REVIEW

HMG versus recombinant FSH plus recombinant LH in ovarian stimulation for IVF: does the source of LH preparation matter?

BIOGRAPHY
Professor Raoul Orvieto is a full professor of Obstetrics and Gynecology and incumbent of the Tarnesby-Tarnowski Chair for Family Planning and Fertility Regulation, at the Sackler Faculty of Medicine, Tel-Aviv University, Israel. Professor Orvieto is the Director of the Division of Reproductive Endocrinology and Infertility, at the Sheba Medical Center.

KEY MESSAGE
In an analysis of prospective and retrospective studies, no firm conclusions can be drawn in favour of one source of preparation containing LH bioactivity over another. Further studies are needed to investigate the effect of the LH source and to identify patients who might benefit from the addition of LH supplementation.

ABSTRACT
Studies on the role of LH supplementation in patients undergoing assisted reproductive technique use different sources of LH bioactivity-containing preparations, daily doses and modes of administration. This review aims to critically present the available evidence comparing the effect of the two commercially available LH preparations (human menopausal gonadotrophin [HMG] and recombinant FSH + recombinant LH) with different sources of intrinsic LH bioactivity (HCG versus LH, respectively) on ovarian stimulation characteristics and IVF cycle outcomes. A literature review was conducted for all relevant articles reporting on IVF and intracytoplasmic sperm injection treatment outcome after ovarian stimulation using HMG or recombinant FSH plus recombinant LH. The available studies are mostly observational, using different daily doses and modes of administration. No statistically significant differences were observed in ovarian stimulation variables and clinical pregnancy and live birth rates when HMG was compared with recombinant FSH + recombinant LH. Moreover, combined analysis of all the available prospective and retrospective studies produced no firm conclusions in favour of either source of LH bioactivity. Further large randomized controlled studies are needed to investigate the effect of the LH source on IVF outcome and to identify patients who are most likely to benefit from the addition of LH bioactivity supplementation.
INTRODUCTION

Ovarian stimulation is considered a key factor in the success of IVF and embryo transfer because it enables the recruitment of multiple healthy fertilizable oocytes (Penzias, 2004). Usually, ovarian stimulation includes the co-administration of gonadotrophin-releasing hormone (GnRH) analogues and gonadotrophins, aiming to prevent premature luteinization and to facilitate folliculogenesis, respectively.

In clinical practice, ovarian stimulation is tailored to the patient’s clinical characteristics (demographic, anthropometric and ovarian reserve profiles) (Alviggi et al., 2016; Santi et al., 2017) and should include the GnRH analogue protocol used (agonist versus antagonist), type (urinary versus recombinant, with or without LH supplementation) (Levi-Setti et al., 2019), daily dose of gonadotrophins (van Tilborg et al., 2012) and the time and mode of triggering final follicular maturation (Orvieto, 2015). In a recently published large, real-world, observational study (Levi-Setti et al., 2019), women with the worst prognostic factors were generally treated with a combination of LH and FSH. Moreover, the addition of LH to FSH during ovarian stimulation was suggested to improve the quality of oocytes retrieved, balancing the differences in patients’ baseline characteristics.

Several gonadotrophin products are available, which are classified according to their source and constitution: (a) urinary derived preparations: menotrophins or human menopausal gonadotrophin (HMG) preparations that contain FSH and LH or urinary FSH; (b) recombinant preparations: recombinant FSH preparation that contain FSH completely devoid of LH, or a combination of recombinant FSH and recombinant LH, usually in a 2:1 ratio (not available in the USA).

The first available source of LH was in HMG, a urinary extract containing both FSH and LH at a fixed combination of 1:1. Subsequently, a second generation of highly purified (HP) HMG was introduced. This product was obtained by adding extra purification steps, such as hydrophobic interaction chromatography and anion exchange, with the consequent reduction in the content of proteins devoid of gonadotrophin bioactivity. Additionally, as LH molecules are preferentially lost during purification, most LH bioactivity in HP-HMG is provided by HCG (Wolfenson et al., 2005).

The second commercially available LH preparation is recombinant LH (lutropin alfa), which is produced in genetically engineered Chinese hamster ovary cells. Recently, recombinant FSH and recombinant LH have been combined in a single product (Pergoveris; follitropin alfa/lutropin alfa 150 IU/75 IU), thereby allowing administration of both gonadotrophins in a convenient single injection at a 2:1 ratio (Bosch, 2010; Gibreel and Bhattacharya, 2010).

