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EMBRYO BIOLOGY

Embryo developmental capability and pregnancy outcome are related to the mitochondrial DNA copy number and ooplasmic volume

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Abstract
Purpose To investigate the correlation between the ooplasmic volume and the number of mitochondrial DNA (mtDNA) copies in embryos and how they may affect fecundity.
Method Using real-time PCR, mtDNA quantification was analyzed in unfertilized oocytes and uncleaved embryos. The size of the ovum was also assessed by calculating the ooplasmic volume at the time of granulosa cell removal for IVF or ICSI. Quantification analysis of the mtDNA in blastomeres was performed by real-time PCR at the 7–8 cell stage of the cleaved embryos at 72 h after oocyte retrieval. We calculated the cytoplasmic volume of the blastomeres.
Result Our studies showed a significantly lower mtDNA copy number in unfertilized oocytes and uncleaved embryos in women who were older than 40 years of age ($p<0.05$). The larger ooplasmic volume was also associated with earlier and more rapid cleavage ($p<0.05$). The ooplasmic volume was also significantly larger in the group achieving pregnancy. We found a significant positive correlation between blastomere volume and the number of mtDNA copies ($r=0.76$, $p<0.01$, from Pearson product–moment correlation coefficient).
Conclusions We have shown that blastomere volume is directly proportional to the number of mtDNA copies. Therefore, larger cytoplasmic volume, with earlier cleavage speed, implies more mtDNA copies. Evaluation of mtDNA quantification and the measurement of ooplasmic and blastomere volume may be useful for selection of high quality embryo and pregnancy outcome.

Keywords Mitochondrial DNA · Blastomere volume · Ooplasmic volume · Aging

Capsule In our studies, higher ooplasmic volume was frequently associated with fecundity. And we found a significant positive correlation between blastomere volume and the number of mtDNA copies.

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Introduction

How to achieve high pregnancy rates is the main challenge that we are facing today in assisted reproductive technology (ART). Gross embryo morphology [1, 2] is one of the most reliable markers of embryo viability. The correlation between blastomere uniformity and a positive outcome of either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures is well established. Other morphological features such as variation in zona thickness [3], the presence of multinucleated blastomeres [4], and the appearance of the cytoplasm, pronuclei, and polar bodies [5, 6] have been shown to affect the implantation rate.

In addition to the morphological assessment, cytoplasmic factors (intrinsic factors) also play an important role in oocyte maturation, fertilization and early development [7, 8]. Live births have been achieved after the transfer of anucleate donor oocyte cytoplasm into recipient eggs [9, 10]. Major subcellular organs in the cytoplasm is mitochondria, and mitochondria account for 23 % of the cytoplasm in the preimplantation human embryo. Mitochondria are maternally inherited organelles that use oxidative phosphorylation to supply ATP to the cell. Unlike other organelles which are produced via transcription and translation of nuclear chromosomal DNA, the genetic information for mitochondria is contained within the organelle itself. Mitochondrial DNA

(mtDNA) is a double-stranded circular DNA molecule of approximately 16.5 kb in all mammals in which it has been sequenced [11]. The mitochondrial genome is not transmitted in a pattern of Mendelian inheritance, rather it passes from one generation to the next by way of the oocyte cytoplasm. Therefore, an individual's mtDNA is entirely derived from his or her mother as any paternal mtDNA is expelled from the cleaving pre-embryo at the two cell stage [12]. Usually all the mitochondrial chromosomes in a cell carry identical copies of mtDNA (homoplasmy) [13]. Due to the location within the mitochondrial matrix as well as the lack of histones and intervening sequences, mtDNA is particularly susceptible to the detrimental effects of reactive oxygen species [14, 15]. The mutation rate of mtDNA is almost 20 times greater than that of nuclear DNA [16]. MtDNA therefore accumulates both point mutations and deletions over time. The proportion of mutant mtDNA varies within different organs in a single individual and among individuals within the same family [17]. Single-cell studies and hybrid-cell studies both show that the proportion of mutant mtDNA must exceed a critical threshold level before a cell expresses a biochemical abnormality of the mitochondrial respiratory chain (the threshold effect) [18]. Individuals with mitochondrial disorders resulting from mtDNA mutations may harbor a mixture of mutant and wild-type mtDNA within each cell (heteroplasmy) [19, 20]. The expression of a mtDNA mutation is not proportional to its degree of heteroplasmy. It is necessary to have a high degree of heteroplasmy in order to observe the clinical signs of the pathology [21]. Regarding the number of mtDNA mutation, deletions increase with age [22].

