Morphologic Features and Morphometric Measurements of Human Oocytes That Failed to Cleave after Intracytoplasmic Sperm Injection (ICSI)

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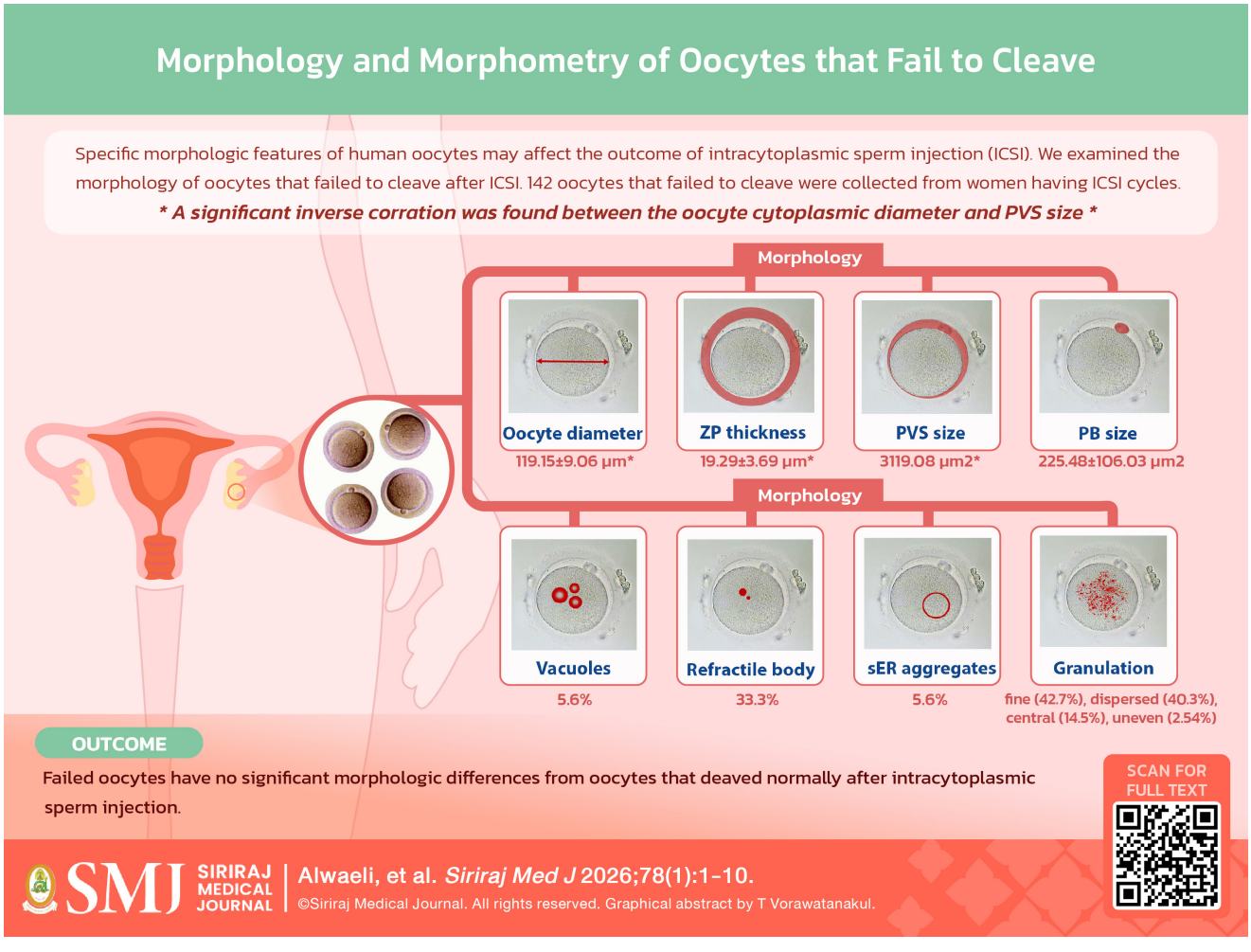

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ABSTRACT

Objective: Human oocytes collected for in vitro fertilization (IVF) vary in morphologic features and measurements. This study aimed to describe the morphology of human oocytes that failed to cleave after intracytoplasmic sperm injection (ICSI).

Materials and Methods: Oocytes that failed to cleave post-ICSI were collected from IVF cycles. The oocytes were microscopically examined and the cytoplasmic diameter, zona pellucida thickness, perivitelline space (PVS) size, and first polar body (PBI) size were measured. The granularity pattern of cytoplasm; the color of cytoplasm; the presence of smooth endoplasmic reticulum (sER) aggregates, vacuoles, refractile bodies, and pronuclei; and, the status of polar bodies were all recorded.

