Intracytoplasmic sperm injection procedure for infertility treatment in couples

Helenyse Mattanó, Renato Koike and Suzana de Fatima Paccola Mesquita*

*Departamento de Biologia Geral, Universidade Estadual de Londrina, Campus Universitário, 86051-970, Londrina, Paraná, Brasil. *Author for correspondence. e-mail: mesquita@uel.br*

ABSTRACT. The ICSI procedure was used in a group of 68 patients (mean age 34,7 years). Ovarian stimulation, preparation of spermatozoa, preparation of the oocytes, direct injection of a single spermatozoon into the ooplasm and embryos replaced in uterus were done. The results of the present work demonstrate that 56% of diagnostic infertility categories were due to female factors. The highest frequency of male and female factors which caused infertility were asthenozoospermia (63%) and tubal factors (32,7%), respectively. From a total of 695 oocyte aspirated, 453 (67,1%) normal zygotes with two pronuclei (2 PN zygotes) were produced; 4 (0,55%), 32 (4,42%) and 2 (0,27%) resulted in 1 PN, 3PN and 4 PN zygotes respectively. The pregnancy rates by cycle and by transference were 25% and 28,9% respectively. Our data suggest that the ICSI procedure has been the method of choice in the treatment of infertility due to different etiology and the results showed that the successful fertilization rates has increased the chances of pregnancy.

Key words: ICSI, infertility, assisted reproduction.

RESUMO. Injeção intracitoplasmática de espermatozóide para tratamento de casais inférteis A injeção intracitoplasmática de espermatozóide (ICSI) foi o procedimento aplicado em 68 pacientes com idade média de 34,7 anos. Foram realizadas as seguintes etapas: indução da ovulação; aspiração folicular; seleção dos oócitos e coleta de sêmen; injeção de espermatozóides e transferência dos embriões ao útero. Os resultados mostraram que 56% dos problemas de infertilidade relacionam-se a fatores femininos. A maior freqüência de fatores masculinos e femininos que causaram infertilidade foram astenozoospermia (63%) e fatores tubários (32,7%), respectivamente. De um total de 695 oócitos aspirados, 453 (67,1%) resultaram em zigotos com 2 pró-núcleos (2PN); 4 (0,55%), 32 (4,42%) e 2 (0,27%) resultaram em zigotos 1PN, 3PN e 4PN, respectivamente. As taxas de gestação por ciclo e por transferência foram 25% e 28,9%, respectivamente. Nossos dados sugerem que a ICSI tem sido o método de escolha no tratamento da infertilidade devido a diversas etiologias, e os resultados mostraram que o sucesso nas taxas de fertilização tem aumentado as chances de gravidez.

Palavras-chave: ICSI, infertilidade, reprodução assistida.

Introduction

Intracytoplasmic sperm injection (ICSI) is a technique done by introducing a single spermatozoon into the ooplasm with a micromanipulation system. In recent years the ICSI procedure has become the method of choice in the treatment of infertility due severe factors (Palermo *et al.,* 1992). ICSI procedures are indicated to treat couples with infertility problems due to severe male-factors such as oligozoospermia and asthenozoospermia (Ahumada *et al*., 1998; De Croo *et al.,* 2000) who could not be helped by in-vitro fertilization (Dale and Elder, 1997) and idiopathic cause of failed fertilization (Van Steirteghem, 1997). The literature has shown that ICSI is one of the most efficient assisted-fertilization techniques in the treatment of severe male-factor infertility (Palermo *et al.,* 1993; Van Steirteghem *et al.,* 1993; Mansour *et al.,* 1995).

The purpose of this article is to provide information about the outcome of ICSI procedure for infertility treatment in couples and characterize the factors that were causing both male and female infertility. The data presented here have been obtained from procedures initiated in September, 1999 through August, 2000 at Cedilon - an assisted reproduction technique center in *Londrina,* state of *Paraná*, Brazil.

Material and methods

A retrospective study was carried out with a total of 88 ICSI cycles (68 patients / maternal age = 34,7 years old) between September 1999 and August 2000 at Cedilon - an assisted reproduction technique center.

