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EMBRYO BIOLOGY

Ultrastructure of cytoplasmic fragments in human cleavage stage embryos

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Abstract
Purpose The goal of this study was to evaluate the ultrastructure of cytoplasmic fragments along with the effect of cytoplasmic fragment and perivitelline space coarse granulation removal (cosmetic microsurgery) from embryos before embryo transfer on ART outcomes.
Methods One hundred and fifty intracytoplasmic sperm injection cycles with male factor infertility were included in this prospective study. Patients were divided into three groups of case ($n = 50$), sham ($n = 50$), and control ($n = 50$). Embryos with 10–50 % fragmentation were included in this study. Cosmetic microsurgery and zona assisted hatching were only performed in case and sham groups respectively. Extracted fragments were evaluated ultrastructurally by transmission electron microscopy (TEM). Rates of clinical pregnancy, live birth, miscarriage, multiple pregnancies, and congenital anomaly in the three groups were also compared.
Results Micrographs from TEM showed that mitochondria were the most abundant structures found in the fragments along with mitochondria-vesicle complexes, Golgi apparatus, primary lysosomes, and vacuoles. There were no significant differences in demographic characteristics, laboratory and clinical data, or embryo morphological features between the groups. The rate of clinical pregnancy in control, sham, and case groups had no significant differences (24, 18, and 18 %, respectively). The rates of live birth, miscarriage, multiple pregnancy, and congenital anomaly were also similar between the different groups.
Conclusions Our data demonstrated that cosmetic microsurgery on preimplantation embryos had no beneficial effect on ART outcomes in unselected groups of patients. As mitochondria are the most abundant organelles found in cytoplasmic fragments, fragment removal should be performed with more caution in embryos with moderate fragmentation.

Keywords Cosmetic microsurgery · Embryo fragmentation · Transmission electron microscopy · Mitochondria

Capsule Mitochondria are numerous structures inside human embryo cytoplasmic fragments, and their removal on moderately fragmented embryos does not improve ART outcomes.

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Introduction

The main goal in assisted reproductive technology (ART) is achieving a healthy singleton live birth. There are several factors that can affect ART outcome, namely the quality of embryos developed in vitro [1–3]. Embryo morphology is a noninvasive selection method that can be easily adopted in an in vitro fertilization (IVF) laboratory for choosing embryos for embryo transfer (ET) [4–6]. Despite certain limitations, morphological evaluation of embryos is routinely used for embryo scoring [7, 8]. In fact, morphology is considered the most important parameter that is assessed in different stages of embryo development.

Embryo fragmentation in in vitro produced embryos is the cornerstone of the embryo grading system. The presence of fragmentation impairs embryo morphology and is associated with a lower embryo viability [9, 10]. Fragmentation usually

occurs during the first cell division in more than 40 % of in vitro generated embryos [11, 12]. The degree of fragmentation may vary from zero to over 50 % of cytoplasmic volume, and it was shown that mean cytoplasmic reduction increases when the degree of fragmentation increases [13]. While the impact of a low degree of fragmentation (<10 %) on ART outcomes seems negligible, severe fragmentation (>50 %) impairs embryo viability [7]. However, the impact of moderate fragmentation on ART outcomes has been a matter of debate for many years. There are several studies regarding the positive effects of fragment removal on embryo viability and pregnancy rates [14–17]. The presence of coarse granulation and debris in the perivitelline space (PVS) is the other embryo dysmorphism. Coarse granulation may originate from ooplasm and coronal cell prolongations [18, 19]. Moreover, it is suggested that coarse granulation may originate from cumulus cells entered into the PVS due to zona pellucida (ZP) abnormality [20]. Extra-cytoplasmic particles such as entrapped cumulus cells could impair the hatching process as well. In addition, these particles may be associated with reduced ART outcomes because of a synchronous inherent oocyte defect or a ZP abnormality [18, 19, 21].

