Plant Archives Volume 20 No. 2, 2020 pp. 6680-6684 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

APPLICATION OF EMBRYO PRODUCTION USING ICSI TECHNIQUE IN CAPRINE

Hussein A. Khamees[1] and Saad A. Hatif[2]

[1]Department of surgery and obstetrics. Collage of veterinary medicine AL-Muthannah University, Iraq.
[2]Department of surgery and obstetrics. Collage of veterinary medicine Baghdad University, Iraq.

Abstract

This study was conducted on goats with the purpose of applying intra cytoplasmic sperm injection (ICSI) in animals as one of the new techniques used to treat fertility impairments and sterility in field animals in Iraq. The study was conducted at the College of veterinary medicine – Baghdad University 2019-2020. Sixty goat ovaries were collected after slaughter and the ova were removed by aspiration. The ova were examined under a microscope, classified according to the surrounding cumulus cell layer, and then matured and incubated for 36 hours. The ova were fertilized using sperms collected from slaughterhouse samples, by illustration semen from the tail of the epididymis and dealing it before fertilization. ICSI was conceded by injecting one sperm into the ovum and then incubating it and follow the development of fertilization and embryo production. The study shows that collection of ova by aspiration is improved than the method of slicing. Collecting ova during the breeding season is improved to process by obtaining ova since the animal is seasonal breeding. The study shows that the using of PVD to control sperm and limit the movement of sperm quickly only without affecting its activity; it is quickly picked up from the prim and collected in another part of the ICSI dish, as well as to re-select the strongest and most active sperm, since sperm that traverses the PVD and reaches the prim is undoubtedly very active, selected, captured and used in the ICSI process. Fixing the ovum in preparation for ICSI; it must be rotating and gently moved using the ends of the pipette until the ovum is stabilized when the polar body is positioned at 12 o'clock or 6 o'clock so as avoiding cutting the chromatin pockets during the insertion of the injection pipette and pushing the sperm. We conclude from this that the factor of experience and practice are very important in increasing the success rate of the injection process and obtaining acceptable and good future results.

Key words: Intracytoplasmic sperm injection, embryo production, factors affecting, caprine.

Introduction

Assisted reproductive techniques offer a unique way of treating infertility by *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). *In vitro* fertilization is indicated in most of the female related infertility, whereas ICSI is mostly employed in couples with male factor infertility (Verheyen *et al.*, 1999) or with a history of fertilization failure (Benadiva *et al.*, 1999).

The advent of IVF embryo transfer enabled patients who were irremediably infertile due to absolute tubal factor to conceive. However, a considerable number of patients remained unable to achieve pregnancy through IVF mainly due to failure of fertilization. Therefore, various micromanipulation techniques have been developed to assist fertilization. These techniques originated from a pre-existing technology of micromanipulation that was developed for various purposes other than assisted fertilization (Edwards *et al.*, 1980).

The ICSI has become method of choice to achieve fertilization. Fertilization is possible in cases in which the sperm motility and ability to penetrate the zona pellucida are impaired. Injection is possible with sperm obtained from ejaculation, microsurgical epididymal sperm aspiration (MESA), per-coetaneous epididymal sperm aspiration (PESA), or testicular sperm extraction (TESE). In addition, indications for ICSI include idiopathic infertility and repeated conventional *in vitro* fertilization (IVF)

failures (Benadiva and Julsen, 1999).

In 1911, Kite discussed a nuclear dissection experiment in which he used micro needles to separate the pronuclei in newly fertilized zygotes (Chambers, 1940). In 1928, Emerson described the first micromanipulator with a joystick design that could directly transmit the movements required by the operator (El-Badry, 2012). In 1934, De Fortune described hydraulic principles that could be used for the joystick manipulators, and also he developed the first micro forge and micro tool making techniques that are still in use today. Failure of male pronuclear formation is one of the main reasons for the embryo development after ICSI. Its application and results have been very successful compared to other species live births have been obtained 21% in the cow (Goto *et al.,* 1990), 8-10% in mouse (Ahmadi *et al.,* 1995), and sheep (one pregnant) (Nakai *et al.,* 2014).

This study was aimed to applying ICSI technique on animals to increase success of fertilization before transfer and transplantation of embryos and expressive what the factors which affect.

