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The impact of oocyte denudation without a pre-incubation on intracytoplasmic sperm injection outcomes

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ABSTRACT

Aims: Debate over the optimal timing of oocyte denudation following oocyte retrieval continues. Multinucleation has been associated with lower embryo quality and implantation rate. Defining oocyte characteristics might increase the chances of better embryo selection. This study aimed to investigate the laboratory and clinical outcomes of intracytoplasmic sperm injection when the oocytes were denuded immediately after oocyte retrieval.

Methods: A prospective randomized sibling-oocyte study was performed. The participants were under 40 years of age with more than 5 oocytes. Patients with male factors were excluded. The study and control groups were formed by simple randomization. The study group underwent oocyte denudation immediately. In the control group, oocytes were routinely incubated in an equilibration solution for 2 h until the removal of the cumulus cells. Outcome measures were normal fertilization (two pronuclei, 2PN), multinucleation rate, the proportion of good quality embryos, blastocyst formation, and pregnancy rate.

Results: A total of 792 oocytes were collected from 54 participants (mean age: 30.6±3.7 years). The fertilization rate was higher in the study group (n=209 in 376) compared with the controls (n=201 in 416) (55.6% vs. 48.3%, p=0.041). The multinucleation rate (6.6% vs. 4.3%, p=0.150), proportion of grade 1 embryos on day 3 (48.7% vs. 43%, p=0.112) and day 5 (27.7% vs. 23.8%, p=0.214), the proportion of embryos reaching blastocyst stage (34.0% vs. 28.1%, p=0.072), and the pregnancy rates (78.6% vs. 71.4%, p=1.000) were similar.

Conclusions: Our results demonstrated that immediate removal of cumulus cells does not have any negative impact on intracytoplasmic sperm injection outcomes.

Introduction

The clinical absence of pregnancy after 12 months of regular and unprotected sex is defined as infertility (1), an issue studied by researchers for years. Since the first successful induction of ovulation followed by pregnancy in 1963, assisted reproductive technologies (ART) have developed rapidly, causing a drastic change to find suitable treatment options for infertile couples (2). Today, intracytoplasmic sperm injection (ICSI) has become a widely employed treatment option in every ART unit. ICSI outcome highly relies on oocyte quality (3), and the evaluation of oocyte characteristics and oocyte behavior during ICSI might assist in enhancing cycle outcome rates (4).

Corona cumulus cells originate from granulosa cells and are located around the oocyte. They are implicated in oocyte growth and maturation, ovulation, and fertilization in mammals (5). These cells are removed in a process called oocyte denudation

to allow the evaluation of oocyte maturation and microinjection during ICSI. Routinely, the oocytes undergo a pre-incubation period for several hours following oocyte retrieval (6). However, no consensus on the timing of oocyte denudation has been established (6-10). Recent studies using oocytes from mice reported that long-term oocyte culture with the surrounding cumulus cells promotes oocyte aging and induces apoptotic changes (11-13). Also, Zhou et al. (10) demonstrated that 4-hour co-incubation of gametes and early removal of cumulus cells improved the clinical outcomes in patients with initial complete fertilization failure. However, abnormal fertilization with more than two visible pronuclei in couples with good-fertilizing capabilities has also increased significantly increased.

Multinucleation corresponds to the presence of more than one nucleus in a blastomere. Multinucleated blastomeres are frequently observed in ART. The incidence positively correlates with factors such as shorter stimulations, a higher number of oocytes retrieved, and a higher follicle-stimulating hormone dose for stimulation (14). Desch et al. (15) showed that embryo multinucleation at the two-cell stage affects birth potential negatively. Xiong et al. (16) compared the effects of denudation of oocytes after 6 h or 20 h post-insemination on the outcomes of in vitro fertilization (IVF) and reported similar results in terms of multinucleation (16). To our knowledge, the effect of early oocyte denudation on multinucleation in patients undergoing ICSI has not been assessed before.

Defining oocyte characteristics might allow the ART facilities to predict its developmental potential and increase the chances of better embryo selection for transfer (17). This study aimed to investigate the laboratory and clinical outcomes of ICSI when the oocytes were denuded at time points 0 or 2 h after oocyte retrieval.

