Reprod Med Biol (2012) 11:129–133
DOI 10.1007/s12522-012-0120-2

ORIGINAL ARTICLE

Combination of recombinant follicle stimulating hormone with human menopausal gonadotrophin or recombinant luteinizing hormone in a long gonadotrophin-releasing hormone agonist protocol: a retrospective study

Gottumukkala Achyuta Rama Raju · S. C. Teng · Prathigudupu Kavitha · Balabomma Kavitha Lakshmi · Cheemakurthi Ravikrishna

Received: 21 November 2011 / Accepted: 23 January 2012 / Published online: 18 February 2012

Abstract
Purpose To assess the effect of supplementation with recombinant human luteinizing hormone (rhLH) for patients treated either with recombinant follicle stimulating hormone (rFSH) plus rhLH or with rFSH plus human menopausal gonadotrophin (HMG) in a long gonadotrophin-releasing hormone (GnRH) agonist-stimulation protocol.
Methods A single-centre, retrospective analysis of patients with hypo responsiveness to a long GnRH agonist protocol ($n = 174$), with consecutive in-vitro fertilization or intracytoplasmic sperm injection cycles, compared the outcomes of long luteal GnRH agonist ovarian stimulation using rFSH combined with HMG ($n = 100$) versus rFSH combined with rhLH ($n = 74$). The endpoints included clinical pregnancy, number of oocytes retrieved, and total gonadotrophin dose.
Results Significantly more clinical pregnancies were achieved after stimulation with rFSH and rhLH than after stimulation with rFSH and HMG (35.1 vs. 19%, $p < 0.01$). More oocytes were recovered (13.1 vs. 11.3, $p = 0.024$) with less FSH utilized in the rFSH and rhLH group than in the rFSH and HMG group (2706.4 vs. 4134.2 U, $p < 0.001$).
Conclusions Use of rFSH combined with rhLH in long GnRH agonist assisted reproductive technology (ART) cycles was associated with more clinical pregnancies, recovery of more oocytes, and reduction in gonadotrophin use, suggesting that the superior purity and consistency of rFSH and rhLH may result in better clinical outcomes.

Keywords ICSI · IVF · LH · Ovarian stimulation · Recombinant FSH

G. A. R. Raju (✉) · P. Kavitha · B. K. Lakshmi · C. Ravikrishna
Krishna IVF Clinic, Zillaparishad Junction, Maharanipeta, Visakhapatnam 530 002, Andhra Pradesh, India
e-mail: krishnaivf@yahoo.com

S. C. Teng
Merck Pte Ltd, 3 International Business, Park #02-01, Singapore 609927, Singapore

Introduction

Women treated with gonadotrophin-releasing hormone (GnRH) agonists or antagonists during ovarian stimulation may experience reduced luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations because of over-suppression of endogenous LH and FSH pituitary secretion. Documented results associate poorer outcomes with patients whose LH concentration was low after gonadotrophin-releasing hormone (GnRH) agonist treatment [1–3]. A minimum concentration of LH is required for optimum theca cell function and subsequent oestradiol synthesis in the granulose cells. Human menopausal gonadotrophin (HMG), a urine-derived preparation containing both FSH and LH is the only available source of exogenous LH. However HMG preparations are subject to wide variation in amount and bioactivity of LH, despite significant improvements in processing techniques. Recombinant human luteinizing hormone (rhLH) is structurally and functionally analogous to endogenous LH. Studies by Lisi et al. [4], and Mochtar et al. [5] confirmed the benefit of adding rhLH supplementation to women who have poor response to ovarian stimulation. There is one literature report on more live births (40.7%) by patients with suboptimum response to stimulation with a GnRH agonist and who received recombinant follicle stimulating

hormone (rFSH) and rhLH than for patients on an rFSH and HMG protocol [6]. Only scant information exists in the literature regarding the beneficial effects of rhLH supplementation for patients with suboptimum response to stimulation with GnRH agonists, and no study from Asia. Hence we initiated a retrospective analysis to investigate whether there is any beneficial effect, in terms of clinical pregnancy, of adding LH supplementation by rhLH or HMG for patients with suboptimum response who are treated with GnRH agonists.

Materials and methods

This retrospective analysis included 174 couples who underwent IVF/ICSI treatment during 2008–2010 at our clinic. The institutional review board gave its approval to review the records of infertile couples. We used the combination protocol, initiating ovarian stimulation after long luteal gonadotrophin-releasing hormone (GnRH) agonist down-regulation with recombinant follicle stimulating hormone (rFSH; Recagon, Organon Schering-Plough, India), and adding luteinizing hormone (LH) activity using either HMG or recombinant human LH (rhLH; Luveris; Merck Serono, India) on stimulation days 6–9. Patients are offered the choice of either HMG or rhLH and make their decision on the basis of multiple factors including cost. This study analysed the outcomes of consecutive assisted reproductive technology (ART) cases in our database in order to draw conclusions about the efficacy and consistency of urinary or recombinant gonadotrophin-stimulated ART cycles.

