Original Article

Central Asian Journal of Medical Sciences
CAJMS

Cent Asian J Med Sci. 2024 June; 10(2): 76-84 https://doi.org/10.24079/CAJMS.2024.02.005

Impact of Oocyte Morphologic Abnormalities on Embryo Development

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Submitted date: January 3, 2024
Accepted date: May 27, 2024

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Running title: Impact of oocyte morphologic abnormalities on embryo development

Objectives: This study aimed to investigate the relationship between oocyte morphologic features and embryo development following an Intracytoplasmic Sperm Injection (ICSI) procedure. **Methods:** In this retrospective study, 485 mature oocytes were obtained from 70 patients undergoing ICSI cycles. Controlled ovarian stimulation was performed using a standard antagonist protocol. Morpho kinetic variables were analyzed using Time-Lapse Monitoring (TLM). The primary outcome measures were fertilization and reasonable embryo rate. **Results:** The patients' age ranged from 22 to 45 years (34.9±4.9 on average). From all retrieved MII oocytes, 285 (59.0%) showed at least one morphologic aberration. The organelle cluster and ovoid shape were related to a lower fertilization rate (OR: 0.35 p-value 0,045 and OR:0.137; p-value 0,048, respectively). Time to 5 cells (t5) and time to 7 cells (t7) were different between normal and abnormal morphologic oocytes (P-value 0.016 and 0.017, respectively). Average Oocyte Quality Index (AOQI) negatively correlated with the number of good embryos. **Conclusions:** In our study, the fertilization potential of organelle clusters and ovoid-shaped oocytes was lower. The t5 and t7 were significantly longer in the abnormal oocyte group.

Keywords: Embryonic development, Oocytes, Sperm injections, Fertilization in Vitro, Assisted reproductive technique

Introduction

Mongolian-based studies on infertility, such as Purevtogtokh et al., are clinically based; they found an infertility rate of 11.6% in 20131 In a population-based study, Odkhuu et al. found an average infertility rate of 8.2% in 20232 In Vitro Fertilization (IVF) is one of several techniques available to help people with fertility problems have a baby. The treatment results depend on the number and quality of the oocytes3 Oocyte quality and morphology depend on factors such as the patient's age, genetic defects, type of ovarian stimulation, culture condition, and nutrition.[4] For example, in women with Polycystic Ovarian Syndrome (PCOS), mild CC stimulation resulted in increased oocytes and viable embryos compared with non-PCOS women5 85%

of the retrieved oocytes following ovarian hyperstimulation are classified as metaphase II (MII), whereas approximately 10% and 5% of these oocytes are germinal vesicle (GV) and MI oocytes. Oocyte morphological assessment in the laboratory is based on nuclear and cytoplasm maturation. Normal oocyte characteristics are normal-looking cytoplasm, a single polar body, appropriate zona pellucida thickness, and proper perivitelline space6 The variations of oocyte morphology have been studied to a lesser extent, and currently, there is no consensus regarding the impact of oocyte morphology on embryo development. Not every morphology abnormality of oocytes may impact their fertilization and embryo development. The primary aim of the present study was to establish the incidence of oocyte morphological abnormalities. A secondary aim was to evaluate the relationship between oocyte morphological abnormalities and quantitative aspects of the fertilization and embryo development of ICSI cycles. Additionally, we collected the embryo morphokinetic variables using the time-lapse monitoring system and evaluated their relation to oocyte morphology.

Material and Method

This retrospective study analyzed 485 mature oocytes obtained from 70 patients undergoing ICSI cycles in our hospital (Creation and Love Women's Hospital, Ulaanbaatar, Mongolia). The exclusion criteria were as follows: oocyte cryopreservation, in vitro fertilization method used, and embryos were cultured in a conventional incubator. Controlled ovarian hyperstimulation was performed using GnRH antagonist protocol. Patients were treated with the GnRH antagonist (Cetrotide, Merck, USA) and recombinant follicle-stimulating hormone (Gonal-F, Merck, USA). Ovarian stimulation was started on day 3 of the cycle with 150-300 IU/day of rFSH. When the largest follicle reached a diameter of 14 mm, the administration of Cetrotide was started at a dose of 0.25 mg SC daily and continued until the day of hCG administration. The ovulation was triggered with 5000-10000 IU hCG when at least two follicles ≥18 mm in diameter were recorded by ultrasound.

