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INTRACYTOPLASMATIC SPERM INJECTION (ICSI): METHOD AND APPLICATION IN HUMAN AND VETERINARY REPRODUCTION

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Summary

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ICSI is the newest and the most successful micromanipulation technique for treating male factor infertility, both, in human and animal species. In our hospital first human ICSI was performed in 2004. In veterinary field this micromanipulation is used for commercial, experimental and for training of our students in IVF lab. From 2004 to the end of 2012 in our private human female hospital were performed 1,979 procedures of IVF, from which 1,608 were ICSI procedures. Last two years all procedures of IVF were ICSI procedures, with better results than previous years. ICSI procedures were performed with Inverted Microscope with two hydro manipulators Nikon 2000. These results are from materials from Private Female Hospital "Fertility" in Bitola, R. Macedonia. From our results we can see that IVF procedures were combined with classic IVF and ICSI procedures with successful rate between 39–51%, depending on age of the females. Last two years all IVF procedures were performed only with ICSI (2011 year/ ICSI (246) – all (246) and 2012 year/ICSI (306) – all (306)) with more higher percentage of successful rates, >50% (50–61%). In patients with unexplained failure of a previous conventional IVF (normal count, motility and morphology), ICSI showed higher incidence of fertilisation as compared with IVF. IVF was initially developed for female infertility due to tubal disease. Several studies showed an increase in the adverse perinatal outcome of pregnancies obtained after ICSI-embryo transfers. Scope of ICSI in veterinary science: 1. Availability of material; 2. Research; 3. Training and 4. The potentiality of ICSI in exotic species for conservation which is yet to be exploited.

Key words: intracytoplasmatic sperm injection, reproduction

INTRODUCTION

In 1992, the first human pregnancies and births after replacement of embryos generated by ICSI procedure of assisted fertilization were reported (Palermo *et al.,* 1992). ICSI resulted in the production of more embryos with higher implantation rates, in comparation with standard IVF procedure. So ICSI has been used worldwide and successfully to treat infertility due to impaired testicular function or obstruction of the excretory ducts resulting in severe oligoasthenoteratozoospermia or even azoospermia in the ejaculate. A successful ICSI programme depends on ovarian stimulation, which is essentially similar to conventional IVF. The combination of gonadotrophin-releasing hormone (GnRH) agonists, human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (HCG) allows the retrieval

of a high number of cumulus-oocyte complexes and ovulation is induced using HCG. Over the years, live birth have been reported with ICSI in mice (Roknabadi *et al.,* 1994*)*, rabbits (Hosoi *et al.,* 1993), cows (Goto *et al.,* 1990) as well as in humans (Palermo *et al.,* 1992). In our hospital first human ICSI was performed in 2004.

Intracytoplasmatic sperm injection as the newest and the most successful micromanipulation technique for treating male factor infertility. It entails the mechanical insertion of a chosen spermatozoon directly in to the cytoplasm of an oocyte. The technique was used in veterinary science for the purpose of clarifying the different steps of fertilization by investigating the fusion and penetration of animal gametes. First mammalian egg injection procedure was reported by Lin in 1966. Later Uehara & Yanagimachi (1976*)* described the microinjection of human and golden hamster spermatozoa into hamster eggs. Hosoi & Iritani (1993) obtained live offspring after transfer of micro fertilized rabbit eggs into the oviduct of pseudo pregnant female rabbits. Based on the successful fertilization of normal animal gametes using ICSI, the procedure was experimentally applied to human gametes. In the veterinary field micromanipulation of murine and domestic animal species has been used for the past two decades both as an experimental tool and in the commercial field. The intracytoplasmatic sperm injection (ICSI) program is offered at Texas A&M as a means of establishing pregnancies from oocytes (eggs) recovered from donor mares. Using ICSI, oocytes are injected with individual sperm from a donor stallion, and the resulting embryos are allowed to develop in the laboratory for approximately one week. Developed embryos are then shipped to a private embryo transfer facility for transfer to a recipient mare, as for standard embryo transfer.

MATERIAL AND METHODS

Patients

During a 9-year period between 2004 and 2012, 1979 patients entered this retrospective study with sibling MII oocytes to compare the incidences of fertilization between ICSI and conventional IVF (Table 1).

Semen preparation

Spermatozoa (fresh or frozen from ejaculate, or from TESE, MESE), for IVF or ICSI were to be prepared using the swim up procedure in Sydney (Cook) sperm medium (K-SISM-20, 50 & 100), supplemented with human serum albumin (10 mg/ml) and gentamycin (0.01 mg/mL).