Results: A total of 142 oocytes were analyzed. The mean cytoplasmic diameter was 119.15±9.06 µm (range: 90.66-137.79 µm). The mean zona pellucida thickness was 19.29±3.69 µm (range: 12.86-33.69 µm). The mean PVS size was 3119.08 µm^2 (range: 765-6448 µm^2). The mean PBI size was 225.48±106.03 µm^2 (range: 53.56-703.65 µm^2). The cytoplasm showed fine (42.7%), dispersed (40.3%), central (14.5%), and uneven (2.54%) patterns of granulation. Other cytoplasmic abnormalities included refractile bodies (33.3%), sER aggregates (5.6%), vacuoles (5.6%), and dark cytoplasm (14.5%). A significant inverse correlation was found between the oocyte cytoplasmic diameter and PVS size.

Conclusion: The morphologic abnormalities in oocytes that failed to cleave after ICSI are not significantly different from those observed in the general population of human oocytes. The oocyte cytoplasmic diameter was found to significantly inversely correlate with PVS size.

Keywords: Morphologic features; morphometric measurements; human oocytes; failed; cleave; intracytoplasmic sperm injection; ICSI (Siriraj Med J 2026;78(1):1-10)

INTRODUCTION

A normal human metaphase II (MII) oocyte generally shows a rounded and clear zona pellucida, a small perivitelline space (PVS) that contains a single oval polar body (or first polar body [PBI]), and a light cytoplasm with moderate granulation and no cytoplasmic inclusions.[1,2] However, most retrieved oocytes after controlled ovarian stimulation have some morphologic abnormalities, including ooplasmic and/or extracytoplasmic features.[3,4] Whether the presence of these visible oocyte morphologic abnormalities can adversely influence the outcome of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) or not has not yet been conclusively established. Some authors suggest that oocytes may be successfully fertilized via ICSI regardless of their morphologic features.[5,6] Other authors suggested that oocyte morphology influences embryo quality and development. One previous study showed that the grade of oocyte (based on PBI morphology, PVS size, and the presence of intracytoplasmic inclusions) correlates with oocyte developmental potential after ICSI.[7] Another study found the added effect of oocyte morphologic anomalies, like cytoplasmic granularity, refractile bodies, vacuoles, inclusions, and central cytoplasmic granulation, to be significantly associated with impaired embryo quality, but the pregnancy rates were not adversely affected.[8] However, a contradictory conclusion was obtained by a different study, which found that the same features mentioned above had no significant effect on the in vitro development of embryos, but that they were associated with lower implantation and pregnancy rates when the embryos developed from oocytes with these cytoplasmic defects.[9]

In the present study, the morphologic and morphometric features and measurements of oocytes that failed to cleave following ICSI were investigated. The results of this study will improve our understanding of whether the presence of morphologic and/or morphometric abnormalities influence the fertilization failure observed in these oocytes by comparing them with the prevalence of similar abnormalities observed in human oocytes before ICSI as mentioned in the literature.

MATERIALS AND METHODS

Fifty-two women, aged 20 to 44 years, were included in this prospective observational study during January 2023 to July 2023 study period. These women had controlled ovarian stimulation with the antagonist protocol, using GnRH antagonist and recombinant FSH. Recombinant FSH was used for its proved safety and efficacy in IVF practice.[10] A total of 142 oocytes that failed to show the first mitotic division (cleavage) after ICSI were enrolled. Study oocytes were collected on day-2 post-

injection and immediately examined and photographed using an inverted light microscope (Olympus IX71; Olympus Corporation, Tokyo, Japan) fitted with a digital camera system that is connected to a personal computer loaded with digital imaging software. Photography and microscopic examination of the morphologic parameters were performed under x200 magnification. The protocol for this study was approved by the Institutional Review Board of Kamal Al- Sameraie Hospital for Infertility Management and In Vitro Fertilization, Baghdad, Iraq (COA no. 879/2022), and all female patient volunteers provided written informed consent permitting the use of their failed oocytes in this study.

Oocyte morphometry

Oocyte morphometric measurements were performed using ImageJ software (ij153-win-java8 version; National Institutes of Health, Bethesda, MD, USA). The measurements included oocyte diameter, polar body (PB) size, perivitelline space (PVS) size, and zona pellucida (ZP) thickness. Oocyte size was estimated by measuring the oocyte cytoplasmic diameter (ooplasmic diameter). This was performed by calculating the mean of 4 different cytoplasmic diameter measurements for each oocyte, and each cytoplasmic diameter measurement was taken at a location at least 45° away from any of the other cytoplasmic diameter measurements. The area occupied by the polar body in the oocyte image was used as an indicator of PB size. The size of the PVS was also estimated by measuring its area in the oocyte image. This was performed by subtracting the oocyte image area from the area bounded by the inner surface of the ZP. ZP thickness was measured as the distance between its outer and inner surfaces. To obtain more accurate results, ZP thickness was measured at 8 different locations around its circumference and the mean measurement was recorded.