Patients

All patients were referred for ICSI because of previously failed pregnancy caused by male (17), female (37), and male and female (34) factors.

Ovarian stimulation

All female patients were stimulated for ovulation using the protocol of gonadotrophin-releasing hormone analogue (GnRH, Reliser®) and follicle-stimulating hormone (FSH, Gonal-F®). Reliser was started with 0,20mL (1mg) from day 2 to day 5 of menstrual cycle and 0,10mL (0,5mg) from day 6 on, administered daily by s.c. injection to the short protocol (used in older women) or 1mg/day by s.c injection to the long protocol (used in younger women). FSH administration was 300UI or 150UI per day by s.c. injection to the short or long protocol, respectively. Ultrasound measurement of follicular development was done on day 7 of the cycle and human chorionic gonadotrophin–HCG 10.000UI (Profasi ®HP) was administered when two or more follicles measuring 17mm in size were observed. Oocytes were collected vaginally under ultrasound guidance 36h later.

Spermatozoa preparation

In all cases semen samples were liquefied on a heated stage (37°C) and then were manually assessed for concentration and motility. The semen samples were recovered by Isolate. The semen was subsequently washed by gentle centrifugation at 200g; the pellet resuspended in 2mL human tubal fluid (HTF) medium. After that, the suspension was centrifuged at 200g for 10 min. The supernatant was removed and washed with 1mL HTF. Sperm concentration and motility of the recovered aliquot were determined and the concentration was adjusted, if necessary, before use for insemination.

Oocyte preparation

Prior to ICSI the oocyte was transferred to HEPES-buffered culture medium containing 80IU/mL hyaluronidase. The oocyte oocyte-cumulus complex was maintained in the hyaluronidase for a maximum of 30 seconds, where upon the oocyte and its attendant corona radiate cell were removed and washed through HEPES culture medium before mechanical removal using finely pulled sterile Pasteur pipettes.

ICSI procedure

The injection procedure was performed in culture dish containing two kinds of droplets: 1) eight droplets of 5μl HEPES medium containing the oocytes; 2) two droplets of polyvinilpirrolidone (PVP). The droplets were overlaid with mineral oil. One drop of PVP was diluted with 3μl of sperm suspension. Another drop of PVP did not contain any sperms. The micro-injection Petri dish was maintained on a heated stage (37°C) on the inverted microscope (Olympus IMT2) during the ICSI procedure. The micromanipulators were mounted with microneedles (holding and injection). A single, living spermatozoon was selected from the sperm suspension droplet and permanently immobilized. The spermatozoon was aspirated tail-first into the microneedle. The oocyte was fixed on the holding microneedle in a way that the polar body was situated at 6 or 12 o'clock while the injection microneedle was pushed through the zona pellucida at the 3 or 9 o'clock position and into the cytoplasm. The oolema was pierced and the spermatozoon deposited in the ooplasm. The oocyte was released from the micropipette, transferred to the incubator for routine embryos culture prior to embryo transfer.

Sixteen to eighteen hours after injection, the state of fertilization was assessed by looking for presence of pronuclei, and 24h later the state of embryo cleavage was recorded. If embryos had been produced, up to four embryos were placed into the uterine cavity about 68h after sperm injection. Abnormally fertilized zygotes (>2PN) were noted and discarded immediately. A ß -hcg test was performed on the patients 15 days after embryo transfer for pregnancy confirmation. Clinical pregnancy was defined as the presence of a gestational sac(s) with a viable embryo shown on vaginal ultrasonography performed approximately 30 days after embryo transfer.

Results

Table 1 shows the results of ICSI obtained from procedures initiated in September, 1999 through August, 2000 at Cedilon - an assisted reproduction technique center. Of these 88 cycles, 816 follicles were obtained and resulted in 695 (85,2%) oocytes retrievals. ICSI was performed in 85,2% oocytes (n = 592). Normal fertilization (2PN) was achieved in 453 oocytes (76,5%) and abnormal fertilization (1 PN or 3 PN) in 0,67%(n = 4) and 5,4% (n = 32) of

cells, respectively. Transfer did not occur in 8 cases (9,1%). There was fertilization failure due to intrinsic oocytes dysfunction like absence of cleavage or broken oocytes. Pregnancy rates per cycle, per transfer and per patient were, 25%, 28,9% and 33,3% respectively.

