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ORIGINAL RESEARCH

Comparison of *in vitro* Fertilization/Intracytoplasmic Sperm Injection Outcomes in Patients receiving Recombinant Human Luteinizing Hormone *vs* Human Menopausal Gonadotropin Supplementation

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ABSTRACT

Objectives: To compare the outcome of recombinant human luteinizing hormone (rh-LH) and human menopausal gonadotropin (hMG) supplementation in women undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) with recombinant follicle stimulating hormone (FSH) in the long gonadotropin-releasing hormone (GnRH) agonist stimulation protocol.

Materials and methods: It was a retrospective analysis of the case records of 90 consecutive women who underwent nondonor IVF/ICSI cycle with long GnRH agonist. All women received recombinant FSH on day 2/3 of the programming cycle. When the level of LH was < 0.5 mIU/mL during any phase of stimulation, then addition of LH either as rh-LH or hMG is given along with recombinant FSH.

Results: The number of oocytes collected, the number of oocytes in metaphase II (MII), and fertilization rate were similar in both groups. In addition, the mean number of embryos produced per cycle and the mean number of frozen embryos per cycle were similar in both groups. The cost of gonadotropin is similar in both groups. The ongoing pregnancy rate at 12 weeks was 20.4% after rh-FSH + hMG and 29.2% after rh-FSH + rh-LH (p-value = 0.092).

Conclusion: Supplementing recombinant FSH with recombinant LH (rh-LH) when compared with hMG does not show statistically significant increase in pregnancy rates. However, this study was a pilot venture to introduce the rh-LH into our practice and further randomized study is required to substantiate its use in assistive reproductive technology.

Keywords: Follicle stimulating hormone, *In vitro* fertilization/intracytoplasmic sperm injection outcome, Recombinant luteinizing hormone.

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INTRODUCTION

The pharmacology of ovarian stimulation has been strongly influenced by the two-cell, two-gonadotropin theory while, historically, stimulation protocols have included both luteinizing hormone (LH) and follicle stimulating hormone (FSH) in an attempt to mimic normal physiology.[1] The introduction of gonadotropin-releasing hormone (GnRH) agonist in the mid-1980s successfully circumvented the problems of a premature LH surge. There has also been a gradual shift from hMG with equal amounts of FSH and LH-like activity over pure urine-derived FSH preparations to recombinant FSH (rh-FSH), without LH activity.

According to the LH threshold theory, if minimum serum LH levels are not maintained, the consequent levels of estradiol will not be sufficient for endometrial proliferation and corpus luteal formation.[2] However, exposure of the developing follicle to inappropriately high concentrations of LH was associated with poor oocytes quality, reduced rate of fertilization and embryo implantation, and high rate of miscarriage. Thus, high levels of LH, which promotes follicular atresia and early miscarriage, led to the concept of a therapeutic window of LH.[2]

In India, assisted reproduction is self-financed and hence cost is a major factor to be considered, when introducing new drugs. This concern prompted us to use the less expensive option of human menopausal gonadotropin (hMG). But variability in LH content and the presence of human chorionic gonadotropin (hCG) as an LH substitute in these preparations made it difficult to provide controlled LH dosing.

The use of recombinant DNA technology has permitted the production of a pure preparation of recombinant LH (rh-LH). This preparation is well characterized, and is subject to tight regulation of product content and quality. This study was done to compare the clinical pregnancy in patients receiving rh-LH *vs* hMG in GnRH agonist cycle.

AIMS AND OBJECTIVE

To compare the outcome of rh-LH supplementation and hMG in women undergoing *in vitro* fertilization (IVF) with rh-FSH in the GnRH agonist protocol.

- The primary outcome was clinical pregnancy rate per embryo transfer and ongoing pregnancy.
- The secondary outcomes included total gonadotropin usage, mean duration of stimulation, number of mature oocytes retrieved, fertilization rate, implantation rate, and cost per cycle.

MATERIALS AND METHODS

The present study was carried out in the Institute of Reproductive Medicine and Women's Health, Madras Medical Mission Hospital in Chennai, Tamil Nadu, India from 2010 to 2011. The study was approved by Institutional Review Board. It was a retrospective analysis of the case records of 90 consecutive women who underwent nondonor *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles in our institute.

Inclusion criteria

- All patients undergoing IVF/ICSI using long agonist protocol.

Exclusion criteria

- Patients undergoing egg donation/embryo donation program.

Ovarian Stimulation

All women underwent controlled ovarian hyper stimulation using long agonist protocol. This involved initial downregulation with GnRH agonist from the mid-luteal phase (day 21) of the preceding cycle and maintained till hCG administration. Downregulation was confirmed by serum estradiol <50 pg/mL, endometrial thickness less than 5 mm, and no follicle >10 mm. Patients were then started on daily subcutaneous rh-FSH to initiate follicular development and recruitment. The initial dose of rh-FSH was based on age, body weight, baseline FSH, previous response, and clinical judgment.