Oocyte morphology

The morphologic features of the oocytes evaluated in this study include the following: **a.** Cytoplasmic granulation: According to the pattern of cytoplasmic granules, oocytes were divided into one of the following four granulation categories[11]: fine granulation (FG), dispersed granulation (DG), central granulation (CG), or uneven granulation (UG); **b.** Cytoplasmic color (light or dark); **c.** The presence or absence of specific intracytoplasmic structures, including refractile bodies, smooth endoplasmic reticulum [sER] aggregates, vacuoles, and pronuclei); and, **d.** Extracytoplasmic features, including PB fragmentation and PVS granulation.

Sample size calculation and statistical analysis

The sample size for this study was calculated using Yamane's formula (1967) n = N/(1+N(e)^2)
where n: sample size, N: population size, e: margin of error (p=0.05) at 95% confidence interval and 30% expected frequency. The Epi Info application (Centers for Disease Control and Prevention [CDC], Atlanta, Georgia, USA) was used to apply these parameters and the calculated sample size was 140-148. The enrolled sample in the present study was N=142.

The results of this study were analyzed using SPSS Statistics version 26 (SPSS, Inc., Chicago, IL, USA) to confirm or deny statistical significance. The continuous data are expressed as mean ± standard deviation, and a correlation between continuous measurements was detected by Pearson's correlation coefficient (r). T-test and analysis of variance (ANOVA) were used to determine the statistical significance of association between non-continuous data. A probability value of $p<0.05$ was considered to reflect statistical significance.[12]

RESULTS

Microscopic examination of the 142 oocytes for the investigated features and parameters revealed the following morphometry and morphology data.

Oocyte morphometry

The oocyte diameter (excluding the ZP) ranged from 90.66 µm to 137.79 µm, with a mean of 119.15 µm. Out of the 124 oocytes included, 122 (98.4%) oocytes had a diameter less than 130 µm (the upper normal limit of metaphase II [MII] human oocyte diameter), and only two (1.6%) exceeded 130 µm (Fig 1a, Fig 2a, b). The thickness of oocyte ZP ranged from 12.86 µm to 33.69 µm, with an average of 19.29 µm. Nine (7.2%) oocytes had a ZP thickness greater than the upper normal limit of 24.9 µm, and no oocytes had a ZP thickness below the lower normal limit of 8.57 µm (Fig 1b, Fig 2c, d). The size of the PVS (as estimated by the area in the image) of the included oocytes showed a wide range of variation, ranging from 765 μm^2 to 6,448 μm^2, with an average of 3,119.08 μm^2 (Fig 1c, Fig 2a, b). All of the studied oocytes had at least one visible PB. The size of the first polar body (PBI) (as estimated by its area in the image) showed broad variation, ranging from 53.56 to 703.65 μm^2, with an average of 225.48 μm^2 (Fig 1d). Fragmented PBI was observed in 33 oocytes (26.6%). A second polar body (PBII) was identified in only 18 oocytes (14.5%). One oocyte had a giant PB (Fig 3b, c, d).