Table 1. Results of ICSI procedure performed between September 1999 and August 2000 at Cedilon - an assisted reproduction technique center.

	ICSI
Cycles initiated	88
Cycles cancelled	4
Cycles without transfer	8
Follicles aspirated (A)	816
Transfers (B)	76
Clinical pregnancies (C)	22
Oocytes collected	695
Oocytes MII (D)	592
Oocytes MI (E)	32
Oocytes VG (F)	27
Oocytes HM (G)	12
Oocytes ZF (H)	21
Oocytes DG (I)	11
1 PN (J)	4
2 PN (K)	453
3 PN (L)	32
4 PN (M)	2
Clinical pregnancies/cycle (%)	25
Clinical pregnancies/transfer (%)	28,9
Clinical pregnancies/patient (%)	33,3

A. Transvaginal follicular aspirations, regardles oocyte retrieval; B. Transfer of one or more embryos to uterus; C. Intrauterine pregnancy confirmed by ultrasound with at least one sac; D. Mature oocytes in meiosis II; E. Immature oocytes in meiosis I; F. Immature oocytes in state of germinal vesicle; G. Post-mature oocytes; H. Oocytes with broken zona pellucida; I. Damaged oocytes; J. Presence of only one pronucleus after fertilization; K. Presence of two pronuclei, showing normal fertilization; L. Presence of three pronuclei after fertilization; M. Presence of four pronuclei after fertilization.

Table 2 analyzes the male factors which caused infertility. As can be seen from this table, asthenozoospermia (63%) was the male-factor that mostly affected reproductive efficacy.

Female- factor infertility is shown in Table 3. Tubal factors (32,7%) were the higher factors which caused infertility in women.

Table 2. Male-factors associated with infertility.

	N° of patients	% frequency
Deferent agenesis	1	3,3
Asthenospermia	19	63,3
Erection failure	1	3,3
Oligospermia	4	13.3
Vasectomy	5	16,6

Discussion

Over the past few decades, remarkable progress has been made in developing modern reproductive technology. In recent years the ICSI procedure (Palermo *et al*., 1992), has become the method of choice in the treatment of severe male infertility and idiopathic causes of failed fertilization (Borges-Jr *et al.,* 2000; Andrews *et al.,* 2001; Zegers-Hochschild *et al*., 2001). The direct injection of the spermatozoon into the ooplasm surpasses all the natural barriers of fertilization that are required during conventional IFV procedures (Fishel *et al*., 1995).

Table 3. Female-factors associated with infertility.

	N. pacientes	% freqüência
Ovarian	2	3,4
Tubal	19	32,7
Ovulatory	4	6,9
Endometriosis	2	3,4
Uterine	2	3,4
Tubal – Endometriosis	2	3,4
Tubal – Uterine	2	3,4
Tubal – Hormonal	1	1.7
Tubal – Ovarian	2	3,4
Uterine – Ovarian	1	1.7
Tubal - Ovarian – Endometriosis	4	6,9
Tubal - Ovarian – Ovulatory	1	1.7
Tubal - Ovarian –Uterine	2	3,4
Tubal - Uterine – Endometriosis	1	1.7
Tubal – Ovulatory	10	17,2
Tubal – Ovulatory – Endometriosis	2	3,4
Tubal - Ovulatory - Uterine - Endometriosis	1	1.7

In the present study, the different causes leading to infertility were the same shown in literature (Borges-Jr *et al*., 2000; Takeuchi *et al*., 2000; Khamsi *et al*., 2001). It has been well established that the ICSI procedure is indicated to treatment of severe male-factor infertility (Van Steirteghem *et al.,* 1993; Fishel *et al.,* 1994).

Hochschild (1998) reported the frequency of the factors which caused infertility: male-factor infertility (40%), female-factor infertility (40%) and both male and female (10%). In this study we found that female-factor infertility was more frequent (56%) than male-factor infertility (14,6%) because the number of women who looked for treatment in this assisted reproduction technique center in Londrina was higher than men.

