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Application of Intracytoplasmic Sperm Injection in Mammals

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ABSTRACT

For more than two decades, the intracytoplasmic sperm injection (ICSI) technique has been used as a valuable tool to provide opportunities for studying fertilization, treating human infertility, and producing transgenic animals. Not only in facilitating fertilization but also in propagating mammalian species, ICSI has enhanced the potential of assisted reproductive technologies in human. Polyspermic fertilization has been one of major problems in pig reproduction, but the ICSI helped to solve the problem, and used widely to generate transgenic piglets. Although the ICSI technique is considered to be a very useful tool in assisted reproductive technologies, including generation of transgenic animals, there are some disadvantages using the technique. In this review, we describe the ICSI technique and its application in animal production and human infertility, and discuss advantage and disadvantage of the technique in mammals.

(Key words : Intracytoplasmic sperm injection (ICSI), Assisted reproduction technique (ART), Review, Application, Mammals)

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) described as a procedure of a single sperm injection into the oocyte cytoplasm has been widely used for propagating mammalian species and providing an opportunity for research into fertilization mechanisms (Cheng *et al.*, 2009; Lee *et al.*, 2004). For more than two decades, ICSI has been known as a valuable technique providing opportunities for studying fertilization, has enhanced the potential of assisted reproductive technologies to facilitate fertilization, treat human infertility and producing transgenic animals (Tian *et al.*, 2006). Not only in facilitating fertilization but also in propagating mammalian species, ICSI has been used and has enhanced the potential of assisted reproductive technologies (Cheng *et al.*, 2009; Lee *et al.*, 2004). In the porcine system, polyspermy has been known to occur very frequently, influencing the embryonic development to the blastocyst stage during *in vitro* fertilization (Jin *et al.*, 2009; Kren *et al.*, 2003; Day *et al.*, 2000). Thus, ICSI is considered to be very useful in assisted reproductive technologies (Binh *et al.*, 2009), is especially well-known for generating porcine transgenesis (Lai *et al.*, 2001). Even though many species have been successfully generated as live ICSI-derived offspring, live ICSI-derived piglets have only been recently reported with the use of *in vivo* and *in vitro* matured oocytes (Martin *et al.*, 2000; Nakai *et al.*, 2003). However, using ICSI, the porcine blastocyst formation rate and quality is poor compared with outcomes in other species. Porcine embryos produced by *in vitro* methods generally show reduced pregnancy rates and increased incidence of abnormal offspring based on low total cell numbers, altered inner cell mass ratios, high nuclear apoptosis and fragmentation (Funahashi *et al.*, 1995; Koo *et al.*, 1997; Abeydeera *et al.*, 2001; Coy *et al.*, 2002). Even though the reasons for the low success rate in porcine research are not clear, culture conditions have been considered an important factor affecting the quality and developmental capacity of porcine embryos produced *in vitro* (Hwang *et al.*, 2008; Gupta *et al.*, 2007; Prather, 2000).

WHAT IS ICSI? WHY TO DO THAT?

Intracytoplasmic sperm injection (ICSI, pronounced "eeksee" or "icksy") is an *in vitro* fertilization procedure which a single spermatozoa is injected directly into an oocyte. In 1976, Uehara and Yanagimachi

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first performed ICSI in mammals by injecting the heads (nuclei) of hamster spermatozoa into mature oocytes; a few hours later, these oocytes had formed male and female pronuclei (Uehara *et al*., 1976). ICSI is a technique for assisted reproduction (ART). This is especially useful for men with very low sperm counts since ICSI ensures that the sperm reaches the egg directly rather than waiting for the sperm to naturally fertilise the egg. The use of ICSI provides an effective treatment for severe male factor infertility (Palermo *et al*., 1992). The negative effects of abnormal semen characteristics and sperm quality on fertility can be overcome with ICSI if viable sperm are available because the technique bypasses the zona pellucida and oolemma to deliver the male chromosomes directly into the ooplasm.

ICSI allows couples with male factor infertility to achieve live birth rates comparable to those achieved with *in vitro* fertilization using conventional methods of fertilization. ICSI can be performed even in men with azoospermia if spermatozoa can be successfully collected from the epididymis or the testis (Schlegel *et al*., 1995; Schlegel *et al*., 1997; Wennerholm *et al*., 2000). ICSI is compatible with normal embryonic development (Bonduelle *et al*., 1994; Palermo *et al*., 1996), and is no longer regarded as an experimental procedure. Currently, several male factor abnormalities, including varying degrees of oligozoospermia, asthenozoospermia, oligoasthenozoospermia and teratozoospermia, are best treated by ICSI (Orief *et al*. 2004).