Subject selection

The inclusion criteria for selection of the patients from the database were: mid follicular (day 6) hyporesponse on long GnRH agonist, no follicles >10 mm, E2 <200 pg/ml, endometrial thickness <6 mm, baseline serum LH <1.2 IU/ml on day 6.

Laboratory and clinical procedures remained consistent during this period. Patient management was handled by the same team of 2 reproductive endocrinologists and 2 embryologists. Patient data were drawn from our in-house database (Filemaker 10). This study was approved by the Krishna IVF Clinic Institutional Review Board.

Ovarian stimulation

A total of 174 patients undergoing consecutive in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles at our clinic between December 2008 and January 2010 were evaluated in this retrospective study. Of 174 patients, 74 received rFSH and rhLH stimulation and 100 patients were stimulated with rFSH and HMG.

The long luteal phase GnRH agonist protocol was used for ovarian stimulation. The long luteal phase GnRH agonist protocol involved depot triptorelin (decapetyl 3.75 mg; Ferring, India) administered on day 21 of the preceding cycle. After 14 days, down-regulation was confirmed by serum oestradiol <50 pg/ml, endometrium thickness <5 mm, and no follicle >10 mm. Patients were then started on daily subcutaneous rFSH (Recagon, Organon Schering-Plough, India) to initiate follicular development and recruitment. The initial dose of rFSH was based on age, body weight, baseline FSH, previous response and clinician judgment. The median dose was 225 U/day.

Patients were maintained on the same dose of rFSH for the first 5 days. Ultrasonography was performed on stimulation day 5 and, depending on the progress of follicular development, the dose of rFSH was adjusted. LH supplementation was given on days 6–9, either through rhLH (Luveris; Merck Serono, India) or HMG (Ferring, India). Patients are offered the choice of either HMG or rhLH and made their decision on the basis of factors which included the cost of the preparation. If patients were started on rhLH, the dose of rFSH was maintained and rhLH 75 U/day was added. If HMG was added, the gonadotrophin was switched on days 6–9 to a daily dose of 225–450 U HMG. Doses of both rFSH and HMG were titrated on the basis of the progress of follicular development, assessed through TVUS once every 2 days. When ≥3 lead ovarian follicles reached a diameter of 18 mm, oocyte 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 TVUS, 34–36 h after administration of hCG. After oocyte aspiration, the follicular fluid was examined for cumulus–corona–oocyte complexes. On the day of oocyte retrieval male partners were asked to produce semen samples for the IVF or ICSI procedure in sterile specimen containers. Semen samples were washed twice using sperm washing media (HEPES media). The resulting pellet was used directly for ICSI or IVF.

Gamete handling, embryo culture, transfer

Oocyte cumulus complexes (OCC) were collected from follicular fluid after observation under a stereo-microscope (Discovery V-20, Zeiss). Excess cumulus was removed immediately, by use of an insulin syringe, washed twice in gamete buffer (Gamete, Vitrolife), 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 h post-retrieval by exposing them to hyaluronidase solution (80 IU/ml, Sigma, USA) for 30 s. Any residual adherent cumulus cells were removed mechanically by use of flexipets of appropriate size. The oocytes were then assessed for maturity by observing the presence of a first polar body. Mature metaphase II (MII) oocytes were subjected to the ICSI procedure (Luigs and Neuman, Germany) and incubated in fertilization media (Sage Biopharma, USA).

In the IVF procedure, the required number of motile spermatozoa were calculated for each oocyte and the fertilization media containing OCC was inseminated.

Fertilization was assessed 16–18 h post-insemination or injection for both IVF and ICSI. Oocytes with two pronuclei (2PN) and having a second polar body were classified as fertilized. The fertilized oocytes were washed twice and cultured in cleavage media (Sage Biopharma, USA) for 48 h. Before transfer, embryos were graded on the basis of morphological condition using the criteria outlined by Veeck et al [7]. From 3–5 embryos were transferred 72 h post retrieval and any surplus grade I and II embryos were cyropreserved by vitrification.