Materials and Methods

The study was conduct in the Baghdad- Iraq, from middle August 2018 to end of October 2019 (inbreeding). The experiment includes 60 caprine ovaries were randomly collected from 60 slaughtered does in the slaughterhouse of in Baghdad. The ages of the doe was known by indentation (1.5-4 years old). The main device used in the experiment is the computer unit for ICSI with *Eppendorf micromanipulator*.

Ovaries and Oocytes collection

Genital organs of doe were collected from slaughterhouse, placed in a cooling box containing a maintain media at 35-33°C, and transferred within 1-2 hours to the laboratory. Only the ovaries were collected and cleaned from all the other tissues. The ovaries were washed for the first time in a distill water to remove blood clots and residual dirt, then wash twice with SOF media, and finally put them in a sterile glass container containing the SOF, Nystatin and Penicillin Streptomycin (Saif & Ihasan, 2018). The oocyte were collected by a sterile syringe (aspiration method) with G18 needle and other by using of slicing the ovaries using a surgical blade, and then transferred into petri dish, containing 5 ml culture media. Oocytes were assessed under the microscope to select mature oocytes to conduct our research.

Oocytes Evaluation

Collected oocytes were examined via inverted microscope, and the quality of oocytes was classified according to (Wani *et al.,* 2000) as Good (Grade A), Fair (Grade B) and Poor (Grade C), based on of cumulus cells and cytoplasmic homogeneity (Chart 1).

Oocytes maturation

In vitro maturation (IVM), involves maturation of oocytes in culture using hormones or soon, a naturally occurring oocyte protein dimmer. After collection, assessment and classification of oocytes, oocytes from class A and B were selected. Oocytes were washed twice in SOF and then incubated in appropriate media at temperature of 38.5 ° C, 5% CO2 and 90% humidity for 36 hours. At the end of the maturation period, plates were examined via inverted microscope, and appearance of the first polar body is a good indication of oocyte maturation. The numbers of mature oocytes were calculated.

Semen collection and Preparation

Sperms were obtained from a slaughtered bucks, where testicles were taken and placed in a cooler box, and then transported within 1-2 to the laboratory. The epididymis was taken by sterile scissors, and the epidermis tail is washed with a protective phosphate solution (PPS) (Saif & Ihasan, 2018) to remove the blood clots and dirt.

The epididymis is placed in a sterile dish. TALP solution was injected into the epididymal tail by using a G-18 needle. The surface of the epididymis tail was removed via sharp scalpel, and epididymis contents were withdrawn by using a sterile syringe of 5 ml (Lone *et al.,* 2011). Samples were tested for semen quality, as well as for the assessment of the individual and collective movement of sperm.

The sperms kept in the incubator at 35°, 5% CO_2 for 6 hours to sperm maturity. The presence of the protoplasm droplet at the end of the sperm tail is evidence of sperm maturity. Heparin is then added to the sperm and incubated at 38° for 45 minutes in according to (Palamo *et al.,* 1999) in order sperm adaptation. Heparin can induced spermatozoa capacitation in any levels through the breeding season while the high level of heparin gave better results, fertilizable index and embryo production are both gave best result via breeding season (Saleh & Abdul-Ameer, 2019).

Preparation of ICSI dish

Several droplets of medium (5 µL to 25 µL TCM) are placed in the center of the cell culture dish. Droplets intended for retrieved spermatozoa are supplemented with PVP before the addition of the sample. Additional droplets containing PVP only might be necessary for storage of selected spermatozoa before injection (Fig. 1).

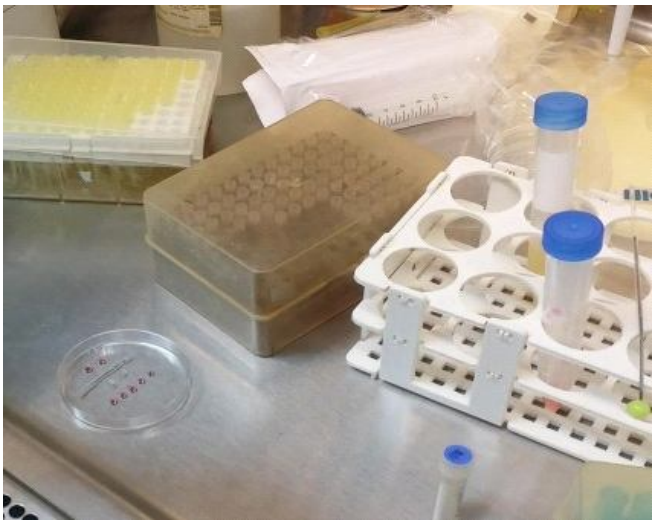

Fig. 1: Preparation of ICSI dish.