Methods

Patients

This prospective randomized sibling-oocyte study included participants from Acibadem Altunizade Hospital, Unit of ART (Istanbul, Türkiye) between 01.11.2020 and 30.08.2021. The inclusion criteria were as follows: Females under 40 years of age, at least 5 oocytes retrieved, and no male factor. The local clinical ethics board of Maltepe University Faculty of Medicine, İstanbul approved this study with an approval number of 2020/900/82 on 21.10.2020. A written consent form was obtained from the patient. The study was conducted in accordance with the principles of the revised Declaration of Helsinki (18).

Ovarian stimulation, oocyte pick up, and denudation

Pituitary downregulation was performed either by a gonadotropin-releasing hormone agonist (GnRH-a) or a GnRH antagonist. GnRHa leuprolide acetate (Lucrin 0.5 mg/mL, Abbott, Madrid, Spain) was injected in the late luteal phase before the treatment cycle. The GnRH antagonist cetrorelix acetate (Cetrotide, Baxter Oncology GmbH, Halle, Germany) injections started on the fifth day of the treatment cycle. Both were applied daily until the trigger ovulation. Before initiating the IVF cycle, baseline ultrasounds were performed. Gonadotropin injections were started in the absence of more than 2 cm cysts on cycle days 2 or 3 and the dose used ranged from 150 international units (IU) to 300 IU.

A human chorionic gonadotropin (hCG) injection was administered when at least 3 follicles equal to or more than 17 mm in diameter were observed. Follicular aspiration was performed 35-36 h after ovulation was triggered.

The oocytes of the patients were divided into two groups by simple randomization using a closed envelope method: (i) control group: cumulus-cell removal was performed 2 h after oocyte pick up (OPU) (time point: 2 h, as in the routine applications), (ii): a study group: cumulus-cell removal was performed immediately (time point: 0). Opaque sealed envelopes with serial numbers contained random allocation cards with the names of the control or study group prepared by an independent laboratory technician. The envelopes were opened sequentially to randomly allocate the sibling oocytes to either group.

The 2-hour incubation of the oocytes of the control group until denudation was performed in equilibration solution (G-IVF, Vitrolife-Swemed/Sweden) in a culture incubator with 5-6% CO_2, 5% O_2, 37 °C and 90% humidity. The bonds of the corona cells were loosened using the hyaluronidase enzyme (HYASE, Vitrolife-Swemed/Sweden) and the cells were cleaned using a denudation pipette. After the decellularization of the oocyte, its maturation was evaluated under a light microscope and the morphological evaluation of the oocytes was performed. ICSI was performed following cumulus-cell removal.

Assessment of fertilization and multinucleation

Fertilization was determined 16-18 hours following ICSI. 2PN and two polar bodies were required for normal fertilization.

To determine the multinucleation status, blastomeres were assessed for the presence of more than one nucleus. Each blastomere was evaluated for multinucleation 42 h, 46 h and 50 h after fertilization and multinucleated embryos were noted.

Evaluation of the embryos on days 3 and 5, and assessment of pregnancy

Embryo quality on days 3 and 5 was determined based on the morphological criteria and on the grading system described by Gardner and Schoolcraft (19) in 1999, respectively. Maternal serum β-hCG ≥10 mIU/mL 10-12 days after embryo transfer was used to define pregnancy.

Statistics Analysis

Statistical analyses were performed using Number Cruncher Statistical System (NCSS) (NCSS LLC, Kaysville, Utah, USA). Comparisons of fertilization rates, multinucleation rates, embryo development on days 3 and 5 and the rates of reaching the blastocyst stage were performed using Pearson's chi-square test. Fisher's exact test was employed to compare the pregnancy rates of the patients. Statistical significance was set with a p-value <0.05.