ICSI IN ANIMALS

ICSI has been reported in several species, including rabbits (Iritani *et al*., 1989), cattle (Goto *et al*., 1990), humans (Lanzendorf *et al*., 1988), mice (Kimura *et al*., 1995), sheep (Catt *et al*.,1996), horses (Cochran *et al*.,1998), cats (Pope *et al*., 1998), Japanese monkeys (Hosoi *et al*., 1998), dogs (Fulton *et al*., 1998), pigs (Kolbe *et al*., 2000) and lions (Damiani *et al*., 2004). The potential applications of ICSI in animals include its use with sperm obtained post-mortem, with samples that have low motility or with samples from individuals of high genetic value or special features, such as transgenic animals or endangered species. With respect to oocytes, ICSI can be useful in the fertilization of oocytes with alterations preventing the normal process of fertilization, such as cryopreserved oocytes, *in vitro* matured oocytes and oocytes obtained from prepubertal females. For example, ICSI in horses is becoming important for fertilizing the limited number of oocytes obtained by ovum pick-up or from cryopreserved oocytes (Jacobson *et al*., 2010). Especially, in pigs, ICSI has shown the most valuable benefit to exclude the possibility of high proportions of polyspermic fertilization during *in vitro* fertilization (Binh *et al*., 2009).

ICSI in Human

Since the report of the first human baby born via ICSI was reported by Palermo *et al.* in 1992, there have been hundreds of thousands of children born by this technique. ICSI has been an important development in human assisted reproduction, and its application and results in humans have been very successful compared to other species: fertilization rates using ICSI are 70~80% and pregnancy rates are close to 45% (Palermo *et al*., 2009). The initial and most important application was in the case of patients with low sperm concentrations, leading to a poor prognosis for the use of *in vitro* fertilization. Other indications were later incorporated, including cases of severe teratozoospermia or asthenozoospermia. Currently, human applications for ICSI include the use of sperm obtained from testicular biopsies and protection from infectious diseases such as human immunodeficiency virus (HIV). In addition, ICSI has spread to other applications that are not directly related to solve sperm pathology, such as its use in the fertilization of *in vitro* mature or cryopreserved oocytes (by vitrification or other cryopreservation techniques), and it has an essential role in preimplantation genetic diagnosis, which avoids the contamination of oocytes with multiple spermatozoa.

DISADVANTAGE OF ICSI

Low Efficiency

Recently, several reports have been introduced the possibility to generate viable piglet from oocytes by this useful technique ICSI (Kolbe *et al*., 2000; Martin *et al*., 2000; Lai *et al*., 2001; Nakai *et al*., 2003; Probst *et al*., 2003). However, it is obvious from these studies that this technique is not ready to be considered as a method of commercial application in pigs because the conditions are immature and un-standardized to be employed yet (Empar *et al*., 2006). Even though many species have been successfully generated as live ICSI-derived offspring, live ICSI-derived piglets have only been recently reported with the

use of *in vivo*- and *in vitro*-matured oocytes (Martin *et al*., 2000; Nakai *et al*., 2003). However, using ICSI, the porcine blastocyst formation rate and quality is poor compared with outcomes in other species. Porcine embryos produced by *in vitro* methods generally show reduced pregnancy rates and increased incidence of abnormal offspring based on low total cell numbers, altered inner cell mass ratios, high nuclear apoptosis, and fragmentation (Fuchimoto *et al*., 2003; Koo *et al*., 1997; Abeydeera *et al*., 2001; Coy *et al*., 2002).

Safety

When ICSI was introduced, there was major concern about its safety. ICSI is indeed a more invasive procedure than routine IVF, since one spermatozoon is injected through the oocyte membrane and since fertilization can be obtained from spermatozoa which could never have been used previously in fertility treatment. Even more questions were raised and concern was again expressed when ICSI with non-ejaculated spermatozoa, either epididymal or testicular, was introduced. Emphasis was put on the fact that the risk of chromosomal aberration might be even higher in men with non-obstructive azoospermia. On the other hand, it was suspected that imprinting might be less complete at the time of fertilization if testicular spermatozoa were used. If this were so, it would be unlikely to impair fertilization and early development, but anomalies might become manifest at birth or only later in life. The safety of this novel procedure of assisted fertilization had, therefore, to be assessed carefully (Steirteghem *et al*. 2002).

CONCLUSION

This review discussed that ICSI is one of the strong assisted reproductive technologies for producing transgenic animals as well as treating infertility in animals and humans. It is a very important technique for treating male subfertility and for basic research. It is also have some disadvantage such as low efficiency and its safety.

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