et al[14] and Lehert et al[15] suggest that the combination between r-LH and r-FSH may be beneficial in poor responder women, although a review and meta-analysis did not demonstrate advantages of r-LH supplementation in comparison with r-FSH alone in GnRH antagonist protocol.

It is well known that LH plays an essential role in physiologic oocyte maturation[16]. Low LH levels in IVF cycles are associated with lower quality of embryos[17] while some studies found that a higher level of LH in follicular fluid was correlated to an increased number of successful pregnancies[18]. The efficacy of r-FSH for ovarian stimulation is well established; however, the role and the efficacy of supplementary r-LH are less clear[19].

Different researches[20-23] have demonstrated that LH exerts several roles in follicular development, ovulation induction and in oocyte maturation process, such as the completion of meiosis and the extrusion of the first polar body. Researchers have compared r-FSH *vs.* highly purified HMG (hp-HMG), a gonadotrophin that has LH like activity. Their results demonstrated a 3% higher ongoing pregnancy rate in the hp-HMG group compared with r-FSH alone[24].

RIF is determined when embryos of good quality fail to implant following several IVF treatment cycles[25]. Different fertility centers practicing IVF may use different definitions for RIF because no formal definition exists, but the most common definition for RIF is the failure of implantation in at least three consecutive IVF attempts, in which 1-2 embryos of high-grade quality are transferred in each cycle[26,27].

In our study, we aim to evaluate if adding r-LH in the late phase of stimulation can improve the clinical outcome in patients with RIF.

Patient and Methods

Patients

The study was conducted at the Biofertility IVF Center in Rome, Italy, between May 2014 and September 2015 on infertile couples due to male factors undergoing ICSI treatment. The study was reviewed and approved by the Institutional Review Board at the Biofertilty IVF Center. Data collection followed the principles outlined in the Declaration of Helsinki; all patients provided their informed consent agreeing to supply their own anonymous information for this and future studies.

ClinicalTrials.gov Identifier: NCT03204253

Patients included in the study had regular spontaneous menstrual cycles (26-39 days) and were aged < 42years. All patients had acceptable follicular phase serum concentrations of FSH (≤ 10 IU/L), LH (< 10 IU/L) and estradiol (< 60 pg/ml), body mass index (BMI) ≤ 30 kg/m^2, presence of both ovaries and normal uterine cavity. Only patients with RIF in at least two previous IVF cycles were included. Patients were excluded from the study if they had any clinically significant systemic disease, polycystic ovarian syndrome (PCOS), a previous history of severe ovarian hyperstimulation syndrome (OHSS), abnormal gynecological bleeding of unknown origin, and history of intolerance to any agents used in the study. This is a prospective, open-label, parallel arm study.

Patients were randomly allocated in two groups (A and B). Randomization was conducted by a computerized random number generator. All patients were stimulated with a GnRH-ant flexible protocol using r-FSH and starting on day 2 of spontaneous or estroprogestin induced cycles. hCG (10000 IU) was administered by intramuscular route when at least 2 follicles reached 18 mm in diameter.

Group A included 29 women stimulated with r-FSH supplemented by r-LH in the late follicular phase started at the same time of GnRH-ant administration.

Those patients received 75 IU of r-LH daily and 150 IU about 12 hours before triggering ovu-

lation with hCG.

Group B included 32 women who were stimulated with only r-FSH. The oocytes were decumulated and assessed for the maturation 2 hours after pick up, which was done 36 hours after hCG injection. Mature oocyte should have an intact first polar body and homogeneously fine granular and light-colored ooplasm[28]. The rate of metaphase II (MII) oocytes was calculated, and the MII oocytes were assessed for cytoplasmic morphology and maturity. MII oocytes with a light color and fine homogeneous granulate ooplasm were considered with normal morphology and classified as oocytes that have completed their cytoplasmic maturation.

Embryo transfer was performed 2 days after ICSI. The pregnancy test was done 12 days after embryo transfer, and clinical pregnancy and number of gestational sacs were assured 2 weeks after positive pregnancy test with the presence of fetal heartbeats.

Statistical Analysis

The mean, SD, *t*-test and x^2-test were used for statistic calculations of the results; $p < 0.05$ was considered to be statistically significant.

Results

A total of 66 infertile couples undergoing ICSI treatment for male factor were included in the study and assigned 1:1 in two study groups, 33 in group A and 33 in group B.

Four patients in group A and one patient in group B were protocol violators and were excluded from the study. Thus, our final population consisted of 61 women, 29 in group A and 32 in group B.

There were no statistically significant differences between the two study groups for any demographic characteristic assessed: woman's age, BMI, basal gonadotropin and estradiol levels, duration of stimulation, number and quality of retrieved oocytes (Table I). The mean number of embryo transferred was 3.7 ± 1.6 and 3.9 ± 1.8 in the r-LH group and r-FSH group, respectively.

Details on oocytes, embryos, and pregnancies are shown in Table II; only in the r-LH group a higher number of positive pregnancy test ($p < 0.03$), gestational sacs ($p < 0.001$), implantation rate ($p < 0.01$) and a lower percentage of abortion rate ($p < 0.01$) was found.