Oocyte retrieval, denuding, and morphological observation

Oocytes were retrieved by transvaginal ultrasound-guided aspiration 35.5 hours after human chorionic gonadotropin (Ovidrel, Merck, USA) administration. The collected oocytes were cultured in IVF media (Vitrolife Co., Switzerland) and incubated at 37oC, under 6% CO2 and 5% O2 for 2–3h. Cumulus cells were removed mechanically using glass pipettes and enzymatically with 75 IU/ml hyaluronidase. Each of the MII oocytes was placed in an ICSI dish for injection. One embryologist performed an oocyte morphology evaluation. The oocyte's morphologic characteristics were classified as extracytoplasmic and intracytoplasmic abnormalities. Extracytoplasmic abnormalities included a fragmented first polar body, abnormal zona pellucida (thick and dark), enlarged perivitelline space, debris in perivitelline, and oval shape. Intracytoplasmic abnormalities included granular cytoplasm, vacuolated cytoplasm, and organelle clustering. The AOQI score was calculated. This score corresponds to the ratio of the total number of abnormalities to the number of MII oocytes.[7]

Assessment of fertilization, embryo quality

Normal fertilization was recognized by the appearance of two pronuclei and two polar bodies 16-18 hours after the ICSI procedure. Oocytes presenting one, three, or more pronuclei were classified as abnormal. Cleavage-stage embryos were evaluated on day 3 after ICSI using CL hospital classification, taking into blastomere count, symmetry, and fragmentation. Embryos, more than 6 evenly sized blastomeres with <20% fragmentation, are classified as good quality.

Time-lapse imaging system

A Primo Vision camera was set to take a picture of the embryos every 10 minutes. The timing of each developmental event was annotated manually by an embryologist according to the European Society of Human Reproduction and Embryology (ESHRE) guideline for the use of time-lapse technology.[8] The time of ICSI was defined as t0, and all time points were reported as hours. The following early kinetic parameters were evaluated: Time to PN fading, time to 2 cells (t2), 3-cells (t3), 4 cells (t4), 5 cells (t5), 6 cells (t6), 7 cells (t7), 8 cells (t8), durations of the second cycle Cc2 (t3-t2), durations of the third cycle Cc3 (t5-t4), period time of complete second synchronous S2 (t4-t3), third synchronous S3 (t8-t5), second cell cycle ECC2 (t4-t2), third cell cycle ECC3 (t8-t4).

Statistical Analysis

Data were analyzed using descriptive statistics (frequency, percentage, mean value, and standard deviation). Binary logistic regression analysis was performed to determine the associations between the dependent variables (fertilization, embryo quality) and independent variables (morphological abnormalities of oocyte). The fertilization and embryo quality variables were dichotomized as combined; 'fertilized or good embryo' was recorded as 1, and 'unfertilized or not good embryo' was recorded as 0. In this analysis, the p-value, odds ratio (OR), and 95% confidence interval (CI) of the OR were calculated to understand how the morphological abnormality was associated with embryo development. We divided the oocytes into two groups with normal morphology and at least one abnormal morphology. An independent t-test was carried out for continuous variables to compare the mean between the groups of oocytes. Pearson's correlation test was performed to verify the relationship between patients' cycle characteristics and several good embryos. Statistical significance was determined at a P-value lower than 0.05. Data were analyzed using the Statistical Package for the Social Sciences computer program (SPSS version 21.0).

Ethical Statement

The study was approved by the Research Ethics Committee of the Mongolian National University of Medical Sciences (No.2023/03- 04).

Results

The mean age of 70 patients included in this study was 34.9±4.9 years (range 22-45). The average body mass index was 24.4±4.4. Patients with primary infertility at 77.1% (n=54) and secondary infertility at 22.9% (n=16). Female infertility rate of 61.4% (n=43), male infertility rate of 20.0% (n=14), unexplained infertility was 1.5%, and the combined rate for couples was 17.1% (n=12). The mean of the infertility period was 65.9±44.4 months (max 156 months). Study population characteristics are presented in Table 1.