Although the addition of LH supplementation was associated with a tendency towards improved IVF outcome in different subgroups of patients undergoing ovarian stimulation for IVF, e.g. patients with hypogonadotropic hypogonadism and elderly patients who responded poorly (European Recombinant Human LH Study Group, 1998; Hill et al., 2012; Musters et al., 2012; Humaidan et al., 2017), it does not seem to improve outcomes in the general IVF population (Baruffi et al., 2007; Kolibianakis et al., 2007; Mochtar et al., 2007; Oliveira et al., 2007). The available studies on the role of LH supplementation in patients undergoing assisted reproductive technology use different preparations, different daily doses and different modes of administration.

Three large systematic reviews that evaluated the effectiveness of HMG versus recombinant FSH in women undergoing ovarian stimulation for IVF demonstrated a significant increase in live birth rate with HMG compared with recombinant FSH (Ali-Irany et al., 2008; 2009; Coomarasamy et al., 2008). The superiority of HMG over recombinant FSH was attributed to the LH activity of the HMG preparations, which was suggested to play an important role in optimizing ovarian stimulation by modulating folliculogenesis and improving embryo quality and endometrial receptivity (Filiciot et al., 2005; Ziebe et al., 2007). In fact, more than two-thirds of participants in the HMG arm of the aforementioned reviews underwent ovarian stimulation using HP-HMG (Ali-Irany et al., 2009), which suggests that the improved IVF outcome using HMG may be a result of its intrinsic LH bio-activity or a result of the increased HCG content in the HP-HMG preparation.

Prompted by these observations, the present study aimed to critically review published studies that compared the effect of the two commercially available LH preparations (HMG and recombinant FSH + recombinant LH) with different sources of intrinsic LH bio-activity (HCG versus LH, respectively) on ovarian stimulation characteristics and IVF cycle outcome. These findings may help to clarify the proper approach to LH preparations in ovarian stimulation and to aid fertility specialists and their patients in the decision-making process.

MATERIALS AND METHODS

Bibliographic databases MEDLINE, PubMed and EMBASE were searched for studies on IVF and intracytoplasmic sperm injection after ovarian stimulation with the use of either HMG or recombinant FSH plus recombinant LH. All eligible studies comparing the use of HMG with recombinant FSH plus recombinant LH were assessed for study characteristics, interventions and outcomes. The search period was up to June 2019.

RESULTS

The search yielded 11 articles, of which three were prospective trials (Pacchiarotti et al., 2010; Requena et al., 2014; Tehrannejad et al., 2017); two were randomized controlled trials (Pacchiarotti et al., 2010; Tehrannejad et al., 2017) and one was a prospective observational study, in which patients were assigned to each treatment group based on a quasi-experimental design comprising consecutive opportunity sampling (Requena et al., 2014). Eight retrospective studies were found (Buhrer and Fischer, 2012; Fâbrege et al., 2013; Dahan et al., 2014; Revelli et al., 2015; Schwarz et al., 2016; Bleau et al., 2017; Renzini et al., 2017; Xia et al., 2019).

The characteristics of all the studies are presented in Table 1.

The studies used different ovarian stimulation protocols (Table 1): long GnRH-agonist (Pacchiarotti et al., 2010;
TABLE 1 CHARACTERISTICS OF INCLUDED STUDIES

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Ovarian stimulation protocol</th>
<th>Gonadotropin preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacchiarotti et al. (2010)</td>
<td>Multicentre, prospective RCT</td>
<td>Long GnRH-ag</td>
<td>HMG (225 IU) versus Pergoveris (225 IU recFSH+112.5 IU recLH) from day 1 of stimulation</td>
</tr>
<tr>
<td>Requena et al. (2014)</td>
<td>Prospective, parallel, observational study</td>
<td>Long GnRH-ag</td>
<td>HP-HMG (75 IU) + uFSH (75 IU) versus Pergoveris (150 IU recFSH + 75 IU recLH) from day 1 of stimulation</td>
</tr>
<tr>
<td>Tehraninejad et al. (2017)</td>
<td>RCT</td>
<td>Long GnRH-ag</td>
<td>recFSH (150–225 IU) from day 1 of stimulation and after 6 days of either HMG (150–225 IU) or recLH (150–225)</td>
</tr>
<tr>
<td>Fábregues et al. (2013)</td>
<td>Retrospective study</td>
<td>Long GnRH-ag</td>
<td>HP-HMG (150 IU) versus Pergoveris (150 IU recFSH + 75 IU recLH)</td>
</tr>
<tr>
<td>Dahan et al. (2014)</td>
<td>Retrospective study</td>
<td>Long or microdose flair</td>
<td>HMG + rec or uFSH (112.5–600 IU) versus recFSH (112.5–600 IU) + recLH in 2:1 ratio</td>
</tr>
<tr>
<td>Schwarze et al. (2016)</td>
<td>Cohort analysis</td>
<td>GnRH-ant</td>
<td>recFSH + HMG vs recFSH plus recLH</td>
</tr>
<tr>
<td>Bleau et al. (2017)</td>
<td>Cohort study</td>
<td>Long GnRH-ag</td>
<td>FSH (112.5–225 IU) from day 1 of stimulation and after 5 days either HMG or recLH were added with FSH to LH ratio of 3:1 to 2:1</td>
</tr>
<tr>
<td>Renzini et al. (2017)</td>
<td>Retrospective study</td>
<td>Long GnRH-ag</td>
<td>HP-HMG (300 IU) versus recFSH (300 IU) + recLH (150 IU)</td>
</tr>
<tr>
<td>Xia et al. (2019)</td>
<td>Retrospective study</td>
<td>Long GnRH-ag</td>
<td>HP-HMG (75 IU) + recFSH (75 IU) versus recFSH (150 IU) + recLH (75 IU)</td>
</tr>
</tbody>
</table>

