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Prognostic value of oocyte denudation on fertilization and embryo quality in patients undergoing intracytoplasmic sperm injection

Berrin YANGIN[1,*], Bülent AYAS[1], Davut GÜVEN[2], Leman TOMAK[3] Aysın Pınar TÜRKMEN[1]

[1]Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[2]Department of Gynecology and Obstetrics, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[3]Department of Bioistatistic and Medical Information, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

A retrospective analysis of data obtained from 2034 oocytes collected from 157 patients with unexplained infertility was performed. Two groups were planned according to the diameters of flexible polycarbonate pipettes used in denudation. Group 1: Partial denudation of oocytes using a 140µm diameter pipette and Group 2: Complete denudation of oocytes using a 135µm diameter pipette. The data obtained were evaluated using statistical methods. It was aimed to investigate the possible effects of denudation on oocyte maturation, oocyte degeneration rate, fertilization rate, embryo quality, embryo development rate, abortion and live birth rates. After partial and complete denudation, there was no statistically significant difference between Group 1 and Group 2 in terms of oocyte maturation, normal fertilization rate, early embryonal development, embryo transfer numbers, blastocyst stage embryo numbers and morphology. In the third day embryos obtained from Group 2 oocytes, the rate of blastomeres incompatible with the day of development, the rate of degeneration among oocytes and the number of patients whose pregnancies ended due to pathologic reasons were found to be high (p=0.036, p=0.037, p=0.035). Live birth rate was higher in pregnancies developing from group 1 oocytes (p=0.039). It was concluded that granulosa cells left around the oocyte by pipettes with standardized diameter opening during denudation increased the success of live birth.

Keywords: granulosa cells, cumulus, intracytoplasmic sperm injection

1. Introduction

The global prevalence of infertility is between 8% and 12% and affects approximately 186 million people (1). With the advancement of modern medicine, in vitro fertilization (IVF) treatment is a solution option for infertility, but it brings a great financial burden to health expenditures and the national economy (2, 3). According to the results of ten years of assisted reproductive technology (ART) in Germany, 52% success rate can be achieved after three cycles (4). The primary outcome recognized as success is live birth after treatment (5). Achieving this outcome is mutifactorial. Obtaining good embryos with the laboratory techniques used is of great importance. To date, the main aim of all applications in ART is to increase implantation success and to increase the rate of bringing a healthy baby to the home. In line with this goal, scientists and physicians desire to provide the most appropriate treatment for patients, to develop the best embryo under in vitro conditions and to select the embryo with the highest implantation potential for transfer (6, 7). Conventional IVF used in embryo development has a higher rate of reaching the blastocyst stage compared to intracytoplasmic sperm injection (ICSI). The blastocyst has a higher implantation capacity than the embryo at the cleavage stage (8). The superiority of blastocyst development in conventional IVF draws attention to the positive effect of cumulus cells (CC) on oocyte maturation and embryo development (9-12). The fertilization rate achieved after ICSI is higher than in conventional IVF (13-15). In order to achieve this goal in blastocyst development, studies have been conducted in which CC is left on the oocyte to make ICSI possible. In these studies, CC was completely or partially removed from the oocyte. It has been concluded that CC has a positive effect on embryo quality, the resumption of meiosis and the completion of oocyte maturation. Communication from the granulosa cells that line the follicle to the CC surrounding the oocyte is provided by connections called gap junctions. These connections enable the entrance and exit of many molecules into the oocyte (16-18).

In previous studies, the effects of CC left around the oocyte after denudation on maturation and embryo development after ICSI were investigated. In these studies, hand-pulled glass pipettes were used due to the technological conditions of the period. Since the diameter opening was adjusted observationally, there are gaps in the methods of both oocyte damage and granulosa cell quantity studies. Since the diameter of the pipette is not standardized in hand-pulled pipettes, the number of cells remaining on the oocyte through the aperture is highly variable. Since counting CC around the oocyte that will form the embryo will increase the exposure of the oocyte to the external environment, the numbers given in previous studies are suggestive.