Results

Clinical characteristics of the patients

This study was conducted with 54 patients. The mean age of the patients was 30.6±3.7 (19-36) and the mean number of retrieved oocytes was 14.7±6.4 (5-39). Two groups of oocytes were formed by simple randomization using a closed envelop method according to the denudation timings o and 2 h, corresponding to the study and control groups, respectively. Three hundred and seventy-six oocytes (47.5%) in the study group and 416 oocytes (52.5%) in the control group were observed as suitable for ICSI with a total number of 792.

The association of early oocyte denudation on fertilization, embryo quality and blastocyst formation

As provided in Table 1, the fertilization rate significantly increased when the cumulus cells were removed immediately after OPU without a pre-incubation than the 2 h incubation (55.6% vs. 48.3%, p=0.041). No significant differences were obtained between the study and control groups regarding the multinucleation rate (6.6% vs. 4.3%, p=0.150), the proportions of Grade 1 day 3 (48.7% vs. 43%, p=0.112), and day 5 embryos (27.7% vs. 23.8%, p=0.214) and the proportion of embryos reaching the blastocyst stage (34.0% vs. 28.1%, p=0.072).

The effect of early oocyte denudation on pregnancy

Embryos of 23 patients were frozen and 10 patients received embryos from both groups during transfer. Therefore, among the 54 patients, 21 patients were included to compare their pregnancy rates (Table 2). Pregnancy was detected in 78.6% of the cases in the study group and 71.4% cases in the control group. There was no statistically significant difference between the pregnancy rates in the groups (p=1.000).

Discussion

Cumulus cells are supporting cells that surround the oocyte and protect it from the microenvironment. Bi-directional communication is responsible for oocyte growth, maturation in the follicles, and early embryonic developmental competence (5). The complex regulation between the oocyte and the cumulus cells results in a coordinated function (20). Oocyte denudation should be performed before ICSI as the cumulus cells affect the microinjection process and a pre-ICSI evaluation of oocyte maturity is required (21). However, the debate over the optimal oocyte denudation timing has been going on. Several studies on mice oocytes reported that apoptotic changes and oocyte aging are induced following a long-term oocyte culture with cumulus cells (11-13). Aged oocytes have a reduced developmental competence (22). However, a systematic review by Wang et al. (21) found that the time from OPU to oocyte denudation did not change ICSI outcomes in most of the studies, though some suggested a short pre-incubation following oocyte retrieval.

Multinucleation is a frequently observed phenomenon in ART. In the study by Xiong et al. (16), a pre-incubation of 6 or 20 h was performed in classical IVF and no difference was reported in terms of multinucleation when the two groups were compared similarly to our findings. Multinucleation is one factor compromising the embryo quality and implantation

Table 1. Fertilization rates, multinucleation rates, proportion of grade 1 embryos on day 3 and on day 5, and blastocyst development in the study group and control group

	Study group (n=376)	Control group (n=416)	[a]p
Fertilization (2PN), n (%)	209 (55.6)	201 (48.3)	0.041*
Multinucleation, n (%)	25 (6.6)	18 (4.3)	0.150
3rd day G1, n (%)	183 (48.7)	179 (43.0)	0.112
5th day G1, n (%)	104 (27.7)	99 (23.8)	0.214
Blastocyst development, n (%)	128 (34.0)	117 (28.1)	0.072

[a]Pearson's chi-square test, 2PN: Two pronuclei, G1: Grade 1

Table 2. Pregnancy rates depending on the timing of oocyte denudation (pregnancy + serum β-human chorionic gonadotropin ≥10 mIU/mL)

	Study group (n=14)	Control group (n=7)	[b]p
Pregnancy (+), n (%)	11 (78.6)	5 (71.4)	1.000
Pregnancy (-), n (%)	3 (21.4)	2 (28.6)	

[b]Fisher's exact test

rate. Therefore, the evaluation of embryo multinucleation is considered an important parameter for embryo transfer (23).

In this prospective randomized sibling-oocyte study, we observed similar multinucleation rates between the control and study groups. Moreover, no statistically significant difference was found in embryo quality on days 3 and 5, blastocyst formation rates, and pregnancy rates when the cumulus cells were removed at time points 0 or 2 h. However, a higher fertilization rate was observed in the study group consisting of the oocytes denuded without a pre-incubation.