All droplets are completely covered with light mineral oil to maintain their stability as well as temperature and pH. Once prepared, the microinjection dish can be placed into the incubator until use.

Selection of spermatozoa

The Dual Speed joystick of the TransferMan 4m has the advantage that it does not need to be re-positioned by declutching if the maximum displacement of the actual path radius has been reached. Instead, it is possible to press the joystick gently against its outer margin and after a short transition phase the dynamic mode is activated and the needle proceeds in the desired direction. The speed of the dynamic movement can be adjusted in relation to the proportional movement.

Using this feature, the needle can be moved carefully in the fine or extra fine (x-fine) speed mode whilst still capable of a considerable range of quick motion once the dynamic, outer zone of the joystick is entered.

Sperm microinjection

The sperm sample is taken in a drop filled with PVP, while the ova are placed in the medium droplets specified in the dish. ICSI transfer bristles are stored to position 1 T and under 200x to 400x magnification

The sperm are withdrawn by either wrapping the ICSI transfer filaments over the tail or by gently pressing the tail at the bottom of the dish.

The sperm, the tail first, are pulled into the capillaries of the ICSI transport as smoothly as possible by turning the Cell Tram 4m oil handle. The joystick switch is then pressed twice to move the sperm-containing capillaries up to the center of the overlay.

The cell culture plate is transferred to a drop containing the ova and the cell is focused. The ovum is

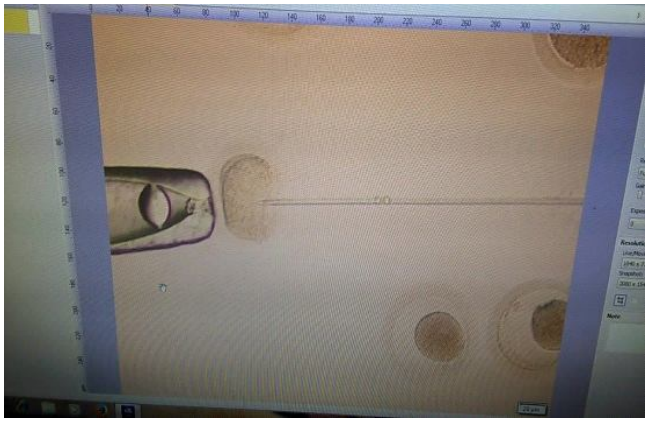

Fig. 2: Polar body at 12 o'clock during sperm injection.

attached gently but firmly to the holding filament by the negative pressure produced by the air system. The ovum is usually injected with the first polar body positioned either at 6 or 12 o'clock (Fig. 2).

To achieve this direction, it may be necessary to flip the ovum, and this can be done with the help of ICSI capillaries to lower back to 1 T and change the negative pressure of CellTram 4m Air slightly until the polar body reaches the desired position.

The capillaries by injection are now concentrated at the same level as the maximum diameter of the ovum becomes clear. By turning the CellTram 4m oil handle, the sperm are gently moved along the capillaries until placed at the tip.

By slightly moving the joystick, the capillaries traveling carefully through the intense region are then pushed through the zona pellucida into the cytoplasm at 3 o'clock. The ovum should be pricked in the middle so that the zona pellucida membrane is gently broken and shocked. To make sure this happens, a small amount of Ooplasm is gently pulled into the capillaries by injection as a sign of rupture of the membrane. The inhaled Ooplasm and spermatozoon are then deposited towards the center of the ovum.

In order to reduce the media size and the PVP inserted into the cytoplasm, the capillaries are gently withdrawn after leaving the sperm cell head capillary. Once this is done, the injected ovum is released from the holding capillaries and both capillaries are returned to position 2 by pressing the joystick key twice.