Since embryos are selected for ET based on their morphological features, a question remains as to whether ART outcomes improve if a poor-looking embryo is refurbished into a good looking one. The cosmetic microsurgery is defined as removal of fragments and coarse granulation from the embryo before ET. Recently, ongoing pregnancy following cosmetic micromanipulation of cleavage-stage embryos in patients with a history of implantation failure has been reported [22]. Transmission electron microscopy (TEM), as a state-of-the-art technology, provides more details about the origin of fragments and their contents. There is lack of data regarding TEM of fragments removed from human embryos produced in vitro. Using TEM, the fine structure of fragments and logic of performing fragment removal in moderately fragmented embryos would be clarified as well. To the best of our knowledge, no comprehensive studies have been carried out up to now on the ultrastructure of fragments removed from human embryos in a clinical setting. Our main goal was to evaluate the ultrastructure of fragments formed during the first cleavages removed from the transferred embryos in a clinical setting. Our group was also, for the first time, introduced a new method for TEM evaluation of fragment using an empty ZP. The second goal was to evaluate the impact of embryo cosmetic microsurgery on the ART outcomes in an unselected group of infertile patients.

Materials and methods

Patient selection

The patients entered into this prospective randomized study were referred to Yazd Institute for Reproductive Sciences. This study was conducted from June 2013 to April 2015. Cleavage-stage embryos with grades B and C (10–50 % fragmentation) were included in this study. The exclusion criteria included patients with less than two embryos or with embryos derived from non-ejaculated spermatozoa, multinucleated embryos, cycles using donor gametes, embryos derived from conventional insemination, advanced maternal age (39 years or older), difficult/complicated ET, or retained embryos at ET. The patients were randomly divided into three groups by computer-generated random numbers: the control group ($n = 50$) included 103 embryos with grades B and C, the sham group ($n = 50$) included 101 embryos with grades B and C undergoing laser-assisted hatching (LAH) only, and the case group ($n = 50$) included 94 embryos undergoing cosmetic microsurgery. This study was approved by the ethics committee of the first author's institute. Written informed consents were obtained from all patients.

Embryo morphology

Embryo cell number, pattern, and percent of fragmentation, blastomere multinucleation and evenness were recorded. The fragmentation pattern was divided into two categories: (a) fragments were around one blastomere and localized and (b) fragments were scattered around two or more blastomeres. The fragmentation was reported as a percentage, and was defined as volume of the embryo cavity occupied by fragments. The embryos were graded into four categories according to their fragmentation index: grade A, equal size blastomeres and less than 10 % fragmentation; grade B, slightly unequal blastomeres with up to 20 % fragmentation; grade C, unequal sized blastomeres, up to 50 % fragmentation and large granules; and grade D, unequal blastomeres with significant fragmentation (>50 %) and large granules. The shape of embryos was categorized as circular or oval.

Cosmetic microsurgery

Micromanipulation of cleavage-stage embryos was performed in Ca-Mg-free culture medium (Vitrolife, Sweden) on embryos selected for ET. A micropipette with the inner diameter of 10–12 μm and a holding micropipette with the inner diameter of 25–30 μm were used in order to perform cosmetic microsurgery. The embryo was held by the holding micropipette with the best orientation of fragments and coarse granulation in PVS for fragment and coarse granulation removal. A 10–12-μm opening was made in ZP using a 1480-nm wavelength infrared diode laser (OCTAX Laser Shot®; MTG, Germany). The micropipette was gently entered and brought in close proximity to the fragments, debris, and granules around the blastomeres. Fragments and granules were gently removed from the embryo using a ×25 objective. LAH was performed only in the sham group. In all embryos, the site of hatching

was selected near the cytoplasmic fragments. The microsurgery was monitored by video recording, and the image of the embryo before and after microsurgery was taken for further analysis. After micromanipulation, the embryo was carefully washed in G-1™ v5 (Vitrolife, Sweden) culture media and incubated at 37 °C in 6 % CO_2 until ET.