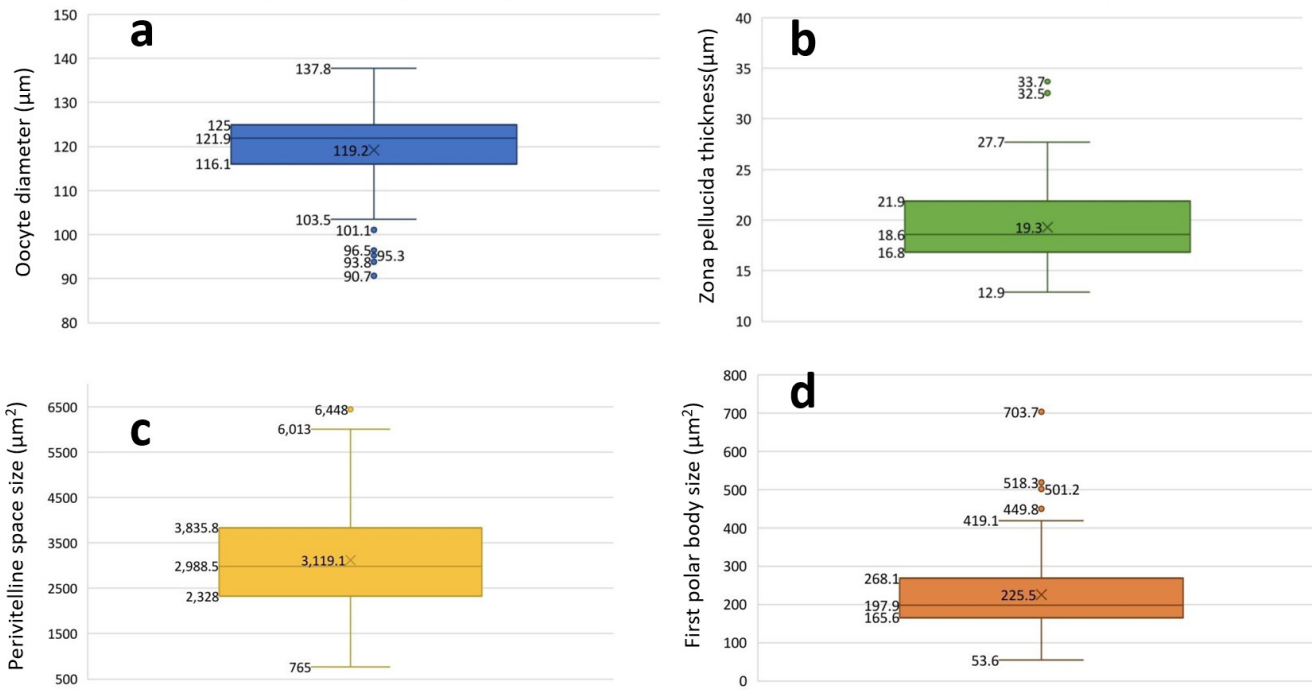

Fig 1. Overall descriptions of oocyte diameter (**a**), zona pellucida thickness (**b**), perivitelline space size (**c**), and first polar body size (**d**).

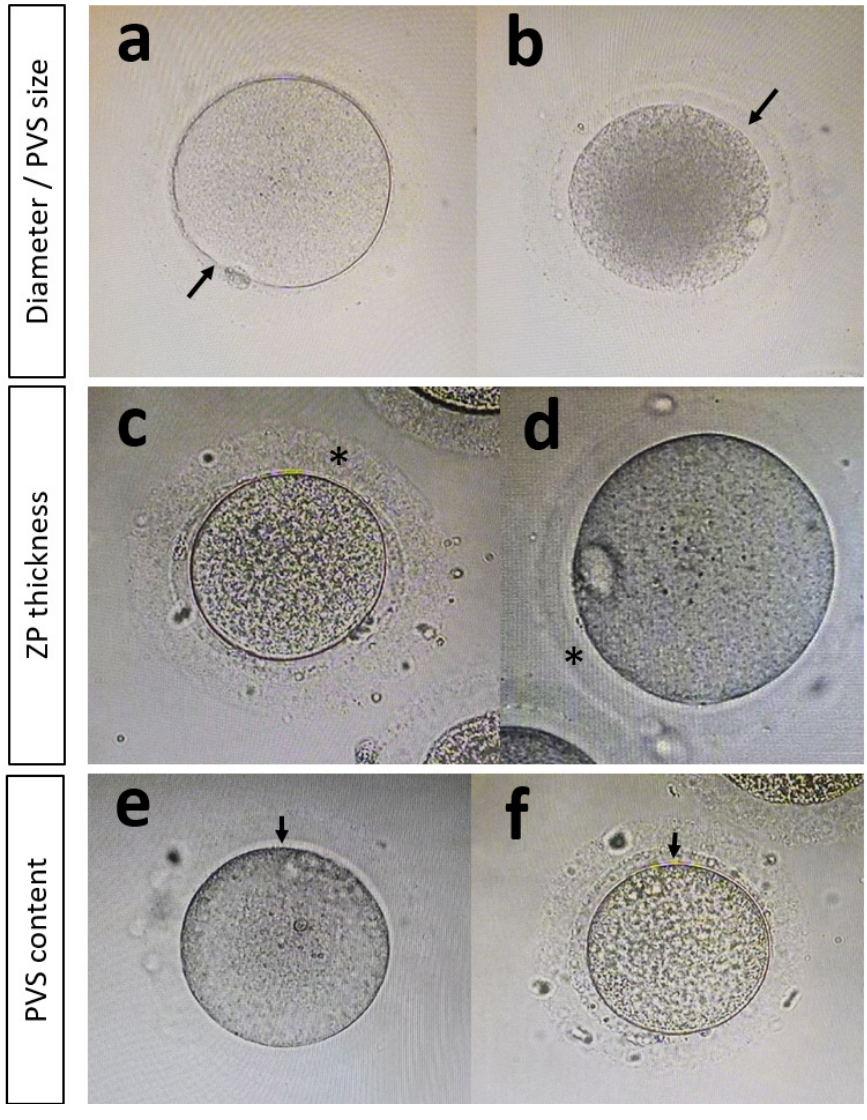

Fig 2. Variations in oocyte morphology and measurements, including oocyte with large diameter and small perivitelline space (PVS) size (a), oocyte with small diameter and large PVS size (b), thick zona pellucida (ZP) (c), thin ZP (d), clear PVS (e), and PVS with fragmentations (f). Arrows indicate PVS. (magnification x200)