*Correspondence: aysin.turkmen@omu.edu.tr

In the present study, the possible effects of cumulus cells left around the oocyte as a result of denudation with standardized plastic pipettes of different diameters on oocyte maturation, fertilization rate, embryo quality, embryo development rate and live birth were retrospectively investigated. The aim of the study was to retrospectively determine and statistically evaluate the effects of cumulus cells left around the oocyte as a result of standard diameter opening on the above parameters and the specific oocyte damage rates according to pipette diameter. As a result of our study, the pipette diameter that does not damage oocytes, the amount of cells that allows ICSI to be performed and the relationship with embryo development could be determined.

2. Materials and Methods

Female patients aged 18-45 years with unexplained infertility who had undergone ICSI at Ondokuz Mayıs University Faculty of Medicine IVF Center were included. Patients who had undergone pelvic and ovarian surgery, had a single ovary, had a family history of early menopause, received chemotherapy treatment, had systemic diseases such as diabetes mellitus, hypertension, thyroid dysfunction, liver failure, hepatitis and infectious diseases caused by human immunodeficiency virus (HIV), autoimmune diseases and smokers were excluded. Women who were under 18 years of age or over 45 years of age, had low ovarian reserve, had an antral follicle count below five (AFC<5), had partners with oligoasthenoteratozoospermia (OAT), responded poorly to gonadotropin stimulation, and had sperm retrieved by TESE from partners with azoospermia were excluded from the study. Patients whose partner's sperm concentration was above 15 million/ml were included in the study. Inclusion and exclusion criteria and other factors that may affect fertilization, embryo development and implantation were excluded from the study. Data were collected retrospectively from embryology laboratory. Forms of the patients who received infertility treatment within the scope of the criteria were evaluated.

2.1. Clinical procedure

Cumulus oocyte complexes (COC) are collected from the aspirated follicular fluid using glass pasteur pipettes with a diameter opening of 200µm. As a standard procedure, each oocyte is removed from the cumulus using hyaluronidase enzyme with pipettes of different diameter apertures (200µm, 140µm, 135µm). Mechanical removal of CC is performed randomly using flexible polycarbonate pipettes with narrower diameters of 140µm and 135µm. In the laboratory routine (based on the experience of denudation of oocytes to be destroyed from TESE-negative partners), 15-20 granulosa cells remain around the oocyte as a result of partial denudation performed 5 times with a 140µm diameter pipette. The 15-20 CCs remaining around the partially denuded oocytes are at the limit that will not interfere with the ICSI procedure and allow maturation evaluation. Complete denudation with a 135µm diameter pipette removes all granulosa cells around the oocyte. In this study, based on the clinical procedure, patient files were retrospectively reviewed over a period of one year. The data were divided into two groups according to the pipette diameters used in oocyte denudation.

Group 1: Group with partial denudation of oocytes with a standard 140µm diameter pipette (SKDG) (n_1= 81)

Group 2: Group with complete denudation of oocytes with standard 135µm diameter pipette (STDG) (n2= 76)

A total of 157 female patients diagnosed with infertility who met the inclusion and exclusion criteria within a one-year period between 01.07.2019 and 01.07.2020 and the data obtained from all 2034 oocytes collected from these patients (Group 1 oocytes= 1045, Group 2 oocytes= 989) were included in the study.

In this study, the effect of CC remaining after partial or complete oocyte denudation on the following parameters was investigated: Oocyte morphology, oocyte maturation, number of immature and mature oocytes, oocyte degeneration rate, comparison of total grades, number of biochemical pregnancies and clinical pregnancies, number of live births, total number of transfers, number of patients with no embryo transfer, comparison of embryos according to day 2 and day 3 embryo scores, blastocyst abortion, ectopic pregnancy, absence of fetal cardiac activity (FCA-) number of fertilized oocytes, number of normally fertilized oocytes (2PN), fetilization rates, number of developing embryos, (number of day 2, day 3, day 4, day 5, day 6 embryos), total number of embryos, number of embryos reaching cleavage and blastocyst stage, number of fresh transfers, comparison of embryos at frozen transfer stage in terms of embryo quality, classification of embryos according to their grades, number of patients whose pregnancies were terminated by dilatation and curettage (D/C) for pathological reasons after transferred embryos.