According to Borges-Jr. *et al.* (2000), the results of ICSI procedure using spermatozoa from partner ejaculate showed that the pregnancy rates/transference were 26,3% whereas Nygren *et al.* (2001) detected 26,4% of the pregnancy rate/transference. Mansour *et al*. (1995) reported that the semen morphology was the only "quality" that affected fertilization and pregnancy rates following ICSI. The high rate of pregnancy per transfer reported here (28,9%) show that ICSI has proven to be an efficient and accurate fertilization process. Those cases where the transfer didn't occur (9,1%) can suggest that oocytes intrinsic factor were interfering with the fertilization and cleavage faults, compromising the initial stages of the embryogenesis.

Its possible to evaluate the oocyte maturation stage at the fertilization moment which should be into metaphase II (Figure 1) with complete

cytoplasmatic and nuclear maturation to permit the fertilization (Bergh *et al*., 1998). From a total of 695 oocytes retrievals, 592 (85,2%) were metaphase-II oocytes and two–pronuclear zygotes were seen in 453 (76,5%) oocytes after ICSI. All the embryos of a good enough quality were transferred 3 days after sperm injection .The quality of the embryos was attested by number and morphology blastomeres and presence of cellular fragmentation previously described by Petersen *et al* (1999).

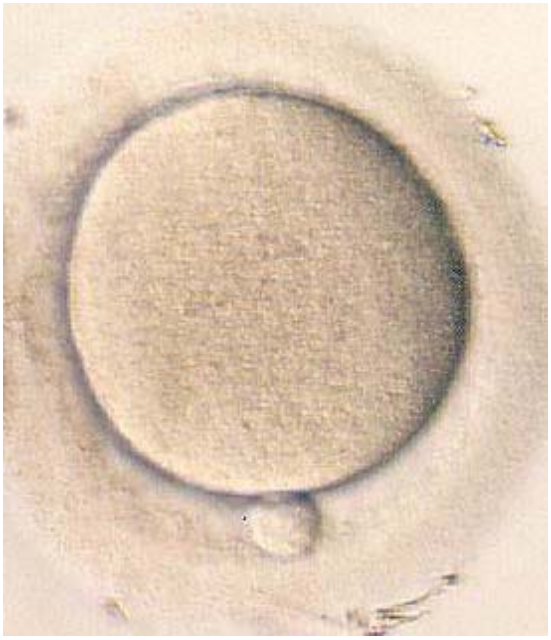

Figure 1. Oocyte in metaphase II.

In agreement with a previous study by Kim *et al.* (2000) we found that the number of oocytes having 2PN after intracytoplasmic sperm injection in the stimulated cycles was 453 (76,5%) whereas the proportion of the oocytes with 1 PN, 3PN and 4PN after ICSI was smaller .

The use of ICSI has produced consistent results in terms of fertilization and pregnancies (Obasaju *et al*., 2000; Andrews *et al.,* 2001). According to Fishel *et al.* (2000) ICSI as a first option offers a higher incidence of fertilization maximizes the number of embryos and minimizes the risk of complete failure of fertilization for all cases requiring in-vitro conception. However, current knowledge of ICSI as an outcome procedure does not provide the confidence to use this process in all cases of IVF (*in vitro* fertilization) for the time being.

Conclusion

Because of the high fertilization and pregnancy rates, we agree that the intracytoplasmic sperm injection (ICSI) may be useful in the treatment of male and female-factor infertility. On the basis of these results we conclude that successful maturation in vitro of oocytes from stimulated cycles and ICSI after maturation ensure a wider range of fertilized oocytes and increase the chances of pregnancy.

References

AHUMADA, A. *et. al*. *Manual de procedimientos*. Santiago de Chile: Red Latinoamericana de Reproducción Asistida, 1998.

ANDREWS, M. M. *et al*. Analysis of intracytoplasmic sperm injection produces related to delayed insemination and ejaculated, epididymal and testicular spermatozoa. *RBM Online*, Cambridge, v. 2, n. 2, p. 89-97, 2001.

BERGH, C. *et al*. Comparison of fertilization, cleavage and pregnancy rates of oocytes from large and small follicles. *Hum. Reprod*., Oxford, v. 13, p. 1912-1915, 1998.

