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Oocyte Degeneration Subsequent Intracytoplasmic Sperm Injection (ICSI)

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ABSTRACT

Objective: Oocyte degeneration Subsequent to ICSI has historically been noteworthy phenomenon associated with the Intracytoplasmic (ICSI) technique. We sought to determine whether oocyte degeneration rates how typically associated with the various factors and how associated with the technician performing the procedure.
Design: Retrospective Randomized and Review study.
Setting: Base Fertility Medical science Pvt Ltd (IVF Center)
Patient(s): Couples undergoing ICSI.
Intervention(s): One thousand and two hundred eighty two injected oocytes were analyzed to determine whether the degeneration rate was technician dependent or various factors dependent (Micro tools, Oocyte quality, Positioning of Pipettes Alignment, Injection Pipettes – Spikes and Bevels, Volume of Injection Vehicle: PVP or Image Eminence). Thirty two articles reviewed and were examined to categorize predictors allied with oocyte degeneration.
Main Outcome Measure(s): Oocyte degeneration rates
Result(s) and Conclusion(s): The studies suggest that oocyte collapse/degeneration subsequent to ICSI is not technician or physician dependent totally. Degeneration is likely a function of the inherent oocyte quality in women who underwent ovarian stimulation and the various factors inevitably involved. The degeneration rate did not appear to be associated solitary on formative factors.

Key Words: Oocyte Degeneration, ICSI, Ovarian stimulation.

1. INTRODUCTION

ICSI is the heart of the Embryology. Intra-Cytoplasmic sperm injection (ICSI) entails injecting a single sperm straight into an egg in order to fertilize it. The fertilized egg that is now an embryo is then transferred to the woman's womb/uterus/fundus for the further development. The major expansion of ICSI means that as long as some sperm can be obtained (yet in very low numbers); fertilization is possible (Wang et al., 2001). The ICSI work system is similar to that for IVF, but as an alternative of fertilization taking place in an ICSI dish, the embryologist picks sperm from the sample and a single sperm is injected directly into each Oocyte gently. In the initial years after ICSI's beginning each practitioner had to build up a procedure that would assure successful fertilization and at the equivalent time, reduces the fabrication of degeneration or abnormally fertilized eggs (Rosen et al., 2006). There has always been an unambiguous learning arch for each new embryologist related with the acquisition of sufficient skill to go through the zona pellucida and the oolemma without irrevocably harming the oocytes (Wilding et al., 2007). Numerous embryologists can possibly remember months of low fertilization rates followed by a steady scale in success as skills recovered. In particular, learning to distinguish when the oolemma has been successfully breached is doubtless the most significant step in mastery of ICSI. Joyfully, skill transfer to the next generation of technical personnel come into views to be happening at a much faster rate today contrast to those initial days, grateful to years of following and guidance from the older invention of embryologists who brought this innovatory modus operandi into their individual laboratories. Intracytoplasmic sperm injection (ICSI) has, since its' progress as a means of assisted reproduction technology (ART), become most likely the major technique in use at the current day (Palermo et al., 1995). It is been estimated that over 1 million children have been born worldwide with this procedure. The technique is applicable to almost all male factor including severe pathologies (Etiologic types of azoospermia: obstructive azoospermia (OA) and (NOA) nonobstructive azoospermia) in ART including semen samples without motility, samples procured from testicular biopsies and in some cases immature spermatids where no mature spermatozoa are present, elongated spermatid, round spermatid and with such immature germ cells, follow-on in the deliverance of healthy offspring. Henceforth, more or less entire male factor infertility diagnoses can now be treated with ICSI. However, the ICSI procedure does not answer in 100% fertilization of oocytes infused with the process. Oocytes sometime stay unfertilized, or degenerate subsequent to the technique. Although the degeneration of oocytes past ICSI is often pointed to the operator, several additional aspect can influence the altitude of oocytes degeneration in a specific cohort of oocytes. Previously these aspects are elucidated the degeneration of oocytes subsequent to ICSI can too be practical as a diagnostic cause intended for oocytes quality.