Transmission electron microscopy

Cytoplasmic fragments were prepared for TEM by the novel method of using empty ZP [23]. Empty ZPs were made from unfertilized oocytes or discarded embryos. The cytoplasm was removed and fragments were transferred into empty ZPs. The fragments were then fixed by glutaraldehyde 2.5 % in a cacodylate buffer solution. After fixation, samples were rinsed in cacodylate, post-fixed with 1 % osmium tetroxide in cacodylate, and rinsed several times. The empty ZPs were then checked for the presence of fragments at the end of each step. The specimens were embedded in agar 1 %, dehydrated using an ascending series of ethanol, and embedded in epoxy resin. Ultra-sectioning was performed by Ultra-cut UCT microscope (Leica Microsystems Inc., Germany). The semi-thin sections (500 nm) were stained with toluidine blue to check the samples. Ultra-thin sections (50–70 nm) were mounted on copper grids and stained with uranyl acetate and lead citrate. The grids were then checked and photographed with TEM (Zeiss, Germany).

Outcome measures

Chemical pregnancy was confirmed by a positive serum beta hCG >30 mIU/ml 14 days after ET. Clinical pregnancy was confirmed by detection of a gestational sac with a fetal heart beat 7 weeks after ET. Miscarriage rate was defined as clinical pregnancy losses before the 20th week of gestation divided by the chemical pregnancies.

Statistical analysis

The data are presented as mean ± SD, median, and percentage. The Shapiro–Wilk test was applied to evaluate normal distribution of the data. Independent sample *t* test, Mann–Whitney *U* test, one-way ANOVA (followed by Tukey), Kruskal–Wallis, and chi-square tests were used appropriately. All hypotheses were two-sided and the significant level was defined as $P < 0.05$. The sample size was calculated by comparison between two proportions. A sample size of 89 embryos (45 cycles) in each group reveals 80 % power to detect to increase of 20 % in clinical pregnancy rate with alpha level of 0.05.

Results

Ultrastructure of fragments

The TEM micrographs showed that the fragments have a distinct, continuous membrane provided with rare microvilli. There were various cytoplasmic organelles inside the fragments. The mitochondria were the most abundant structures observed. These were mainly intact, apparently healthy mitochondria, with a moderately electron-dense matrix. Residual mitochondria-vesicle complexes represent a peculiar association between mitochondria and round vesicles that are filled with a flocculent and slightly electron-dense material. They are commonly found in the human mature oocyte and pronuclear eggs. In addition, membranes belonging to Golgi apparatus were also seen. Remnants of regressing mitochondria, small clear vacuoles, and primary lysosomes were occasionally found (Figs. 1 and 2).

Demographic and clinical data between different groups

The demographic data of patients are shown in Table 1. There were no significant differences for male age, female age, and duration of infertility between the three groups. Also, most of the patients had primary infertility, and the type of infertility was similar in different groups as well. Table 2 shows the comparison of clinical data between control, sham, and case groups. The majority of patients underwent antagonist protocol for ovarian stimulation. The levels of estradiol, FSH, and LH in female serum were the same as well. Previous implantation failure rate was not different among the three groups.

Laboratory data

Semen quality was similar between the different groups. In addition, the number of aspirated COCs, rates of mature

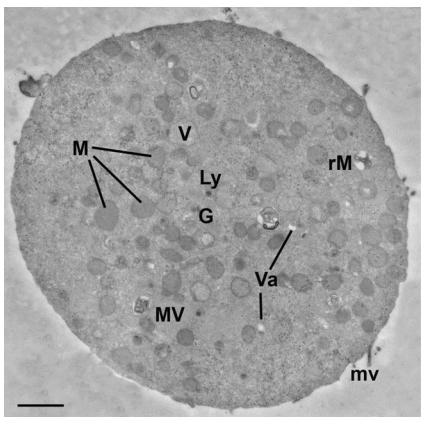

Fig. 1 Morphology and organelle microtopography of an embryo fragment inside the empty ZP by TEM. *M* mitochondria, *rM* remnant of regressing mitochondrion, *V* vesicle, *MV* mitochondria-vesicle complex, *Ly* primary lysosome, *G* Golgi apparatus, *mv* microvilli, *Va* vacuoles; *scale bar* = 2 μm