Table 1. General characteristics of the study population

Variables	Mean ± SD
Women's age (years)	34.9±4.9
BMI (kg/m2)	24.4±4.4
Infertility duration (months)	65.9±44.5
AFC	7.3±3.8
AMH (ng/ml)	2.2±1.4
FSH (mIU/ml)	8.4±3.5
Total dose of rFSH (IU)	2514.3±990.1
Duration of gonadotropin administration (days)	8.9±2.1
Estrogen level in trigger day (pg/ml)	2221.5±860.0
LH level in trigger day (mIU/ml)	5.4±3.9
Count of oocytes retrieved	8.8±4.7
Count of MII oocytes	6.9±3.9

BMI: body mass index; AFC: antral follicle count; AMH: antimullerian hormone; FSH: follicle-stimulating hormone; rFSH: recombinant follicle-stimulating hormone; LH: luteinizing hormone; MII: metaphase II; SD: standard deviation.

During this study, 614 oocytes were retrieved in 70 cycles, out of which 485 (78.9%) were MII stage, 59 (9.7%) were MI stage, and 70 (11.4%) were GV oocytes. The mean number of oocytes per patient was 8.8±4.7. Our study demonstrated that 200 (41.0%) of MII oocytes had normal morphology, and 285 (59.0%) were abnormality. Abnormalities in the extracytoplasmic area were observed in 58.3% of the cytoplasmic in 41.7% of the analyzed MII oocytes. In particular, granular cytoplasm was present in 30.9% of the oocytes and vacuolated cytoplasm in 7.2%. Polar body fragmentation was observed in 15.1% of

the analyzed oocytes. In 17.7% and 9.7% of the oocytes, debris in perivitelline space (PVS) and enlarged PVS were identified. Abnormalities of zona pellucida, organelle clustering, and oval shape were relatively rare (6.6%, 2.1%, and 1.7% of analyzed oocytes, respectively). The AOQI score was 0.89 (Table 2).

Table 2. Evaluation of the morphological parameters in mature oocytes

Variables	N (%)
Normal oocytes	197 (40.6%)
Abnormal morphological oocytes	288 (59.4%)
Intra-cytoplasmic abnormality	195 (40.2%)
Granular cytoplasm	150 (30.9%)
Vacuolated cytoplasm	35 (7.2%)
Organelle clustering	10 (2.1%)
Extra-cytoplasmic abnormality	246 (50.7%)
Debris in perivitelline space	86 (17.7%)
Enlarged perivitelline space	47 (9.7%)
Fragmanted first polar body	73 (15.1%)
Abnormalities of zona pellucida	32 (6.6%)
Ovoid shape	8 (1.7%)

As shown in Figure 1, no correlations were found between age, body mass index, anti-mullerian hormone level, antral follicle count, total dose of FSH, and the number of good embryos. On the other hand, we observed that FSH level on menstrual cycle day three and AOQI negatively correlated with good embryo count (r -0.405 and -0.26, P-value 0.001 and 0.03, respectively). The number of retrieved oocytes and estrogen level on trigger day correlated positively with an increased count of good embryos (r= 0.616 and 0.458, P-value<0.001, respectively).