2. MICRO TOOLS

Poor labors frequently blame their tools, as it is important that embryologists have good tools to do ICSI. There are several commercially accessible IPs industrially made to suite different specifications (lumen sizes, bevels, angles, and with non-spiked or spiked tips). The IP diameter disturbs ICISI because a slight diameter diminishes disturbance, whereas an outsized diameter causes more disturbance and escalates the probability of oocytes damage. IP diameter is dictated by the magnitude of the sperm to be injected and is the typical magnitude for the majority of the commercially accessible IPs for clinical ICSI.

The IP angle relays on distinct preference, and should not mark the lyses providing the position of penetration is acceptable. Ideal penetration ideally should be vertical to the equator of the oocyte. This can be confirmed by moving the IP onto the zona, applying a significant force onto the oocyte and witnessing equivalent bend in both halves of the oocyte above and below the IP. ICSI can be executed at other latitudes, but the menace of irreversible breaks is expected more with growing space from the equator. The holding pipette (HP) has to be of adequate magnitude to secure the oocyte throughout ICSI, deprived of collapsing the oocyte form. Nevertheless, a new survey of interspecies ICSI by using murine oocytes stated the use of an improvised HP with a trumpet-shaped opening (Lyu et al., 2010). This allowed the oocyte contour to be partly distorted by the aspiration action into the HP, thus letting a propounded injection in the oocyte. This considerably enhanced oocyte existence and fertilization in a species with an extremely greater deterioration rate after comparison with the classical ICSI.

3. OPERATOR SKILL & LEARNING CURVE

Machinist-induced harm can occur while preparing the oocyte and during the real ICSI process. For cumulus denudation before the ICSI, the time hyalurodinase gets in contact with the oocyte the time needs to be as less as possible and should comprise adequate washing phases to make sure the proper t removal of the acid post-denudation. The concentration of the hyalurodinase also needs to be carefully maintained; as the stock solution may need dilution before exposing the oocyte to it .Equally both over-exposure and excessive concentration can badly disturb oocyte quality. Cumulus exclusion needs mechanical handling of the cumulus oocyte complex; the lumen size

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of the denuding pipette needs to be carefully measured and decided. Aspiring an oocyte bounded by numerous tightly held layers of cumulus cells into a denuding pipette with a small lumen (e.g. 135µm diameter) may impact high pressure leading to zona rupture. Few embryologists hence use numerous denudation pipettes of various lumen sizes to make sure that the oocyte gets minimal stress while steadily removing the cumulus cells. While denuding the cumulus and the real ICSI, the oocyte temperature should be sustained at 37°C. While some temperature variations are unavoidable while the transfer of oocytes between dishes, the heated stages should be accustomed in such a way that the temperature of the media in the droplets should be 37°C. This requires a minor surge to the heated stage so that no account for thermal loss is occurred due to transmission through the base of the dish in which the oocytes are kept. Few embryologists accomplish any 'out of the incubator' manipulations by using buffered medium to preserve the pH. Nevertheless, subjected to the practitioner skills and technique, both cumulus elimination and ICSI can be achieved in pre-equilibrated culture medium without a buffer. This depends on the effective timing of the process to make sure the pH does not alter. This has the added advantage of not exposing the oocytes to any alteration in medium type, and safeguards the procedures are time-bound. The main disadvantage of using buffered medium is long exposure of the oocytes to the buffer during micromanipulations, which may badly affect the oolemmas .With any microsurgery; there is a skill level that needs to be achieved in order to perform ICSI to a suitable standard. While the initial ICSI key performance indicator (KPI) is oocyte survival post-injection, other KPIs such as fertilization rate, embryo development and implantation rate need to be taken into consideration. Variations to the ICSI technique by various embryologists may bring more refined differences to the injected oocytes beyond survival. Alterations between embryologists performing ICSI are a controversial topic. The survival rate after ICSI has been reported as both dependent and independent of the embryologist, while an important inter-embryologist difference in the fertilization rate has been stated (Shen et al., 2003).