Patients were maintained on the same dose of rh-FSH for the first 5 days. Ultrasound was done on the stimulation day 6, and depending on the progress of the follicular development, the dose of rh-FSH was adjusted. Luteinizing hormone supplementation was given on days 6 to 9, either through rh-LH or hMG whenever there is hyporesponse, i.e., no follicle >10 mm, E2 <200 pg/mL, baseline serum LH <0.5 IU/L. If the patient is started on rh-LH, the dose of rh-FSH was maintained and rh-LH 37.5 U/day was added. Doses of both rh-FSH and hMG were titrated based on the progress of follicular development, assessed through ultrasound once in every 2 days. When ≥ 3 lead ovarian follicles reached a diameter of 18 mm, oocytes maturation was initiated with an intramuscular injection of 10,000 U of hCG.

Oocyte Retrieval, Sperm Processing, and IVF/ICSI

Oocyte retrieval was performed under intravenous sedation guided by transvaginal ultrasound, 35 hours after administration of hCG. After oocytes aspiration, the follicular fluid was examined for cumulus-corona-oocytes complexes. On the day of oocytes retrieval, the male partners were asked to produce semen samples for the IVF or ICSI procedure in sterile specimen containers. Semen samples were washed using sperm washing media. The resulting pellet was used directly for ICSI or IVF.

Gamete Handling, Embryo Culture, Transfer

Oocyte-cumulus complexes were collected from follicular fluid after observation under a stereomicroscope. Excess cumulus was removed immediately, washed in IVF media, and transferred to fertilization media. The oocytes were then placed in the CO_2 incubator until the denudation procedure. Oocytes were fertilized using either conventional IVF or ICSI.

In the ICSI procedure, oocytes were subjected to the denudation procedure 3 hours post retrieval by exposing them to hyaluronidase solution for 30 seconds. Any residual adherent cumulus cells were removed mechanically by use of flexipets of appropriate size. The oocytes were assessed for maturity by observing the presence of a first polar body. Mature metaphase II (MII) oocytes were subjected to the ICSI procedure and incubated in fertilization media.

In the IVF procedure, the required numbers of motile spermatozoa were calculated for each oocytes and the fertilization media containing oocytes-cumulus complex was inseminated.

Fertilization was assessed 16 to 18 hours post insemination or injection for both IVF and ICSI. Oocytes with two pro nuclei and having a second polar body were classified as fertilized. The fertilized oocytes were washed and cultured in cleavage media for 48 hours. Before transfer, embryos were graded based on the morphological condition. Oocytes with expanded cumulus, radiant corona, distinct zona pellucida, clear cytoplasm, unfragmented first polar body, and those without debris in the perivitelline space were considered as good. From three to four embryos were transferred 72 hours post retrieval and any surplus grade I and II embryos were cryopreserved by slow-freezing method.

At our center, our embryo transfer policy was to transfer 3-day three embryos. The number of embryos transferred was decided in consultation with couple. The embryo transfers were done using soft catheter (labotect) under ultrasound guidance.

Luteal support was provided by use of micronized vaginal progesterone pessaries in a dose of 400 mg twice daily for 18 days post oocyte retrieval. In addition, 100 mg intramuscular (IM) progesterone and 6 mg of estradiol valerate was administered. Serum beta hCG was done on 18th day following oocyte retrieval and if positive, a transvaginal ultrasound was done 7 days later to detect and confirm intrauterine pregnancy.

Statistical Analysis

For quantitative variables the parametric t-test was used to compare means between the rh-LH and hMG groups. For qualitative variables, the chi-squared test was used to compare difference between the two groups. Statistical Package for the Social Sciences (SPSS) was used for statistical analysis. $p < 0.05$ was considered significant.

RESULTS

The baseline variables that affect ovarian response to stimulation, including age, body mass index (BMI) between the two groups, were listed in Table 1. There was no statistically significant difference in age between the two groups. The BMI was significantly less in the rh-LH group (mean BMI of 24.6 *vs* 27 for hMG).

