

Intracytoplasmic Sperm Injection (ICSI) and its Applications in Veterinary Sciences: An Overview

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ABSTRACT

Background: Intracytoplasmic Sperm Injection (ICSI) is one of the methods of assisted reproductive technology that aims to successfully treat male factor infertility in humans and domestic animals. The technique involves mechanically injecting a single sperm or sperm nucleus with the help of a micromanipulator into the cytoplasm of a mature metaphase II oocyte. **Results:** In domestic animals, ICSI has been used to study ways to surmount problems that are faced during *in vitro* fertilization such as non-motile sperm, inability of spermatozoon to penetrate oocyte, capacitation etc. Moreover, ICSI technique can also be applied to preserve biodiversity and to produce transgenic animals. **Conclusion:** Several modifications have been done in conventional ICSI so as to enhance success rate, however, there are still number of problems regarding practical use of ICSI in domestic animals. This study aims to review the historical development, its applications and research needs for its improvement.

Key words: Assisted reproductive technologies, intracytoplasmic sperm injection, livestock

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INTRODUCTION

In the livestock sector, infertility related problems have been one of the major impediments. Assisted Reproductive Technologies (ART) refer to the use of reproductive technology to treat infertility. ART such as Artificial Insemination (AI), *In vitro* Fertilization (IVF), *in vivo* Embryo Transfer (ET), embryo sexing, embryo splitting, cloning and Intracytoplasmic Sperm Injection (ICSI) have been extensively used to overcome infertility in animals and to enhance production efficiencies. Moreover, the use of ART procedures not only eliminates the risk associated with animal transport but have also been useful in propagating valuable genetics. The death of a genetically superior animal or inability to serve (e.g., Loss of libido, injury to hindquarter, etc.) leads to the loss of valuable germplasm. However, this loss could be circumvented by harvesting germ cells from the genetically superior animals and using ART procedures, such as AI or ICSI to propagate it. ICSI

involves direct injection of a single sperm or sperm head (nucleus) into the ooplasm, by passing natural process of sperm oocyte interaction. Hence, fertilization process taking place via ICSI is different from *in vivo* or *in vitro* fertilization. It is a microfertilization technique that is used to cure the male infertility problems in animals and also in cases where eggs are not easily penetrated by sperm. ICSI is considered as a last resort when all other conventional methods of insemination fail. This technique is considered to be efficient than IVF and AI in terms that in ICSI only one intact spermatozoon is sufficient to fertilize an ovum while AI and IVF require millions of spermatozoa. ICSI also provides exciting opportunities for studying the cell cycle control and the basic mechanisms involved in sperm induced oocyte activation, ooplasmic factors leading to transformation of a sperm nucleus into male pronucleus, fertilization and early embryo development. However, in comparison to humans, success rates in cattle using ICSI are quite less. In the veterinary field, micromanipulation in domestic animal species e.g. bovine, equine, ovine etc., has been used for the past two decades as an experimental means and in the commercial field. There are some problems

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related to ICSI in the domestic animals that are required to be resolved before its widespread applicability in livestock sector. There are many outstanding reviews on current status and future of ICSI in mice¹ and cattle² and this study reviews the historical aspects that led to the development of ICSI, potential applications in animals and major problems resulting in limited success in animal models.