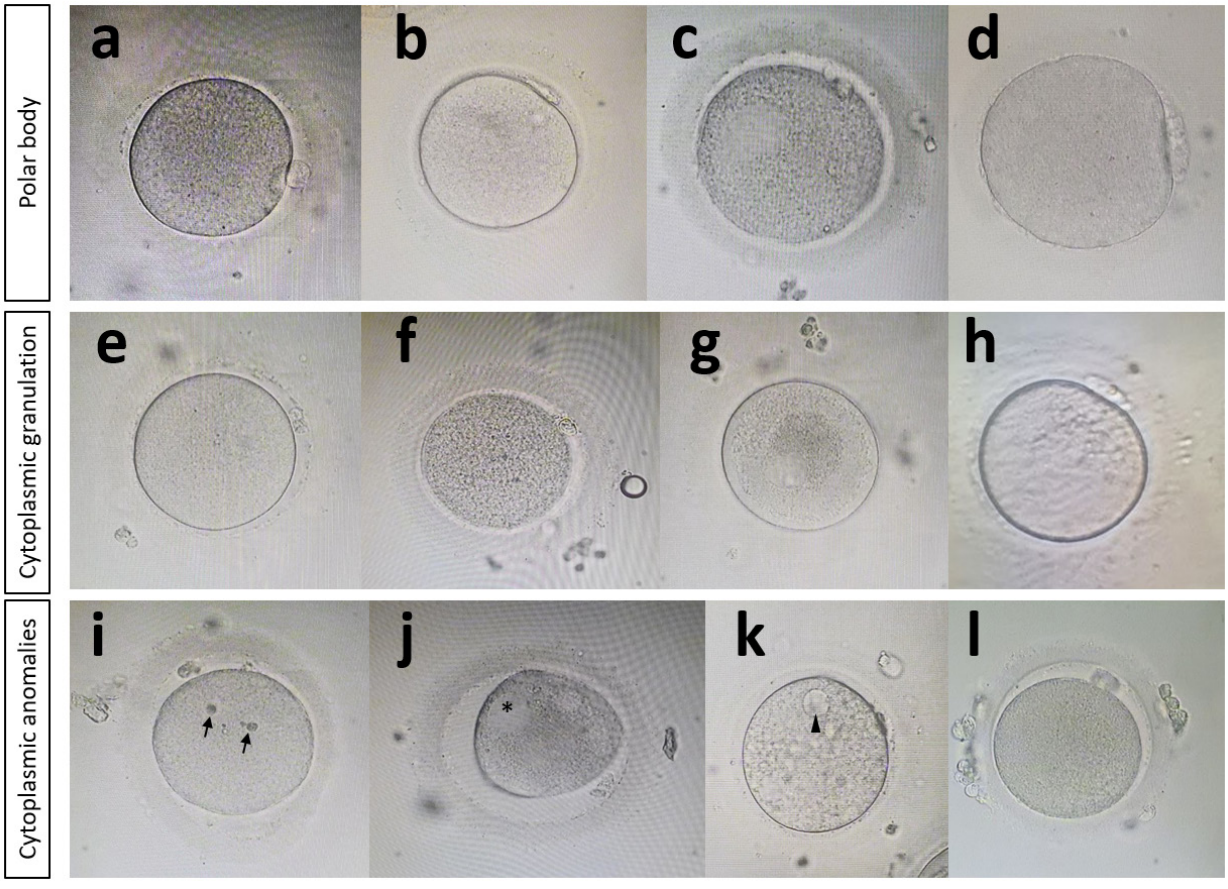

Fig 3. Polar body variations, including regular polar body (PB) (a), fragmented PB (b), double polar bodies (c), and giant PB (d); cytoplasmic granulation patterns: fine (e), dispersed (f), central (g), and uneven (h); cytoplasmic abnormalities: refractile bodies (arrows) (i), smooth endoplasmic reticulum (sER) aggregates (asterisks) (j), vacuoles (arrow head) (k), and dark cytoplasm (l). (magnification x200)

Oocyte morphology

Regarding the pattern of cytoplasmic granularity, the normal fine granulation (FG) pattern of the cytoplasm was seen in 53 (42.7%) oocytes, while the remaining 71 (57.3%) oocytes had one of the abnormal cytoplasmic granulation patterns, including dispersed (50 oocytes; 40.3%), central (18 oocytes; 14.5%), and uneven (3 oocytes; 2.4%) (Fig 3e, f, g, h). Concerning the other morphologic features, refractile bodies were observed in the cytoplasm of 41 oocytes (33%), aggregates of smooth endoplasmic reticulum (sER) were seen in 7 oocytes (5.6%), cytoplasmic vacuoles were observed in 7 oocytes (5.6%), and dark-colored cytoplasm was seen in 18 (14.5%) of oocytes (Fig 3i, j, k, l). PVS with fragmentations was found in 59 (47.6%) oocytes (Fig 2f).