The causes for infertility are listed in Table 2. Male factor was the most common cause of subfertility in both the groups (40%). The clinical and laboratory outcomes are listed in Table 3. The total gonadotropin dose was significantly higher in the rh-LH group, with a mean total FSH dose of 2191 *vs* 1819.7 U for the hMG group (p = 0.033). The recombinant human LH dose was significantly less, with a mean total rh-LH dose of 392 *vs* 1233.8 U for the hMG group (p = 0.000). There was no statistically significant difference in the length of stimulation and cost of total gonadotropin required between the two groups. The follicles which are more than 16 mm were significantly higher in the rh-LH group, with a mean of 9.8 *vs* 8.0 for the hMG group (p = 0.013). There was no significant difference in the mean number of oocytes collected between the two groups (p = 0.983). The number of oocytes collected, the number of oocytes in MII, and fertilization rate were similar in both groups. In addition, the mean number of embryos produced per cycle and the mean number of frozen embryos per cycle were similar in both groups.

A total of 45 women were studied from hMG group and 45 women from rh-LH group. Out of 45 women, who underwent ovarian stimulation in hMG group, only 44 women had fresh embryo transfer and one woman had elective cryopreservation of embryos in view of severe ovarian hyperstimulation syndrome (OHSS).

In the rh-LH group, 45 women had ovarian stimulation but only 41 women had embryo transfer and the remaining four women had elective cryopreservation of embryos in view of OHSS (Table 4).

Table 1: Age and BMI of the patients

	hMG (n = 45)	*rh-LH (n = 45)*	*p-value*
Mean age (SD)	31.4 (3.6)	30.4 (3.6)	0.163 NS
Women ≤ 35 years (n, %)	38 (84.4)	41 (91.1)	
Women > 35 years (n, %)	7 (15.6)	4 (8.9)	
BMI (mean, SD)	27 (4.3)	24.6 (3.0)	0.002 S

NS: Nonsignificant; S: Significant

Table 2: Causes of infertility

	hMG (n = 45)	*rh-LH (n = 45)*
Causes of infertility		
Male, n (%)	12 (26.6)	24 (53.3)
Anovulation, n (%)	5 (11.1)	3 (6.6)
Tubal, n (%)	12 (26.6)	4 (8.8)
Endometriosis, n (%)	2 (4.4)	4 (8.8)
Unexplained, n (%)	5 (11.1)	3 (6.6)

Table 3: Total dose of FSH, total dose of HMG/rh-LH, days of stimulation, total cost of FSH + HMG/rh-LH, follicles ≥ 16 mm, estrogens on the day of hCG, oocytes aspirated, number of MII oocyte, number of embryos fertilized, and endometrial thickness

	hMG (n = 45)	*rh-LH (n = 45)*	*p-value*
Total dose of FSH mean (SD)	1819.7 (655)	2191.0 (952.8)	0.033 S
Total dose of hMG/ rh-LH mean (SD)	1233.8 (860.4)	392.0 (279.7)	0.000 S
Days of stimulation mean (SD)	10.6 (1.6)	10.4 (1.5)	0.692 NS
Total cost of FSH + HMG/rh-LH, mean (SD)	69085.8 (25357.8)	69894.7 (30211.1)	0.890 NS
Follicles ≥ 16 mm, mean (SD)	8 (3.9)	9.8 (2.6)	0.013 S
Estrogen on the day of hCG, mean (SD)	2135.9 (1268.6)	2291.8 (1687.4)	0.621 NS
Oocyte aspirated, mean (SD)	10.3 (5.0)	10.3 (5.3)	0.983 NS
No. of MII oocyte, mean (SD)	7.5 (3.5)	6.7 (4.3)	0.340 NS
No. of embryos fertilized, mean (SD)	7.7 (3.9)	6.9 (4.6)	0.383 NS
Endometrial thickness, mean (SD)	10.3 (1.3)	10.3 (1.2)	0.855 NS

S: Significant; NS: Nonsignificant

Table 4: Elective cryopreservation of embryos and OHSS

	hMG (n = 45)	*rh-LH (n = 45)*	*p-value*
No. of patients for whom elective cryopreservation of embryos	1	4	
No. of OHSS, n (%)	3 (6.6)	4 (8.8)	0.694 NS

NS: Nonsignificant

Table 5: Number of embryos transferred, frozen, and pregnancy rate

	hMG (n = 44)	*rh-LH (n = 41)*	*p-value*
No. of embryos transferred, mean (SD)	3.5 (0.9)	3.2 (1.2)	0.219 NS
No. of embryos frozen, mean (SD)	1.6 (4.1)	1.7 (3.9)	0.917 NS
Pregnancy rate, n (%)	16 (36.3)	14 (34.1)	>0.05 NS
Ongoing pregnancy rate, n (%)	9 (20.4)	12 (29.2)	>0.05 NS

NS: Nonsignificant

There were no statistically significant differences in the clinical pregnancy between both the groups (36.3 *vs* 34.1%; p > 0.05). But the ongoing pregnancy was high in the rh-LH group when compared to hMG but not statistically significant (29.2 *vs* 20.4%; p-value > 0.05) (Table 5).