BORGES JR. *et al*. Tratamento da infertilidade conjugal com a técnica de injeção intracitoplasmática de espermatozóide (ICSI). *Reprod. Clim*., Ribeirão Preto, v. 15, n. 4, p. 228-232, 2000.

DALE, B.; ELDER, K. Micromanipulation techniques. In: DALE, B.; ELDER, K.. *In vitro fertilization*. New York: Cambridge University Press, 1997. Cap 11, p. 228-268.

DE CROO, I. *et al*. Fertilization, pregnancy and embryo implantation rates after ICSI in cases of obstructive and non-obstructive azoospermia. *Hum. Reprod*., Oxford, v. 15, n. 6, p. 1383-1388, 2000.

FISHEL, S. *et al.* Micro-assisted fertilization in patients who have failed sub-zonal insemination. *Hum. Reprod*., Oxford, v. 9, p. 725-738, 1994.

FISHEL, S. *et al*. Systematic examination of immobilizing spermatozoa before intracytoplasmic sperm injection in the human. *Hum. Reprod*., Oxford, v. 10, p. 497-500, 1995.

FISHEL, S. *et al*. Should ICSI be the treatment of choice for all cases of in-vitro conception? *Hum. Reprod*., Oxford, v. 15, n. 6, p. 1278-1283, 2000.

HOCHSCHILD, F. Z. Registro latinoamericano de reproducción asistida, 1998. Disponível em: <http://www.redlara.com/reg_1998asp>. Acesso em: 10.out.2002.

KHAMSI, F. *et al*. Intracytoplasmic sperm injection increased fertilization and good quality embryo formation in patients with non-male factor indications for in vitro fertilization: a prospective randomized study. *Fertil. Steril*., New York, v. 75, n. 2. p. 342-347, 2001.

KIM, B.K. *et al*. In vitro maturation, fertilization, an development of germinal vesicle oocytes collected from stimulated cycles. *Fertil. Steril*., New York, v. 74, n. 6, p. 1153-1158, 2000.

MANSOUR, RT. *et al*. The effect of sperm parameters on the outcome of intracytoplasmic sperm injection. *Fertil. Steril*., New York, v. 64, p. 982-986, 1995.

NYGREN, K. G. *et al*. Assisted reproductive technology in Europe, 1997. Results generated from European registers by ESHRE. *Hum. Reprod*., Oxford, v. 16, n. 2, p. 384-391, 2001.

OBASAJU, M. *et al*. Pregnancies for single normal embryo transfer in women older than 40 years. *RBM Online*, Cambridge, v. 2, p. 98-101, 2000.

PALERMO, G. *et al*. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet, New York, v. 340, p. 17-18, 1992.

PALERMO, G. *et al*. Sperm characteristics and outcome of human assisted fertilization by sub zonal insemination and intracytoplasmic injection. *Fertil. Steril*., New York, v. 59, n. 4, p. 826-835, 1993.

PETERSEN, C. G. *et al*. Seleção precoce de embriões após ICSI: quais são os melhores critérios? *Jornal Brasileiro de Reprodução Assistida*, São Paulo, v. 3, p. 7-13, 1999.

TAKEUCHI, S. *et al*. In vitro fertilization and intracytoplasmic sperm injection for couples with unexplained infertility after failed direct intraperitoneal insemination. *J. Assist. Reprod. Genet*., Bethesda, v. 17, n. 9, p. 515-520, 2000.

VAN STEIRTEGHEM, A. Intracytoplasmic sperm injection. *Clin. Obstet. Gynaecol*., Baillieres, v. 11, p. 725-738, 1997.

VAN STEIRTEGHEM, A.C. *et al*. Higher success rate by intracytoplasmic sperm injection than by sub zonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum. Reprod*., Oxford, v. 8, p. 1055-1066, 1993.

ZEGERS-HOCHSCHILD, F. *et al*. Results of assisted reproduction techniques in Latin America. *RBM Online*, Cambridge, v. 2, n. 2, p. 129-137, 2001.

Received on November 08, 2002.
Accepted on March 18, 2003.