A comparative study of the effect of ovarian stimulation protocols with different gonadotropin preparations on the biological and clinical parameters of the outcome of intracytoplasmic sperm injection

P. Drakakis, D. Loutradis, K. Kallianidis, R. Bletsa, S. Milingos, A. Dionyssiou-Asteriou[1], S. Michalas

IVF Unit, ALEXANDRA Maternity Hospital, 1st Dept. of OB/GYN, Athens University Medical School
[1]Dept. of Biological Chemistry, Athens University Medical School, Athens (Greece)

Summary

Intracytoplasmic sperm injection (ICSI) is widely employed today in cases of severe male factor infertility. This technique requires denuding the oocytes from the surrounding granulosa cells prior to sperm injection. One can thus assess oocyte maturity more accurately and can study the effects of various ovarian stimulation protocols on egg maturation and the rest of the parameters of the outcome of ICSI.

The aim of the present study was to compare the outcome of ovarian stimulation using human menopausal gonadotropin (hMG) with that achieved by using highly purified follicle stimulating hormone (pFSH). The biological and clinical parameters of the outcome of ICSI in 99 subfertile couples were studied. Group A consisted of 46 patients to whom hMG was administered and Group B consisted of 53 patients to whom pFSH was employed for ovarian stimulation. The fertilization rate was significantly higher in the pFSH group but all other factors were similar, including the percentage of mature oocytes and pregnancy rate. The latter does not seem to be affected by the gonadotropin preparation employed for ovarian stimulation. This is very helpful for the physician to know since a gonadotropin with a lower cost can be employed and, in addition, shortage of some preparations of gonadotropins occurs frequently.

Key words: Ovarian stimulation; Gonadotropins; Intracytoplasmic sperm injection (ICSI).

Introduction

In intracytoplasmic sperm injection (ICSI), a single sperm is deposited directly into the cytoplasm of a mature metaphase II oocyte [1-4]. ICSI, thus, bypasses sperm-oolemma fusion, bypassing, accordingly any fertilization defects in these steps [5]. It has therefore provided a great number of infertile couples due to severe male factor with the opportunity to give birth to a healthy child.

Proper ovarian stimulation is very important for providing mature oocytes for fertilization either in IVF-ET or in ICSI. After so many years, there is still some controversy as to the most efficient method of ovarian stimulation in order to promote the development and growth of a large number of follicles and to, consequently, yield a large number of mature, good quality oocytes for fertilization in vitro [6].

On the other hand, an important part of the intracytoplasmic sperm injection (ICSI) procedure [11,12] involves the removal of the cumulus-corona complex so that the oocyte can be stabilized by suction against a holding pipette. This stripping off of the vestments of the oocyte also allows a precise determination of the stage of progression of the oocyte through meiosis and, therefore, the maturity of the oocyte can be determined more accurately [7], something that we are not able to do in standard IVF-ET.

Recombinant FSH (r-FSH) is available nowadays but the cost is high and results are not always better than those obtained by urinary gonadotropins. Urinary gonadotropins differ, also, among themselves in ratio of FSH and LH and in cost. The present study was carried out to compare the effect of ovarian stimulation using two different preparations of urinary gonadotropins (human menopausal gonadotropin and highly purified follicle stimulating hormone) on the outcome of ICSI.

Material and methods

The stimulation protocol for all patients was briefly as follows [8]: On day 21 of the previous cycle, a baseline ultrasound scan was performed and buserelin (D-Ser-T-B46-LHRH-1-9 ethylamide, Hoechst, Germany) intranasal spray was commenced at a dose of 100 μg 5 times daily (every 4 h, omitting the 3 am dose). For all patients, the extent of ovarian suppression was evaluated by ultrasound scan and serum E2 levels (≤ 40 pg/ml) before starting exogenous gonadotropin administration.

