Abstract. – Objective: To compare the pregnancy outcome in patients undergoing in vitro fertilization-embryo transfer (IVF-ET) cycles, using human derived follicle-stimulating hormone (FSH) or recombinant FSH for ovarian stimulation protocols.

Design: Prospective, multi-centre, randomized controlled trial.

Patients: 115 infertile patients undergoing a first attempt of in vitro fertilization and embryo transfer were included in the study. The inclusion criteria were: female age <37 years and use of GnRH agonist (GnRH-a) for pituitary down-regulation.

Interventions: Long Protocol-controlled ovarian stimulation with human derived FSH or recombinant FSH for IVF-ET.

Main Outcome Measures: Primary endpoints were implantation rate, clinical pregnancy rate and spontaneous abortion rate. 

Secondary end-points were total units of FSH injected, days of stimulation, peak estradiol levels at point of hCG administration, mean number of oocytes at pick-up, fertilization rate and cleavage rate.

Results: No statistically significantly differences in pregnancy outcomes were found in the patients receiving hFSH in comparison to patients receiving rFSH.

Conclusions: This study did not demonstrate a difference between the use of h-FSH vs r-FSH for ovarian stimulation in terms of pregnancy outcome, in good prognosis patients undergoing their first IVF-ET procedure.

Key Words:

Human derived FSH, Recombinant FSH , IVF-ET Cycles, COH.

Introduction

In vitro fertilization (IVF) is an established assisted reproduction technique that requires ovarian stimulation to increase the number of mature oocytes available for fertilization. Several different types of gonadotropins have been used for ovarian stimulation. One of the first used in IVF-ET was the urinary-derived human menopausal gonadotropin (hMG). It’s role is still debated considering the fact that hMG, having a low specific activity and containing significant amounts of luteinizing-hormone (LH) may lead to poor oocyte quality, reduced fertilization rates, lower embryonic viability and early pregnancy loss1,2.

The more recent use of other human-derived FSH preparations (hFSH) containing smaller quantities of LH was associated with higher pregnancy rates compared to the use of hMG in IVF cycles3,4.

Recently was shown that hMG treatment did not result in superiority in ongoing pregnancy rates in first-cycle IVF-intracytoplasmic sperm injection if compared with rFSH, and in particularly highly purified hMG resulted in retrieval of fewer oocytes, a lower incidence of hyperresponse, and comparable pregnancy rates.

The development of recombinant follicle stimulating hormone (rFSH), totally free from LH, activity was thought to represent a significant improvement for ovulation induction. Despite its theoretical advantages, clinical results in IVF using rFSH were not significantly better5.
Recombinant human FSH preparations, produced by inserting the DNA encoding the alpha and beta subunits of FSH into a Chinese hamster ovary cell line, are free from urinary proteins, do not contain any LH activity nor any other extraneous human proteins. Production of the product is independent of the vagaries of urine collection, thus ensuring a constant FSH supply and guaranteed batch-to-batch consistency.

Large-scale clinical trials and meta-analyses have been performed in order to investigate any differences between rFSH and hFSH in IVF cycles. No significant differences in the rates of spontaneous abortion, multiple pregnancy or ovarian hyper-stimulation syndrome (OHSS) were found between IVF cycles using rFSH and hFSH. The total dose of FSH required to meet criteria for hCG administration was lower with rFSH, but there was no significant difference in the number of follicles, serum estradiol levels on hCG day or in the number of oocytes retrieved.

Furthermore, a meta-analysis comparing the efficacy of recombinant LH (rLH) supplementation for ovarian stimulation in IVF/intracytoplasmic sperm injection cycles demonstrates that the presence of rLH with rFSH may prevent a decrease in oestradiol levels after antagonist administration and that a significantly higher number of mature oocytes was available for embryology work.

The aim of physicians working in the field of reproductive medicine remains to optimise the number of good quality oocytes available while giving due consideration to cost-effectiveness.

Published studies suggest that serum FSH levels are a prognostic factor for the quantity of oocytes obtained with elevated levels indicating reduced ovarian reserve and a poor response to ovarian stimulation in IVF. While age generally defines the quality of the oocyte: it is this difference in quantity, and not in quality, which makes the difference between cases of poor and normal response to IVF.

The aim of this prospective, randomized controlled, study was to compare the pregnancy outcome of IVF-ET cycles in which either hFSH or rFSH were used for controlled ovarian stimulation (COH) when GnRH agonist is used in for pituitary desensitization in “good prognosis” patients aged <37 years.

**Materials and Methods**

All patients treated in our IVF units between January 2008 and September 2008 were invited to participate in the study.

As shown in Table I, the indications for IVF-ET treatment were mainly moderate male factor, tubal defects, idiopathic infertility with previous failed intrauterine insemination attempts. All patients were age <37 years, with a basal FSH <12 mIU/ml.