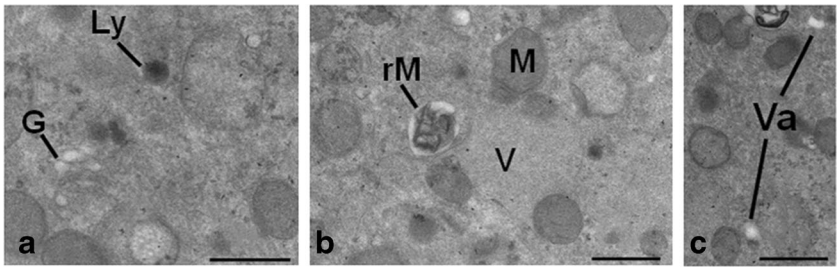

Fig. 2 Morphology and organelle microtopography of an embryo fragment inside the empty ZP by TEM. An enlargement of selected areas of Fig. 1 is provided. *M* mitochondria, *rM* remnant of regressing mitochondrion, *V* vesicle, *Ly* primary lysosome, *G* Golgi apparatus, *Va* vacuoles; *scale bar* = 1 μm

oocytes at metaphase II, fertilization, and embryo development were the same (Table 3).

Embryo morphology, pregnancy, and live birth rates

The most common dysmorphism of the embryos in the control group was fragmentation. The number of embryos with even and uneven blastomeres in the different groups showed insignificant differences ($P = 0.1$). The rate of fragmentation and cell number were similar in the different groups. The groups showed similar results for pattern of fragmentation. The rates of pregnancy, miscarriage, live birth, multiple pregnancy, and congenital anomaly had no significant differences in the three groups (Table 4).

Discussion

From an ultrastructural point of view, our data showed that cytoplasmic fragments have a distinct and intact membrane with some remnants of the microvilli of the oolemma. Some researchers believe that large fragments (up to 45 μm in diameter in day 2 and 40 μm in day 3) are associated with the loss of a large volume of the blastomere's cytoplasm that contains essential organelles such as mitochondria, mRNA, and proteins, resulting in embryo development arrest [24]. Therefore, removing fragments in the early stages of development can deprive the embryo of such essential organelles. Our data showed that the mitochondria were the most frequent organelle inside the fragments. The finding of abundant, well-preserved mitochondria in the fragments suggests that these have enzymatic activities. The number and shape of mitochondria also depends on the type and degree of cell differentiation, the mitochondria being more numerous and elongated in the embryo blastomeres as cleavage progresses [25]. The mitochondria is the power house of the egg, and embryo development relies on the energy (ATP) produced by these organelles. Losing mitochondria by fragmentation, on the one hand, and exposure of embryos for extended time out of the incubator to remove these fragments, on the other hand, may threaten the embryo viability and its implantation potential. Other morphodynamic changes which occur in the early human embryo during preimplantation development are the reduction in number and dimensions, up to disappearance, of mitochondria-vesicle complexes and de-novo formation of Golgi membranes [25, 26]. Mitochondria-vesicle complexes are firstly detected in the immature, germinal vesicle oocyte. They appear as small and numerous in the mature oocyte, become enlarged in aged/cultured oocytes, and just after fertilization, in the pronuclear eggs [27]. They disappear in the early embryo, thus showing a high recycling/remodeling activity. It has been suggested that these complexes play a role in the transport system from the nucleus to the developing mitochondria [28]. Chi et al. evaluated the ultrastructure of fragmented embryos using TEM. They found that the number of mitochondria is lower in anucleated fragments compared to normal blastomeres, suggesting depletion of fragments from ATP, resulting in cell lysis [9]. Thus, in our samples (Fig. 2), the occasional finding of small vacuoles and primary lysosomes in the fragments, along with the presence of regressing mitochondria with dilated cristae and altered membranes, may be considered ultrastructural markers of early degeneration.