Correlations and associations between morphologic and/or morphometric features

There was a statistically significant moderately inverse linear correlation between oocyte diameter and PVS size (Fig 4). No statistically significant correlation was found between other measurable morphologic features, including ZP thickness, PVS size, or PBI size. Larger oocyte diameter was significantly associated with the presence of fragmentations in the PVS, and smaller oocyte diameter was significantly associated with uneven cytoplasmic granulation, and with dark-colored oocytes (Fig 5a). Larger PBI size was significantly associated with PB fragmentation (Fig 5b). Smaller ZP thickness was significantly associated with uneven granulation pattern of cytoplasm. Larger PVS size was associated with dark oocytes, and with the presence of a second polar body (PBII) (Fig 5c). Light cytoplasmic color was associated with significantly higher rates of fine cytoplasmic granulation, and dark cytoplasmic color was associated with significantly higher rates of dispersed granulation (Fig 5d).

DISCUSSION

The aim of this study was to investigate the specific morphologic features in an oocyte population that failed to cleave after ICSI, and to compare the prevalence of those features with the features and prevalence observed in the general oocyte population described in the literature.

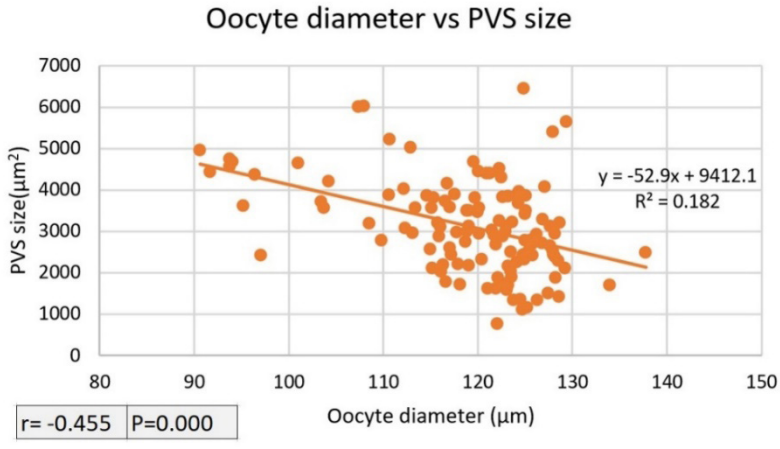

Fig 4. Linear correlation between oocyte diameter and perivitelline space (PVS) size.

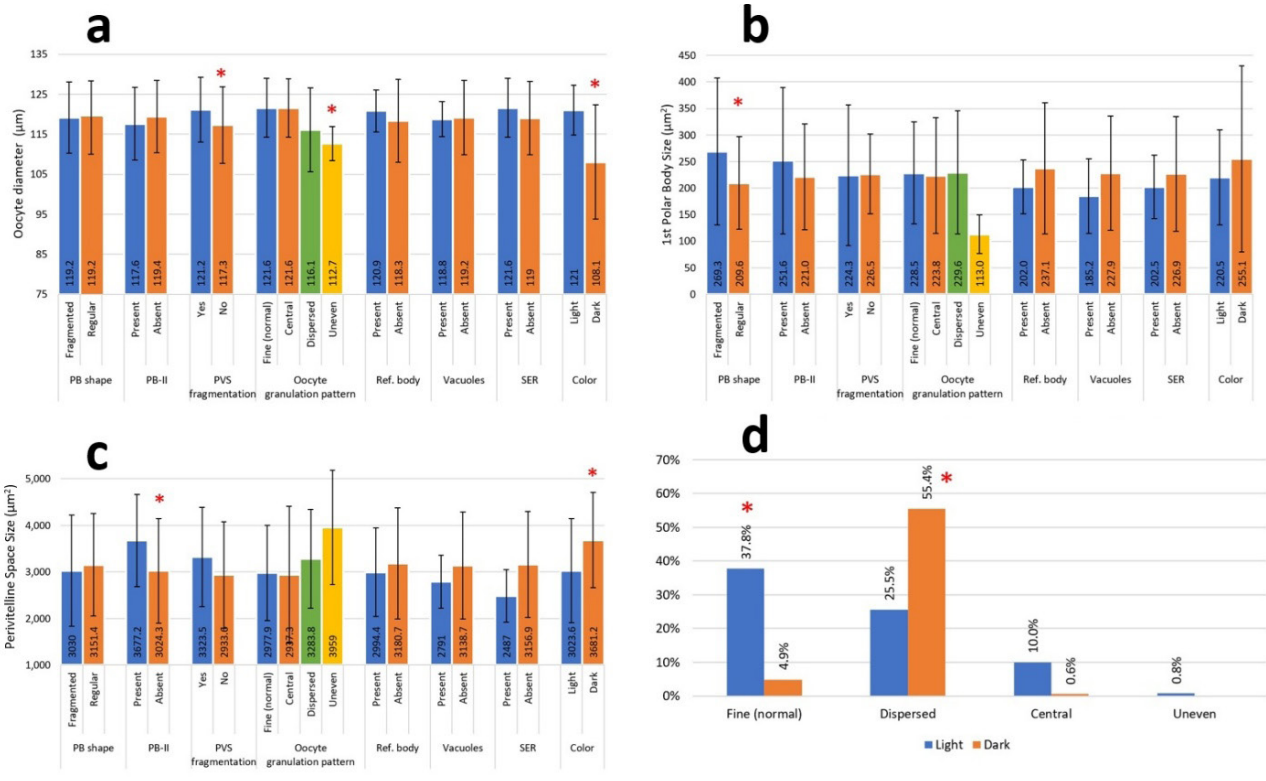

Fig 5. Associations among oocyte morphologic and morphometric parameters, including association between oocyte diameter and oocyte morphologic features (a), association between first polar body size and oocyte morphologic features (b), association between perivitelline space size and oocyte morphologic features (c), and association between oocyte cytoplasmic granulation and oocyte cytoplasmic color (d). An asterisk indicates statistical significance with a p-value <0.05.

