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## USING ICSI IN DIFFERENT CATEGORIES OF MALE FACTOR INFERTILITY

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### ABSTRACT

The aim of our study was to test whether a high percentage of morphologically abnormal sperms in the male ejaculate can be eliminated by assisted reproduction using the method of Intracytoplasmic Sperm Injection (ICSI). The treatment success was evaluated by comparing fertilization rates, clinical pregnancy rates and baby rates in males with heavy teratospermia ( $\leq 1\%$  of morphologically normal spermatozoa) versus males with a higher percentage ( $> 1\%$ ) of morphologically normal sperm forms. One hundred and seventy four patients who had underwent 174 ICSI cycles were evaluated retrospectively. We detected a lower number of fertilized oocytes in patients with the heavily impaired sperm morphology ( $P=0.038$ ). On the other hand, neither gravidity nor delivery (baby rates) of the partners differed between the patients with the heavily impaired sperm morphology and the patients with the mildly impaired sperm morphology.

**Key words:** intracytoplasmic sperm injections, sperm morphology, pregnancy rate, baby rate

## INTRODUCTION

Male infertility is common. The majority of cases are idiopathic in origin and medical treatment has limited, if any, value. Intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male infertility and allowed couples whose only prior options were donor insemination or adoption to procreate using their own gametes (El-Toukhy T., Braude P. 2012). Morphological abnormalities of spermatozoa are often identified in males with problematic fertility. The quality of spermatozoa has an essential influence on the fertilization of the oocyte and on the subsequent evolution of the embryo. A direct relation exists between abnormal sperm morphology and embryo morphology at the later stage of cleavage (Parinaud J. *et al.* 1993). The first two cycles of the embryo cell cleavage are controlled by the maternal factor. The paternal effect begins to apply in the embryo from the four-cell stage (Braude P. *et al.* 1988). The quality of DNA in sperms is valued as an absence/incidence of fragmentations in the late embryonic development – late paternal effect (Tesarik J., Mendoza C. 2004; Tesarik J. 2005; Cohen-Bacrie P. 2008). The first pregnancy and child birth after the application of ICSI method was recorded in 1992 (Palermo *et al.*, 1992). The Intracytoplasmic Sperm Injection (ICSI) method brought higher prosperity in the IVF treatment of couples with the male sterility factor. In our study, we focused on fertilization rates, pregnancy rates (PRs) and baby rates by using the ICSI method in males with the high percentage of morphologically abnormal spermatozoa. Teratospermia is one of key parameters in choosing sperms suitable for the ICSI method. The aim of this study was to ascertain whether the ICSI method is a good perspective for infertile males with heavy teratospermia ( $\leq 1\%$  of normal spermatozoa).

## MATERIAL AND METHODS

### *Patients*

The method of intracytoplasmic sperm injection (ICSI) was used to treat infertility in 174 couples presenting at ReproGenesis clinic in Brno, Czech Republic. In this group, the success of the treatment was valued from the viewpoint of male factor – male infertility. A total number of 174 males were subjected to ICSI cycles. The male patients were divided into a group of 137 individuals with mildly impaired or normal sperm morphology ( $>1\%$  of normal spermatozoa) and a group of 37 individuals with heavily impaired sperm morphology ( $\leq 1\%$  of normal spermatozoa).

### **Semen analysis**

The spermatozoa were sampled through masturbation after sexual abstinence lasting 3-5 days. Prior to the analysis, the sample was incubated for 20 minutes at 37 °C for fluidization. The concentration, motility and morphology of the sperms were assessed according to standard WHO (1999) guidelines for morphology, motility, and concentration. Data were gained through the visual assessment of samples under the microscope.

### **Sperm processing**

The spermatozoa gained from ejaculation were processed by using the swim-up method (Enginsu M.E. *et al.* 1993) and incubated at a temperature of 37 °C (5%O<sub>2</sub>, 6%CO<sub>2</sub>, 89%N<sub>2</sub>) in the Sydney IVF Sperm Medium (Cook IVF Cell Culture Media, Australia).

### **Ovarian stimulation and oocyte retrieval**

Ovarian stimulation was induced by long protocol in all 174 cycles: Protocol GnRH $\alpha$  (Triptorelin, Ferring) and FSH (Metrodin, Serono) and HMG (Humegon, Organon). The development of follicles was stimulated by FSH and HMG injections. The dose of gonadotropins was individual, respecting the age of the female patient, previous stimulation or response to stimulation.

### **Oocyte handling**

Cumular cells were removed from the oocytes with a denudation pipette with using hyaluronidase (80IU/ml in Sydney IVF Fertilization Medium) for 10-15 seconds. After the partial removal of cumular cells, the oocytes were further denudated (Sydney IVF Fertilization Medium) until complete denudation. The sperm was injected into the oocytes 2-3 hours after the ovum- pick up. Oocytes used for the ICSI method were those in the metaphase II with a cleaved P-element.

### **ICSI procedure**

ICSI was conducted on the inverted Olympus microscope with using the Research Instruments micro-manipulator and Eppendorf injectors. The oocytes were placed individually into 10µl micro-drops of Sydney IVF Fertilization Medium, and one micro-drop with the Sydney IVF PVP medium was injected with a 2µl suspension of spermatozoa. The sperms were selected, immobilized, sucked into the ICSI pipette and inserted into the oocyte cytoplasm at a 400-x expansion with using Hoffman's modulation contrast. Prior to the injection, the morphological structure of sperms was assessed.