Once the injection procedure is complete, the ova are placed in an appropriate culture medium and incubated overnight. Injected ova were incubated at 38.5 ° C, 5% carbon dioxide and 90% moisture for 30 hours

Zygote Evaluation and *in vitro* Culture

Zygotes were evaluated after 24 hours of fertilization

based on the presence of the second polar body or presence of the sperm head within the oocyte cytoplasm. The number of zygotes was counted. The zygotes were incubated at 38.5 ° C, 5% CO_2, and 90% Humidity. The embryonic development was monitored every 24 hours with the replacement of 50% of the media with a new sterile medium every 24-hour.

Results and Discussion

The study shows that collection of ova by aspiration is improved than the method of slicing (Chart 1 and 2). Collecting ova during the breeding season is improved to process by obtaining ova since the animal is seasonal breeding (Chart 3). The study shows that the using of PVD to control sperm and limit the movement of sperm quickly only without affecting its activity; it is quickly picked up from the prim and collected in another part of the ICSI dish, as well as to re-select the strongest and most active sperm, since sperm that traverses the PVD and reaches the prim is undoubtedly very active, selected, captured and used in the ICSI process. Fixing the ovum in preparation for ICSI; it must be rotate and gently moved using the ends of the pipette until the ovum is stabilized when the polar body is positioned at 12 o'clock or 6 o'clock so as to avoid cutting the chromatin pockets during the insertion of the injection pipette and pushing the sperm.

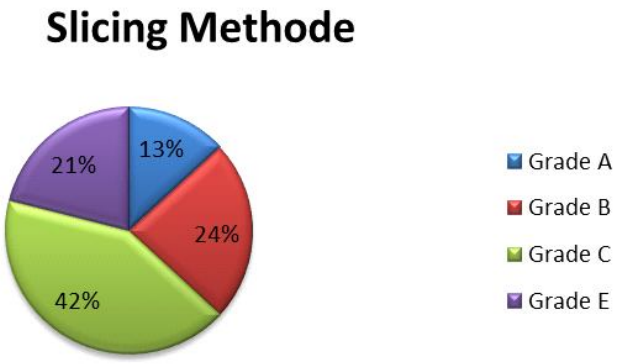

Chart 1: Number of ova using slicing method.

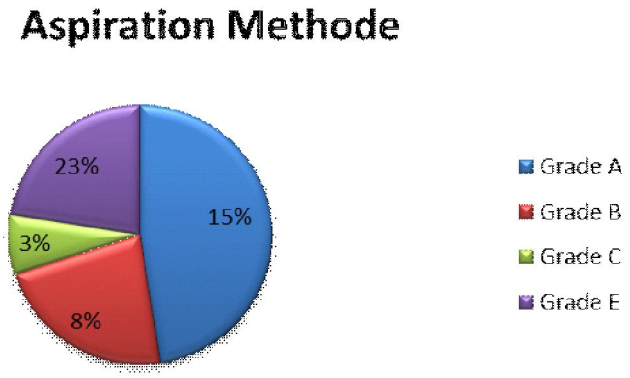

Chart 2: Number of ova using aspiration method.

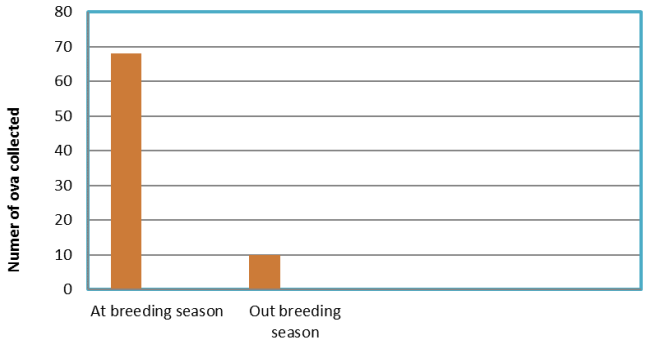

Fig. 3: Number of ova that collected in different season of goat.

We conclude from this that the factor of experience and practice is very important in increasing the success rate of the injection process and obtaining acceptable and good future results.