Patients were randomized prospectively into two groups: In Group A (n = 46), human menopausal gonadotropin (FSH:LH 1:1) was employed for ovarian stimulation. Either Pergonal (Serono, Switzerland) or Humegon (Organon, The Nederlands) was employed. Stimulation commenced with a starting dose of 3 amp daily for four days and the dose was readjusted according to daily measurements of serum estradiol levels and folli-

Revised manuscript accepted for publication March 18, 2002

cle development. In group B (n = 53), two ampules of highly purified FSH (Metrodin HP, Serono, Switzerland) along with one ampule of hMG (Pergonal or Humegon) was administered for four days and the dose was readjusted the following days only with Metrodin HP ampules, i.e., a total of four hMG ampules was employed and the rest were purified FSH preparations. The difference in the number of patients in the two groups was due to the availability of the preparations.

GnRH-analog administration was continued until human chorionic gonadotropin (hCG) administration (Pregnyl, Organon, The Netherlands). When the mean diameter of at least two leading follicles was ≥ 18 mm and serum E_2 was rising, 10,000 IU were given intramuscularly. The inverval between the last gonadotropin injection and hCG was no more than 24 h. Thirty-five to 36 hours after hCG, ovum retrieval was performed by transvaginal echo-guided ovarian puncture. The luteal phase was supported with 2,500 IU of hCG injected on the days of ovum retrieval, embryo transfer and day 4 after replacement.

Embryology Laboratory Procedures

Semen assessment and preparation

Semen samples were produced by masturbation on the day of egg retrieval following an abstinence period of at least three days. Assessment regarding sperm density and motility was undertaken according to the recommendations of the World Health Organization [9]. Evaluation of sperm morphology was performed according to the strict criteria by Kruger [10]. Prior to ICSI, semen assessment was carried out at least once in order to ensure whether enough motile-viable sperm were present in the ejaculate.

Sperm was prepared by the method of a discontinuous Percoll gradient [11]. Finally, sperm were resuspended in Ham's medium at a concentration of approximately $7x10^6$ sperm/ml in most cases and incubated in a 37 °C, 5% Co_2 incubator – until the ICSI procedure.

Oocyte preparation

Approximately four hours after egg collection, removal of the cumulus and corona cells was performed by incubating the eggs in Ham's F-10 medium with 80 IU/ml hyaluronidase (type VII, 320 IU/mg; SIGMA) for 30 sec. The oocytes were then transferred to fresh medium and further removal of adhering corona cells was obtained by mechanical pipetting. Intracytoplasmic sperm injection was performed only on mature eggs that had extruded the first polar body (metaphase II).

Micro injection procedure

Holding and injection pipettes were prepared from microcapillary tubes (Narishige, Japan) by standard procedures [4]. The procedure was performed on the heated stage of an inverted microscope (Diaphot, Nikon, Japan) at 400x magnification using Hoffman modulation contrast optics. The microscope was equipped with two coarse positioning manipulators (3D-motor drive coarse manipulator, MM-188, Narishige-Nikon) and a Joystick hydraulic micromanipulator Mo-188 (Narishige-Nikon). Suction and release of medium through the holding and ICSI pipettes were adjusted using the micro-injectors IM-188 and IM-6 (Narishige-Nikon), respectively.

ICSI was performed as previously described [13]. After injection, the oocytes were washed in fresh culture medium and incubated in Ham's F-10 medium until assessment for presence of pronuclei. The further handling of the injected ova was similar to our standard IVF program [12].

Statistics

Data were analyzed with the x^2-test.

Results

The hormonal profile (baseline values) of the patients was comparable in the two groups (Table 1).

