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Research Article

Impact of Oocytes Insemination Timing on Fertilisation and Embryo Development Rate

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Abstract

Background: Pre-incubation period between oocyte retrieval and injection in Intracytoplasmic sperm injection (ICSI) cycles improved the percentage of mature oocytes, the preincubation of oocytes prior to *in vitro* fertilization (IVF) improves the fertilization rate and the quality of embryo formation.

Objective: The study was to analyze whether the preincubation of oocytes and microinjection timings on the outcome of ICSI increases rates of fertilization and the quality of embryo formation and also to evaluate the relationship between different pre-incubation periods of oocytes.

Design: Retrospective randomized controlled trial.

Methods: A total of 129 patients undergoing their Intracytoplasmic sperm injection cycles, retrieved oocytes were preincubated for 2 - 6h prior to injection, the injected oocytes were cultured in Culture NX media, fertilization and Embryo development assessment was performed on day 1 and day 3, Patients were randomly divided into different groups.

Results: Neither 2 - 3 hour and 3 - 4 hour insemination time of post oocyte retrieval had no significant influence on the fertilisation rate (FR) and showed no significant improvement in Embryo development, fertilization rate showed a significant improvement after inseminating at 2 - 3 hour gives 86% fertilization rate and 95% embryo development rate while FR significantly decreased with the increase of insemination time. Also, inseminating oocytes at 3 - 4 hour post oocyte retrieval gives 81% fertilization rate and 97% embryo development rate.

Keywords: *Pre-Incubation; Intracytoplasmic Sperm Injection; Fertilization Rate; Embryo Quality*

Introduction

Assisted reproductive technology (ART) has been widely accepted as a part of infertility treatment in the present decade, resulting in the birth of millions of children. Intracytoplasmic sperm injection (ICSI) is the treatment of choice for infertility couples. The ICSI technique has been consistent but there is no typical for the specific timings of all the procedures. it has been shown that preincubation of oocytes for 2 - 6h before *in vitro* fertilization (IVF) improves fertilization, embryo formation, and pregnancy rates, but there are some conflicting results regarding the timing of ICSI. It has been reported that a pre-incubation period between oocyte retrieval and injection in ICSI cycles improved the percentage of mature oocytes, the fertilization rate, and the quality of embryo formation.

In the IVF, the oocytes are surrounded by the cumulus cells at the time of insemination but in ICSI the surrounded cumulus cells have been removed by standardized way of using mechanical and

enzymatic procedures so that the oocytes can be injected. The maturity of the oocytes can be evaluated and the oocytes which have extruded the first polar body (metaphase-II Oocytes) and thus reached nuclear maturity are injected.

Objective of the Study

The study was to analyze retrospectively the preincubation of oocytes and microinjection timings on the outcome of ICSI in a selected population to carry out corrective measures to improve the results. This study is also to evaluate the relationship between different pre-incubation periods of oocytes, and the outcome of the ICSI.

Materials and Methods

Ovarian stimulation can have a major impact on the outcome. In the natural cycle, many follicles commence to developing which depends on many aspects including the patient's age, her hormonal status and the complete health of her ovaries. The antagonist protocol is chosen to optimize the number of retrieved eggs and to maximize the fertilization rate. The follicle undergoes meiosis during the 35 hours that precede ovulation or retrieval. After 35 hours post hCG trigger shot, the follicles are retrieved by using oocyte retrieval needle with a single lumen or double lumen by transvaginal ultrasound-guided. Dishes for oocyte culture were prepared on the day of trigger by placing 40 µl droplets of Continuous Single Culture-NX (Irvine Scientific) in Oosafe 35 mm dish layered with culture oil (Surelife media). The dishes were kept at 37°C with 6%CO_2, 5%O_2, 89%N_2. After oocyte retrieval, the cumulus-oocyte complex is placed in Fertilization media (Surelife media). the Incubation of cumulus-oocyte complex is continued until denudation. The removal of cumulus cells from oocyte is performed at different time intervals for each ICSI cycle. Oocyte complexes were briefly exposed to dilute hyalase (Surelife Hyaluronidase) at the concentration of 80% (800 µl) of fertilization media and 20% (200 µl) of Hyaluronidase. The surrounding cumulus cells are mechanically removed by denuding Flexipet with diameters of 175 µm and 140 µm. The oocyte maturation was checked before ICSI. A standard procedure of sperm injection was performed.