In the context of fertility, an association exists between maternal age and the rate of mtDNA deletion in human oocytes and granulosa cells [14, 23, 24]. There have been studies that show the relationship between mtDNA deletion and the oocyte diameter in human [25],and the relationship between oocyte diameter and the developmental competence of oocytes [26, 27]. But to our knowledge, there has been no study that shows how the ooplasmic volume affects fertility and fecundity in human. Therefore, the importance of mtDNA content and the ooplasmic volume to human fertilization outcome and embryonic development needs to be clearly determined. We focused on the correlation between the ooplasmic volume and the number of mtDNA copies, and investigated the fertility and fecundity of human oocyte by quantitative analysis of the mtDNA and by the ooplasmic volume.

Materials and methods

Collection of oocytes, embryo and blastomeres

Human oocytes were donated with informed consent by 19 patients (31–44 years old) undergoing IVF at Keio University Hospital from August, 2005 to January, 2011. The research procedure was approved by the Research Ethics Committee of Keio University School of Medicine. Follicular growth was stimulated with FSH/hMG under the administration of GnRH agonist. Oocytes were collected after administration of hCG and fertilized under conventional IVF and ICSI.

We used unfertilized oocytes (with one or no polar body) and uncleaved embryos (with two polar bodies) ($n=29$) (31–44 years old), which had failed to undergo cleavage by 44 h after oocyte retrieval. Under an inverted microscope the unfertilized oocytes and uncleaved embryos were rinsed in a drop of PBS, and transferred into individual PCR tubes containing cell lysis solution. We grouped subjects according to age <40 years old (from 31 to 39 years old, $n=15$) and age ≥40 years old group (from 40 to 44 years old, $n=14$); and we compared the numbers of mtDNA copies between the two groups.

We used degenerated oocytes ($n=9$) (33–41 years old), which were classified as degenerated either before or after IVF or ICSI procedures. Degenerated oocytes were characterized by multiple abnormal morphologic aspects, such as darkened, vacuolated, and irregular ooplasm [28, 29] (Fig. 1a, b). Under an inverted microscope the degenerated oocytes were rinsed in a drop of PBS, and transferred into individual PCR tubes containing cell lysis solution.

Four cleaved embryos at the 7–8 cell stage were analyzed at 72 h after oocyte retrieval (Veeck's classification Grade1,3). We used a vitrification kit (KIAZATO®) to thaw frozen cleaved embryos. Then, the embryos were irradiated by a non-contact 1.48 μm diode laser system (OCTAX Laser Shot®:MTG, Germany) to pierce the zona pellucida. Two or three short pulses (2.9 ms) were applied, and single blastomeres were aspirated with a biopsy pipette. The diameter of each blastomere was measured under an inverted microscope (OLYMPUS IX71 Cronus®, U.S.A.). The long axis (D) and minor axis (d) of each blastomere were measured under an inverted microscope. We calculated the blastomere volume using ($\pi d^2 D \div 6$, $\pi=3.14$) [30]. Under an inverted microscope the blastomeres were rinsed in a drop of PBS and transferred into individual PCR tubes containing cell lysis solution. Whole blastomeres from each embryo were examined.

Measurement of oocyte cytoplasmic volume

To assess the size of the ovum, the ooplasmic volume was calculated. Human oocytes were donated with informed consent by 48 patients (28–44 years old) undergoing IVF. Follicular growth was stimulated with FSH/hMG administered in conjunction with a GnRH agonist. Oocytes were collected after the administration of hCG and fertilized with conventional IVF or ICSI.