At our centre, our embryo transfer policy was to transfer three day 3 embryos into women who were aged <35 years undergoing their first ART attempt. For women older than 35, or women undergoing their second, third, and subsequent ART cycle, we transferred 3–5 embryos. The numbers of embryos transferred was decided in consultation with each couple. In our centre, patients were often referred from other ART centres after their first failed ART cycle. This, combined with a cultural preference for maximizing success with every fresh ART cycle, meant that patients often chose to implant more than 3 embryos in each cycle.

Luteal support was provided by use of progesterone vaginal suppositories (Uterogestan; Laboratoires Besins International, Paris, France) starting from the day of oocyte retrieval and continued for up to 5 weeks after a biochemical pregnancy was confirmed.

Statistical analysis

For quantitative variables the parametric *t*-test was used where normality and homogeneity assumptions were satisfied to compare means between the rhLH and HMG groups. For qualitative variables, the chi-squared test was used to compare differences between the 2 groups. SPSS was used for statistical analysis. $p < 0.05$ was considered significant.

Results

There were no statistically significant differences in baseline variables that affect ovarian response to stimulation, including age, body mass index (BMI), and antral follicle count (AFC) between the 2 groups (Table 1).

The clinical and laboratory outcomes are listed in Table 2. The length of stimulation was shorter in the rFSH + rhLH group (mean stimulation duration of 11.3 vs. 11.9 days for HMG). The total gonadotrophin dose was significantly higher in the HMG group, with a mean total FSH dose of 4134.2 versus 2706.4 U for the rhLH group ($p < 0.001$). Mean serum E2 on the day of hCG administration was higher in the HMG group but this difference did not result in a statistically significant difference in endometrial thickness assessed by TVUS. There was a statistically significant difference in the number of oocytes retrieved, with more oocytes in the rhLH group (13.1 vs. 11.3 in the HMG group, $p = 0.024$). There were no statistically significant differences in the mean number of MII oocytes or in occurrence of fertilization. In the rFSH + rhLH group, there were 5.4 oocytes per 1000 U FSH compared with 3.6 oocytes per 1000 U FSH in the rFSH + HMG group ($p < 0.001$).

The number of embryos transferred was statistically lower in the rhLH group than in the HMG group (Table 2). There were no significant differences in the numbers of embryos that were cryopreserved (Table 2).

Clinical pregnancy was achieved significantly more often in the rFSH + rhLH group than in the rFSH + HMG group (35.1 vs. 19.0%; $p < 0.01$). The incidence of miscarriage was significantly less in the rFSH + rhLH group than in the rFSH + HMG group (7.6 vs. 26.3%; $p < 0.02$) (Table 3).

Table 1 Baseline variables of 174 patients undergoing consecutive IVF/ICSI cycles

Baseline variable	rFSH + rhLH	rFSH + hMG	*p* value
Age (years)	30.6 ± 3.6	30.9 ± 3.7	NS
BMI (kg/m^2)	26.9 ± 4.0	26.95 ± 4.5	NS
AFC	10.6 ± 2.5	10.5 ± 2.5	NS
Etiology			
Female factor (%)	19.7	16.1	
Male factor (%)	50.6	55.3	
Mixed factor (%)	29.7	28.6	

Values are given as mean ± SD unless otherwise indicated

AFC antral follicle count, *BMI* body mass index, *hMG* human menopausal gonadotrophin, *ICSI* intracytoplasmic sperm injection, *IVF* in-vitro fertilization, *rFSH* recombinant follicle-stimulating hormone, *rhLH* recombinant human luteinizing hormone

Table 2 Clinical outcomes of 174 IVF/ICSI cycles

	rFSH + rhLH ($n = 74$)	rFSH + hMG ($n = 100$)	p value
Mean duration of stimulation (days)	11.3 ± 1.1	11.9 ± 1.4	<0.003
Total FSH dose (U)	2706.4 ± 651.1	4134.2 ± 1589.0	<0.001
Mean E2 on hCG day (pg/ml)	1449.2 ± 980.9	1780.58 ± 981.1	0.03
Endometrium thickness on day of hCG (mm)	11.8 ± 2.1	11.4 ± 2.0	NS
Mean no. retrieved oocytes	13.12 ± 4.7	11.36 ± 5.2	0.024
No of oocytes/ 1000 U of FSH	5.4	3.6	<0.001
Mean no. retrieved MII oocytes	10.2 ± 4.3	9.3 ± 4.7	NS
Fertilization rate (%)	80	82	NS
Number of embryos transferred	3.8 ± 1.1	4.2 ± 1.1	0.034
Number of embryos preserved	0.6 ± 1.5	0.5 ± 1.3	NS