HISTORY DEVELOPMENT OF ICSI

The first study reported using ICSI was performed in starfish³. In 1962, embryological development following injection of live spermatozoa into the protoplasm of unfertilized sea urchin oocyte was reported⁴. This study inspired researchers to implement same technique in higher animals as well. Later on, this technique was first reported in amphibians^{5,6}. In mammals, the first successful fertilization achieved by direct intra-ooplasmic sperm transfer was documented in hamster⁷. In 1988, first normal fertilization after injection of a single spermatozoon into human oocyte was reported⁸. They demonstrated the capability of human oocyte to develop upto pronuclear stage. Using ICSI, live offsprings in rabbit and cattle were also obtained^{9,10}. In 1992, a major advancement in the field of assisted reproductive technology was made when healthy human babies were produced successfully by ICSI in Brussel, Belgium¹¹. It was a spectacular medical achievement after that ICSI has undergone explosive growth. However, after that, numbers of critical steps have been identified and several modifications are made in ICSI so as to escalate its success rate. Later it was reported that higher fertilization and pregnancy rates could be achieved by injecting live spermatozoa¹². Conditions involving severe morphological abnormalities such as cryptozoospermia, total asthenozoospermia and teratozoospermia do not influence success rate in ICSI¹³. ICSI has gradually developed over the century with the contribution of many researchers. Later on technique of piezo assisted ICSI in mouse which proved to be highly successful in enhancing fertilization rates in comparison to conventional methods was introduced¹⁴. The use of piezo micromanipulator in achieving higher fertilization and pregnancy rates has been supported in many studies^{15,16}. On microinjecting tail cut fresh spermatozoa into the ooplasm of goat oocyte using a piezo micropipette driving system (PiezoDrill) it was concluded that using tail-cut spermatozoa along with Piezodrill and oocyte holding pipette is efficacious approach in caprine ICSI¹⁷.

APPLICATIONS/INDICATIONS FOR ICSI IN LIVESTOCK

- ICSI is recommended when all other techniques of assisted reproduction fail. It is a method of

choice to obtain monospermic zygotes if IVF fails to produce fertilized oocytes

- ICSI has become a useful technology in cases when semen availability is less e.g. if semen is sex sorted¹⁸
- Semen collected from deceased animal. Epididymal sperms can be collected and used via ICSI if a genetically superior male dies
- Sub-fertility related to abnormalities in ejaculated semen e.g., oligozoospermia (low sperm concentration), teratozoospermia (sperms with abnormal morphology), asthenozoospermia (sperms with reduced motility), necrozoospermia (Dead spermatozoa)
- Azoospermia i.e., No sperms in ejaculate. It may be obstructive or non-obstructive. In such cases, ICSI may be combined with epididymal or testicular sperm retrieval
- Presence of antisperm antibodies
- Ejaculatory disorders
- Congenital (cystic fibrosis or unilateral renal agenesis leading to congenital absence of vas deferens) or acquired abnormalities (fibrosis) of reproductive tract
- Transgenic animal production
- Conservation of endangered species

MAJOR IMPEDIMENTS AFFECTING OUTCOME OF ICSI IN LIVESTOCK

Male pronucleus formation: During ICSI sperm acrosome and plasma membrane are introduced into the oocyte which averts male pronucleus formation. Sperms have been subjected to various treatments viz., Triton X 100¹⁹, dithiothreitol²⁰, repeated freezing and thawing without cryoprotectant²¹. These treatments were aimed to remove sperm membrane so as to improve male pronuclear formation and to speed up the availability of sperm-borne oocyte activation factor to the oocyte. Recently, it has been reported that selecting sperm after combined heparin-glutathione pretreatment may improve ICSI outcome in bovines²². However, in pigs sperm pretreatment has been documented to cause reduction of oocyte-activating capacity²³.

Role of polyvinylpyrrolidone (PVP): Is a polymer (molecular weight of 360,000) used in ICSI procedure to impede sticking of spermatozoa to inner surface of injection pipette and decrease their motility. PVP has a primary detrimental action on plasma, acrosomal and mitochondrial membrane of human spermatozoa²⁴. Better fertilization rates can be achieved using a modified method of ICSI free of PVP than ICSI with use of PVP²⁵. However, in a study no harmful effect on the rate of normal fertilization and embryo quality in bovine embryos after injecting small amount (2-3 pl) of PVP was found²⁶. Till date, PVP remains the solution

of choice for ICSI; however there is still need to evaluate its potential detrimental effects with further studies.

ICSI equipments: Size, shape, sharpness and diameter of ICSI needle are very important criterion determining success of ICSI. In one study, it was documented that fertilization and embryo development after ICSI were affected by diameter of injection needle²⁷. Fresh epididymal sperm collected from a fertile male rabbit to compare different sperm injection methods into oocytes were also used²⁸. It was documented that the fertilization rate was highest when the needle tip was pushed across half the diameter of the oocyte and when oolemma breakage was achieved by repeated aspiration and expulsion.