There are several reports on reduced pregnancy outcomes from transferring embryos with poor morphology [29, 30]. Some embryo dysmorphisms, however, like cytoplasmic fragments and coarse granulation in PVS, could be removed from

Table 1 The demographic data of patients in different groups

Variables	Groups			*P* value
	Control	Sham	Case	
Male age[a]	33.64 ± 5.3 33 (23–47)	35.56 ± 6.7 35 (25–64)	35 ± 6.7 34 (23–62)	0.4
Female age[a]	29.1 ± 3.7	31.4 ± 4.9	29.6 ± 6	0.06
Duration of infertility[a]	5.08 ± 3.3 5 (1–17)	5.88 ± 3.2 5.5 (1–15)	5.82 ± 3.3 6 (1–16)	0.3
Type of infertility (%)				
Primary	85	83	88	0.4
Secondary	15	17	12	

[a] Data are presented as mean ± SD, median (min–max)

Table 2 The comparison of clinical data between three groups

Variables	Groups			*P* value
	Control	Sham	Case	
COH protocol (%)				
Agonist	6	10.2	12.2	0.5
Antagonist	94	89.8	87.8	
Estradiol (pg/ml)[a]	1581.74 ± 777.2	1659.49 ± 773.7	1916.53 ± 1154.7	0.4
	1300 (544–4600)	1600 (500–3620)	1800 (395–5334)	
FSH (mIU/ml)[a]	6.18 ± 3.4	6.75 ± 2.7	6.4 ± 3.1	0.2
	5.1 (2.4–18.9)	6.8 (9–13.9)	5.5 (1.6–15.6)	
LH (mIU/ml)[a]	5.06 ± 2.8	5.11 ± 2.6	5.18 ± 3.7	0.8
	4.4 (1.5–14.5)	4.9 (0.2–13.9)	4.3 (0.1–20)	
Ampoules[a]	26.64 ± 6.6	29.26 ± 7.7	27.71 ± 8.5	0.06
	26 (13–46)	29 (6–56)	25 (19–64)	
Previous implantation failure (%)				
Yes	20	22	20	0.9
No	80	78	80	

COH controlled ovarian hyperstimulation

[a] Data are presented as mean ± SD, median (min–max)

the cleavage-stage embryo prior to ET. It is unclear if making a poor-looking embryo into a good-looking one would improve the pregnancy outcome. In this study, we defined a cosmetic microsurgery for the cleavage stage embryo as removal of fragments and coarse granulation. Our data showed that cosmetic microsurgery in moderately fragmented embryos does not improve the ART outcome in unselected patients undergoing ICSI.

In theory, fragment removal may improve the viability and development potential of poor-quality embryos by improving cell-to-cell interactions. It could also prevent the release of probable harmful compounds by fragments, which may lead

Table 3 Comparison of laboratory data between different groups

Variables	Groups			*P* value
	Control	Sham	Case	
Sperm abnormality (%)				
Single	34	31.2	34.7	0.7
Oligo/astheno/terato-zoospermia				
Double	22	33.3	22.4	
Oligoastheno/asthenoterato/oligoterato-zoospermia				
Triple	44	35.4	42.9	
Oligoasthenoteratozoospermia				
Aspirated COCs[a]	9.28 ± 4.3	8.5 ± 3.4	10.56 ± 5.2	0.08
	9 (2–23)	7.5 (2–19)	10 (2–23)	
MII oocytes[a]	7.9 ± 3.8	7.02 ± 3.3	8.98 ± 4.9	0.07
	8 (2–21)	6 (2–17)	9 (2–22)	
Fertilized oocytes[a]	4.98 ± 3	4.46 ± 2.3	5.18 ± 3.5	0.8
	4 (1–17)	4 (1–12)	4 (2–15)	
Embryo formation[a]	4.26 ± 2.64	3.98 ± 2	4.76 ± 3.5	0.9
	4 (1–13)	4 (1–10)	4 (2–15)	
Transferred embryos[a]	2.06 ± 0.4	1.98 ± 0.4	1.9 ± 0.4	0.2
	2 (1–3)	2 (1–3)	2 (1–3)	