4. OOCYTE QUALITY

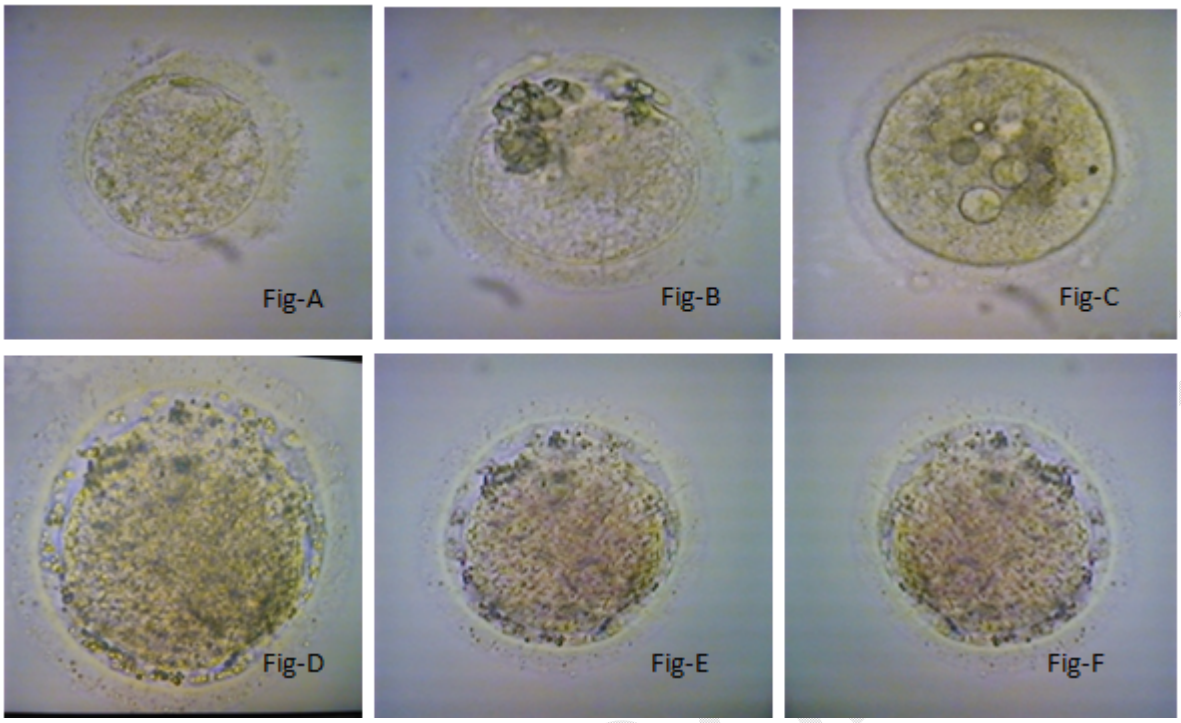
The basic nature of different oocytes of different patients states that some oocytes are less likely to last the ICSI process. Metaphase II oocytes differ in physical features such as shape and size, the thickness and regularity of the zona, the size of the peri-vitelline space, the size and shape of the first polar body, and the consistency of cytoplasmic granularity. The Other qualities such as oolemma elasticity cannot be evaluated until and unless they are enthusiastically tried, most possibly in response to the early insertion of the IP (Strassburger et al., 2004). Deprived quality oocytes might have fragile zonae and oolemmas which breakdown effortlessly upon IP insertion. Further, oocytes may have thicker zonae and excessively resistant oolemmas which require a high negative pressure than the usual to be applied on the IP, causing in a huge amount of cytoplasm entering into the IP. The two types of oocytes are prone to a high deterioration degree after ICSI. Familial oocytes may exhibit related properties. The application of laser-assisted ICSI has been stated for patients with atypical oolemma elasticity (Demirol et al., 2006). This technique uses a laser to make a bore into the zona instantaneously before IP insertion. The IP is then delivered through the hole and into the oolemma as per classical ICSI, thus decreasing the oocyte firmness and the peril of harm.

