European Review for Medical and Pharmacological Sciences 2015; 19: 2162-2167

The use of r-hFSH in treatment of idiopathic male factor infertility before ICSI

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Abstract. – OBJECTIVE: To evaluate the efficacy of pre-treatment of idiopathic oligozoospermic patients with r-hFSH to improve the clinical results of ICSI.

PATIENTS AND METHODS: 82 infertile couples due to male factor who attended our center were included in the study. Thirty-six were randomized to the treatment group (group A) and forty-six to the control group (group B). The male partners in group A were treated with recombinant human FSH (r-hFSH; Gonal F®) 150 IU subcutaneously three times a week for a 3-months period. The control group (group B) did not receive any treatment. After the treatment couples of both groups underwent a cycle of ICSI.

RESULTS: The fertilization rate was comparable in both groups. However, in the treatment group (group A), the clinical pregnancy rate was significantly higher (42%) compared to the control group (group B) (20%) ($p < 0.02$). Also, the implantation rate was significantly higher in treatment group (26%) compared to the control (15%) ($p < 0.04$). Miscarriage rate was lower (15.7%) in the treatment group than in the control (43.7%), and this difference was statistically significant ($p < 0.05$).

CONCLUSIONS: Treatment of idiopathic male factor infertility with r-hFSH before ICSI improves clinical pregnancy rate, increases implantation rate and decreases the early pregnancy loss.

Key Words:
Idiopathic male factor infertility, FSH therapy, ICSI.

Introduction

Whilst male factor alone is responsible for approximately 30% of cases of infertility, a combination of male and female factors affect another 20%. Thus, it is estimated that the male factor contributes to 50% of infertile couples[1].

Unfortunately, many aspects of male factor infertility are still poorly understood, and most patients are classified as having idiopathic oligoteratoasthenozoospermia[2,3].

Intracytoplasmic sperm injection (ICSI) is now established as the treatment of choice for infertile male patients with severe oligozoospermia. However, the poor quality sperm structure may impede ICSI outcomes[4]. Low fertilization rates associated with poor quality sperm may be due to acrosomal dysfunction or to disturbed axonemal or DNA integrity[5,6].

The importance of good quality sperm for embryonic development is now beginning to be appreciated. The use of ICSI as the treatment of choice for male factor infertility should not prevent us from improving sperm quality and, thereby, achieving better results in both IVF and ICSI.

There is a general consensus on the need for FSH in the regulation of spermatogenesis[7,8]. Because of the physiologic role of FSH in spermatogenesis, various attempts have been made to treat idiopathic oligozoospermic patients with this hormone. However, the results obtained are still controversial. Several uncontrolled studies have shown an improvement of seminal parameters and/or an increase of pregnancy rates in infertile men treated with different doses of FSH[9,10].

In this work, we have conducted a prospective randomized controlled study to evaluate the efficacy of pre-treatment of idiopathic oligozoospermic patients with r-hFSH to improve the clinical results of ICSI.

Patients and Methods

Patients Selection

The study was conducted at the IVF/ICSI program of the Biofertility IVF Center, Rome, Italy, between May 2013 and April 2014 on infertile couples due to idiopathic male factor infertility undergoing ICSI treatment.

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The study was reviewed and approved by the Institutional Review Board at the Biofertility IVF Center, Rome, Italy. All patients undergoing ICSI and participating to the study gave informed consent.

A total of 92 infertile couples who met the inclusion criteria were enrolled in our study.

But only 82 couples completed the study. Ten of the treated group were excluded because seven of them decided to stop treatment and do ICSI, another three did not return to us after treatment. So, thirty-six were randomized to the treatment group (group A) and forty-six to the control group (group B).

Inclusion criteria were; a history of infertility for at least 2 years; sperm count > 10×10^6/ml according to World Health Organization; idiopathic male factor infertility with exclusion of common conditions, such as history of cryptorchidism, post-mumps orchitis, testicular torsion or trauma, varicocele, seminal tract infections, antisperm antibodies karyotypic abnormalities and y chromosome microdeletions; normal plasma levels of FSH (range, 2-12 IU/L), LH (range, 2-12 IU/L), and testosterone (range, 10-30 nmol/L). Exclusion of clear female factors, such as ovulatory disorders, tubal factor, endometriosis, and endocrine abnormalities as evaluated by endocrine evaluation, pelvic ultrasound examination, and hysterosalpingography.

The male partners in group A were treated with recombinant human FSH (r-hFSH; Gonal F®, Serono) 150 IU subcutaneously three times a week for a 3 months period.

The control group (group B) did not receive any treatment

Ovarian Stimulation

The female partners were treated with a long protocol in which GnRH-analogue (Buserelin subcutaneous injection, 0.4 mg daily) was given as a pre-treatment and r-hFSH administration (Gonal-F 225 IU daily) took place when pituitary desensitisation was established.