### **Assessment of fertilization, embryo cleavage and establishment of pregnancy**

The oocytes were checked after 16-18 hours to verify fertilization. Fertilized oocytes were separated and tested for the occurrence of the 2PN (two pronuclei) stage. The cleavage phase of the embryo was established subsequently after 25-27 hours from the oocyte fertilization and the early embryo cleavage was assessed (Petersen C.G. *et al.* 2001). The early paternal effect of the sperm shows before the main activation of embryonic genome expression – it starts between the fourth and the eighth cell stage of embryo preimplantation development (Tesarik *et al.*, 2004; Tesarik, 2005). Embryos of the highest quality were transferred within 72-96 hours from the sampling of oocytes. The mean number of transferred embryos was 2 embryos per transfer. In the case of positive chemical gravidity, HCG was detected on the fourteenth day following the embryo-transfer. Clinical gravidity was defined as an intrauterine finding of the gestational sac with a heart function. Abortion was defined as gravidity terminated before the twentieth week of pregnancy.

### **Statistical analysis**

A comparison was made of pregnancy rates and baby rates. The normality of data was tested by the Anderson-Darling normality test and by the visual inspection of histograms. Since some parameters exhibited a non-normal distribution, the Mann-Whitney U-test was used to compare the continual data. Fisher's exact test was applied to compare categorical data. Significance was established at a level of  $P > 0.05$ .

## **RESULT AND DISCUSSION**

Oocytes injected in the MII phase totalled 2811 and subsequent fertilization (two pronuclei – 2PNs) was recorded in 2303 oocytes (82%). The transfer was implemented in all 174 couples. A total number of transferred embryos amounted to 349 with an average count of two embryos per transfer. Clinical gravidity per transfer was achieved in 92 cases (53%). A resulting number of births was 83 (48%) with 108 live-born infants. Tab. 1 summarizes the character of the studied group.

Tab.1 Basic Characteristics of subjects and their partners during intervention

	UNIT	X ± S <sub>x</sub>
AGE man	years	34.9 ± 2.8
AGE woman	years	30.5 ± 2.7
<b>NORMAL SPERMATOZOA</b>	%	11 (3 - 21)
<b>OOCYTES</b> (total number)	pieces	25.1 ± 7.7
<b>MATURE OOCYTES</b> (total number)	pieces	16.2 ± 6.1
<b>FERTILIZED OOCYTES</b> (total number)	pieces	12 (9 - 17)

Tab. 2 is already divided into a group of males with the mildly impaired sperm morphology and a group with the heavily impaired sperm morphology. In this Table, a statistical evaluation is made of the age of males whose sperms were used for the ICSI method, mean count of oocytes sampled from their female partners, number of oocytes in the MII phase suitable for fertilization, number of fertilized oocytes, number of clinical gravidities and number of the deliveries of live-born children. The Table shows that while the number of fertilized oocytes in the patients with the heavily impaired sperm morphology was significantly lower (P=0.038), neither gravidity nor delivery of the partners differed in the patients with the heavily impaired sperm morphology as compared with the rest of the group. This suggests that the lower number of fertilized oocytes was not related to the overall result (Table 1, Table 2).

Tab. 2 Characteristics and the outcome of fertilization in patients with mildly and heavily defective spermatozoa

	Heavily defective	Mildly defective	P - value
	( ≤ 1% of normal spermatozoa )	( > 1% of normal spermatozoa )	
AGE man	35.3 ± 3.1	34.8 ± 2.7	0.339
<b>OOCYTES</b> (total number)	26.2 ± 7.2	24.7 ± 7.8	0.271
<b>MATURE OOCYTES</b> (total number)	15.3 ± 5.8	16.2 ± 6.1	0.192
<b>FERTILIZED OOCYTES</b> (total number)	10 (9 - 13)	13 (9 - 17)	<b>0.038</b>
<b>PREGNANCY RATES</b> (present/absent)	23/14	69/68	0.266
<b>DELIVERY</b> (present/absent)	22/15	61/76	0.138

The number of couples who had formerly no chance for their biological offspring has considerably increased since the icsi method was introduced in 1992 (first gravidity and delivery of a child after the icsi method applied in a female). Following this first pregnancy and birth of a healthy child with using the icsi method, the procedure started to be widely used to treat infertility especially in males (palermo g. *Et al.* 1992). Compared with the conventional method of *in vitro* fertilization (ivf), this method yields higher fertilization rates as well as higher counts of cleaved embryos (lucas h. *Et al.* 2010). One of main differences between the conventional ivf and the icsi method is a possibility to choose just one sperm and to insert it mechanically into the egg cytoplasm (shoukir y. *Et al.* 1998). Aytoz a. *Et al.* (1998) and palermo g.d. *et al.* (1999) recorded absence of a significant difference between the pregnancy rates in normal and abnormal ejaculates with using the icsi method. Their results were corroborated in our study. The method of selecting a suitable sperm markedly increased the count of fertilized oocytes in the ivf/icsi treatment and made it possible to find a general solution for the problem of heavy teratospermia in males who had no chance of a successful treatment with his partner female. The need of the sperm donor was remarkably reduced.

## CONCLUSIONS

The presented study points to a significant difference between fertilization rates in the group of males with heavily impaired sperm morphology ( $\leq 1\%$  of normal spermatozoa) and in the group of males with mildly impaired sperm morphology ( $> 1\%$  of normal spermatozoa). The result of a successful treatment is the achieved pregnancy and the birth of a healthy child. The statistical evaluation showed no difference between the two groups in this respect. The lower number of fertilized oocytes was not linked to the overall outcome of fertilization in our group and that patients with the heavily impaired sperm morphology enjoy the same benefit from ICSI as patients with the only mildly impaired sperm morphology.

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