5. MECHANISM OF OOCYTES COLLAPSE AFTER ICSI

Plasma membrane of the oocytes, as for all cell kinds, is intended to sustain equilibrium sandwiched between the cells' inner milieu (the cytoplasm) and the extracellular milieu (which in the case of human fertilization will be the fluids of the fallopian tube and follicular fluid). This is entailed to avoid the dissipation of the cell substances, assist in the establishment of energy for utilized in cell homeostasis and maturity and to act as a fence to pathogens. Cells accomplish this through a selection of structures. In the case of human, the oocyte plasma membrane is retained integral through a large actin-tubulin cytoskeleton that acts as a scaffold to maintain the lipid bilayer in place. During the ICSI process, the plasma membrane is cracked, causing to an epigrammatic influx of extracellular environment. This medium is extremely toxic to the oocyte while it holds a high concentration of sodium and calcium ions. The oocyte cytoplasm is as a result briefly depolarized and uncovered to a short-lived peak of calcium. Flourishing oocyte repair after ICSI then depends on the fast restoration of the plasma membrane and the ionic stability between internal and external environment (Figures A-F). As can consequently be expected, numerous aspects can contribute to the victorious conclusion of the ICSI technique.

6. THE ICSI TECHNIQUE

Variations to ICSI technique may outcome in not injecting the complete aspirate quantity of cytoplasm or the sperm and injecting extra medium/fluid/PVP into the oocytes. Cytoplasmic outflow or sperm 'falling out' of the injection spot may be scrutinized with the previous, whilst large fluid-filled gaps within the oocyte may result with the latter. Deepness of injecting pipettes insertion requires consideration. There is a peril that the distal oolemma on the



reverse side of the oocyte may be by mistakenly breached if the IP is pushed too deep into the oocyte, causing to inevitable irreparable membrane damage. In opposition, if the depth of insertion is too superficial, then the oolemma may not be sufficiently extended to permit a non-fatal breach. Quite a few embryologists monitor the initial oolemma infringe, but then go on to aspire additional cytoplasm into the IP 'to confirm a specific breach'. However, this may not be finest practice as would-be too much cytoplasm may source important intracellular harm, as well as possibly causing a hazardously huge breach in the oolemma. Traumatic injection and removal is an additional variable. If the movement is too rapid, then the ICSI may not be totally accomplished before IP removal. Conversely, if the motion is too leisurely, the peril of cytoplasmic outflow from the induced oolemma membrane injury may amplify. Conventional ICSI modus operandi include 'priming ICSI', whereby the IP is outset dropped into the oolemma to prime the membrane devoid of penetration (Shen et al., 2003). The IP is then removed and a second incorporation is used to break through the oolemma in a procession analogous to the first insertion. The 'before-stretching' of the oolemma may aide a well breach as an end result. An alternative technique is that piezo-ICSI (Yanagida et al., 1999). This occupy puncture of the zona by means of a flat-tipped IP enthused by a piezo-electrically activated contact tool called a piezo-drill. The puncture is sourced by a swift piezo-pulse of changeable frequency, duration and amplitude. Piezo-ICSI decreases oocyte deformation through ICSI and has been revealed to get better endurance or survival rates in mouse (Kimura and Yanagimachi et al., 1995).