We also reported the statistically significant correlations/associations that we identified between some oocyte morphologic and/or morphometric features.

Cytoplasmic granularity is a common finding in metaphase II (MII) oocytes; however, the previously published studies in the literature do not report the exact numbers regarding the incidence of cytoplasmic granulations. The 4 patterns of cytoplasmic granulation are reported to occur in highly variable proportions in oocytes retrieved for IVF. The present study investigated oocytes that failed to cleave, and the cytoplasmic granulation patterns among those oocytes included fine (42.7%), dispersed (40.3%), central (14.5%), and uneven (2.4%). In a large recent study[11], fresh oocytes showed cytoplasmic granulation in the following percentages: uneven (62.1%), fine (20.1%), dispersed (9.9%), and central (7.9%). Most of the other studies investigated the pattern of central granulation in particular, and reported a higher incidence

of central granulation compared to the rate found in our study.[13,14,15] The presence of central granulation pattern had a negative effect on ICSI outcomes in most studies[13,15-17]; however, one study[14] reported no difference in ICSI outcomes between oocytes with central granulation and morphologically normal oocytes. We did not compare the granulation patterns in failed oocytes with those in successfully cleaved oocytes; however, we did find the incidence of CG pattern to be relatively low in failed oocytes.

The association between fine granulation with light-color cytoplasm, and dispersed granulation with dark-color cytoplasm can be explained by the visual effect of the coarse, large-size granules that characterize dispersed granulation pattern and that give the cytoplasm a darker tint compared to that conferred by fine granules. However, not all dark-color oocytes had a dispersed cytoplasmic granulation, and no similar association was mentioned in the studies we searched during our literature review.

The current literature describes the presence of specific cytoplasmic dysmorphisms, including refractile bodies, sER aggregates, and vacuoles, in the oocytes retrieved for IVF, but none of those studies mention the specific rate of incidence for each one. Refractile bodies were commonly seen in our study (33.3% of studied oocytes), which is a higher rate than the previously reported rate for oocytes before performing ICSI (26.6% according to Takahashi, *et al.* 2019.[18] Smooth endoplasmic reticulum aggregates and cytoplasmic vacuoles were rare (both 5.6%), but were within the range mentioned in the literature (4-23% according to Wang, *et al.* 2023.[19] The prevalence of cytoplasmic vacuolation in our study (5.6%) is also within the range previously reported for MII oocytes (5-12% according to Fancsovitz, *et al.* 2011.[20] The effect of the presence of these morphologic abnormalities on ICSI outcome is related to the number and severity of these dysmorphisms rather than their mere existence.[2]

The mean oocyte cytoplasmic diameter in our study was comparable to the diameter described in the literature for MII oocytes retrieved for intracytoplasmic sperm injection.[21-24] While all of these studies described the oocyte diameter in all oocytes collected for ICSI, our study included only oocytes that failed to cleave after ICSI, and our results show that the diameter of failed oocytes is not significantly different from that of oocytes that were successfully fertilized.

There was a moderate inverse correlation between ooplasmic diameter and the size of the PVS in our study. This was most evident in oocytes with a diameter <100 µm, which was the oocyte subpopulation that had the largest size of PVS. A possible explanation is the early degeneration of these oocytes, which results in oocyte shrinkage and increased PVS size. We were unable to find a similar previously reported correlation in the literature to which we could compare our result.

Giant oocytes are characterized as having both approximately twice the size of normal oocytes and abnormal ploidy, which is defined as diploid, triploid, or tetraploid instead of the normal haploid number of chromosomes. Kitasaka, *et al.* (2022)[24] set a diameter of >130 µm to define giant oocytes. In our study, two oocytes had a diameter slightly larger than 130 µm; however, they didn't have extra sets of chromosomes when examined by immunofluorescence stain. They were, therefore, not categorized as giant oocytes. Larger oocyte diameter was found to be significantly associated with higher rate of fragmentation in the PVS in our study. Fragmentation in the PVS has been associated by some authors to high doses of follicle-stimulating hormone (FSH) used during ovarian hyperstimulation in IVF cycles.[25] Another study found association between FSH use and a number of oocyte morphologic and morphometric criteria, including larger ooplasmic diameter.[26] So our finding may be explained by the use of FSH during controlled ovarian hyperstimulation.

