Highly purified-hMG versus rFSH in ovarian hyperstimulation in women undergoing elective fertility preservation

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Research Article

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Abstract

**Purpose:** To compare recombinant FSH (rFSH) with highly purified-human menopausal gonadotrophin (hp-hMG) on ovarian response in women undergoing elective fertility preservation (FP).

**Methods:** This retrospective study included 456 women who underwent elective FP with gonadotropin-releasing hormone (GnRH) antagonist or progestin-primed ovarian stimulation (PPOS) protocols between 01/2017-12/2021. Three-hundred and forty-one women were stimulated with rFSH and 115 with hp-hMG, and the ovarian stimulation outcomes were compared between the two groups. A multivariate linear regression analysis assessed the impact of age, basal FSH level, antral follicle count, and protocol type on the ovarian stimulation outcomes.

**Results:** Women in the rFSH group were significantly younger, and their antral follicle count was significantly higher than those in the hp-hMG group (35.50±2.12 vs. 35.99±2.13 years, \(P = 0.034\) and 13.76±6.08 vs. 11.84±6.06, \(P = 0.002\)). There were no significant group differences in the amount (\(P = 0.645\)) and duration (\(P = 0.265\)) of FSH stimulation. The peak estradiol level was significantly lower for the rFSH group compared to the hp-hMG group (2547.18±1648.21 pg/mL vs. 3468.02±2497.69 pg/mL, \(P < 0.001\)), while peak progesterone level was significantly higher (1.33±0.75 ng/mL vs. 1.01±0.52 ng/mL, \(P = 0.001\)). The numbers of retrieved and MII oocytes were significantly higher for the rFSH group compared with the hp-hMG group (16.82±10.95 vs. 13.25±9.66, \(P = 0.02\), and 13.22±9.13 vs. 9.76±7.11, \(P = 0.005\)), while the maturity rates were comparable (\(P = 0.103\)).

**Conclusion:** rFSH was demonstrated to have superior oocyte yield compared to hp-hMG in ovarian hyperstimulation for women undergoing elective FP.

Introduction

Recombinant follicle stimulating hormone (rFSH) and highly purified-human menopausal gonadotrophin (hp-hMG) are widely and successfully used for ovarian stimulation in infertile women undergoing assisted reproductive technology (ART) procedures. hp-hMG is a mixture of FSH, luteinizing hormone (LH), and human chorionic gonadotropin (hCG with LH-like properties) that are collected, extracted, and purified from the urine of post-menopausal women. The usual preparation contains a 1:1 ratio of FSH and LH (e.g., 75 IU each) [1]. rFSH is manufactured by recombinant DNA technology using Chinese hamster ovary cell lines transfected with the genes encoding for the two human FSH subunits or genes derived from a cell line of human fetal retinal origin. rFSH is completely free of any LH [2–3].

Many studies have compared the outcome of rFSH and hMG for ovarian stimulation, with most having been conducted on infertile women undergoing pituitary down-regulation with a gonadotropin-releasing hormone (GnRH) agonist long protocol. Two meta-analyses and one Cochrane review showed a better outcome in terms of the live birth rate when hp-hMG was used compared with rFSH in the GnRH agonist long protocol [4–6]. A recent systemic review and meta-analysis supported that conclusion but found the evidence of a difference in the cumulative live birth rate to be insufficient [7].

The GnRH antagonist protocol is one of the most popular of the protocols used to treat women undergoing ART. GnRH antagonists induce a rapid decrease in LH and FSH levels, thereby preventing spontaneous LH surges. Previous studies have compared the effectiveness of rFSH with that of hp-hMG in infertile women undergoing in-vitro fertilization (IVF) by means of an antagonist protocol and found no benefit of one over the other [8–12].
Progestin-primed ovarian stimulation (PPOS) is a new ovarian stimulation protocol that can block the LH surge through the use of progesterone instead of the traditional down-regulation with a GnRH antagonist in order to achieve multi-follicle recruitment [13], and women undergoing fertility preservation (FP) are mostly treated with these two protocols. A major advantage of using the GnRH antagonist and the PPOS protocols in women who are considered fertile is the possibility of preventing ovarian hyperstimulation syndrome [14–15] by triggering ovulation with a GnRH agonist [16–17].