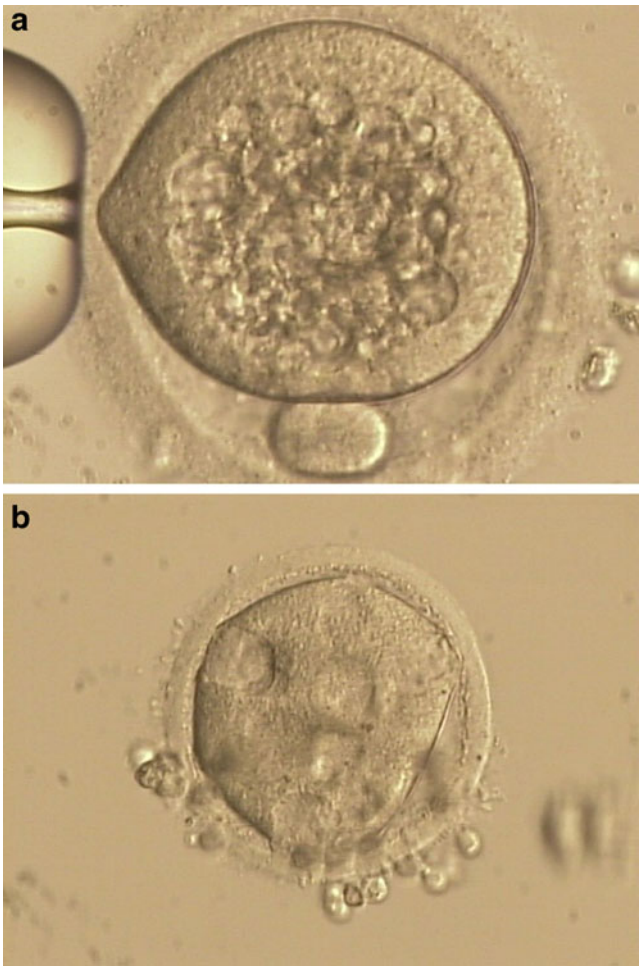

Fig. 1 **a**, **b**. Degenerated oocyte. Degenerated oocytes were characterized by multiple abnormal morphologic aspects, such as darkened, vacuolated, and irregular ooplasm [28, 29]. Differential interference contrast microscopy :objective (x400)

The diameter of each ooplasm cytoplasm was measured under an inverted microscope (OCTAX Laser Shot®:MTG, Germany) at the time of granulosa cell removal. The long axis (D) and minor axis (d) of each ooplasm were measured under an inverted microscope. We calculated the ooplasmic volume using ($\pi d^2 D \div 6$, $\pi = 3.14$).

MtDNA quantification by real time PCR

Quantification of mtDNA was performed by real-time PCR. We used a cell line derived from a single human osteocarcinoma cell with a fixed 9100 mtDNA copy number (ATCC, 143B; CRL-8303) [31]. We diluted it with purified water, and manufactured dilute solutions of 100×10^4copy/μl, 50×10^4copy/μl, 25×10^4copy/μl, 10×10^4copy/μl, 5×10^4copy/μl, 1×10^4copy/μl, 5000 copy/μl, 1000copy/μl, and 500copy/μl.

Quantitative and qualitative analysis for mtDNA were performed by previous established protocol with real-time PCR analysis [32]. Real-time PCR primers and fluorescent probes (TaqMan® MGB probe; Applied Biosystems, USA) corresponding to sequences of ATPase6 gene were prepared. The forward primer (5′-CGAAACCATCAGCCTACTCAT TCAA-3′) spanned from nt 8958 to nt 8982. The reverse primer (5′-CCTGCAGTAATGTTAGCGGTTAGG-3′) spanned from nt 9026 to nt 9003. The probe for wild sequences (8993T; CCAATAGCCC[T]GGCCGT) had "VIC" fluorochrome and the probe for mutant (8993G; AATAGCCC[G]GGCCGT) had the "FAM" fluorochrome. 8993 point is in the ATPase6 gene which is responsible for major energy production for embryo development. The T8993G point mutation was commonly found and is responsible for Leigh syndrome.

Each 25 μl PCR reaction was prepared with the following final concentrations: 12.5 μl TaqMan Universal PCR Master Mix, 5 μl distilled water, 1 μl×2 TaqMan MGB Probe, 2.25 μl×2 PCR primer, 1 μl specimen or 1 μl dilute solutions of 9100 mtDNA copy with distilled water or 1 μl TE buffer (negative control).

The reactions were performed with the following conditions: initial denaturation at 50°C for 2 min and 95°C for 10 min, and 35 cycles at 92°C for 15 s (denaturation), 60°C for 1 min (annealing and extension). The allelic discrimination assay using real-time PCR (ABI PRISM 7000) was used to measure each fluorescence signal.

Statistical analysis

Continuous data were compared using Student's *t*-test or Welch's *t*-test. $p<0.05$ was considered statistically significant.

Results

The number of mtDNA copies in unfertilized oocytes and uncleaved embryos

The minimum mtDNA copy number found in unfertilized oocytes and uncleaved embryos was 277,059, and the maximum was 1045,000. Copy number was highly variable both within and between patients. Our studies have shown that the average number of mtDNA copies in unfertilized oocytes and uncleaved embryos was 697,176±37,057. The average number of mtDNA copies was 784,307±48,379 for age <40 years and 603,822±46,153 for age ≥40 years. Our studies in unfertilized oocytes and uncleaved embryos in women who were older than 40 years of age showed significantly fewer mtDNA copies ($p<0.05$) (Fig. 2).