Smaller oocyte diameter was significantly associated with darker-color oocytes, and also with uneven oocyte cytoplasmic granulation in our study. The first association may be due to early degeneration and shrinkage of the oocyte. The data and findings from the very few studies in darker-color oocyte cytoplasm are not enough to support or deny this assumption. The same can be said about the second association in addition to the small number of oocytes with uneven cytoplasmic granulation (3 oocytes), which is insufficiently robust enough to draw confident and reliable conclusions.

The mean ZP thickness of oocytes in this study is consistent with the ZP thickness data reported in the literature.[27,28] Those studies measured ZP thickness in the entire cohort of oocytes collected for ICSI, whereas our study included only oocytes that failed to cleave. This supports the assumption that ZP thickness is a parameter that will likely not differ significantly between those that failed to cleave and the general oocyte cohort.

The method used to estimate the size of the PVS in this study (by obtaining the area of the PVS on the oocyte 2-dimensional image) is probably more accurate than what is described by some authors. Shi, *et al.* (2016)[28] and Faramarzi, *et al.* (2017)[29] measured the maximum distance between the ooplasm and the inner surface of the ZP to estimate the PVS size. Alternatively, Yoshida and Niimura (2011)[30] subtracted the ooplasmic diameter from

the inner ZP diameter and took half of that measurement as the PVS size. Another study assessed and measured the PVS size subjectively.[25] The wide variation in the PVS size observed in our study is also described in many other studies.[28,30-33] Approximately one-third of all retrieved oocytes show a large PVS[7,34,35]; however, the published literature doesn't mention a numerical measurement to define what the term "large PVS" actually characterizes. In our study, oocytes with a normal-looking PVS size measured less than 3000 μm^2, so we considered oocytes with a PVS size >3000 μm^2 to have a large PVS. Accordingly, 60 (48.4%) out of 124 oocytes had a large PVS in our study, and PVS fragmentation was seen in 59 (47.6%) oocytes. These findings suggest a higher chance of failed oocytes having PVS abnormalities (i.e., large PVS and/or PVS fragmentation) compared to all oocytes. This result agrees with previous studies that found a relationship between a large PVS or PVS granulation and a low fertilization rate[33,34] and disagrees with the studies that found no effect of the size or fragmentation of the PVS on the fertilization rate.[31] In our study, a larger PVS was found to be associated with dark-colored cytoplasm, and also with the presence of 2 polar bodies. The first finding may be due to early oocyte degeneration that causes both darker ooplasm and ooplasmic shrinkage (and hence larger PVS) since large PVS has been associated with oocyte degeneration in previous studies.[4] As for the second finding, the extrusion of a second polar body requires more space to accommodate it, so this finding is rational from a mathematical perspective.

The size of every polar body observed in this study was measured to investigate for giant polar body phenomenon, which was observed in one oocyte. Moreover, we found larger PBI size to be significantly associated with PBI fragmentation. A polar body tends to fragment over time, so many authors consider a fragmented PB to be a sign that an oocyte has exceeded it optimal maturity.[31,35,36] This may explain the association between large (overly mature) polar bodies and PBI fragmentation.

The most notable limitation of this study is the relatively small number of oocytes included in our investigation and analysis. Despite our finding of several important statistically significant correlations or associations, further study of oocyte morphology in a much larger sample of oocytes is needed to shore up the findings of this study, and to further elucidate characteristics like giant oocytes and giant polar bodies.

CONCLUSIONS

Morphologic abnormalities in oocytes that failed to cleave after ICSI showed no significant differences compared to the general population of human oocytes. Central cytoplasmic granulation is less frequently encountered, and dispersed cytoplasmic granulation is associated with dark cytoplasmic color. Refractile bodies are more commonly observed in failed oocytes. The oocyte cytoplasmic diameter significantly inversely correlates with the PVS size.

Data Availability Statement

The data supporting the findings of our study are available upon request from the corresponding author, Ali Mohsin Alwaeli, with the following contact information: ali.alwaeli@uomustansiriyah.edu.iq

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DECLARATIONS

Grants and Funding Information

This was an unfunded study.

Conflict of Interest

All authors declare no personal or professional conflicts of interests.

Registration Number of Clinical Trial

None.

Author Contributions

Conceptualization and methodology, A.M.A.; Investigation, A.M.A. and S.S.A.; Formal analysis, M.H.A.; Visualization and writing – original draft, A.M.A.; Writing – review and editing, M.H.K. All authors have read and agreed to the final version of the manuscript.

Use of Artificial Intelligence

No form of AI was used to conduct or report the results of this study.

Ethics Approval

Our study was approved by the Institutional Review Board of Kamal Al- Sameraie Hospital for Infertility Management and In Vitro Fertilization, Baghdad, Iraq under approval number (COA no. 879/2022), and all female patient volunteers provided written informed

consent permitting the use of their failed oocytes in this study.

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