DISCUSSION

The present study was carried out in the Institute of Reproductive Medicine and Women's Health, Madras Medical Mission Hospital Chennai, Tamil Nadu, India from 2010 to 2011. It was a retrospective analysis of the case records of 90 consecutive women who underwent nondonor IVF/ICSI cycles in our institute. A total of 45 women were studied from hMG group and 45 women from rh-LH group.

There were no statistically significant differences in the clinical pregnancy between both the groups (36.3 *vs* 34.1%; p = 0.83). But the ongoing pregnancy was high in the rh-LH group when compared to hMG but not statistically significant (29.2 *vs* 20.4%; p = 0.09).

In our study, majority of women (48%) were in the 30 to 35 years age group. Age has a definite effect on pregnancy rate. A higher conception rate in younger women may be attributed to their fertility potential. There was no statistically significant difference in age between the two groups.

The BMI was significantly less in the rh-LH group when compared to hMG group (mean BMI of 24.6 *vs* 27 for hMG). That may be one of the reasons for increased requirement of total gonadotropin in the hMG group.

Male and female factors each account for approximately 35% of cases. Often, there is more than one factor, with male and female factors combined causing 20% of infertility. In the remaining 10% of cases, the etiology is unknown. In our study, it was the male factor (40%) that accounted for the most common reason for undergoing subfertility treatment in both the groups.

In our study, the results showed that the total gonadotropin required was lower for the combination of rh-FSH and rh-LH than for the rh-FSH and hMG treatment procedure. This may be because of two reasons, i.e., the group receiving hMG had higher BMI (27) when compared with the group receiving rh-LH (24.6). The other reason being superior consistency, purity, and accuracy of dosing of the rh-FSH and rh-LH preparations and it is consistent with previous randomized controlled trials (RCTs) in ovulation induction, as reported by Hugues et al[3] who used recombinant follitrophin alpha, and in assistive reproductive technology (ART), as reported by Bergh et al[4] who compared follitrophin alpha with highly purified (HP)-uFSH.

We found differences in clinical pregnancy rate between patients treated with rh-FSH combined with rh-LH and those treated with rh-FSH and hMG, though not statistically significant. We postulate that this could be because of differences between the effects of rh-LH and hMG on oocyte quality and, ultimately, embryo quality.

A recent Cochrane meta-analysis on RCTs comparing rh-FSH only *vs* rh-FSH and rh-LH stimulation procedures reported no evidence of statistically significant pregnancy outcomes when rh-LH was used.[5] However, the authors concluded that further large RCTs should be undertaken using long GnRH agonist downregulation procedures, because all pooled pregnancy estimates, although not statistically different, probably because of the small numbers, point toward a beneficial effect of co-treatment with rh-LH, particularly with regard to pregnancy loss and poor responders.

Ferraretti et al[6] reported difference in clinical pregnancy success of 54% (rhLH) *vs* 11% (hMG) in an RCT that compared addition of rh-LH or hMG for a group of ART patients with a suboptimum response in a long GnRH agonist stimulation cycle. Likewise, after a recent RCT, Carone et al[7] reported clinical pregnancy success of 57.9% in hypogonadotropic hypogonadism (HH) patients stimulated with a fixed-dose combination of 150 U rh-FSH and 75 U rh-LH *vs* 17.2% clinical pregnancy success for HH patient stimulated with a hMG 150 U (n = 24, p = 0.003).

In a RCT, Grøndahl et al[8] reported that mRNA expression of the LH receptor and other genes involved in cholesterol and steroid biosynthesis was reduced in the granulosa cells of the patients treated with hMG. Meta-analysis by Lehert et al[9] has evidenced that high exposure to hMG is related to altered endometrial receptivity due to premature progesterone elevation. This consequently led to reduced implantation and pregnancy rates.

Although these results must be confirmed by larger RCTs, there seems to be a growing body of evidence of a positive effect of rh-LH in some ART patients. Based on extensive evidence, the Asia Pacific Fertility Treatment Advisory Group now recommends the use of rh-LH in women with prior poor response to controlled ovarian hyperstimulation, women with suboptimal response in an ongoing treatment cycle, and women aged > 35 years.

Use of rh-FSH combined with rh-LH in long GnRH agonist ART cycles was associated with more ongoing pregnancies. Other parameters like oocytes retrieval, gonadotropin requirement, total days of stimulation, estrodial level on the day of HCG, fertilization rate, and the cost of the cycle were similar between both the groups.

However, this study was a pilot venture to introduce the recombinant LH into our practice and further randomized study are required to substantiate its use in ART. Future studies will have to further define the subgroups of patients whose cycle has to be managed with additional LH.

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