The number of mtDNA copies in degenerated oocytes

The average number of mtDNA copies for the nine degenerated oocytes was 330,513±78,002. In this result, the number of mtDNA copies in degenerated oocytes showed a

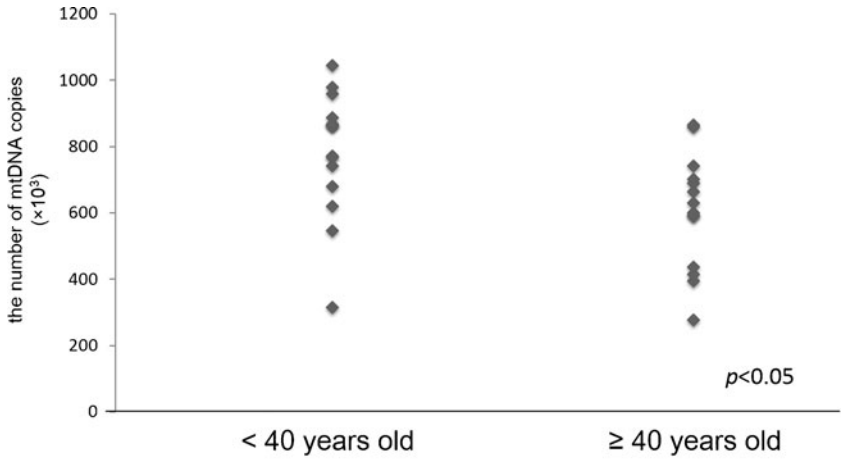

Fig. 2 The number of mtDNA copies in unfertilized oocytes and uncleaved embryos. Quantification of mtDNA was performed by real-time PCR. We grouped subjects according to age <40 years old (from 31 to 39 years old, $n=15$) and age ≥40 years old group (from 40 to 44 years old, $n=14$); and we compared the numbers of mtDNA copies between the two groups. The average number of mtDNA copies was 784,307±48,379 for age <40 years and 603,822±46,153 for age ≥40 years. Our studies in unfertilized oocytes and uncleaved embryos in women who were older than 40 years of age showed significantly fewer mtDNA copies ($p<0.05$)

remarkable decrease in comparison with unfertilized oocytes and uncleaved embryos ($p<0.01$) (Fig. 3).

The number of mtDNA copies in blastomeres

The average number of mtDNA copies in blastomeres from7 to 8 cell embryos at 72 h after oocyte retrieval was 673,722±12,952. The number of mtDNA copies varied widely among individual blastomeres in an embryo. We evaluated the relationship between the volume and the number of mtDNA copies in isolated blastomeres from 7 to 8 cell embryos. We found a significant positive correlation between blastomere volume and the number of mtDNA copies ($r=0.76$, $p<0.01$, from Pearson product–moment correlation coefficient) (Fig. 4).

The T8993G point mutation was not detected in any unfertilized oocytes, degenerated oocytes or blastomeres in our studies (data not shown).

Ooplasmic volume

We measured the ooplasmic volume in order to evaluate the correlations with other factors. The speed of embryo cleavage and the ooplasmic volume were compared. In embryonic

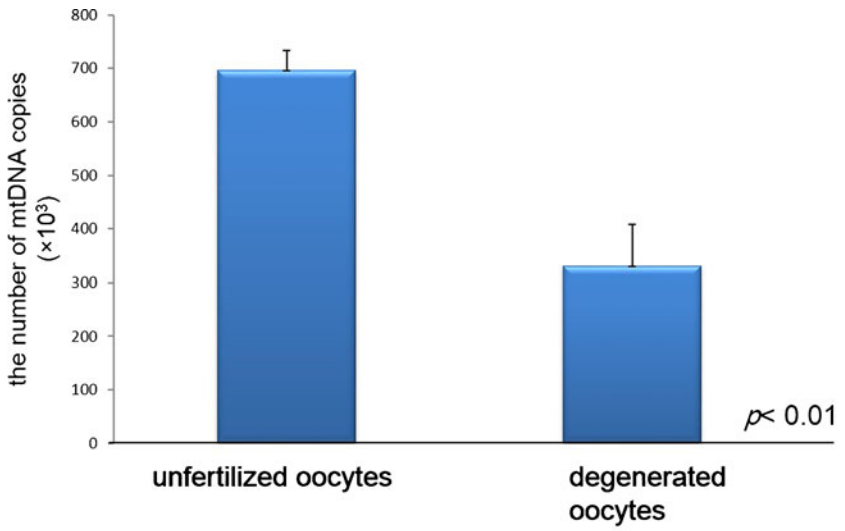

Fig. 3 The number of mtDNA copies in unfertilized oocytes and degenerate oocytes. Quantification of mtDNA was performed by real-time PCR. Our studies have shown that the average number of mtDNA copies in unfertilized oocytes and uncleaved embryos (from 31 to 44 years old, $n=29$) was 697,176±37,057. The average number of mtDNA copies for the nine degenerate oocytes (from 33 to 41 years old) was 330,513±78,002. The number of mtDNA copies in degenerated oocytes showed a remarkable decrease in comparison with unfertilized oocytes and uncleaved embryos ($p<0.01$)