FP is carried out for both medically indicated and elective reasons. The former includes young women with low ovarian reserve or those at risk for early ovarian failure (cancer patients, genetic conditions, severe endometriosis, unexplained background, family background) and the latter is generally age-related [18]. It is well known that advanced maternal age (> 35 years) [19–20] is associated with a decline in both follicular pool number and oocyte quality, as well as posing higher risks of fetal chromosomal abnormalities that result in fetal loss, representing the leading causes of age-related fertility decline [21–23]. Currently, the proportion of women delaying childbearing until the late 3rd to early 4th decade of life has greatly increased, especially in Western societies [24–25]. Oocyte cryopreservation affords women the chance to conceive and deliver their genetic offspring at a future date [18]. These are characteristically healthy, ostensibly fertile women who wish to postpone motherhood for various reasons, such as educational or career ambitions, or because they had not yet found a partner [26–27].

The present study aimed to compare the ovarian stimulation outcomes of hp-hMG versus r-FSH in women undergoing elective FP treatment by means of the GnRH antagonist and PPOS protocols.

**Materials And Methods**

**Ethical approval**

This study was approved by the ethics committee (Helsinki) of the Tel Aviv Sourasky Medical Center. (#0325-22-TLV). Informed consent was waived for this retrospective and anonymous analysis.

**Study population and participant recruitment**

This retrospective study was performed between January 2017 and December 2021 at the IVF Unit, Fertility Institute, Tel Aviv Sourasky Medical Center, a tertiary university-affiliated medical center. Four-hundred and fifty-six women who underwent their first elective FP treatment with either a GnRH antagonist or a PPOS protocol were included. Ovarian stimulation was carried out with rFSH \((n = 341)\) or hp-hMG \((n = 115)\). Only the first treatment cycle of each woman was included in this study.

**Data collection**

All relevant data were collected from the computerized database of the hospital. The data in the electronic charts included the following: clinical details [age, body mass index (BMI), marital status, number of children, thyroid-stimulating hormone (TSH) levels, and prolactin levels], fertility potential markers [basal FSH, antral follicle count (AFC)], ovarian stimulation details (protocol type, ovarian stimulation duration, total FSH dose, and type of ovulation trigger), and outcomes [peak serum estradiol (E2), peak serum progesterone, number of retrieved oocytes, number of metaphase II (MII) oocytes, and maturation rate (derived from the number of MII oocytes/number of aspirated oocytes)].

**Ovarian stimulation data and outcomes**
Controlled ovarian stimulation was carried out by the GnRH antagonist or PPOS protocols. Either rFSH (Gonal-F, Merck) or hp-hMG (Menopur, Ferring Pharmaceuticals) was used for ovarian stimulation. Ovulation was triggered with 0.2 mg of triptorelin (Decapeptyl; Ferring Pharmaceuticals) or 250 mcg of choriogonadotropin α (Ovitrelle; Serono, Geneva, Switzerland), or by a combination of both when at least three follicles achieved a diameter of 18 mm. Ovum pickup was performed 36 hours later, and the embryologists determined the total number of oocytes retrieved and the MII oocytes per cycle. All MII oocytes were cryopreserved.

Statistical analysis

Data were analyzed with SPSS version 27.0 (SPSS, Inc., Chicago, IL, USA). The data were summarized as mean ± standard deviation or number of responders (percentage) according to the variables. Continuous variables between groups were compared with the t test. Comparison of ovarian stimulation data and outcomes between patients undergoing stimulation with rFSH or hp-hMG were made after ANCOVA with AFC as a covariate. A multivariate linear regression analysis was performed to control for age, basal FSH level, and protocol type as confounders for the estradiol (E2) level, number of retrieved oocytes, number of MII oocytes and maturity rate. Significance was tested with the t-test, Mann-Whitney U test, χ² test, and Fisher’s exact test as appropriate. A P value of < 0.05 was considered significant.

Results

Clinical characteristics of the study participants

A total of 456 women who underwent elective FP were included in this analysis, of whom 341 were stimulated with rFSH and 115 with hp-hMG. The clinical characterizations of the entire cohort are detailed in Table 1. The women in the rFSH group were significantly younger than the women in the hp-hMG group (35.50±2.12 years vs. 35.99±2.13 years, P = 0.034) and their AFC was significantly higher (13.76±6.08 vs. 11.84±6.06, P = 0.002).