7. POSITIONING OF PIPETTES ALIGNMENT

Pipettes must be set up and united so that a straight plane stripe can be drawn from end to end tip sections of both the injection and holding pipette when observed through the ICSI phase contrast microscope. This confirms that, as soon as the injection pipette is pressed into the oocyte, it is not going at an angle virtual to the holding pipette, rendering increased tension to be positioned on the oolemma and raising the probability of tearing the oolemma. Moreover, if they are not in same plane, the oocyte may rotate off the holding pipette as the injection pipette is passed into the oocyte, and yet again, the oolemma stretches and is further prone to rupture. Comparable tension happens if the bevel tip of the holding pipette is not in a plane parallel to the bottom surface of the Petri dish. Significantly despite of, holding the oocytes will become difficult, as the base of the dish avoid the oocyte from meeting squarely on to the holding pipette. To hold the oocytes firmly therefore, advanced air/oil suction has to be used, and a petite portion of the oocytes is repeatedly sucked into the holding pipette. This causes the tension in the membrane surrounding the oocytes and thus, when the injection pipette is pushed against the oolemma, it may

rupture. These may seem to be moderately negligible considerations when performing numerous ICSI procedures in a day, though, those oocytes with somewhat delicate membranes will be the foremost to succumb if the pipettes are badly aligned. Vigilant alignment of pipettes not merely makes the system much easier, but enhances the possibility of the majority, if not the entire, oocytes viability.

8. INJECTION PIPETTES – SPIKES AND BEVELS

The spike conformation on injection pipettes is predominantly essential. The spike wants to be sharp and spiky adequate to go through the zona pellucida, devoid of causing too much distortion of the oocyte, but not so spiky that it will right away pierce the oolemma. Usually pipettes that had very fine spikes globally caused oocyte lysis. A very fine spike should assist in the insertation of the zona pellucida and reduces oocyte twisting, however, a very clean spike will slash the oolemma on touch, and the oocyte cytoplasm will straight away leak out. The oolemma has negligible prospect to heal itself under these circumstances. The typical ICSI technique has been to compel the oolemma to shatter, maybe at a labile junction, as the membrane is being aspirated reverse into the injection pipette. Since the cytoplasm is being redeposited to the oocyte, the strain on this fraction of the membrane is unrestricted and the liability in this region of the membrane permits for fast healing. As the oolemma is strained into the injection pipette it creates a sharp "V" surrounding the shaft of the pipette. The moment the oolemma ruptures, it relaxes around the pipette. In an oocyte in which the oolemma has been pierced prior to suction by a fine spike, the oolemma will not become taut around the injection pipette. This oocyte will almost certainly lyse. It is possible to circumvent the problem of fine spikes, prior to injection, by pressing the point of the injection pipette against the holding pipette and breaking the very tip of the spike away. Blunt injection pipettes will not go through the zona pellucida with no trouble, and amplify the distortion of the oocyte throughout ICSI. Even extremely blunt spikes are not simply recognized in outline, and the spike appears somewhat square quite than sharp. Amplified twisting of the oocyte throughout ICSI enhances the rate of oocyte lysis. These three making imperfection are not easy to recognize in the laboratory, as once the injection pipette is set up for ICSI, it can only be viewed in the microscope. Examination of pipettes before to ICSI may diminish the probability of inappropriate pipettes being used, but in practice, this is time utilizing and should not be required if superior quality injection pipettes are used for the purpose.

9. INJECTION PIPETTES - ADDITIONAL THOUGHT

If the material used to fabricate injection pipettes is lipophilic (lipid loving nature), usually as a result of insufficient washing, the pipette stick to lipid in the oolemma, and also to membrane-bound organelles such as Golgi and endoplasmic reticulum. If organelles are pulled out of the oocyte with the injection pipette as it is pulled from the oocyte, the oolemma will not be able to heal around the withdrawn organelles. More essentially, it can reason the oolemma to tear as it is stretching over the sharp irregular surface The pipette will also adhere to the plasma membrane of the sperm as the sperm membrane are PUFA.