Ag, agonist; ant, antagonist; RCT, randomized controlled trial; rec, recombinant; GnRH, gonadotrophin releasing hormone; HP, highly purified; HMG, human menopausal gonadotrophin, uFSH, urinary FSH.

Buhrer and Fischer, 2012; Fábregues et al., 2013; Requena et al., 2014; Bleau et al., 2017; Renzini et al., 2017; Tehraninejad et al., 2017; Xia et al., 2019; GnRH-antagonist (Schwarze et al., 2016), both (Revelli et al., 2015), or long GnRH-agonist and microdose flair GnRH-agonist (Dahan et al., 2014). Considering the type and source of gonadotrophin preparations, eight used HMG (Pacchiarotti et al., 2010; Buhrler and Fischer, 2012; Dahan et al., 2014; Revelli et al., 2015; Schwarze et al., 2016; Bleau et al., 2017; Tehraninejad et al., 2017; Xia et al., 2019) and three used HP-HMG (Fábregues et al., 2013; Requena et al., 2014; Renzini et al., 2017). Moreover, nine used LH preparation during the stimulation period (Pacchiarotti et al., 2010; Buhrler and Fischer, 2012; Fábregues et al., 2013; Dahan et al., 2014; Requena et al., 2014; Revelli et al., 2015; Schwarze et al., 2016; Renzini et al., 2017; Xia et al., 2019), whereas two studies (Bleau et al., 2017; Tehraninejad et al., 2017) added the LH preparation only after 5 and 6 days of FSH treatment, respectively. The daily dose of the LH-activity in the HMG preparations was reported in eight studies and ranged between 75 and 300 IU, whereas, in recombinant LH, it was 75–300 IU (table 1).

The prospective and retrospective studies (Supplementary Tables 1–3) were analysed separately, however, no statistically significant between-group differences were observed in patients’ age, total and daily dose of gonadotrophin used, stimulation variables and clinical pregnancy and live birth rates, between the HMG and the recombinant LH groups.

The prospective and retrospective studies together were analysed together; 5440 patients in the recombinant LH and 14433 in the HMG groups (Supplementary Tables 1–3) revealed no statistically significant between-group differences in the aforementioned variables, although values seemed to differ for daily dose of LH used (93.75 ± 31.37 IU versus 150 ± 94.87 IU), number of oocytes retrieved (10.12 ± 4.44 versus 8.74 ± 4.1; borderline significant, P = 0.06) and percentage of mature oocytes (575 ± 199% versus 64.7 ± 16.2%, P = 0.24) in the recombinant LH versus HMG groups, respectively. Moreover, ongoing and live birth rates were 25.9 ± 17.3% versus 29.5 ± 16.0%, and ovarian hyperstimulation syndrome rates were 16.4 ± 7.49% versus 11.8 ± 6.16% in patients using recombinant LH compared with HMG, respectively.

DISCUSSION

In the present review of the role of the different sources of LH activity supplementation given to patients undergoing assisted reproductive technology during ovarian stimulation, the available studies were found to be mostly observational, using the long gonadotrophin releasing hormone (GnRH) agonist protocol, with different preparations containing ‘FSH activity’, different daily doses and different modes of administration. Moreover, combined analysis of the available prospective and retrospective studies showed retrieval of a higher number of oocytes and a lower percentage of mature oocytes in the recombinant LH versus the HMG groups and lower ongoing and live birth rates and higher ovarian hyperstimulation syndrome rate; however, the differences failed to reach statistical significance. In fact, prospective studies revealed no statistically significant between-group differences in the total and daily dose of gonadotrophin used, stimulation variables and clinical pregnancy and live birth rates, when comparing the use of HMG with recombinant LH.