The injected oocytes were cultured in Culture NX media at 37°C with 6% CO_2, 5% O_2, 89% N_2. The fertilization assessment was performed 18 - 20 hours after ICSI. The zygotes with two distinct pronuclei (PN) were cultured for an additional 48 hours. Embryo development assessment was performed on the morning of Day 3 including blastomeres number, size and the percentage of fragmentation. Grade A embryos consist of symmetrical blastomere of equal size with no fragmentation. Grade AB embryos had blastomeres of equal size and less than 10% fragmentation covering the embryo surface. Grade B embryos had blastomeres of distinctly unequal sizes and variable fragmentation. Grade C embryos had few blastomeres of any size, and severe fragmentation covering greater than 50% of the volume of embryos.

Results

This table 1, gives the information on the 129 couples in the study. There was no significant difference between 2 - 3 hour and 3 - 4 hour insemination time of oocytes post oocyte retrieval. This is explained by the fact that inseminating at 2 - 3 hour after retrieval gives 86% fertilization rate and 95% embryo development rate. Also, inseminating oocytes at 3 - 4 hour post oocyte retrieval gives 81% fertilization rate and 97% embryo development rate.

S. No	ICSI Time	No of cases	Fertilization	Embryo development	Grade A	Grade AB	Grade B	Grade C
1	0-1 hr	9	76%	96%	20%	38%	31%	11%
2	1-2 hr	27	81%	94%	42%	25%	29%	4%
3	2-3 hr	32	86%	95%	39%	25%	28%	8%
4	3-4 hr	31	81%	97%	35%	33%	25%	7%
5	4-5 hr	16	85%	94%	65%	30%	25%	10%
6	5-6 hr	13	76%	86%	30%	25%	30%	15%
7	6-7 hr	1	60%	91%	0%	25%	35%	40%

Table 1: Fertilization rate and Embryo Development rate outcome based on timing of oocyte insemination.

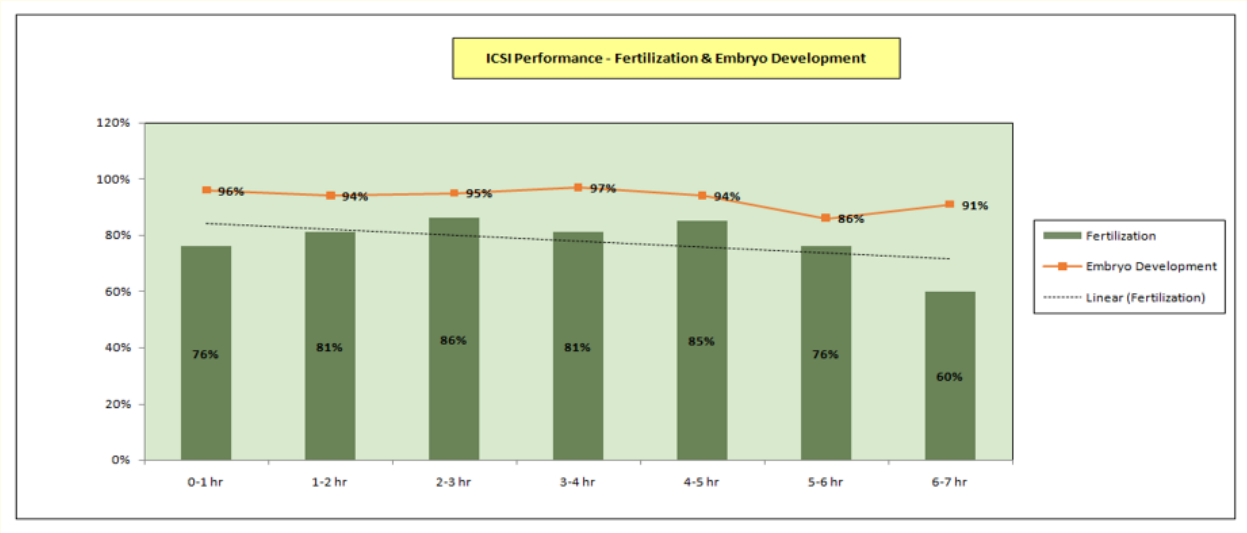

Figure 1

Conclusion

The evidence described in our study indicates that inseminating oocytes post oocyte retrieval at 2 - 3 hour and 3 - 4 hour gives good fertilization rate and embryo development rate with grade A and grade AB embryos. Inseminating oocytes at 6 - 7 hours after oocyte retrieval leads to less fertilization rate and grade B and grade C embryos [1-6].

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