10. HOLDING PIPETTES

The holding pipette should embrace the oocyte tenderly, but firmly, throughout injection. Pipettes with an outsized flat face, somewhat slighter in width than that of the zona whole oocyte, offer added constancy and avoid excessive distortion of the oocyte during the course of ICSI. Guarantying that the oocyte is in touch with the petri dish gives a second point of hold and raise the steadiness of the oocyte during injection. This can avert the oocyte undulating off the pipette during injection, and minimizes tension in the oolemma that may result in shattering and oocyte lysis.

11. VOLUME OF INJECTION VEHICLE: PVP

PVP is mostly used to reduce the progression of the sperm so that immobilization and catching can be done with no trouble performed. Unavoidably, little PVP will go into the oocyte during injection. Whereas oocyte lysis amplified significantly as large amount PVP was injected, embryo usage and implantation rate of the surviving potency of the oocytes was not unlike. The raise in oocyte lysis pace was owing to a more complicated injection which was reproduced by the quantity of PVP injected into the oocyte and consequent interruption of the cytoplasm, or trauma to the oolemma and not the incidence of PVP per se (Payne et al.,1998).

12. IMAGE EMINENCE

Well successful ICSI relays on watchfully operating the process and that in turn sits on being competent enough to preface clearly see what is occurring. Unwanted distortion of the oocyte, aspirating excessively much cytoplasm, and rough unturned, uncontrolled injection entirely lead to an amplify rate of oocytes lysis, and this can happen when image quality is deprived and not clear. The point of the injection pipette must be visible in the equivalent plane as

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the oolemma so that the position of the tip and the association of the oolemma and cytoplasm can be clearly and cleanly visualized during ICSI. If the oolemma cannot be seen as a clearly delineated fine clear line then the optical arrangement is improper, or the focus is inaccurate. Optical section achieved when the oocyte is correctly focused allows for finer proper judgment throughout the course of ICSI since the cytoplasm and plasma membrane can be more effortlessly delineated. The image quality achieved with DIC (Differential Interference Contrast) is superior to HMC (Hoffman's Modulation Contrast) and therefore has always been my selection.

13. ADDITIONAL ASPECTS

Seldom may it be essential to re-inject an oocyte, if the deposition of sperm was failed during the first ICSI. A subsequent ICSI might increase the danger of oocyte lyses, as the cell membrane may still be in a state of repair following the foremost injection. Somewhat an instantaneous re-injection, a feasible approach to increase endurance might be to tolerate the oocyte a phase of instance to improve before performing a recurrent ICSI. Precaution must be taken to only inject the spermatozoa through ICSI using a clean uncontaminated IP. This is chiefly vital when choosing sperm from a biopsy (testicular biopsy) which may be contaminated with cellular wreckage and fragments that can attach to the IP.

14. CONCLUSIONS

Oocyte degeneration typically after ICSI is associated with degeneration of a noteworthy percentage (5-10%) of the injected oocytes (Mansour et al., 2009; Richter et al., 2006). This article has conversed ways to reduce degeneration subsequent to ICSI, concluding adjusting the injection skill to account for oocytes of contrary quality. If unable to clearly visualize the gametes (oocyte and sperm) and pipettes, or the precise point of contact the injection pipette craft with the cell plasma membrane, using reduced quality pipettes, having a bad oocyte that is subject matter to deformation during ICSI, and not expressive when the membrane has ruptured can all contribute. Oocyte degeneration subsequent to ICSI is a physical as well as physiological modus operandi .The eminence of equipment, micro tools and embryologist is elementary to the positive upshot of the skill. Conversely, oocyte nature (Metaphase -1 or Metaphase -2) and quality is also an eminent factor. Oocyte quality can be defined in conditions of the properties of the oocyte plasma membrane, as well as the capability of the oocyte to recuperate from the grievance sustained during the process. Here in cooperation with the preparation of the patient/recipient for the ICSI procedures as well as the physical completion of the procedure are elementary formative factors.

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DISCLAIMER

The author has nothing to declare.

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