2.2. Data Collection and Statistical Analysis

In this study, female patients aged 18-45 years with unexplained infertility who presented to XX University IVF Center between 01.07.2019 and 01.07.2020 were selected and data were collected retrospectively. Two different groups were formed according to the diameter opening of the pipettes. Statistical analyses were performed with SPSS 21.0 program. Data were presented as mean ± standard deviation (SD), median (min-max) and frequency (%). Shapiro-Wilk test was used to check the assumption of normal distribution of the data. Mann-Whitney U test was used to compare the data of two groups that did not have normal distribution. Frequencies were compared using Pearson Chi-square, Chi-square with continuity correction and Fisher Exact test. $P<0.05$ was accepted for statistical significance.

3. Results

The groups were compared for equivalence at baseline according to age, FSH, LH, E2, progesterone, prolactin, follicle number, follicle volume, total follicle volume, number of oocytes collected, and oocyte morphology parameters.

Demographic data and oocyte morphology of the groups were similar (p>0.05; Mann-Whitney U, Chi-square with Continuity Correction). The groups were compared in terms of oocyte number, oocyte maturation and oocyte degeneration.

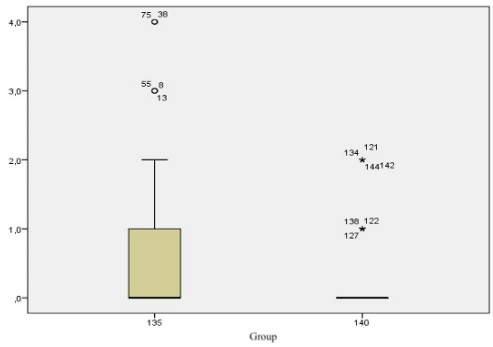

Fig. 1. Shows degenerated oocytes in Group 1 and Group 2 (p=.018)

The number of degenerated oocytes (Fig. 1) and the ratio of degenerated oocytes to total oocytes were significantly higher in Group 2 compared to Group 1 (p=0.018, p=0.036; Mann-Whitney U). There were no statistically significant differences in the number of M1 oocytes, the ratio of M1 to total oocytes, the number of M2 oocytes, the ratio of M2 to total oocytes, the number of GV oocytes, the ratio of GV to total oocytes, the number of empty zona and the ratio of empty zona to total oocytes. No statistically significant difference was found when the fertilization rates of the groups and embryo development according to days for 6 days were compared (p>0.05; Mann-Whitney U).

Table 1. Comparison of embryos with incompatible blastomere number between day 3 and day of development between groups

Day 3 embryo grade	Group 1		Group 2		
	AM± S.D.	Median(Min-Max)	AM± S.D.	Median(Min-Max)	
Blastomer D (Number of blastomeres incompatible with day)	1.32±1.532	1.00(0-6)	1.88±2.290	1.00(0-12)	**0.131**
Ratio of blastomere D to total number of day 3 embryos	0.224±0.247	0.181(0.000-1.000)	0.321±0.293	0.250(0.000-1.000)	**0.037**

* Mann-Whitney U, n: Number of patients, AM: Arithmetic mean, SD: Standard deviation

In group 2 (STDG), the ratio of the number of day 3 embryos with inconsistent blastomere number and size (Table 1) to the total number of day 3 embryos was statistically significantly different compared to group 1 (STDG) (p=0.037; Mann-Whitney U).

When Group 1 (SKDG) and Group 2 (STDG) were compared, there was a statistically significant difference in the number of embryos that reached early embryonal development and blastocyst stage (p=0.301 p=0.695; Mann-Whitney U), the number and ratio of embryos that developed good and bad phases (p=0.431 p=0.810 p=0.496 p=0.810; Mann-Whitney U), and the number of embryos transferred (p=0.961 p=0.160 p=0.225 p=0.804 p=1.000 p=0.383 p=0.928 p=0.231; Mann-Whitney U). There was no statistically significant difference between the groups in terms of biochemical pregnancy and clinical pregnancy (p=0.613-0.613; Pearson Chi-square).

