Learning Experience in embryology Laborarory during Clinical Intra Cytoplasmic Injection of Sperm

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ABSTRACT

Introduction: Intracytoplasmic sperm injection is a process of in vitro fertilisation (IVF) which utilizes micromanipulation technique. It involves injecting a single sperm into the centre of a mature oocyte under a microscope and then the injected ovum is then monitored in the laboratory for signs of fertilisation. The present study describes embryology laboratory experience during clinical intracytoplasmic sperm injection procedure commenced at a fertility centre.

Material and Methods: The study comprised of 59 unfertilized and immature oocytes. The study estimated time duration of various steps of ICSI. Data so obtained was analyzed using the SPSS Version 17 software and was arranged according to characteristics and was expressed as mean and standard deviation.

Result: The study revealed that the mean± standard deviation of time taken for dish preparation was 2.38±0.478, time taken for micro manipulator setting was 5.5±1.29, time taken for sperm immobilization was 2±0.408 and time taken for sperm aspiration to injection was 1.75±0.288.

Conclusion: The long-term effects of ICSI on the children born with this procedure is yet to be known, since as the first successful ICSI-conceived child was born in 1992, and most long-term ICSI studies have not yet completed data collection.

Keywords: Assisted Reproductive Technologies; Intracytoplasmic sperm injection; In vitro fertilisation

INTRODUCTION

Assisted Reproductive Technologies that includes *in vitro* fertilization, intracytoplasmic sperm injection (ICSI) provide treatment options and hope for infertile couples; a problem that is estimated to affect 15% of world population.[1] Intracytoplasmic sperm injection is a micromanipulation method used in the process of in vitro fertilisation (IVF). This method employs injection of a single sperm into the centre of a mature oocyte under a microscope and ovum is then observed in the laboratory for signs of fertilisation.[2] This technique is helpful in the case of male factor infertility where sperm counts are very low or failed fertilization occurred with previous IVF attempts. It was developed in 1992 to treat cases with sperm abnormalities i.e. azoospermia, oligozoospermia, asthenozoospermia or teratozoospermia.[3] The present study describes embryology laboratory experience during clinical intracytoplasmic sperm injection procedure commenced at a fertility centre.

MATERIAL AND METHODS

The present study regarding clinical intracytoplasmic sperm injection (ICSI) was conducted at Narmada Fertility Centre, Secunderabad over a period of one month from May 2016 to July 2016. Ethical permission was taken from the institutional committee for the commencement of the study. Data was collected in 4 groups. Oocytes were divided in 4 groups (table-1) and ICSI procedure was carried accordingly.

The procedure of ICSI was carried as follows:

Dish preparation

For this ICSI project, unfertilized and immature oocytes nearly about 59 in number were used. The ICSI machine used was a Nikon 71 X with pnumatic and oil control system. Sperm preparation was carried out using pure sperm media and the process of sperm preparation was carried using double density with swim up technique. For dish preparation, vitrolife G-plus media, oil and polyvinylpyrrolidone (pvp) were used accordingly. The holding and injection needles were from cook company with 35 degree angle. The dish was prepared at room temperature and a thin streak of pvp and two droplets of pvp (2 ul) were made. Each on either side of pvp streak culture medium were used in droplets (5 ul). 2 ul of motile sperms suspension was placed near the lower end of pvp streak and was connected to pvp with small bridge. The dish was covered with mineral oil and was incubated for 15 minutes at 37 degrees temperature and 6% CO_2. The migration of sperms was observed to pvp streak and then the denuded oocytes were transferred to culture droplets and setting up of micromanipulator was started. For micromanipulator setting, first all manipulators were set to be in maximum range of movement in the field of microscope by adjusting incenter. The oil column flow was corrected continuously in the tube and injection needle and holding needle was put in pipette holders of micro injectors.

Micromanipulator Setting: After setting the micromanipulator, the oocytes were denuded with hyalurinadase in the central well dish for 30 seconds. All oocytes were washed and all oocytes were transferred in wash dish and then incubated at 37 degrees in incubator.

Sperm immobilization and aspiration: After alignment of injection and placing holding needle under low magnification 4x, morphologically healthy sperm was searched under 20 x magnification. The sperm tail was focused, immobilized and was aspirated in zero pressure after equilibration of ICSI needle in pvp.

ICSI procedure: The first polar body was aligned in 12 o'clock position with holding pipette, the injection needle was brought

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	Group 1 two weeks		Group 2 two weeks		Group 3 two weeks		Group 4 one week
	16 oocytes		11 oocytes		15 oocytes		12 oocytes
a	4	a	3	A	4	a	3
b	3	b	2	B	4	b	4
c	5	c	3	C	4	c	2
d	4	d	3	D	3	d	3

Table-1: Distribution of oocytes in 4 groups

Groups	A	B	C	D		
Sperm quality					Mean	SD
Pre wash	30 million	20 Million	10 million	30 million	22.5	9.574271
Post wash	18 million	11 Million	8 Million	20 million	14.3	5.678908
Time taken for ICSI						
Dish preparation	3 mins.	2-3 mins.	2 mins.	2 mins.	2.38	0.478714
Time taken for micro-manipulator setting	6 mins.	7 mins.	5 mins.	4 mins.	5.5	1.290994
Time taken for sperm immobilization	2 mins.	2.5 mins	1.5 mins	2 mins.	2	0.408248
Time taken for sperm aspiration to injection	2 mins.	1.5 mins	1.5 mins	2 mins.	1.75	0.288675

Table-2: Mean and Standard Deviation for various steps of intracytoplasmic sperm injection (ICSI)

Figure-1: Observation of procedure under microscope

down and pierced the zona, slight suction of oolemma was done on the holding side and slight aspiration of ooplasm inside the injection pipette and then the sperm was released in the middle of the oocyte. The injection was slowly removed, injection needle and suction were released from the holding needle side of the oocyte. After the process was finished all oocytes were kept in the incubator. After 17 to 18 hours oocytes were checked for cleavage.