Conclusions

The use of microscopic injection technology or ICSI as one of the applications of new techniques for reproduction in animals opens the way for treating cases of infertility or low fertility and decrease pregnancy rates as well as treatment male infertility problems such luck of sperms or the poor quality semen producer.

The application of microscopic injection technology to goats provides the opportunity to raise the reproductive efficiency of this economic animal, as it is a pluriporous animal with a high economic benefit in many countries that are celebrated for breeding goats.

The microscopic injection technology allows the fetus produced under our hands, which allows us to apply other techniques such as sex determination, embryo transfer, genetic testing before the transfer, and others.

References

Ahmadi, A., S.C. Ng, S.L. Liow, J. Ali, A. Bongso and S.S. Ratnam (1995). Fertilization and early embryology: Intracytoplasmic sperm injection of mouse oocytes with 5 mM Ca^{2+} at different intervals. *Human Reproduction*, **10(2):** 431-435.

Benadiva, C.A., J. Nulsen, L. Siano, J. Jennings, H.B. Givargis and D. Maier (1999). Intracytoplasmic sperm injection overcomes previous fer-tilization failure with conventional *in vitro* fertilization. *Fertile. Sterile.*, **72:** 1041–1044.

Benavida, C.A., H. Julsen and L. Siano *et al.*, (1999). Intracytoplasmic sperm injection overcomes previous fertilization failure with convention *in vitro* fertilization. *Fertil., Steril.*, **72:** 1041–1044.

Chambers, R. (1940). The micromanipulation of living cells. The Cell and Protoplasm. *Am. Assoc. Adv. Sci.*, **14:** 20–30.

De Fonbrune, P. (1934). Demonstration d'un micromanipulator

pneumatique et d'un apparel pour la fabrication des micro instrument. *Ann. Physiol. Physiochemical.*, **10:** 4.

Edwards, R.G., P.C. Steptoe and J.M. Purdy (1980). Establishing full-term human pregnancies using cleaving embryos grown *in vitro*. *Br. J. Obstet. Gynaecol.*, **87:** 737–756.

El-Badry, H.M. (2012). Micromanipulators and micromanipulation. Springer Science & Business Media, **3**.

Goto, K., A. Kinoshita, Y. Takuma and K. Ogawa (1990). Fertilization of bovine oocytes by the injection of immobilized, killed spermatozoa. *The veterinary record*, **127(21):** 517-520.

Lone, F. A., R. Islam, M.Z. Khan and K.A. Sofi (2011). Effect of transportation temperature on the quality of cauda epididymal spermatozoa of ram. *Animal reproduction science*, **123(1-2):** 54-59.

Nakai, M., M. Ozawa, N. Maedomari, J. Noguchi, H. Kaneko, J. Ito and K. Kikuchi (2014). Delay in cleavage of porcine embryos after intracytoplasmic sperm injection (ICSI) shows poorer embryonic development. *Journal of Reproduction and Development*, 2013-100.

Palomo, M. J., D. Izquierdo, T. Mogas and M.T. Paramio (1999). Effect of semen preparation on IVF of prepubertal goat oocytes. *Theriogenology*, **51(5):** 927-940.

Saif, A.H., T. Mohammed and A.T. Ihasan (2018). Maturation, Fertilization and Implantation of Cattle Ova Cultured in Different Media. *The Eurasia Proceedings of Science Technology Engineering and Mathematics*, **3:** 181-188..

Saleh, W.M. and A. Abdul-Ameer (2019). Evaluation of *in vitro* Fertilization Index by Caudal Spermatozoa Capacitation with Different Heparin Concentration in Iraqi Sheep. *The Iraqi Journal of Veterinary Medicine (IJVM)*, **43(1):** 171-182.

Verheyen, G., H. Tournaye, C. Staessen, A. De Vos, M. Vandervorst and Van Steirteghem (1999). Controlled comparison of conventional in-vitro fertilization and intracytoplasmic sperm injection in patients with asthenozoospermia. *Hum. Reprod.*,**14:** 2313–2319

Wani, N.A., G.M. Wani, M.Z. Khan and S. Salahudin (2000). Effect of oocyte harvesting techniques on *in vitro* maturation and *in vitro* fertilization in sheep. *Small Ruminant Research*, **36(1):** 63-67.