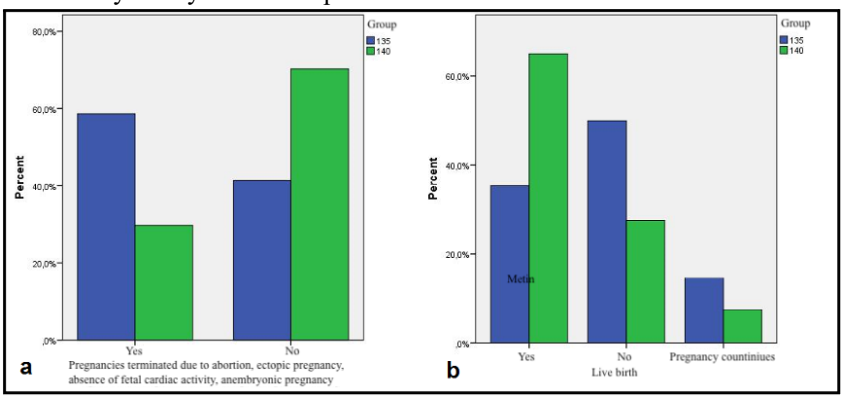

Fig. 2. Comparison of pregnancies terminated due to abortion, ectopic pregnancy, absence of fetal cardiac activity, anembryonic pregnancy according to groups (a) and comparison of live births according to groups (b)

The number of patients whose pregnancies were terminated due to pathologic reasons (abortion, ectopic pregnancy, FCA(-) D/C, anembryonic pregnancy D/C) was significantly lower in Group 1 (SKDG) as shown in Fig. 2a (p=.039; Pearson Chi-square, p=0.035; Continuity Adjusted Chi-square). Live birth rate was significantly higher in Group 1 than in Group 2 (p=0.039; Pearson Chi-square) (Fig. 2b).

4. Discussion

In routine practice, denudation is performed randomly with different pipette diameters. The data of 2034 oocytes denuded with pipettes with standardized diameter aperture in today's technology were collected in two groups. The aim was to retrospectively determine and statistically evaluate the possible effects of similar amounts of residual cumulus cells on oocyte and embryo parameters and possible damage rates specific to pipette diameter after oocyte manipulation. Group 2 oocytes with narrower aperture (135µm) were fully denuded.

Degeneration was significantly higher in these oocytes. The degeneration results found in the literature are from after ICSI. These degeneration data include mechanical damage related to sperm injection (19, 20). Our data on degeneration is specific only after denudation. This result can be interpreted as the removal of cumulus cells from around the oocyte by pipettes with 135µm diameter opening will cause degeneration (p=.018). The proportion of day 3 embryos with inconsistent blastomere numbers was significantly lower in group I. Although the cells supported blastomere development, their effects were not reflected in embryo classification. It is known that co-culture of cumulus complex with oocytes increases embryo quality and fertilization rates in many species (21, 22). Our assumption was that more cumulus cells remained on the oocyte in the group with a larger diameter (140µm) pipette compared to the group with complete denudation. We presumed that a similar number of cells would remain on the oocytes through the standard diameter opening. The hypothesized effect of cumulus cells remaining around group I oocytes was not statistically significant compared to group II in oocyte maturation, early embryonal development, blastocyst and implantation stages contrary to the literature (7, 23-25). Among the developing day 3 embryos, the ratio of embryos with incompatible blastomeres to the total number of day 3 embryos was significantly lower in group I. Consistent with the literature, in the group in which cumulus cells remained around the oocyte, the effect of the cells was not reflected in the embryo classifications, even though the effect of the cells was to support blastomere development. This may be due to the small number of remaining cumulus cells and the inability to observe their effect on short-term morphologic development. Live birth is the ultimate goal in ART (5). In this study, the live birth rate was higher in Group I (p=0.039; Pearson Chi-square). The support of cumulus cells to the embryo during development and its relationship with live birth is promising.

Although retrospective analysis of data over a one-year period allows us to see the relationship between denudation effects and live birth rate, analysis of data over a longer period of time is recommended in retrospective studies. In a prospective study plan, we consider that morphologic effects on oocyte maturation and embryo development can be explained by increasing the number of cells after denudation, to the extent that ICSI is feasible. With the help of flexi pipettes, the minimum number of cells for maximum effect can be determined. We believe that planning a prospective study among sibling oocytes will add value to future research.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.P.T., Design: A.P.T., B.A., D.G., Data Collection or Processing: B.Y.D., Analysis or Interpretation: L.T., Literature Search: B.Y.D., Writing: B.Y.D., A.P.T.

Ethical Statement

Ethical committee permission was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee (2020/588 decision dated 08.10.2020). Data were obtained from Ondokuz Mayıs IVF Center with the approval of Ondokuz Mayıs University Health Application and Research Center Directorate dated 14.10.2020 and numbered 15374210-302.08.01-E.17842.

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