STATISTICAL ANALYSIS

Data so obtained was analyzed using the SPSS Version 17 software and was arranged according to characteristics and was expressed as mean and standard deviation.

RESULT

The present study comprised of 59 unfertilized and immature oocytes. Oocytes were divided into 4 groups and ICSI procedure was conducted accordingly. The present study revealed that mean± standard deviation of number of sperms at pre-wash was 22.5±9.57, at post-wash was 14.3±5.67. The mean± standard deviation of time taken for dish preparation was 2.38±0.478, time taken for micro manipulator setting was 5.5±1.29, time taken for sperm immobilization was 2±0.408 and time taken for sperm aspiration to injection was 1.75±0.288. Table-2 describes mean and standard deviation for various steps of intracytoplasmic sperm injection (ICSI)

DISCUSSION

Intracytoplasmic sperm injection is the novel 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.[4] However, development of this technique raises concerns about the safety of this method as severely infertile men are known to display an increased incidence of genetic abnormalities; secondly, conventionally used semen parameters do not provide information on the quality of the DNA of the spermatozoa and thirdly, injection of the spermatozoa directly into the oocyte bypasses the natural selection processes mediated by the egg's envelope.[5] The present study describes the various steps of intracytoplasmic sperm injection (ICSI) technique and reports the mean duration of each step and thus narrates a learning experience at a fertility centre.

ICSI is novel hope for the couples with severe male factor infertility. However, a spermatozoon containing a functional genome and centriole is required for the generation of normally fertilized oocytes after ICSI. In case of obstruction of the seminal excretory ducts, this procedure can be commenced with sperm taken from the epididymis and testis.[6]

By performing ICSI, the three barriers a sperm cell has to cross in natural fertilization and conventional IVF are overcome: the cumulus-corona cells are removed in order to determine the maturity of the oocytes prior to injection and in order to be able to inject. The oocytes are denuded in two steps using the enzyme hyaluronidase in the first step and in the second step mechanically by pipetting.[7] Similarly, in the present study, after setting the micromanipulator, the oocytes were denuded with hyalurinadase enzyme in the central well dish for 30 seconds. The first polar body was aligned in 12 o'clock position with holding pipette, the injection needle was brought down and pierced the zona, slight suction of oolemma was done on the holding side and slight aspiration of ooplasm inside the injection pipette and then the sperm was released in the middle of the

oocyte. Direct injection overcomes the two remaining barriers, the zona pellucida and the oolemma.[7]

The use of intracytoplasmic sperm injection, with both ejaculated and non-ejaculated sperm, bypasses the process of natural selection barriers against physiologically or genetically abnormal sperms. The technique of direct injection of the sperm into the vitellus of the oocyte during metaphase II can be technically harmful to the oocyte and thus can lead to chemical or mechanical damage, henceforth, because of disturbances of the meiotic spindle, chromosome pairing errors can occur.[8]

A successful ICSI programme depends on ovarian stimulation, which is essentially similar to conventional IVF. In conventional IVF, oocytes are inseminated while they are lodged within the cumulus complexes. Prior to fertilization by means of micromanipulation, oocytes need to be denuded from the surrounding cumulus and corona cells, not only accurate injection into the oocytes, as well as observing for their maturity, which is of essential for ICSI procedure. A combination of enzymatic (hyaluronidase) and mechanical procedures are used to remove cumulus and corona cells. Denuded oocytes can be observed under the inverted microscope (in order to assess nuclear maturity.[9] The similar procedure was followed in the present study. Hyaluronic acid (HA) is a linear polysaccharide that enhances the long-term motility and velocity of spermatozoa in normozoospermic, oligozoospermic and frozen–thawed sperm samples. Additionally, HA-selected spermatozoa have no DNA degradation or active caspase-3, which greatly contributes to the abnormal apoptosis process associated with some immature spermatozoa.[10]

The present study estimated time duration of various steps of ICSI and revealed that the mean± standard deviation of time taken for dish preparation was 2.38±0.478, time taken for micro manipulator setting was 5.5±1.29, time taken for sperm immobilization was 2±0.408 and time taken for sperm aspiration to injection was 1.75±0.288. Morken NH[11] compared in vitro fertilization (IVF) pregnancies with intracytoplasmic sperm injection (ICSI) pregnancies and reported that IVF pregnancies were associated with increased risk of moderately iatrogenic preterm delivery as compared to ICSI pregnancies. The infertility services aims to help people with genetic conditions or fertility problems by providing solutions to their reproductive plans by assisted reproductive technologies. These assisted reproductive methods are also of great importance for normally fertile couples suffering from inherit genetic diseases which can pass to children. Counselling is an essential part of all the treatment plans.[12,13]

CONCLUSION

For couples who do not find success with natural conception or IVF, ICSI can truly be a blessing. However, owing to the invasive nature of the procedure, there is great potential for complications of all degrees of severity. Additionally, the long-term effects of ICSI on the resulting children remain largely unknown, since the first successful ICSI-conceived child was born in 1992, and most long-term ICSI studies have not yet completed data collection.

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