COC cumulus oocyte complexes, *MII* metaphase II

[a] Data are presented as mean ± SD, median (min-max)

Table 4 Comparison of embryo morphology and pregnancy rate between different groups

Variables	Groups			*P* value
	Control	Sham	Case	
Type of dysmorphism (%)				
F	46.5	32.5	45	0.06
F + CG	10	10	10	
Time of microsurgery (min)	–	–	6.6 ± 4.9	–
Evenness (%)				
Yes	46.6	44.6	57.4	0.1
No	53.4	55.4	42.6	
Grade (%)				
B	46.6	46.8	43	0.1
C	53.4	53.2	57	
Fragmentation (%)[a]	18.1 ± 10.2 15 (10–50)	19.5 ± 10.2 20 (10–50)	16.5 ± 7.9 15 (10–50)	0.1
Cell number[a]	5.46 ± 1.9 5 (2–10)	5.43 ± 2.2 4 (2–12)	6.04 ± 2.1 6 (2–12)	0.052
Pattern (%)				
Localized	58.9	54.6	56.8	0.8
Distributed	41.1	45.4	43.2	
Shape	Circular	Circular	Circular	–
Chemical pregnancy (%)	(20/50) 40	(14/50) 28	(14/50) 28	0.3
Clinical pregnancy (%)	(12/50) 24	(9/50) 18	(9/50) 18	0.6
Miscarriage rate (%)	(3/20) 15	(0/14) 0	(2/14) 14.3	0.3
Live birth rate (%)	(10/50) 20	(9/50) 18	(6/50) 12	0.5
Multiple pregnancy (%)	(3/50) 6	(4/50) 8	(1/50) 2	0.3
Congenital anomaly (%)	(0/50) 0	(1/50) 2	(1/50) 2	0.6

F fragmentation, *CG* coarse granulation

[a] Data are presented as mean ± SD, median (min-max)

to degeneration or lysis of healthy neighboring blastomeres [14]. It is believed that fragment removal in embryos with 0–15 % or >35 % fragmentation may be associated with no improvement in clinical outcome [31]. Alikani et al. divided the embryos based on their fragmentation into five grades: (I) less than 5 %, (II) 6–15 %, (III) 16–25 %, (IV) 26–35 %, and (V) more than 35 %. They retrospectively followed homogeneous transfers of fragmented embryos and showed that implantation was significantly reduced after transfer of embryos with >15 % fragmentation, despite fragment removal before ET [14]. However, the present study was a prospective one, in which only the embryos with 10–50 % fragmentation were included. The fate of low and high level of fragmentation could be predicted. While the absence of fragmentation is associated with a higher pregnancy outcome, severe fragmentation (>50 %) is accompanied with low pregnancy rates and high risk of congenital malformation [7, 32]. Performing cosmetic microsurgery for these two groups of embryos does not seem to be beneficial, because embryos with less than 10 % fragmentation have a similar pregnancy rate compared to non-fragmented embryos. In addition, in severely fragmented embryos, cosmetic microsurgery can only make the embryo grading change, but the embryo development potential may not improve.