Table 1. — *Hormonal profile of the patients in the two groups.*

	Group A (hMG)	Group B (pFSH+hMG)	
FSH	6.3 ± 2.7*	6.7 ± 2.9	NS**
LH	7.0 ± 3.7	5.7 ± 4.0	
Prolactin	10.5 ± 6.2	10.3 ± 5.0	
Δ_4A	2.7 ± 2.4	2.1 ± 1.1	
DHEA-S	1763 ± 1165	1601 ± 208	
Ftesto	2.6 ± 4.3	2.8 ± 5.4	

*mean ± standard deviation
**p > 0.10 (NS) for all values (x^2 test)
FSH: Follicle stimulating hormone, LH: Luteinizing hormone
Δ_4A: Δ_4-androstendione, DHEA-S: Dehydroepiandrosterone sulfate,
Ftesto: Free testosterone

The two groups did not differ in mean age (31.3 ± 14.2 versus 33.2 ± 3.8). The cancellation rate was similar in both groups (2.2 vs 1.9%). All patients in both groups had circulating E_2 levels ≤ 40 pg/ml before starting gonadotropin stimulation. The need for exogenous gonadotropins and the length of the stimulation period were similar in the two groups (47.6 ± 14.2 ampules of hMG were injected in 9.7 ± 0.8 treatment days in group A patients; 50.4 ± 16.2 ampules of hMG+pFSH were administered in 9.9 ± 0.8 days to group B patients). Serum E_2 on the day of hCG administration was similar in the two groups (Table 2).

Table 2. — *Ovarian stimulation outcome for the patients in the two groups.*

	Group A (n = 46) (hMG)	Group B (n = 53) (pFSH+hMG)	
Age (y)	31.3 ± 3.6*	33.2 ± 3.8	NS**
Cancellation rate (%)	2.2	1.9	
Serum estradiol after desensitization (pg/ml)	32.6 ± 12.1	31.8 ± 16.6	
No of gonadotropin ampules	47.6 ± 14.2	50.4 ± 16.2	
Duration of stimulation (days)	9.7 ± 0.8	9.9 ± 0.8	
Serum E2 on the day of hCG	2151 ± 182	1980 ± 201	
No of oocytes retrieved per cycle	12.6 ± 3.0	10.9 ± 4.6	
Percentage of mature oocytes (%)	73.0	73.2	

* Mean ± standard deviation
** For all values p > 0.10 (NS) (x^2 test)

The mean number of oocytes retrieved was 12.6 ± 3.0 for group A and 10.9 ± 4.6 for group B. There was no difference in the proportion of mature (metaphase II) oocytes collected (73% vs 73.2% respectively; $p > 0.10$ (Table 2). There was a higher fertilization rate in group A (81.0% vs 73.2% 2 PN embryos respectively; $p < 0.05$). Cleavage rate (at least first division) and the mean number of embryos replaced per patient was not significantly different between the two groups.

Intrauterine pregnancies were confirmed in all cases by ultrasound scans. The clinical pregnancy rate achieved per transfer was 26.7% and 25.0% for the two groups ($p > 0.1$).

Table 3. — *Fertilization, cleavage and clinical pregnancy rates for the two groups.*

	Group A (n = 46) (hMG)	Group B (n = 53) (pFSH+hMG)	
Fertilization rate (%)	81.0	73.2	$p < 0.05$
Cleavage rate (%)	98.5	99.3	NS**
No of transferred embryos	4.7 ± 1.1	4.1 ± 1.2	
Pregnancy rate per transfer (%)	26.7	25.0	

*mean ± standard deviation
**$p > 0.10$ (NS) for all values (x^2 test), unless otherwise mentioned.

Discussion

ICSI is a successful treatment for male factor infertility, the results of which compare favorably to those of IVF cycles [1, 14, 15]. On the other hand, the presence of multiple, good quality oocytes and, consequently, the availability of many good quality embryos for transfer is a very important factor that increases pregnancy rates in IVF-ET or ICSI. This is true especially in the latter, in which the importance of male factor subfertility is overcome by the procedure [13, 16]. Therefore, it is necessary to stimulate the growth and maturation of several follicles, and this is achieved by the exogenous administration of gonadotropins.

Various forms of urinary gonadotropin preparations exist that differ in the proportion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Human menopausal gonadotropin (hMG) has a 1:1 ratio of FSH and LH. It is extracted from postmenopausal urine [17]. By advances in the purification technique, hMG can be further purified and FSH can be immunoextracted using monoclonal antibodies. With this method, a highly purified preparation of FSH (Metrodin HP, Serono, Switzerland) is available for ovarian stimulation [6, 18, 19].