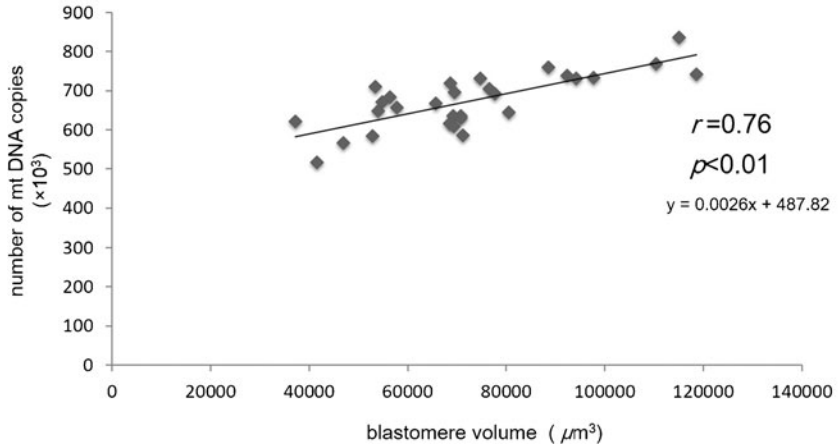

Fig. 4 Blastomere volume and the number of mtDNA copies at 72 h after oocyte retrieval. Four cleaved embryos at the 7–8 cell stage were collected for study at 72 h after oocyte retrieval (Veeck's classification Grade1,3). Each single blastomeres ($n=29$) were aspirated with a biopsy pipette. The diameter of each blastomere was measured under an inverted microscope. The long axis (D) and minor axis (d) of each blastomere were measured under an inverted microscope. We calculated the blastomere volume using ($\pi d^2 D \div 6$, $\pi=3.14$). Quantification of mtDNA was performed by real-time PCR. The average number of mtDNA copies in blastomeres was 673,722±12,952. We found a significant positive correlation between blastomere volume and the number of mtDNA copies ($r=0.76$, $p<0.01$, from Pearson product–moment correlation coefficient)

development, 3–4 cell embryos showed a normal (early) cleavage speed. At 44 h after oocyte retrieval, 348 embryos had reached the 3–4 cell stage and the remaining 326 had not. The ooplasmic volume was compared between these groups.

At 44 h after oocyte retrieval, the average ooplasmic volume of the 3–4 cell embryos was 615,222±2,604 μm^3 and that of those with fewer cells was 605,402±3,145 μm^3 ($p<0.05$) (Fig. 5). This significant difference in volume implies that embryos that cleave at a faster rate have larger ooplasmic volume. Previous reports have also shown that in the early cleavage group there was a higher rate of pregnancy per transfer compared with the late cleavage group [33, 34], suggesting that early cleavage is a strong indicator of better embryo quality. We then adopted Veeck's classification to further analyze 348 embryos which have reached the normal (early) cleavage speed at 44 h after oocyte retrieval. The Veeck's classification and the average ooplasmic volume were as follows, respectively: Veeck 1 ($n=45$), 626,774±6,264 μm^3; Veeck 2 ($n=64$), 625,191±5,844 μm^3; Veeck 3 ($n=75$), 611,083±5,549 μm^3; Veeck 4 ($n=164$), 610,055±3,938 μm^3. When comparing Veeck 1 and Veeck 4, the ooplasmic volume was significantly different; the ooplasmic volume of Veeck1 was significantly larger than that of Veeck 4 ($p<0.05$) (Fig. 6a). The comparison between