The dosage was adjusted according to estradiol (E2) levels in serum and follicular development on ultrasound.

Recombinant hCG (Ovitrelle® Serono250 microgram equivalent 6500 IU) was administered when the follicular diameter reached ≥ 18 mm, 36 hours before oocyte retrieval.

Semen Analysis and Preparation

All semen samples were obtained after 4-5 days of sexual abstinence. Semen evaluation was performed by routine semen analysis using light microscopy. Routine semen analysis included measurements of sperm concentration, motility, viability, and morphology according to the World Health Organization guidelines[11]. Semen samples were prepared with the Swim-up method.

ICSI Procedure

The holding and injection pipettes were made from glass capillaries with an outside diameter of 1 mm and inside diameter of 0.6 mm. ICSI procedures were carried out on the heated stage (37°C) of an inverted microscope at X400 magnification using the Hoffman Modulation Contrast System. The system included Narishige micromanipulators and a video graphic printer. Only metaphase II oocytes were selected for micromanipulation which was performed in all patients according to the published procedures[12,13]. Oocytes with two pronuclei and two polar bodies 18 hours after ICSI were considered as normally fertilized. Assessment of embryonic development was undertaken after 44 hours post insemination. Embryo transfer was performed 48 hours after oocyte retrieval with an embryo transfer catheter.

Establishment of Pregnancy

The luteal phase was supported with vaginal progesterone 200 mg (Progeffik®, Effik, France) three times a day. Serum levels of β-hCG were measured two weeks after embryo transfer. Clinical pregnancy was confirmed by seeing fetal heart beat on ultrasound examination.

Statistical Analysis

The parameters analyzed were, fertilization rate, clinical pregnancy rate, implantation rate and early miscarriage rate.

The results are expressed as mean ± SD. Comparisons between groups were performed using *t*-test and Chi-square as required. Probability (p) values of < 0.05 were considered statistically significant.

Results

The women of the two study groups were similar for age, body mass index, basal levels of FSH and E2 in the 3rd day of the cycle (Table I).

Table I. Clinical and laboratory data for female partners in both groups expressed as mean ± SD.

Parameters	Group A (n = 36)	Group B (n = 46)	*p* value
Age (yr)	35.69 ± 4.1	36.23 ± 4.7	NS
BMI (kg/m^2)	21.5 ± 5.9	23.3 ± 5.9	NS
Basal FSH (mIU/ml)	7.5 ± 2.4	7.8 ± 2.5	NS
Basal LH (mIU/ml)	5.7 ± 3	5.3 ± 2.8	NS
Basal estradiol (pg/ml)	48 ± 20.4	50.6 ± 21.7	NS
Duration of stimulation (days)	11.1 ± 1.2	11.3 ± 1.3	NS
Total dose of FSH required (IU)	2721 ± 1048	2872 ± 1057	NS
Estradiol on day of hCG administration (pg/ml)	1709 ± 848	1691 ± 773	NS
Follicles day hCG	10.2 ± 5.1	8.8 ± 5	NS

NS: not significant.

Table II. Clinical and hormonal features of men with male factor infertility in treated group A compared to control group B, expressed as mean ±SD.

Parameters	Group A (n = 36)	Group B (n = 46)	*p* value
Age (yr) of male	36.9 ± 5.1	38.4 ± 5.2	NS
No. of years of infertility	7.4 ± 3.3	7 ± 4	NS
FSH (mIU/ml)	6.5 ± 2.5	6.4 ± 2.3	NS
LH (mIU/ml)	4.7 ± 1.9	5.1 ± 2	NS

NS: not significant.

Table III. Seminal parameters evaluated before treatment in Group A and compared to group B, expressed as mean ± SD.

Parameters	Group A	Group B	*p* value
Sperm count (×10^6/ml)	7.59 ± 4.3	7.2 ± 3.1	NS
Total motility (%)	17.2 ± 7.39	18.7 ± 9.4	NS
Forward motility (%)	6.77 ± 4.1	7.52 ± 6	NS
Normal morphology (%)	9 ± 5.47	10 ± 7	NS

NS: not significant.

Table IV. Number of oocytes retrieved and embryo transferred in treated group A compared to control group B, expressed as mean ± SD.

Parameters	Group A	Group B	*p* value
No. of oocytes retrieved	7.8 ± 5.2	6.5 ± 3.7	NS
No. of embryo transferred	2.6 ± 0.59	2.4 ± 0.7	NS

NS: not significant.

The two groups showed comparable data for total units of FSH administered, E2 levels at HCG day, days of stimulation, number of follicles (Table I). The male partners were comparable as regards age, hormonal and semen parameters before treatment (Tables II and III). Number of oocytes harvested, and number of embryos transferred (Table IV) were comparable in both groups. The present study indicates that recombinant human FSH treatment improves sperm count, motility and morphology, although they did not reach the normal range (Table V).