Immobilization of spermatozoa

The sperm was examined in PVP drop (polyvynil pyrrolydone, Sydney-Cook PVP K-SIPV) to reduce sperm motility, at 200x magnification and selected morphologically acceptable sperm, which are swimming slowly along the base of peridish lid. After selecting the sperm with injection pipette it was aspirated with tail in front and transferred with injection pipette to 5 oil drops (Sydney IVF culture oil, K-SICO-50 & 200) containing oocyte.

Oocyte preparation

The follicular fluid was aspirated from follicles and collected into sterile tubes which are maintained in a water bath at 38 ^{o}C. Collected oocytes were examined under stereomicroscope equipped with a heating stage set.

Table 1. Numbers of procedures per year for ICSI and classic IVF

Year	IVF-classic	SR%	ICSI	SR%	All	SR*%
2004	68	36	60	39	129	37.5
2005	41	37	83	40	124	39.0
2006	79	56	89	44	168	40.0
2007	79	37	132	45	211	41.0
2008	53	38	174	47	227	42.5
2009	41	37	256	50	297	43.5
2010	10	38	262	51	272	44.5
2011	–	–	246	50	246	50.0
2012	–	–	306	61	306	61.0
	371		1608		1979	

*SR =successful rate.

Technique of ICSI

The general set up included inverted microscope Nikon 2000, Japan with differential interference contrast (DIC) optics a Penerspex environmental chamber, heater and a CO_2 controller (Fig. 1). The chamber was maintained at 37 ^oC and a constant humidified atmosphere of 5% CO_2 in air.

Fig 1. Inverted microscope Nikon 2000, Japan.

Intracytoplasmatic sperm injestion

The oocyte is held flat against the holding pipette using gentle suction. The oocyte should be in contact with the bottom of the dish to provide an extra axis of support during injection. The injection pipette should be positioned in a way such that the oocyte plasma membrane and the tip of the injection pipette are both in sharp focus. This will ensure injection through the middle of the oocyte. The polar body is oriented at 12 o'clock and the level of injection pipette faces 6 o'clock. Then the injection pipette was steadily pushed against the zona pellucida until it pierced the ZP and was situated well within the oocyte, surrounded by a shallow furrow. (Fig. 2, 3, 4). Sometimes the oocyte plasma membrane did not rupture by this process. To ensure the rupture, the oocyte cytoplasm was aspirated back into the injection pipette, which indicates whether the membrane is ruptured. The oocyte cytoplasm was pushed back into the oocyte along with the spermatozoon and a minimal volume of PVP solution was withdrawn with the injection pipette leaving behind the spermatozoon in the oocyte. the oocyte was examined under 200× approximately 17 h post injection for evidence of fertilization.

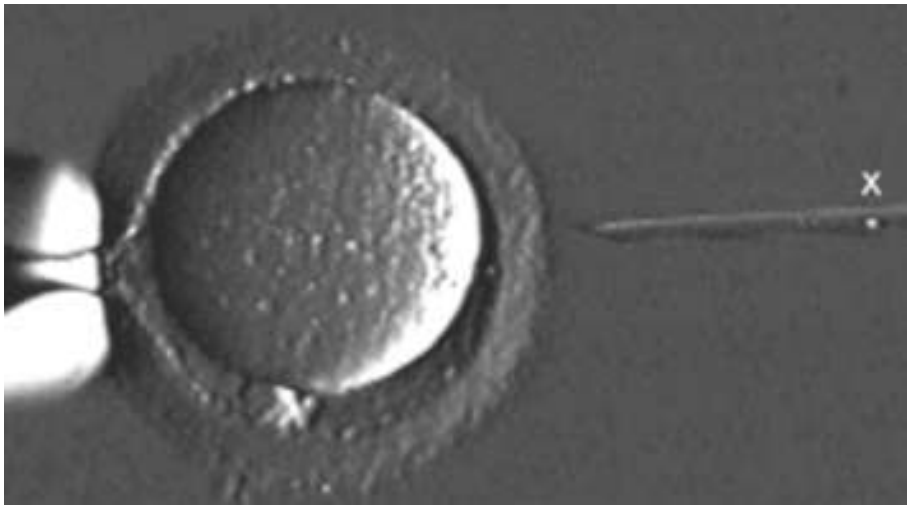

Fig. 2. ICSI, step I.