Values are given as mean ± SD unless otherwise indicated

E2 oestradiol, *FSH* follicle-stimulating hormone, *GnRH* gonadotrophin-releasing hormone, *hCG* human choriogonadotropin, *hMG* human menopausal gonadotrophin, *ICSI* intracytoplasmic sperm injection, *IVF* in-vitro fertilization, *MII* metaphase II, *rFSH* recombinant follicle-stimulating hormone, *rhLH* recombinant human luteinizing hormone

Table 3 Pregnancy outcome of 174 IVF/ICSI cycles

	rFSH + rhLH ($n = 74$)		rFSH + HMG ($n = 100$)		p value
	%	n	%	n	
Biochemical pregnancy success	1.3	01	3.0	3	NS
Clinical pregnancy success	35.1	26	19.0	19	0.01
Live birth success, single pregnancy	17.5	15	9	9	NS
Live birth success, twin pregnancy	12.1	9	5	5	NS
Miscarriages	7.6	2	26.3	5	<0.02

Discussion

These results show that the FSH dose and duration of stimulation was lower for the combination rFSH and rhLH than for the rFSH and HMG treatment procedure. This may be because of the superior consistency, purity, and accuracy of dosing of the rFSH and rhLH preparations, and is consistent with previous RCTs in ovulation induction, as reported by Hugues et al. [8], who used recombinant follitrophin alpha (Gonal-f), and in ART, as reported by Bergh et al. [9], who compared follitrophin alpha (Gonal-F) with HP-uFSH (Metrodin). It is important to note that the protocol used in our centre utilized rFSH for both the rhLH and HMG groups in the first 6–9 days of stimulation and HMG was added only after stimulation day 6–9. Therefore, it seems that the consistency and potency of the FSH/LH preparation used is important, not just in recruiting a synchronous cohort of follicles but also in maintaining the steroidogenesis milieu for the developing cohort of follicles to grow appropriately and mature.

We found statistically significant differences in clinical pregnancy success between patients treated with rFSH combined with rhLH and those treated with rFSH and HMG. We postulate this could be because of differences between the effects of rhLH and HMG on oocyte quality and, ultimately, embryo quality. A recent Cochrane meta-analysis on RCTs comparing rFSH only versus rFSH and rhLH stimulation procedures reported no evidence of statistically different pregnancy outcomes when rhLH was used [5]. However, the authors concluded that further large RCTs should be undertaken using long GnRH agonist down-regulation 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 rhLH, particularly with regard to pregnancy loss and poor responders.

Luteinizing hormone is important in regulating steroidogenesis throughout follicular development; adequate LH is particularly important for oocyte maturation [10]. Previous RCTs corroborate our findings of a difference between the in-vivo LH bioactivity of rhLH and HMG preparations. Ferrareti et al. [6] reported difference clinical pregnancy success of 54% (rhLH) versus 11% (HMG) in an RCT that compared addition of rhLH 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. [11] reported clinical pregnancy success of 57.9% in hypogonadotrophic hypogonadism (HH) patients stimulated with a fixed-dose combination of 150 U rFSH and 75 U rhLH versus 17.2% clinical pregnancy success for HH patients stimulated with a HMG 150U ($n = 24, p = 0.003$). Another retrospective observational study evaluating ART patients undergoing stimulation with an antagonist procedure in a Boston IVF centre [12], reported clinical pregnancy success of 36.0% for patients aged >38 years treated with rFSH and rhLH compared with 19.1% ($p = 0.048$) for those stimulated with rFSH and HMG.

The "LH window" theory of ovarian function proposed recently by Shoham et al. [13] states that in the absence of sufficient LH, E2 production will be inadequate and endometrial proliferation poor. If the LH threshold is exceeded it causes atresia of ovarian follicles and cessation

of follicular development. It is useful to interpret our results from the perspective of the LH therapeutic window. We postulate that perhaps excessive or inconsistent LH activity from the hCG component in HMG may affect oocyte maturation in the latter half of the ovarian stimulation cycle, giving rise to the differences in numbers of oocytes retrieved and success of pregnancy. A retrospective, matched cohort study reported a euploidy difference between patients stimulated with rFSH and those stimulated with HMG in antagonist ART cycles (FSH, 29.4% vs. FSH/HMG, 25.7%) [14]. In a separate prospective RCT, Grondahl et al. [15] reported that mRNA expression of the LH receptor and other genes involved in cholesterol and steroid biosynthesis was reduced in the granulosa cells of patients treated with HMG. Conversely, Pezzuto et al. [16] compared a regimen of rFSH and rFSH combined with rhLH in an RCT in ART patients and found significant differences in favour of the combination of rFSH and rhLH. Lower follicular fluid VEGF levels in the rhLH group suggested there was less granulosa cell apoptosis in the rhLH group [16]. Although these results must be confirmed by larger RCTs, there seems to be a growing body of evidence of a positive effect of rhLH in some ART patients and we propose this may be an interesting topic to pursue in a larger RCT. In conclusion, this study has shown greater clinical pregnancy success, recovery of more oocytes, and a reduction in gonadotrophin with use of rFSH combined with rhLH in long GnRH agonist ART cycles suggesting that the superior purity and consistency of rFSH and rhLH may result in better clinical outcomes.