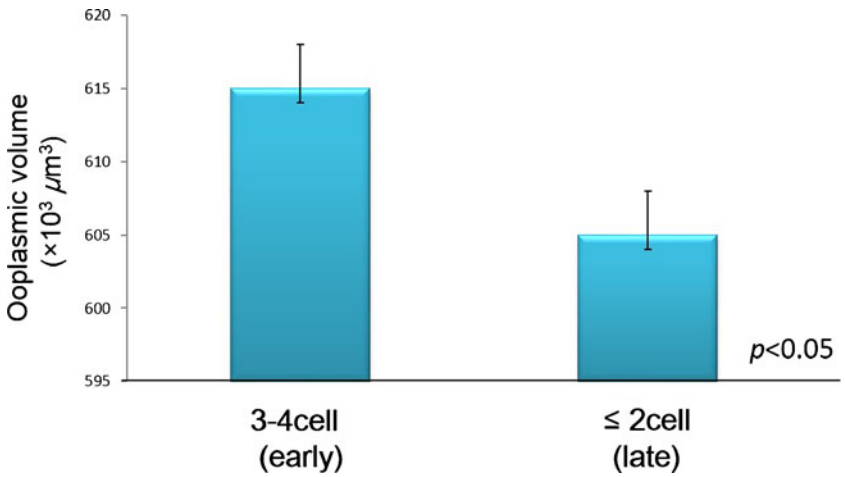

Fig. 5 Comparison of ooplasmic volume at oocyte retrieval and the speed of embryo cleavage at 44 h after oocyte retrieval. In embryonic development, 3–4 cell embryos showed a normal (early) cleavage speed. At 44 h after oocyte retrieval, 348 embryos had reached the 3–4 cell stage and the remaining 326 had not. The ooplasmic volume was compared between these groups. At 44 h after oocyte retrieval, the average ooplasmic volume of the 3–4 cell embryos was 615,222±2,604 μm^3 and that of those with fewer cells was 605,402±3,145 μm^3 ($p<0.05$)

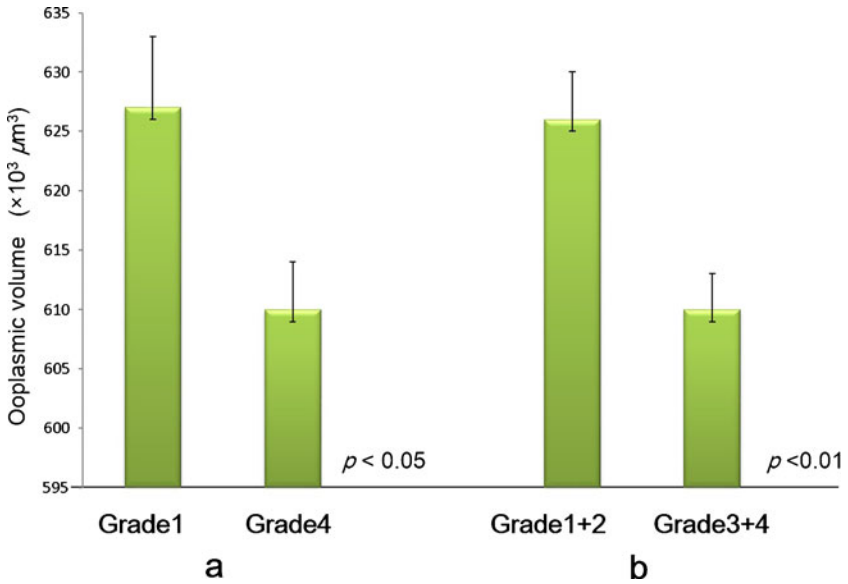

Fig. 6 **a** Comparison of ooplasmic volume at oocyte retrieval and morphological classification of embryo developed at 44 h after oocyte retrieval:Grade1 vs Grade4. There was a total of 348 embryos at 44 h after oocyte retrieval which reached the normal (early) embryonic development stage. The Veeck's classification and the average ooplasmic volume were as follows, respectively: Veeck 1 (n=45), 626,774±6,264 μm^3; Veeck 2 (n=64), 625,191±5,844 μm^3; Veeck 3 (n=75), 611,083±5,549 μm^3; Veeck 4 (n=164), 610,055±3,938 μm^3. When comparing Veeck 1 and Veeck 4, the ooplasmic volume was significantly different; the ooplasmic volume of Veeck1 was significantly larger than that of Veeck 4 (p<0.05). **b** Comparison of ooplasmic volume at oocyte retrieval and morphological classification of embryo developed at 44 h after oocyte retrieval: Grade1+2 vs Grade3+4. The comparison between Veeck 1+2 (n=109), and Veeck 3+4 (n=239) also showed a significant difference in the ooplasmic volume; the ooplasmic volume of Veeck 1+2 (625,845±4,278 μm^3) was significantly larger than that of Veeck 3+4 (610,377±3,208 μm^3) (p<0.01)

Veeck 1+2 and Veeck 3+4 also showed a significant difference in the ooplasmic volume; the ooplasmic volume of Veeck 1+2 was significantly larger than that of Veeck 3+4 (p<0.01) (Fig. 6b).