In this study, it was shown that most biological parameters and the clinical end-point of pregnancy rate were similar in the two groups. Serum E_2 on the day of hCG administration, the mean number of oocytes retrieved, the proportion of mature (metaphase II) oocytes collected and cleavage rate were comparable in the two groups. Oocyte maturation could be assessed clearly since oocytes are denuded before ICSI is performed [16]. Fertilization rate, though, was significantly higher in the pure FSH group. The fertilization potential of an oocyte depends not only on nuclear maturity (which can be assayed after denuding the oocyte from the granulosa cells) but also on cytoplasmic maturity. The maturational stage of the cytoplasm is partially controlled by the sperm nucleus but it is, also, essentially controlled by other cytoplasmic constituents. It has been demonstrated that oocytes require a specific intrafollicular steroid environment for the inductive signals of meiotic resumption and the completion of the full maturation process [20, 21]. Steroids exert a significant role on the synthesis of cytoplasmic factors that induce normal decondensation of the sperm head and formation of the male pronucleus [22]. Pure FSH preparations may have a beneficial influence on local factors that control steroidogenesis, cytoplasmic maturation and consequently, fertilization.

The mean duration of ovarian stimulation and the mean total number of ampules used was similar for the two groups. The same was true for the number of embryos transferred and the clinical pregnancy rate which is the important parameter that we all look for to improve. This latter does not seem to be affected by the gonadotropin preparation used for ovulation induction, as long as three to four good quality embryos are available for transfer. The same seems to be true for other more recent gonadotropin preparations, such as recombinant FSH [6, 23]. Although some parameters may be influenced, clinical pregnancy rate seems to be unaffected by the type of gonadotropin employed. This is mainly true in ICSI programs in which male factor is the only or the main subfertility factor and in ages of women below 35 years. The comparable pregnancy rates with different preparations is a fact that becomes even more important to know nowadays since there is a shortage from time to time of some preparations of gonadotropins and a physician has to rely on whatever form is available. Moreover, gonadotropins with a higher cost seem to yield the same clinical results compared to those with a lower cost and this has also to be taken into account since the expenses of an ICSI program itself are significant for the patients.

Conclusion

The outcome of ovarian stimulation using human menopausal gonadotropin (hMG) with that achieved by using highly purified follicle stimulating hormone (pFSH) in ICSI procedures was compared. It was shown that most biological parameters and the clinical end-point of pregnancy rate were similar in the two groups. Therefore, a gonadotropin with a lower cost, such as hMG, can be employed in ICSI without affecting the outcome adversely and, if shortage of either of these preparations of gonadotropins occurs, the physician can replace them with the other with equally good results.

References

[1] Palermo G., H. Joris, P. Devroey *et al.*: "Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte". *Lancet*, 1992, *340*, 17.

[2] Palermo G., H. Joris, M. P. Derde *et al.*: "Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection". *Fertil. Steril.*, 1993, *59*, 826.

[3] Van Steirteghem A., J. Liu, H. Joris *et al.*: "Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles". *Hum. Reprod.*, 1993, *8*, 1055.

[4] Van Steirteghem A., Z. Nagy, H. Joris *et al.*: "High fertilization and implantaion rates after intracytoplasmic sperm injection". *Hum. Reprod.*, 1993, *8*, 1061.

[5] Svalander P., A. S. Forsberg, A. H. Jakobsson *et al.*: "Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility". *Fertil. Steril.*, 1995, *63*, 828.

[6] Bergh C., C. M. Howles, K. Borg *et al.*: "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.

[7] Greenblatt E. M., J. S. Meriano, R. F. Casper: "Type of stimulation protocol affects maturity, fertilization rate and cleavage rate after intracytoplasmic sperm injection". *Fertil. Steril.*, 1995, *64*, 557.