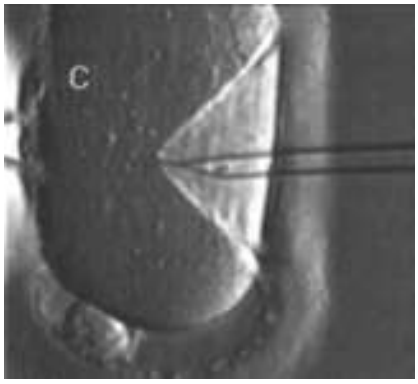

Fig. 3. ICSI, step II.

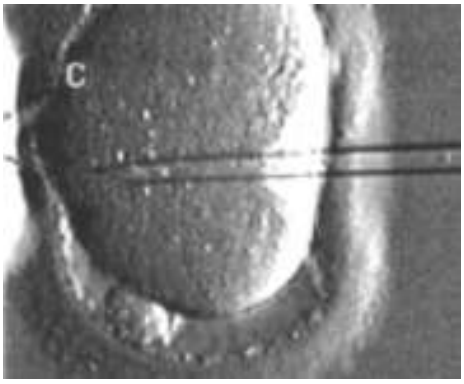

Fig. 4. ICSI, step III.

RESULTS AND DISCUSSION

In private females hospital "Fertility" in Bitola, R. Macedonia during nine years (from 2004 to 2012) were performed 1979 IVF procedures, from which 1608 were ICSI (79%) procedures, last two years only ICSI. In table 1 showed numbers of procedures per year for ICSI and classic IVF).

From our results [2004 year/IVF-classic (68):ICSI (60) – all (128); 2005 year/IVF-classic (41):ICSI (83) – all (124); 2006 year/IVF-classic (79):ICSI (89) – all (168); 2007 year/IVF-classic (79):ICSI (132) – all (211); 2008 year/IVF-classic (53):ICSI (174) – all (227); 2009 year/IVF-classic (41):ICSI (256) – all (297) and 2010 year/IVF – classic (10):ICSI (262) – all (272)], we can see that IVF procedures were combined with classic IVF and ICSI proce-

dures with successful rate (SR%) between 39–51%, dependence of age of the females. Last two years all IVF procedures were performed only with ICSI (2011 year/ ICSI (246) – all (246) and 2012 year/ICSI (306) – all (306)) with more higher percentage of successful rates (SR%), >50% (50-61%).

Indications for ICSI are the following: 1) Sperm concentration $<2\times10^6$; 2) Sperm motility <5%; 3) Strict criteria normal morphology <4%; 4) Use of surgically retrieved spermatozoa; 5) Failure of fertilization in a previous IVF cycle.

In review of literature there are a lot of studies which showed that ICSI procedure is the most successful procedure over classic IVF or other fertilization procedures. Our study correlate with these findings in literature, suggests and recommends only ICSI procedures to be performed for IVF.

Scope of ICSI in Veterinary Science

1. Availability of material: Ovaries in animals are primarily obtained from the abattoir where they are essentially waste material, hence the oocyte recovery is economical and their use is ethical.

2. Research: Virtually nothing is known about what happens to the spermatozoon and the oocyte within few hours following sperm injection. Research with human material is necessarily limited, so the use of domestic species should rapidly promote research in to the fundamental mechanism of ICSI.

3. Training: Technical competency with ICSI takes time and experience. ICSI with domestic species is technically similar to human and thus its suitable as a training tool.

4. The potentiality of ICSI in exotic species for conservation is yet to be exploited: There are two areas where ICSI is already having a direct impact. One is direct propagation. For example, a premium bull might have excellent characteristics with the exception of poor quality of semen. In extreme cases the semen can be unsuitable for artificial insemination (AI) or even IVF, but ICSI can be successfully used to generate embryos. In such cases, an embryo may be worth much, that the initial investment can be commercially viable.

In Texas, a equine intracytoplasmatic sperm injection (ICSI) program is offered at Texas A&M as a means of establishing pregnancies from oocytes (eggs) recovered from donor mares. Using ICSI, oocytes are injected with individual sperm from a donor stallion, and the resulting embryos are allowed to develop in the laboratory for approximately one week. Developed embryos are then shipped to a private embryo transfer facility for transfer to a recipient mare, as for standard embryo transfer. This contract with TAMU (Texas A&M University) includes recovery of oocytes, fertilization, embryo culture and shipment of resulting embryos. All charges related to the transfer of resulting embryos to recipient mares will be billed to you, the client, by the embryo transfer facility performing the transfer and are not included in this contract.

ICSI is powerful micromanipulation tool for achieving fertilization and development independent of semen characteristics, collection methods and storage. Subfertile spermatozoa, epididymal sperm, testicular sperm and even only sperm head can be successfully used for ICSI.

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