Discussion

Our findings demonstrated that the addition of r-LH to r-FSH, in the late phase of ovarian stimulation and until a few hours before hCG administration, is associated with significantly higher clinical pregnancy and implantation rates in patients with RIF. Our data also showed that lower amount of gonadotrophins and fewer days of stimulation were required in the r-LH group. This means higher and quicker response to r-FSH stimulation; this was confirmed also by higher estradiol levels on the day of hCG. Probably this result is mediated by activation of granulosa cell LH receptors. Considering the strict relationship between oocyte and cumulus granulosa cells, the higher cytoplasmic maturity observed in the

Table I. Demographic characteristic.

	Group A rFSH+rLH (n = 29)	Group B rFSH (n = 32)	*p*-value
Age (yr)	40 ± 4.2	39.4 ± 3.7	ns
BMI (kg/m^2)	21.5 ± 5.9	23.3 ± 5.9	ns
Basal FSH (mIU/mL)	7.5 ± 2.4	6.9 ± 2.5	ns
Basal LH (mIU/mL)	5.7 ± 3	5.3 ± 2.8	ns
Basal estradiol (pg/mL)	48 ± 20.4	50.6 ± 21.7	ns
Duration of stimulation (d)	10.1 ± 1.2	11.3 ± 1.3	< 0.05
Total dose of FSH required (IU)	3122.6 ± 525	3852.5 ± 664	< 0.01
Estradiol on day of hCG administration (pg/mL)	1779 ± 848	1591 ± 673	< 0.01
Retrieved oocytes	7.2 ± 4.8	7.3 ± 5.3	ns
Mature oocytes	5.8 ± 4	5.9 ± 4.3	ns
Fertilized oocytes	5.6 ± 3.7	5.4 ± 3.3	ns
Grade I embryos	3 ± 2.1	2.9 ± 1.5	ns
Embryo transferred	3.7 ± 1.6	3.9 ± 1.8	ns

Table II. Outcomes differences between treatment with rFSH plus r-Lh and rFSH alone.

	Group A rFSH+r-Lh (n = 29)	Group B rFSH (n = 32)	*p*-value
Total no. of oocytes retrived	203	236	ns
Total no. of Metaphase II oocytes (%)	164 (80.7%)	191 (80.9%)	ns
Total no. of MII with cytoplasmi maturation (%)	146 (89.02%)	155 (81.15%)	< 0.01
Total no. of fertilized	159	174	ns
Fertilization rate	96.9%	91%	ns
Total no. of embryo transferred	104	129	ns
No. of pregnancy test positive	14 (48.3%)	8 (25%)	< 0.03
No. of gestional sac	20	9	< 0.001
Implantation rate	19%	7%	< 0.01
Abortion rate	21% (3/14)	37.5% (3/8)	< 0.01

oocytes of the r-LH group is not surprising. LH starts to increase many hours before its surge for ovulation triggering. LH surge is responsible for completing meiosis and oocyte maturation with extrusion of the first polar body; furthermore, LH plays a key role in the intermediate-late phases of folliculogenesis[29]. Moreover, LH activity has an important role during early follicular recruitment by increasing the FSH receptors as well as maintaining follicular development during later follicular maturation, by enhancing steroid precursors[30].

It has been observed that some subgroups of women might benefit from LH addition during the ovarian stimulation[31]. Moreover, some authors found improved outcomes with r-LH supplementation in an unselected group of women undergoing follicular stimulation for IVF[32].

Mendoza et al[18] corroborated these findings demonstrating higher levels of LH in the follicular fluid of oocytes that implanted successfully in IVF cycles. Of note, in this study, hp-HMG stimulation protocol was used. Despite LH activity is present in this type of gonadotrophins, we cannot rule out that r-LH molecule has a specific function in oocyte quality and maturation. If this is true, adding r-LH during ovarian stimulation may be useful. Furthermore, an LH antiapoptotic effect on follicles and oocytes treated with LH has been recently described[33,34].

Our results are in line with previous work that shows the benefit of LH supplementation in selected patient's populations. In particular, we demonstrated that in women treated with ICSI due to male factor infertility and diagnosed with recurrent implantation failure, addition of LH in the late phase of ovarian stimulation significantly improves the clinical outcome when compared to FSH treatment alone.

In patients with RIF, oocyte meiosis during maturation may be impaired and result in aneuploid oocytes, a hypothesis that should be confirmed by genetic screening on oocytes. In the future, it will be interesting to investigate if a correlation exists between oocyte aneuploidies and hormonal patterns, including LH levels, during ovarian stimulation with r-LH. Moreover, investigating the physiology of younger women, instead of advanced maternal age patients[35], could enhance our understanding of the more natural and physiological processes that lead to the formation of competent oocytes.

Conclusions

These preliminary data demonstrate that adding r-LH during the late phase of ovarian stimulation improves the clinical outcome of patients with RIF.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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