Ovarian stimulation data and outcomes

No significant differences between the rFSH group compared to the hp-hMG group were found in the total FSH dosage (P = 0.645) or the duration of gonadotrophins treatment (P = 0.265). The peak E2 level was significantly lower in the rFSH group compared to the hp-hMG group (2547.18±1648.21 vs. 3468.02±2497.69 pg/mL; P < 0.001), while the peak progesterone level was significantly higher in the rFSH group compared to the hp-hMG group (1.33±0.75 vs. 1.01±0.52 ng/mL; P = 0.001). The numbers of retrieved and of MII oocytes were significantly higher in patients stimulated with rFSH compared to those stimulated with hp-hMG (16.82±10.95 vs. 13.25±9.66; P = 0.02, and 13.22±9.13 vs. 9.76±7.11; P = 0.005), while the oocyte maturity rates were also similar (P = 0.103) (Table 2)

A multivariate linear regression analysis (Table 3) showed a negative effect of advanced age, high levels of basal FSH and low AFC on the number of MII oocytes. A high basal FSH level and low AFC were also negatively correlated with the total number of retrieved oocytes, while there was no comparable correlation between these variables and the maturity rates. A high AFC was positively correlated with E2 levels. The protocol type (GnRH antagonist or PPOS) was not found to affect the various ovarian stimulation outcomes.

Discussion

The present study is the first to compare rFSH with hp-hMG for controlled ovarian hyperstimulation following an GnRH antagonist and a PPOS protocols in the setting of first-cycle elective FP. The findings demonstrated the
superiority of rFSH over hp-hMG in yielding a higher number of both retrieved oocytes and MII oocytes, although the proportion of mature oocytes to total retrieved oocytes was similar in the two groups.

Our results are in agreement with previous reports [8, 10-12, 28] in which follicular recruitment and development appeared to be more pronounced with rFSH compared with hp-hMG in combination with a GnRH antagonist. These outcomes may be explained by the potency of rFSH being higher than FSH isoforms in hp-hMG, resulting in the retrieval of significantly more follicles and oocytes. Indeed, basic FSH isoforms, such as rFSH, have a higher receptor affinity in vitro compared to more acid isoforms. Several studies suggested that given the higher biopotency in vitro, rFSH might also be more potent in vivo [29-31]. This possible outcome could also be explained by the potential atretic effect on non-dominant follicles attributed to the LH/hCG effect [32-34]. Nevertheless, we observed that hp-hMG treatment resulted in a similar maturity rate as that achieved with rFSH.

Despite the significantly lower oocyte number retrieved with hp-hMG, the E2 levels were substantially higher after treatment with hp-hMG compared with FSH. This finding has already been reported in GnRH antagonist cycles [8, 10-11] and is attributed to the continuous exposure of follicles to the LH activity in the hp-hMG protocol, which induces higher levels of aromatizable androgens leading to higher E2 concentrations in the second half of the follicular phase [35]. Alternatively, the difference in E2 levels could be explained by different elimination kinetics of the FSH isoforms in the two gonadotropins preparations. On the other hand, serum progesterone concentrations were significantly higher in the rFSH group, a finding that had been already related to the administration of FSH in GnRH antagonist cycles [8]. This increase was explained by the stimulation of granulosa cell activity by FSH [8, 36-38], while LH may induce progesterone catabolism to androgens at the level of theca cells. Even minor elevation in progesterone levels at the end of stimulation negatively affects the endometrium and therefore implantation and ongoing pregnancy rates [37-38].

Progestin for pituitary suppression during ovarian stimulation is an equivalent alternative to the GnRH antagonist protocol in women undergoing FP [13, 15]. This protocol has gained considerable popularity, making it essential to compare the ovarian response outcomes with different gonadotropins when following the PPOS protocol. We found no correlation between the protocol type and the ovarian stimulation outcomes in our current study, which included a small number of participants who had been treated with the PPOS protocol. Larger studies to confirm our preliminary findings are needed.