Acknowledgments We would like to thank Dr K. Murali Krishna for his help in the preparation of this manuscript.

Conflict of interest G.A. Ramaraju, Kavitha, Kavitha Lakshmi and Ravikrishna have no conflicts of interest to declare. S.C Teng is an employee of Merck Pte Ltd.

References

1. Humaidan P, Bungum L, Bungam M, Andersen C. Ovarian response and pregnancy outcome related to midfollicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. Hum Reprod. 2002;17:2016–21.
2. Lahoud R, Al-Jefout M, Tyler J, Ryan J, Driscoll G. A relative reduction in mid-follicular LH concentrations during GnRH agonist IVF/ICSI cycles leads to lower live birth rates. Hum Reprod. 2006;21:2645–9.
3. Nakagawa K, Ohgi S, Nakashima A, Horikawa T, Sugiyama R, Saito H. The ratio of late-follicular to midfollicular phase LH concentrations efficiently predicts ART outcomes in women undergoing ART treatment with GnRH-agonist long protocol and stimulation with recombinant FSH. J Assist Reprod Genet. 2008;25:359–64.
4. Lisi F, Rinaldi L, Fishel S, Lisi R, Pepe G, Picconeri MG. Use of recombinant follicle-stimulating hormone (Gonal F) and recombinant luteinizing hormone (Luveris) for multiple follicular stimulation in patients with a suboptimal response to in vitro fertilization. Fertil Steril. 2003;79:1037–8.
5. Mochtar MH, van der Veen F, Ziech M, van Wely M Musters A. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. Cochrane Database Syst Rev. 2007;18:CD005070.
6. Ferraretti AP, Gianaroli L, Magli MC, D'angelo A, Fafalli V, Montanaro N. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. Fertil Steril. 2004;82:1521–6.
7. Veeck L. Atlas of the human oocytes and early conceptus. Baltimore: Williams and Wilkins; 1991. p. 46.
8. Hugues JN, Bstandig B, Bry-Gauillard H, Uzan M, Cedrin-Durnerin I. Comparison of the effectiveness of recombinant and urinary FSH preparations in the achievement of follicular selection in chronic anovulation. Reprod Biomed Online. 2001;3:195–8.
9. Bergh C, Howles CM, Borg K, Hamberger L, Josefsson B, Nilsson L, Wikland M. Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F®) versus highly purified urinary FSH (Metrodin HP®): results of a randomized comparative study in women undergoing assisted reproductive techniques. Hum Reprod. 1997;12:2133–9.
10. Hillier SG. Paracrine support of ovarian stimulation. Mol Hum Reprod. 2009;15:843–50.
11. Carone D, Vizziello G, Vitti A, Chiappetta R. Clinical outcomes of ovulation induction in WHO Group I anovulatory women using r-hFSH + r-hLH in a 2:1 ratio compared to hMG. Hum Reprod. 2010;25:i285–321.
12. Thornton K, Alper MM, Ryley D, Ezcurra D. Outcomes of GnRH antagonist IVF cycles using LH supplementation for COH: FSH/rhLH vs. FSH/hMG. Fertil Steril. 2006;86:S411.
13. Shoham Z. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. Fertil Steril. 2002;77:1170–7.
14. Weghofer A, Munne S, Brannath W, Chen S, Barad D, Cohen J, Gleicher N. The impact of LH-containing gonadotropin stimulation on euploidy rates in preimplantation embryos: antagonist cycles. Fertil Steril. 2009;92:937–42.
15. Grondahl ML, Borup R, Lee YB, Myrhoj V, Meinertz H, Sorensen S. Differences in gene expression of granulosa cells from women undergoing controlled ovarian hyperstimulation with either recombinant follicle-stimulating hormone or highly purified human menopausal gonadotropin. Fertil Steril. 2009;91:1820–30.
16. Pezzuto A, Ferrari B, Coppola F, Nardelli GB. LH supplementation in down-regulated women undergoing assisted reproduction with baseline low serum LH levels. Gynecol Endocrinol. 2010;26:118–24.