The fertilization rate was also comparable in both groups (Table VI).

Table V. Results of ICSI in treated group A compared to control group B, expressed as percentage.

Parameters	Group A	Group B	*p* value
Fertilization rate	89.6%	88.2%	NS
β-hCG positive (%)	52.77%	34.78%	NS
Implantation rate	26%	15%	$p < .05$
Clinical pregnancy rate	42%	20%	$p < .05$
Abortion rate	15.7%	43.7%	$p < .05$

NS: not significant.

Table VI. Seminal parameters evaluated before and after treatment in Group A, expressed as mean ±SD.

Parameters	Group A	Group B	*p* value
Sperm count ($\times 10^6$/ml)	7.59 ± 4.3	11.33 ± 7.95*	< 0.05
Total motility (%)	17.2 ± 7.39	34.7 ± 13.78*	< 0.05
Forward motility (%)	6.77 ± 4.1	11.86 ± 8.6*	< 0.05
Normal morphology (%)	9 ± 5.47	15.36 ± 9.6	NS

NS: not significant.

However, in the treatment group (group A), the clinical pregnancy rate was significantly higher (42%) compared to the control group (group B) (20%) ($p < 0.02$). Also, the implantation rate was significantly higher in the treatment group (26%) compared to the control (15%) ($p < 0.04$).

The miscarriage rate was lower (15.7%) in the treatment group than in the control (43.7%), and this difference was statistically significant ($p < 0.05$) as shown in Table VI.

Discussion

The technique of ICSI bypasses the physiological selection processes, allowing even sperm with severe structural abnormalities to fertilize oocytes. FSH plays a critical role in promoting quantitative spermatogenesis, as demonstrated by several reports[14]. Patients with an inactivating mutation of FSH receptor or mutations of the FSH-b subunit show a variable degree of spermatogenetic damage[15,16]. Mutations and polymorphisms in the genes encoding gonadotropins and their receptors could explain, at least in part, the presence of normal blood levels of gonadotropins in presence of oligoteratoasthenozoospermia. In fact, often these patients showed FSH and LH in normal range despite a low number of spermatozoa. Several publications have been demonstrated a positive effect of recombinant FSH therapy in infertile males with normal gonadotropins levels suggesting a functional defect in circulating FSH[17-20]. Strehler et al[19] reported that FSH treatment may restore sperm structure. Foresta et al[17] had demonstrated that highly purified FSH could be the appropriate treatment for patients with oligozoospermia when inhibin B and FSH plasma levels are in the normal range and the testicular tubular structure is characterized by hypospermatogenesis without maturation disturbances. Ben-Rafael et al[18] in his randomized study, have shown that FSH treatment for men with oligoteratoasthenozoospermia before IVF improves fertilization rate after treatment (20%) compared to (5.8%) in the study control cycles. This effect may be related to improvements in subcellular components of the sperm (mainly the acrosome, nucleus, and axoneme). In recent years, biotechnology has made available recombinant human FSH preparation, which guarantees the clinical availability of the most constant and biochemically pure FSH preparation. More recently, investigators reported the use of recombinant human FSH (r-hFSH) treatment in men with idiopathic infertility and in oligozoospermic patients, with a significant improvement of sperm parameters in the latter[19,20].

Foresta et al[22] in his controlled, randomized clinical study showed that treatment with recombinant hFSH can improve seminal parameters and also spontaneous pregnancy in selected

group of idiopathic oligozoospermic patients (those with normal FSH plasma levels and absence of maturation arrest). Ashkenazi et al[23] concluded that purified FSH therapy in male partners before ICSI improves implantation rate and there was a trend of higher number of better quality embryos per transfer in the treated group.

In our study we used the recombinant human FSH as pre treatment for male factor infertility befor ICSI, and our findings indicate that recombinant human FSH treatment improves sperm count, motility and morphology, although they did not reach the normal range (Table V). Also, there is a significant increase in clinical pregnancy rate and implantation rate in the treated group versus the control (42% versus 20% and 26% versus 15% respectively). The rate of abortion was lower in the treated group versus the control group (15.7% versus 43.7%). With treatment we may have more chance to find good quality sperm and inject it inside the oocyte. This may improve embryo development, so increases implantation and clinical pregnancy rates and also decreases the rate of abortion.

Conclusions

Treatment of idiopathic male factor infertility with r-hFSH before ICSI improves clinical pregnancy rate, increases implantation rate and decreases the early pregnancy loss. The use of ICSI as the treatment of choice for male factor infertility should not prevent us from improving sperm quality and thereby achieving better results in both IVF and ICSI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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