Importantly, all of the above-cited studies that compared the outcome of rFSH and hp-hMG were performed on infertile women undergoing treatment for IVF. Several differences that might affect ovarian response and future pregnancy outcomes should be considered in the setting of FP:

1) The women undergoing FP are ostensibly fertile.

2) The frozen oocytes will be fertilized later by means of intracytoplasmic sperm injection (ICSI). Previous studies could not identify any significant differences in the fertilization rates, pregnancy rates, and pregnancy outcomes between patients treated with rFSH or hp-hMG in ICSI cycles [8, 39-40]. One explanation could involve the interaction between oocytes and cumulus cells after oocyte retrieval that occurs only in IVF cycles. It has been proposed that the beneficial effect of the exogenous LH activity that is associated with the use of menotropins from the start of ovarian stimulation may materialize during the first hours of insemination in the IVF procedure and may be related to an enhanced effect of the cumulus cells on the oocytes resulting from increased LH activity in the stimulation period when menotropins are administered.
3) The parameter of the supraphysiological environment created by ovarian stimulation protocols that may be detrimental to both embryo implantation and placentation does not exist in FP.

4) The effect of the various gonadotropins on the endometrium does not affect future pregnancy outcomes.

Finally, as in most countries, the number of elective FP cycles is constantly increasing in Israel [41]. Women and physicians need to consider that the procedure is not funded, that rFSH is more expensive per unit than hp-hMG, and that there are fewer numbers of retrieved and mature oocytes associated with the hp-hMG protocol.

The present study has several limitations. Firstly, its retrospective design limits the ability to obtain more details about factors that are known to impact fertility, including additional ovarian reserve markers, such as anti-Müllerian hormone and antral follicle count. Secondly, others [42] reported that adding LH to rFSH in the antagonist protocol for older patients may improve ART outcomes. Since the age distribution in our study was similar for both study protocols, we cannot address that issue. Thirdly, the relevance of obtaining a high number of retrieved oocytes is in the acquisition of as many good-quality embryos as possible to increase the chances of pregnancies and live births. All previous studies with one exception [28] demonstrated comparable pregnancy and live birth rates when using either rFSH or hp-hMG for controlled ovarian stimulation in an antagonist protocol [8-12]. However, they all involved infertile women. Too few women have thus far used their cryopreserved oocytes in our IVF to enable us to report pregnancy and delivery outcomes. That information awaits the findings of further studies.

In conclusion, rFSH was demonstrated as being superior to hp-hMG in terms of the number of retrieved and mature oocytes when used in ovarian stimulation with GnRH and PPOS protocols for women undergoing elective FP. Further studies are required to explore the pregnancy and birth outcomes with diverse gonadotropins among women who undergo elective FP. Additional factors, such as availability, convenience, cost, and patient preferences should also be considered when choosing gonadotropins.

**Declarations**

**Funding** No external funding was either sought or obtained for this study.

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Availability of data and material** Data are available upon request.

**Code availability** Not applicable.

**Authors’ Contribution** T.I. and H.A. were involved in the conception, study design, data acquisition and analysis and manuscript preparation. N.S. and S.B. collected the data and managed the database. A.G. and F.A. contributed to the interpretation of the data and provided their expertise in the critical reading of the manuscript. All contributors reviewed and edited the manuscript and gave their approval of the final version.

**Ethics approval:** This study was approved by the institutional review board (Helsinki) of the Tel Aviv Medical Center (#0325-22-TLV).

**Consent to participate:** Anonymous questionnaire - no consent form is required. Approved by the institutional review board (Helsinki) of the Tel Aviv Medical Center.

**Consent for publication:** Not applicable.
References


### Tables
### Table 1
Comparison of clinical parameters between patients undergoing ovarian stimulation with rFSH or hp-hMG