The 115 patients included in the study were randomized to two groups, to receive either hFSH (n=62) or rFSH (n=53) for COH. No statistically significant differences between the groups in terms of mean age, body mass index, mean duration of infertility and the causes of infertility were identified (Table I). The Local Ethics Committee approved the protocol, and all patients gave written informed consent before entering the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: hFSH</th>
<th>Group B: rFSH</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>62</td>
<td>53</td>
<td>–</td>
</tr>
<tr>
<td>Mean (± SD) age (year)</td>
<td>32 ± 4.1</td>
<td>32 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mean (± SD) duration of infertility (months)</td>
<td>47.2 ± 17.6</td>
<td>41.9 ± 10.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>23.6 ± 6.5</td>
<td>22.7 ± 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Basal FSH</td>
<td>6.5 ± 2.7</td>
<td>6.9 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Causes of infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulatory factor* (%)</td>
<td>6 (9.7)</td>
<td>5 (9.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Endometriosis (%)</td>
<td>2 (3.2)</td>
<td>2 (3.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Male factor (%)</td>
<td>25 (40.3)</td>
<td>22 (41.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Tubal factor (%)</td>
<td>19 (30.6)</td>
<td>18 (34.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained (%)</td>
<td>10 (16.1)</td>
<td>6 (11.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

No statistical differences were found between groups, thus P values (P<0.05) are not shown.

Note: NS = not significant; *PCO; clomiphene-resistant; anovulatory/hormogonadotropic.
Pregnancy outcome following in vitro fertilization-embryo transfer (IVF-ET) oil. The embryo transfer was performed at the 2- to 4-cell stage, 40-44 h after insemination. No more than three embryos were transferred. Spare mature oocytes were cryopreserved according to protocols described in previous studies15.

Pregnancy was detected by measuring β-hCG levels. A clinical pregnancy was determined by the visualization of an embryo with cardiac activity at 6-7 weeks of pregnancy. A spontaneous abortion is registered, when the loss of the pregnancy between the fifth and the twelfth week of gestation occurred.

Statistical Analysis

The statistical package SPSS KIT SigmaStat for Windows V2.03S was used for the data analysis. Clinical characteristics were analyzed using the unpaired Student’s t-test or the Mann-Whitney rank sum test. All other analyses were performed using χ2 analysis of Fisher’s exact test. Results with \( P < 0.05 \) were considered significant.

Results

During the study period, 115 patients conforming to the inclusion criteria were randomized into two groups as previously described.

The outcome of ovulation induction is reported in Table II.

Statistical Analysis

The statistical package SPSS KIT SigmaStat for Windows V2.03S was used for the data analysis. Clinical characteristics were analyzed using the unpaired Student’s t-test or the Mann-Whitney rank sum test. All other analyses were performed using χ2 analysis of Fisher’s exact test. Results with \( P < 0.05 \) were considered significant.

Results

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The outcome of ovulation induction is reported in Table II.

In the r-FSH group there was both a significant reduction in the number of ampoules of FSH required (20.7 ± 8.2 vs 24.1 ± 7.7, \( p=0.04 \)) and a significantly increased number of oocytes collected at pick-up (10.9 ± 3.31 vs 9.8 ± 4.12, \( p=0.04 \)).

Table II. Outcome of COH and laboratory.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: h-FSH n = 62</th>
<th>Group B: r-FSH n = 53</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of stimulation (d)</td>
<td>11.3 ± 0.9</td>
<td>10.4 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>No. of ampoules (75 IU) of FSH (Mean ± SD)</td>
<td>24.1 ± 7.7</td>
<td>20.7 ± 8.2</td>
<td>0.04</td>
</tr>
<tr>
<td>17 β-estradiol level on the day of hCG administration (pg/mL)</td>
<td>2232.1±510</td>
<td>2713.3±595</td>
<td>0.02</td>
</tr>
<tr>
<td>Cancelled cycles (Estradiol level &gt; 4000 pg/ml)</td>
<td>1</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>No. of retrieved oocytes (Mean ± SD)</td>
<td>9.8 ± 4.12</td>
<td>10.9 ± 3.31</td>
<td>0.04</td>
</tr>
<tr>
<td>No patients with &gt; 3 spare mature oocytes</td>
<td>57 (91.9%)</td>
<td>46 (86.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of cryopreserved oocytes (Mean ± SD)</td>
<td>5.92 ± 2.7</td>
<td>6.65 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>No of embryo-transferred (Mean ± SD)</td>
<td>2.1 ± 0.6</td>
<td>2.2 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>
Despite those finding, no statistically significant difference between the two groups were noted in IVF outcome in terms of mean number of embryos transferred (2.1 ± 0.6 vs 2.2 ± 0.3, \( p=\text{NS} \)), number of patients with spare mature oocytes, suitable for cryopreservation (57 vs 46, \( p=\text{NS} \)) and mean number of oocytes cryopreserved (5.92 ± 2.7 vs 6.65 ± 2.4, \( p=\text{NS} \)).