The relationship between the rate of pregnancy and ooplasmic volume was analyzed. The number of embryos leading to pregnancy was 44, and the average ooplasmic volume was 625,019±7,448 μm^3. The pregnancies were achieved by either fresh or frozen embryos transfer with conventional IVF or ICSI. The number of embryos leading to pregnancy excluding miscarriage was 35, and the average ooplasmic volume was 631,911±8,255 μm^3. The number of non-pregnant embryos was 630, and the average ooplasmic volume was 609,456±2,112 μm^3. The number of non-pregnant embryos excluding frozen embryos that were not thawed was 549, and the average ooplasmic volume was 608,569±2,246 μm^3. The ooplasmic volume of the pregnant group was significantly larger (Table 1).

We also analyzed the association between age and ooplasmic volume. The probability of clinical pregnancy declines with age, with lower rates in women aged 35–39 in comparison to women aged 30–34 [35]. In the present study, the former age group (n=209, volume 597,634±3,731 μm^3) showed significantly lower ooplasmic volume when compared to the latter age group (n=146, volume 608,572±3,997 μm^3) ($p \leq 0.05$) (Fig. 7).

Discussion

It is commonly known that ovarian aging is associated with the impairment of specific functions of oocytes and granulosa cells, along with more global cellular dysfunction resulting from changes in gene and protein expression profiles, and energetic failure [36–41]. Embryo development in advanced reproductive age may be jeopardized by altered mitochondrial activity. Our studies showed significantly fewer mtDNA copies in unfertilized oocytes and uncleaved

Table 1 Comparison between the ooplasmic volume at oocyte retrieval—Pregnant group vs Non-pregnant group

Group	No. of oocytes	Mean volume ± SE	
Pregnancy	44	625,019±7,448[a]	(511,026–723,604)
Pregnancy (excluded miscarriage)	35	63,911±8,255[b]	(537,143–723,604)
Miscarriage	9	598,217±14,787	(511,026–659,671)
Non-pregnancy	630	609,456±2,122[c]	(443,232–767,134)
Non-pregnancy (excluded frozen embryos that do not thaw)	549	608,569±2,246[d]	(443,232–767,134)

[a,d] p<0.05, [b,c] p<0.05, [b,d] $p \leq 0.01$, μm^3

embryos from women who were older than 40 years of age. This suggests that the number of mtDNA copies decreases with aging. This low mtDNA copy number may result from the accumulation of mutations in the mtDNA or an inherent property of oocytes recruited later in the reproductive lifespan [42]. These observations allude to a relationship between mtDNA and oocyte quality. Our studies have shown that the average number of mtDNA copies in unfertilized oocytes and uncleaved embryos was 697,176±37,057. Previous studies reported it as ranging average from 314,000 to 795,000 [23, 42–44]. Our results may be technically more accurate than those of previous studies, since our quantitation was based on a single standardized human cell with 9,100 mtDNA copies [31]. Previous study [23] has also reported that the number of mtDNA copies decreases with aging, which is the same as our study.

In a study performed in mouse, low mtDNA copy number did not significantly affect fertilization [45]. While others have shown that mitochondrial activity is crucial for the activation of development and for embryonic survival [46]. Mitochondrial dysfunction resulting from a variety of intrinsic and extrinsic influences, including genetic abnormalities, hypoxia and oxidative stress, can profoundly influence ATP generation in oocytes and early embryos, which in turn may result in aberrant chromosomal segregation or developmental arrest. In our studies, the number of mtDNA copies in degenerated oocytes showed a remarkable decrease in comparison with unfertilized oocytes (Fig. 2), suggesting that the extreme low mtDNA copy number present was insufficient to provide the necessary energetic reserves during follicular growth, thereby leading to oocyte degeneration.

While the low mtDNA copy does not negatively impact fertilization in the mouse, it does result in a dramatic reduction in post-implantation embryonic viability [45]. The post-implantation state is characterized by a high energy requirement, rendering the embryo particularly susceptible to deficiencies in mitochondrial function during this period. In the mouse, a minimal threshold for mtDNA copy number has been identified, below which normal post-implantation embryonic development does not occur. No minimum, however, has been defined for the human oocyte. The number of mtDNA copies could be a useful indicator of oocyte quality in experimental models. Although the method used to determine the number of mtDNA copies is retrospective analysis, identifying the standardizing thresholds for mtDNA number may prove useful in the evaluation of patients with IVF failures.