[8] Loutradis D., P. Drakakis, S. Michalas *et al.*: "GnRH-analogues in ovarian stimulation protocols for patients with various causes of infertility". *The Releaser*, 1995, *1*, 10.

[9] World Health Organization. Laboratoty manual for the examination of human semen and sperm-cervical interaction. New York, Cambridge University Press, 1993, 3, 27.

[10] Kruger T. F., R. Menkvelt, F. S. H. Stander *et al.*: "Sperm morphologic features as a prognostic factor in in vitro fertilization". *Fertil. Steril.*, 1986, *46*, 1118.

[11] Guerin J. F., C. Mathieu, J. Lornage *et al.*: "Improvement of survival and fertilizing capacity of human spermatozoa in an IVF programme by selection on discontinuous Percoll gradient". *Hum. Reprod.*, 1989, *4*, 798.

[12] Loutradis D., K. Kallianidis, P. Drakakis *et al.*: "Successful pregnancy in human IVF usin BSA as a protein source in the transfer medium". *ARTA*, 1992, *3*, 233.

[13] Loutradis D., P. Drakakis, K. Kallianidis *et al.*: "The effect of the duration of GnRH-agonist down regulation before ovarian stimulation on the biological and clinical outcome after intracytoplasmic sperm injection". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1998, *80*, 251.

[14] Tucher M. J., G. Wright, P. C. Morton *et al.*: "Practical evolution and application of direct intracytoplasmic sperm injection for male factor and idiopathic fertilization failure infertilites". *Fertil. Steril.*, 1995, *63*, 820.

[15] Oehninger S., L. Weeck, S. Lanzendorf *et al.*: "Intracytoplasmic sperm injection: achievement of high pregnancy rates in couples with severe male infertility is dependent primarily upon female and not male factors". *Fertil. Steril.*, 1995, *64*, 977.

[16] Loutradis D., P. Drakakis, K. Kallianidis *et al.*: "Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection". *Fertil. Steril.*, 1999, *72*, 24.

[17] Guidice E., C. Crisci, A. Eshkol *et al.*: "Composition of commersial gonadotrophin preparations extracted from human post-menopausal urine: characterization of non-gonadotrophin proteins". *Hum. Reprod.*, 1994, *9*, 2291.

[18] Le Cotonnec J. Y., H. Porchet, V. Beltrami *et al.*: "Comparative pharmacokinetics of two urinary human follicle stimulating hormone preparations in healthy female and male volunteers". *Hum. Reprod.*, 1993, *8*, 1604.

[19] Howles C. M., E. Loumaye, D. Giroud *et al.*: "Multiple follicular development and ovarian steroidogenesis following subcutaneous administration of a highly purified urinary FSH preparation in pituitary desensitized women undergoing IVF: a multicentre European phase III study". *Hum. Reprod.*, 1994, *9*, 424.

[20] Moor R. M., C. Polge, S. M. Willandsen: "Effect of follicular steroids on the maturation and fertilization of mammalian oocytes". *J. Embryol. Exp. Morphol.*, 1980, *56*, 319.

[21] Ainsworth L., B. K. Tsang, B. R. Downey *et al.*: "Interrelationships between follicular fluid steroid levels, gonadotropic stimuli, and oocyte maturation during preovulatory development of porcine follicles". *Biol. Reprod.*, 1980, *23*, 261.

[22] Thibault C., M. Gerard, Y. Menezo: "Acquisition par l' ovocyte de lapine et de veau du facteur de decondensation du nuyau du spermatozoide fecondant (MPGF)". *Ann. Biol. Amin. Biochim. Biophys.*, 1975, *15*, 705.

[23] Staessen C., C. Janssenwillen, E. Van Den Abeel *et al.*: "Avoidance of triplet pregnancies by elective transfer of two good quality embryos". *Hum. Reprod.*, 1993, *8*, 1650.

Address reprint requests to:
P. DRAKAKIS, M.D.
Byzantiou 55
Papagou 156 69
Athens (Greece)