Patrat et al. (8) determined that a pre-incubation of 2 h may not increase the proportion of mature oocytes, but lead to the optimal combination of fertilization and implantation rates. However, the inclusion criteria of this study were different from those in our study: attempt rank 1 or 2 of ICSI; female age more than 36 years old; patients with male factor infertility. The cytoplasmic maturity process is not well known. The authors suggest that retrieved oocytes from stimulated cycles are cytoplasmically immature although they reach the Metaphase 2 stage, and still require cumulus cells for cytoplasmic maturation (8,24,25). However, several other studies reported no influence of the timing of oocyte denudation after OPU on the fertilization rate (6,26,27).

One of the most important processes in ART is the selection of the best embryo for transfer. The purpose of embryo grading is to select the embryo with the highest implantation potential (28). In our study, we hypothesized that oocytes that were denuded immediately after oocyte retrieval would have a higher development capacity based on the relation between cumulus cells and oocyte aging (11-13). However, no difference was observed between the study group and the control group in terms of embryo quality on days 3 and 5. However, Mizuno et al. (6) obtained a significantly higher percentage of good-quality blastocysts when the oocytes were denuded 2 h after OPU compared to those denuded at time point 0. Wang et al. (21) reviewed the studies focusing on the relationship between OPU-oocyte denudation time and embryo quality. The researchers reported that most studies did not demonstrate any effect on embryo quality, while some suggested an extended pre-incubation to increase the rate of good-quality embryos. Few studies also evaluated the blastocyst formation rates. While Hassan (29) reported a higher blastocyst formation rate after a pre-incubation with intact cumulus cells, Ishikawa et al. (30), Mizuno et al. (6) and Naji et al. (27) demonstrated no significant differences related to OPU-oocyte denudation time. The differences in methodologies, such as hCG-OPU time, inclusion criteria, or sample size might account for the different effects obtained in the studies.

We also included pregnancy rates in our study. There was no significant difference between the two groups in terms of pregnancy rates. Although the number of patients was limited, our result is in accordance with the studies with a higher sample size (6,10,27,31,32). Bárcena et al. (32) explained this result with high-quality oocytes, which may withstand aging *in vitro.* Others obtained higher pregnancy rates when the oocyte culturing with cumulus cells was performed and proposed that cumulus cells should be maintained during a pre-incubation period (8,26).

Study Limitations

To our knowledge, this is the first report in the literature regarding a relationship between the timing of oocyte denudation and multinucleation in patients undergoing ICSI. Therefore, evaluation of the association between early oocyte denudation and multinucleation in patients undergoing ICSI is the major strength of our study. However, the small number of participants to evaluate the pregnancy outcome is a major limitation. Additionally, the effect of longer periods of culture between OPU and denudation was not assessed. Further multi-center randomized controlled studies with a larger sample size are necessary to confirm the higher fertilization rates obtained with the oocytes denuded immediately after oocyte retrieval and better understand the effects of cumulus removal on embryo quality and developmental fate.

Conclusion

In conclusion, we demonstrated a higher fertilization rate when the oocyte denudation was performed immediately after the oocyte retrieval compared to an incubation period of 2 h. However, other parameters, including multinucleation rates, embryo quality on days 3 and 5, blastocyst formation rates, and pregnancy rates were similar between the groups. Our results indicate that the removal of cumulus cells immediately after OPU does not have any negative impact on ICSI outcome.

Ethics

Ethics Committee Approval: Ethical approval was obtained by the Maltepe University Clinical Research Ethics Committee (approval number: 2020/900/82, date: 21.10.2020).

Informed Consent: All patients were informed about the study and the consent document received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Design: Z.B.D., M.C., Y.D.Ç., B.S., Design: Z.B.D., M.C., Y.D.Ç., B.S., Data Collection, or Processing: Z.B.D., Ş.K., Analysis, or Interpretation: Z.B.D., Ş.K., M.C., Y.D.Ç., B.S., Literature Search: Z.B.D., Y.D.Ç., Writing: Z.B.D., M.C., Y.D.Ç., B.S.

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