It has been shown that there is a negative relationship between blastocyst formation rate and the level of cytoplasmic fragmentation [33]. In a prospective study, it was reported that removal of fragments at cleavage stage led to improving the quality of blastocysts compared to the control group [15]. In our pilot study, we found that blastocysts could be obtained from discarded embryos with over 50 % fragmentation after cosmetic microsurgery (data not shown). In a retrospective study, the rates of implantation, clinical pregnancy, spontaneous abortion, live birth, and fetal defects following transfer of poor-quality embryos after fragment removal reported to be similar to top-grade embryos [16]. The authors only performed fragment removal for embryos with more than 10 % fragmentation, but did not define the upper limit for percentage of fragmentation. The impact of fragment removal on cleavage-stage embryos with moderate fragmentation derived from unselected patients was assessed in another study [34]. The authors showed that in patients younger than 39 years having embryos with 10–20 % fragmentation, defragmentation did not improve the rates of implantation or live birth.

They also reported that the rates of multiple pregnancy and neonatal anomaly were the same between control and study groups.

The effect of fragment removal on IVF outcomes is controversial. Retrospective studies are in favor of the beneficial effect of defragmentation while prospective studies, like ours, do not clearly support its beneficial effects on subsequent embryo development and pregnancy rates. Our results showed that cosmetic microsurgery does not improve pregnancy rate in embryos with fragmentation of 10–50 %, derived from young patients with male factor infertility.

It has been shown that increased maternal age is associated with increased embryo fragmentation [16]. It appears that fragment removal is favorable for advanced age and does not improve the pregnancy outcomes in young patients with good prognosis [14, 34]. It could be suggested to perform cosmetic microsurgery on selected cases, and embryos derived from patients with implantation failure seem to be good candidates for cosmetic micromanipulation [22].

If we consider a fragmented embryo "abnormal" or at least "subnormal" or "non-ideal," then we admit that something should be wrong in the embryo that causes fragmentation. The cause is either related to the embryo itself or to the patient [35, 36]. Our data showed that fragment removal by microsurgery was unable to eliminate the underlying cause of fragmentation. It would be worthwhile to get more information about the formation and resorption of fragments using a new and valuable technique called time-lapse. Our results showed that the fragments contain numerous mitochondria while the time-lapse photography studies have shown that the fragments will be recycled into the mother blastomeres and disappear in the next cleavages [37, 38]. Removing fragments deprives the blastomeres from mitochondria, resulting in decreased ATP production which is necessary for further embryo development. This technique would also help to compare the embryo development between fragmented and non-fragmented embryos. Our next project is evaluation of morphokinetic parameters of early embryo development following fragment removal via time-lapse monitoring.

Debris in PVS is one of the extra-cytoplasmic dysmorphisms of mammalian oocytes with incidence of 10.6 % in human mature oocytes [39, 40]. PVS granularity may have different causes, including exocytosis of cortical granules, gonadotropin overdose, advanced maternal age, and ZP abnormality [20, 40–42]. One study showed that cleavage rate, embryo quality, rates of implantation, and pregnancy were the same between two groups of oocytes with more than 50 % and less than 50 % granules [42]. In contrast, Farhi et al. evaluated the role of coarse granulation in oocytes on IVF and ICSI clinical outcomes. They showed that there were no significant differences for fertilization and cleavage rates and embryo quality between the two groups, but implantation and pregnancy rates were significantly higher in the control group, suggesting this factor as an "egg factor infertility" [21]. When the oocyte is fertilized and developed, these granularities can be found in the subzona area next to the blastomeres. One specific goal in our study was to remove the subzona granularity as a part of cosmetic microsurgery per se. However, these coarse granulations may be attached to the ZP, requiring more manipulation to remove them from the embryos. In this study, we tried to remove the granulation which was loosely attached to ZP, and aggressive manipulation was avoided.

Conclusion

From an ultrastructural stand point, the fragments have signs of both viability and degeneration. Cosmetic microsurgery on preimplantation embryos with moderate fragmentation had no beneficial effect on pregnancy, miscarriage, and live birth rates in an unselected group of patients. Numerous mitochondria in the fragments suggest that fragment removal should be performed with caution, at least in embryos at cleavage stage with moderate cytoplasmic fragments.

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