The IVF outcome of IVF in both group was evaluated in terms of implantation rate (number of embryos implanted/total number of embryos transferred), clinical pregnancy rate (number of clinical pregnancies/number of patients), spontaneous abortion rate (number of spontaneous abortions/total number of clinical pregnancies) (Table III).

No statistically significant differences in any of these clinically significant outcomes were found.

**Discussion**

During the last decade, follicle-stimulating hormone (FSH) treatment to induce follicular development in anovular women and multiple follicular development for assisted conception has involved the use of either human derived or recombinant preparations. The human derived preparations of gonadotropins have been progressively replaced in clinical practice by the new recombinant products which are completely devoid of any LH activity and other extraneous human proteins which contaminated the “first generation” of urinary-derived preparations. However, it must be stressed that the new “highly purified” urinary gonadotrophins have been previously proven to be safe and efficient preparations for use in ovarian stimulation. FSH and LH are both required for appropriate follicular and steroidogenesis and in hypogonadotropic women, the addition of some LH activity (human menopausal gonadotropin, human chorionic gonadotropin or rLH) is obligatory to achieve appropriate follicular development, adequate estradiol levels and pregnancy. The role of LH in ovulation induction in normogonadotropic women is still a matter of debate. The availability of the highly purified and recombinant products raised questions as to whether better results can be achieved in terms of efficacy and cost effectiveness. For this reason several studies have been carried out to determine whether r-FSH is able to improve IVF outcomes compared to those obtained using urinary derived products. The parameters considered were not only the comparative efficacy of the product in terms of IVF outcomes (live births per cycle), but the higher medical costs of the newer recombinant products led many researchers to carry out trials to explore the efficacy and the relative cost-effectiveness of alternative treatments in assisted reproduction techniques. Meta-analyses and “cost-effectiveness” models failed to reveal whether one or the other preparation was associated with a better pregnancy rate and with concomitant lower costs in IVF cycles. However, it should be acknowledged that recombinant products showed a tendency to have better results in terms of oocytes retrieved and embryos available for cryopreservation, even though further studies, using the new ‘high purity’ human derived FSH formulations, showed opposite results.

The chance of a woman successfully delivering a baby after IVF-ET treatment depends on her fertility rate, the risk of spontaneous miscarriage and the chance of chromosomal abnormalities; all factors which are strongly dependent on the age of the woman. Fertility as a function of women’s age has also been clearly demonstrated in donor insemination programmes in which the cause of infertility was related to male factors alone and the women were assumed to have normal fertility potential.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: hFSH</th>
<th>Group B: rFSH</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation rate (%)</td>
<td>35/128a (27.3)</td>
<td>32/110a (29.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical PR (%)</td>
<td>24/62b (38.7)</td>
<td>21/53b (39.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous abortion rate (%)</td>
<td>3/24 (12.5)</td>
<td>2/21 (9.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

PR = pregnancy rate; NS = not significant; atotal number of embryo transferred; bnumber of patients.
Better ovarian response is consistently associated with higher pregnancy rates and hence different hormonal and ultrasound parameters known as ovarian reserve markers have been developed in ART to predict ovarian response to gonadotrophins. The basal level of follicle-stimulating hormone (FSH) is a better predictor of ovarian responsiveness than the age of the woman but ongoing pregnancy and livebirth rates are influenced significantly by age.

In this study all women enrolled were considered to have normal fertility and screening did not identify any recognised female factors that would militate against conceiving with IVF-ET.

The use of r-FSH demonstrated increased ovarian recruitment of follicles compared with h-FSH, as shown by improved number of oocytes retrieved at ovum pick-up, and this was associated with a small but still statistically significant reduction in units of gonadotrophins used.

However, if interest was focused on the gamete and embryo quality and consequently on IVF outcome, no difference was found in terms of fertilization rate, number of embryo-transferred, chance to cryopreserve spare mature oocytes and mean number of cryopreserved oocytes.

Similarly no difference emerged in pregnancy outcome when both h-FSH and r-FSH preparations were compared.

These excellent and strictly comparable implantation, pregnancy and ongoing-pregnancy rates reflect the good prognosis for the study group in terms of female age and ovarian reserve.

For these reasons our data support the need for a choice of which compound to offer a positive outcome in an IVF programme and indicate the need to consider cost-effectiveness of the therapy depending on the category of patients undergoing treatment.

References

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