Opacity of bovine oocytes: Cytoplasm of bovine oocyte is dark in appearance due to high lipid content which makes it difficult to identify intracellular structures and prevents normal visualization of meiotic status²⁹. It also makes delivery of spermatozoon exceedingly difficult and large volume of vehicle solution may be introduced inside ooplasm. Centrifugation has been practised to remove excess lipid content, however, it may sometime lead to activation of oocyte and extrusion of second polar body³⁰.

Large sperm head: In bovines the sperm head is larger than human sperm head leading to a considerable damage to oolemma.

Difficulty in bovine oocyte activation: Bovine matured oocytes are difficult to activate by sham injections³¹ i.e., process of oocyte injection without sperm. A number of methods have been attempted to artificially activate oocyte after ICSI like ethanol³², electric stimulation³³, ionomycin, cyclohexamide³⁴. Recently, it has been documented that sperm injection into bovine oocytes produces abnormal (Ca^{2+}) responses and oocyte activation. It is thought that the release or activation of the sperm factor is compromised after ICSI, thus leading to incomplete (Ca^{2+}) oscillations and leading to premature termination of embryo development³⁵.

Low cleavage rates: Low cleavage rate has been one of the major obstacles in bovine ICSI.

Poor technical proficiency: ICSI is a technically demanding skill that may need months of practice before technical proficiency is achieved. Selecting a single spermatozoa, aspirating it into a microinjection needle and injecting it into the ooplasm of metaphase II oocyte require great deal of adeptness. Ways by which zona

pellucida and oolemma are ruptured can largely affect result of injection procedure. Thus, success of ICSI is majorly dependent on the technical expertise of the handler.

INNOVATIONS IN ICSI

Use of sex sorted sperms: Sperm sorting is an advanced technique that sorts sperm *in vitro* by use of flow cytometry. Dr. Glenn Spaulding was the first to sort viable whole human and animal spermatozoa using a flow cytometer and used the sorted motile rabbit sperm for artificial insemination. Flow cytometry is laser based technique applied to distinguish X and Y chromosomes in sperms for the gender preselection. In one study, birth of 13 male piglets from sex-sorted frozen-thawed epididymal sperm and ICSI was reported¹⁸. Y-chromosome bearing sperm were sorted using flow cytometry technique and were injected into *in vivo* matured oocytes, activated with CaCl_2 solution. Fertilized oocytes were transferred surgically into four recipient females.

ICSI-mediated transgenesis (ICSI-Tr): Since the last decade, there have been enormous attempts with varying degrees of success, to use spermatozoa as a vector for transgenesis in mammals and other vertebrates. Sperm Mediated Gene Transfer (SMGT) technique is based on the ability of spermatozoa to bind and internalize foreign DNA and transfer that into the oocyte during fertilization³⁶. This is a new method used to produce transgenic animals. ICSI-mediated transgenesis (ICSI-Tr) was first reported in mice³⁷ and later developed in pigs^{38,39}. Later on, it was documented that SMGT-treated spermatozoa retain good quality and fertilization potential for at least escalating the possibility to apply transgenesis in field conditions in swine⁴⁰. An active method has been devised termed as 'active transgenesis that combines ICSI-Tr with recombinases or transposases to enhance transfection efficiency. In caprine, it has been reported that sperm status and DNA concentration to some extent play key roles in sperm/ICSI-mediated gene transfer and embryo development⁴¹.

CONCLUSION

ICSI in animals has emerged as a valuable tool. However, there is still a long way to go in order to resolve underlying problems and practically implement this technique in cattle and other domestic animals. The factors which ultimately influence the survival of sperm and oocyte must be considered and all measures must be taken to minimise them. This technique can be implemented from experiment to conservation level in animal sector. A deep knowledge of animal physiology,