<table>
<thead>
<tr>
<th></th>
<th>rFSH (n = 341)</th>
<th>hp-hMG (n = 115)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35.50 (2.12)</td>
<td>35.99 (2.13)</td>
<td>0.034</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.34 (3.91)</td>
<td>23.85 (5.01)</td>
<td>0.354</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td>0.543</td>
</tr>
<tr>
<td>Single</td>
<td>318 (93.3)</td>
<td>108 (93.9)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>5 (1.5)</td>
<td>3 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>18 (5.3)</td>
<td>4 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td>0.071</td>
</tr>
<tr>
<td>0</td>
<td>338 (99.1)</td>
<td>111 (96.5)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (0.9)</td>
<td>4 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Basal FSH (mIU/mL)</td>
<td>8.02 (2.7)</td>
<td>8.67 (3.80)</td>
<td>0.059</td>
</tr>
<tr>
<td>aTSH (µIU/mL)</td>
<td>1.99 (1.06)</td>
<td>1.86 (1.00)</td>
<td>0.113</td>
</tr>
<tr>
<td>aProlactin (mIU/L)</td>
<td>271.90 (154.36)</td>
<td>259.46 (131.15)</td>
<td>0.573</td>
</tr>
<tr>
<td>aAFC (n)</td>
<td>13.76 (6.08)</td>
<td>11.84 (6.06)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are presented as mean (standard deviation) or number (%). A P value of < 0.05 was considered significant.

*aThe P value was calculated after log transformation for normal distribution.

rFSH, recombinant follicle-stimulating hormone; hp-hMG, highly purified-human menopausal gonadotropin; BMI, body mass index; FSH, follicle-stimulating hormone; TSH, thyroid-stimulating hormone; AFC, antral follicle count
Table 2  
Comparison of ovarian stimulation data and outcomes between patients undergoing stimulation with rFSH or hp-hMG*  

<table>
<thead>
<tr>
<th></th>
<th>rFSH (n = 341)</th>
<th>hp-hMG (n = 115)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian stimulation duration (days)</td>
<td>11.33 (1.60)</td>
<td>11.02 (1.46)</td>
<td>0.265</td>
</tr>
<tr>
<td>^aGT total dose (mIU/mL)</td>
<td>3157.36 (919.35)</td>
<td>3263.48 (811.20)</td>
<td>0.645</td>
</tr>
<tr>
<td>^aPeak E2 (pg/mL)</td>
<td>2547.18 (1648.21)</td>
<td>3468.02 (2497.69)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>^aPeak P (ng/mL)</td>
<td>1.33 (0.75)</td>
<td>1.01 (0.52)</td>
<td>0.001</td>
</tr>
<tr>
<td>^aOocytes retrieved</td>
<td>16.82 (10.95)</td>
<td>13.25 (9.66)</td>
<td>0.02</td>
</tr>
<tr>
<td>^aMII oocytes</td>
<td>13.22 (9.13)</td>
<td>9.76 (7.11)</td>
<td>0.005</td>
</tr>
<tr>
<td>Maturity rate</td>
<td>77.98 (16.63)</td>
<td>75.10 (21.22)</td>
<td>0.103</td>
</tr>
</tbody>
</table>

*After ANCOVA with antral follicle count (AFC) as a covariate.

Values are presented as mean (standard deviation) or number (%). A P value of < 0.05 was considered significant.

^aThe P value was calculated after log transformation for normal distribution.

rFSH, recombinant follicle-stimulating hormone; hp-hMG, highly purified-human menopausal gonadotropin; GT, gonadotropins; E2, estradiol; P, progesterone; MII, metaphase II

Table 3  
A Multivariate linear regression analysis for various ovarian stimulation outcomes

<table>
<thead>
<tr>
<th></th>
<th>Peak E2 (pg/mL)</th>
<th>Oocytes retrieved (n)</th>
<th>MII oocytes (n)</th>
<th>Maturity rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standardized coefficient</td>
<td>P value</td>
<td>Standardized coefficient</td>
<td>P value</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.036</td>
<td>0.496</td>
<td>-0.064</td>
<td>0.152</td>
</tr>
<tr>
<td>Basal FSH (mIU/mL)</td>
<td>-0.036</td>
<td>0.496</td>
<td>-0.189</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AFC</td>
<td>0.445</td>
<td>&lt; 0.001</td>
<td>0.461</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Protocol type</td>
<td>-0.038</td>
<td>0.456</td>
<td>0.040</td>
<td>0.357</td>
</tr>
</tbody>
</table>

A P value of < 0.05 was considered significant.

FSH, follicle-stimulating hormone; E2, estradiol; MII, metaphase II; AFC, antral follicle count