Statistical analysis showed that the presence of organelle

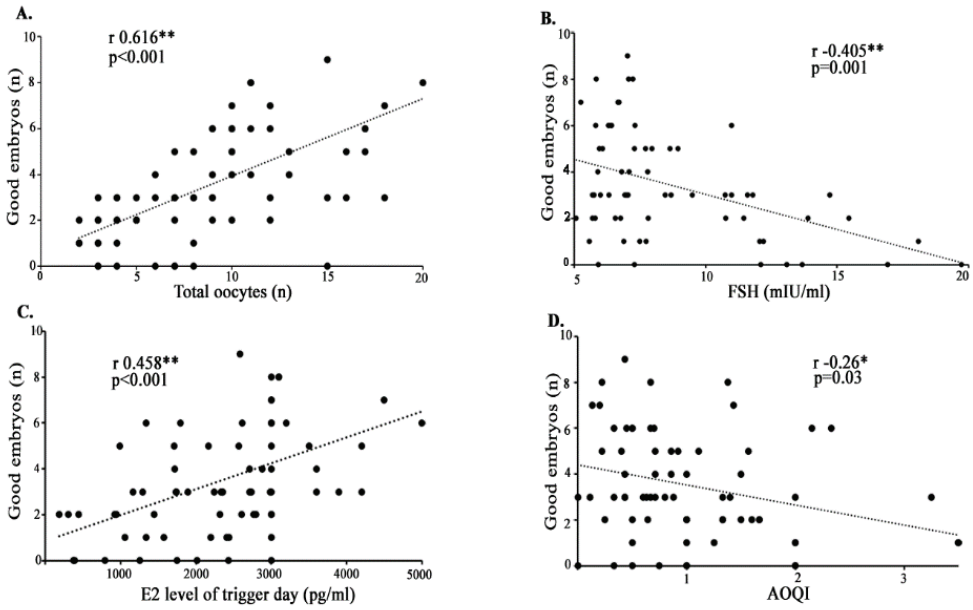

Figure 1. Relationship between oocyte morphology and fertilization rate

clusters and ovoid shapes significantly correlated with decreased fertilization rate. The fertilization rate obtained was 60% for oocytes with organelle clusters (OR 0.35, 95% CI 0.03-0.87) and 50% for ovoid-shaped oocytes (OR 0.137, 95% CI 0.02-0.98). No impact of the other abnormalities on fertilization rate was observed (Table 3).

Relationship between oocyte morphology and embryo quality

Embryo quality was not affected by oocyte morphological abnormalities. In vacuolated cytoplasm oocytes, the incidence of good-quality embryos was lower than other abnormalities but not statistically significant (Table 3).

Table 3. Binary logistic regression analysis of the fertilization and embryo development

Variables	Fertilized oocytes (% per injected oocytes)	Odds ratio (95% CI)	P-value	Good quality embryos (%per fertilized oocytes)	Odds ratio (95% CI)	P-value
Intra-cytoplasmic abnormality						
Granular cytoplasm	116 (77.3%)	OR:1.105 CI: (0.63-1.93)	0.728	71 (61.2%)	OR: 1.38 CI: (0.77-2.46)	0.278
Vacuolated cytoplasm	30 (85.7%)	OR:1.617 CI: (0.58-4.47)	0.355	12 (40.0%)	OR: 0.49 CI: (0.20-1.18)	0.112
Organelle clustering	6 (60.0%)	OR: 0.350 CI: (0.03-0.87)	0.045	3 (50.0%)	OR: 0.99 CI: (0.65-2.09)	0.564
Extra-cytoplasmic abnormality						
Debris in perivitelline space	72 (83.7%)	OR:1.222 CI: (0.60-2.47)	0.577	47 (65.3%)	OR:1.47 CI: (0.75-2.92)	0.264
Enlarged perivitelline space	40 (85.1%)	OR: 2.103 CI: (0.61-7.17)	0.235	28 (70.0%)	OR: 1.53 CI: (0.52-4.54)	0.441
Fragmanted first polar body	64 (87.7%)	OR: 1.808 CI: (1.38-1.47)	0.345	42 (65.6%)	OR: 1.27 CI: (0.63-2.59)	0.507
Abnormalities of zona pellucida	26 (81.3%)		0.497	13 (50.0%)	OR:0.59 CI: (0.24-1.47)	0.261
Ovoid shape	4 (50.0%)	OR: 0.137 CI: (0.02-0.98)	0.048	2 (50.0%)	OR:0.57 CI: (0.04-8.51)	0.687

CI: confidence interval; OR: odds ratio

Relationship between oocyte morphology and embryo morphokinetic variables.

There was mean time to 2-8 cells 28.60±4.48, 37.61±4.81, 40.87±5.34, 50.17±6.80, 54.24±7.25, 57.11±6.98 and 60.7±7.42 hours. Time-lapse monitoring demonstrated a significant relationship between oocyte morphology and embryo morphokinetic variables (t5, t7) (Table 4).