Regarding the ooplasmic volume, we found that higher cytoplasmic volume correlates to younger age and higher pregnancy rate. Higher ooplasmic volume was also frequently associated with better morphology, and earlier cleavage. It is likely therefore that ooplasmic volume affects fecundity. Larger oocyte diameter is a determinant factor for completion of meiosis and acquisition of full competence for embryo development in the goat [47]. It has been reported that a relationship between higher cytoplasmic volume and higher fertilization outcome in the pig [48]. There has been no study in humans correlating ooplasmic volume with fertility and fecundity. Oocyte diameter has been linked to developmental competence during the germinal vesicle (GV) stage in the ferret [26] and in human [27]. The configuration of the GV chromatin correlates with the developmental competence of oocytes. Chromatin condensation is related to the sequential achievement of meiotic competencies during oocyte growth and differentiation, and GV condensation corresponds to an increase in oocyte diameter. The relationship between ooplasmic volume and the optimization of nuclei is considered to be the next subject.

We also evaluated the relationship between the blastomere volume and the number of mtDNA copies in isolated blastomeres from 7 to 8 cell embryos. Blastomere volume was directly proportional to the number of mtDNA copies ($r=0.76$, $p<0.01$). This is in accordance with previous reports in the mouse [49]. Additionally, increased

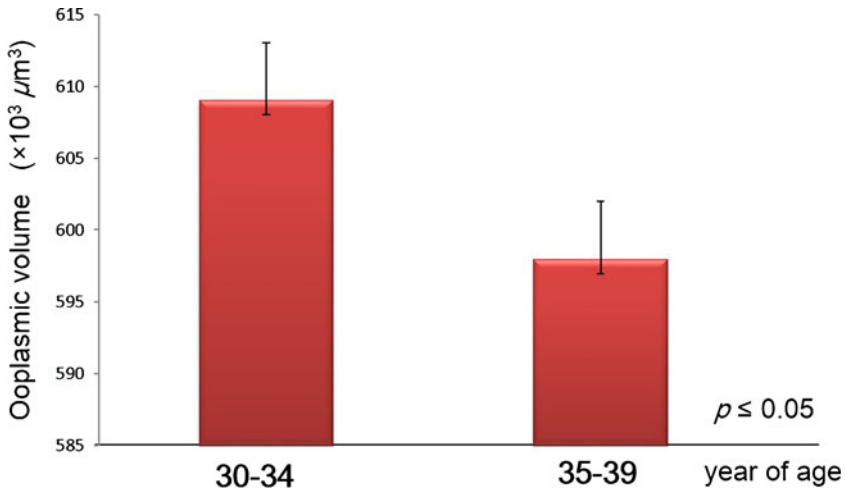

Fig. 7 Ooplasmic volume at oocyte retrieval between two different age groups: 30–34 vs 35–39. We analyzed the association between age and ooplasmic volume. The 35–39 year age group ($n=209$, volume 597,634±3,731 μm^3) showed significantly lower ooplasmic volume when compared to the 30–34 year age group ($n=146$, volume 608,572±3,997 μm^3) ($p \leq 0.05$)

mitochondrial number correlates with cytoplasmic volume in cattle [50], implying that a sufficient supply of ATP is necessary for the development of proper blastomeres. In our study, the number of mtDNA copies in each blastomere from 7 to 8 cell-stage embryos was uneven. The inter-blastomere variation of mtDNA contents may occur due to intrinsic factors, or may be the result of external influences to which each individual embryo and blastomere are exposed [51, 52]. The asymmetric polar distribution of the mitochondria in mature oocytes may be retained through cleavage divisions, resulting in blastomeres with a varying mitochondrial load [46].

Conclusions

We conclude that higher fecundity is associated with an increased number of mtDNA copies in the embryo. The mitochondrion inherited maternally is an important organelle for reproduction, and mtDNA is a key determinant of its function. Evaluation of mtDNA quantification might be an underlying factor to explain the developed oocyte quality, and this may eventually lead to treatment. In the United Kingdom recently there is an ethical issues concerning reproductive-gene-therapy techniques that could prevent children from inheriting certain genetic diseases caused by faulty mitochondria. Mitochondrial therapy at the oocytic level is current burning topic to be discussed all over the world. Moreover so as it includes the issues about oocyte aging and capability for fecundity and we need to be prepared for dealing with this ethically sensitive matter. We found a significant positive correlation between blastomere volume and the number of mtDNA copies. So, low-invasive quantification of ooplasmic and blastomere volume is a novel factor, may be convenient and advantageous predictor for successful clinical outcome in selecting embryos to be transferred.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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