Previous studies (Trew et al., 2010; van Wely et al., 2012), demonstrated that recombinant gonadotrophins are able
to induce the retrieval of more oocytes than HMG. Similarly, Santi et al. (2017) found that the administration of FSH alone during ovarian stimulation retrieved a higher number of oocytes than either recombinant LH supplementation or HMG use. Moreover, although HMG improves the yield of mature oocytes, number of embryos, and increases implantation rate, addition of recombinant LH leads to higher pregnancy rate.

Three prospective studies have compared the use of HMG versus recombinant LH in patients undergoing ovarian stimulation for IVF. Pacchiarotti et al. (2010) conducted a multicentre, prospective, randomized controlled trial comparing HMG versus recombinant FSH plus recombinant LH (Pergoveris, Merck, Darmstadt, Germany) in 122 patients undergoing the long GnRH agonist protocol for IVF, of whom 111 patients underwent oocyte retrieval. 58 in the HMG and 53 in the recombinant LH groups. Significant statistical differences were found in HMG compared with recombinant FSH and recombinant LH in days of stimulation (14.1 ± 1.6 and 10.9 ± 1.1, respectively), total units of FSH administered (3.525 ± 232 5 versus 4.800 ± 345, respectively) and total number of oocytes retrieved (41 ± 1.2 versus 78 ± 11, respectively). Moreover, they demonstrated significantly higher percentage of mature oocytes (48.2% versus 34.7%, respectively) in the HMG group, with comparable results of the main IVF outcomes (clinical pregnancy rate, implantation rate, oocyte, and embryos quality), and with an increased risk of ovarian hyperstimulation syndrome in the Pergoveris group.

Requena et al. (2014) compared the endocrine profiles of 48 oocytes donors who received either recombinant LH plus recombinant FSH or HMG plus urinary FSH. Although more oocytes were retrieved, in agreement with Pacchiarotti et al. (2010), a lower proportion of metaphase II oocytes were obtained using the recombinant LH plus recombinant FSH group. In the recipients, implantation and ongoing pregnancy rates were the same in both groups.

In contrast to these prospective studies, Tehraninejad et al. (2017) investigated the effect of ovarian stimulation with recombinant FSH plus recombinant LH versus recombinant FSH plus HMG on IVF outcome. In their randomized controlled trial, both groups underwent the long GnRH agonist protocol and received recombinant FSH from day 2-5 of stimulation; on day-6, patients were randomized to the group that additionally received either HMG or recombinant LH. No between-groups differences were observed in the number of follicles, total number of oocytes or metaphase II oocytes retrieved, quality of embryos and the total number of embryos achieved; However, live birth rate was higher in the recombinant FSH plus HMG group compared with the recombinant FSH plus recombinant LH group.

Of the retrospective studies, Revelli et al. (2015) evaluated the IVF performance of 848 IVF patients classified as expected ‘poor’ or ‘normal’ responders who underwent ovarian stimulation for IVF with either recombinant FSH plus recombinant LH (2:1 ratio) or HMG. Data were collected under real-life practice circumstances and the clinical and ongoing pregnancy rates with fresh embryos were calculated by stratifying patients according to the number of oocytes retrieved. When comparing patients who responded well with more than eight oocytes retrieved, the recombinant group had a significantly better pregnancy rate outcome. They speculated that the reason(s) for this are unknown, but a more favourable effect on oocyte quality, endometrial receptivity, or both, could be involved.

Although recombinant LH and HP-HMG activate the LH receptor, the activation and downstream effects of the receptor binding seem to be different. One possible explanation is that the effect of recombinant LH and HMG at the intracellular level is likely mediated by different binding affinity of the two molecules with the receptor, leading in turn to the activation of different pathways. The crucial question is whether their addition to ovarian stimulation may affect IVF outcome differently.

In conclusion, considering the randomized controlled trials and retrospective studies discussed above, no firm conclusions can be drawn in favour of a particular source of preparation containing ‘LH activity’. Although published randomized controlled trials and retrospective studies were analysed, no significant differences were found despite an apparently higher number of oocytes retrieved, lower percentage of mature oocytes, lower ongoing and live births and higher OHSS rates in the recombinant LH compared with the HMG groups. Furthermore, large randomized controlled trials are needed to investigate the true effect of the source of LH supplementation on IVF outcome and to identify the selected groups of patients who are most likely to benefit from the addition of ‘LH activity’ supplementation to their ovarian stimulation protocol.

**SUPPLEMENTARY MATERIALS**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2019.08.010.
Trinchard-Lugan, I., Khan, A., Porchet, H.C., Munato, A. Pharmacokinetics and pharmacodynamics of recombinant human
Wolfenson, C., Groisman, J., Couto, A.S., Hedenfalk, M., Cortvrindt, R.G., Smitz, J.E.


Received 29 July 2019, received in revised form 22 August 2019, accepted 30 August 2019.