Table 4. Comparison of morphokinetic parameters in oocyte groups with and without

Kinetic parameters (h)	Oocytes with normal morphology	Oocytes with abnormal	P-value
tPNf (n=352)	24.68±4.58	25.77±6.00	0.056
t2 (n=345)	27.83±5.59	28.18±5.18	0.251
t3 (n=278)	36.41±6.58	37.58±6.93	0.164
t4 (n=302)	40.02±7.87	40.23±6.00	0.311
t5 (n=276)	47.32±9.23	50.03±9.09	0.017
t6 (n=267)	52.66±8.97	54.37±8.51	0.124
t7 (n=227)	55.09±7.69	57.84±8.62	0.014
t8 (n=208)	59.21±8.76	61.56±9.06	0.064
Cc2 (t3-t2)	11.06±8.26	11.80±8.82	0.717
Cc3 (t5-t4)	14.31±11.30	14.23±11.43	0.723
S2 (t4-t3)	12.13±17.20	9.95±15.17	0.393
S3 (t8-t5)	18.21±17.04	18.92±16.38	0.420
ECC2 (t4-t2)	14.44±9.21	13.90±8.72	0.602
ECC3 (t8-t4)	26.44±12.98	27.13±13.48	0.344

Values are presented as mean ± standard deviation (SD). Statistical significance ($p<0.05$) was determined using an independent t-test.

Discussion

The impact of oocyte morphology on the results of IVF treatment is still debated.[9] Nonetheless, oocyte quality is the most essential factor in female fertility, playing a crucial role during fertilization and subsequent embryo development.[10] The assessment by light microscopy can display the presence of the first polar body (PBI) in the perivitelline space (PVS), which is considered to be a marker of nuclear maturation.[11] Oocyte cytoplasmic maturation is less well-defined than nuclear maturation but equally essential. Cytoplasmic maturation entails proper relocation of organelles, synthesis of proteins, and post-translational modifications of mRNAs accumulated during oogenesis required to complete meiosis, subsequent fertilization, and preimplantation embryo development.[12] Disturbances of oocytes' nuclear and cytoplasmic maturation may lead to various morphologic abnormalities[13] Morphologic abnormalities are considered detrimental factors in assessing the quality of oocytes, and some abnormalities affect embryo development in ICSI cycles.[14] In this study, to identify oocyte morphological abnormalities of predictive value for further embryo development, 485 MII oocytes from 70 couples were analyzed. We found that 59% (285) of the mature MII oocytes had at least one feature of morphological abnormalities. The overall incidence of morphological abnormalities in human oocytes after controlled ovarian hyperstimulation has been reported to be as high as 60-70% .[15-17] In 2022 Dmitri, et al. review study, which combined the results of 52 studies, revealed cytoplasmic granular changes in 19%, polar body fragmentation in 37%, perivitelline space enlargement in 18%, and granular PVS in 21%, which were higher than our study. However, the percentage of cytoplasmic vacuoles and cytoplasmic organelle clustering cases was higher.[18] In a previous report, researchers noted a 58% (58 of 100) incidence of dysmorphic oocytes in 35 intracytoplasmic sperm injection (ICSI) cycles, each yielding at least one dysmorphic oocyte. However, when considering all 154 ICSI cycles, including those that produced only oocytes of standard form, the incidence rate dropped to 10.7% (58 of 541).[19] Oocyte morphological abnormalities may result from intrinsic factors, such as genetic defects and age, as well as extrinsic factors, including ovarian stimulation protocols.[20] Most studies similar to ours did not report the ovulation induction protocol or did not differentiate participants by agonist or antagonist protocol. We included the patients who were performed using only GnRH antagonist protocol. The antagonist protocol is a more time-efficient option

than the long protocol. It involves directly stimulating the ovaries with FSH injections without the initial suppression of the natural menstrual cycle.

Successful fertilization is a more complex process, related not only to sperm penetration but to many other processes inherent to oocyte quality that are crucial and responsible for regulating most molecular and cellular mechanisms required for successful fertilization.[21] The previous meta-analysis claimed that some oocyte morphological abnormalities (first polar body and perivitelline space enlarged, refractive bodies, and intracytoplasmic vacuoles) are significantly associated with fertilization rates.[22] Our data suggest that only organelle clustering and ovoid shape are associated with a reduced considerably fertilization rate. Organelle clustering is also described as very condensed centralized granularity.[23] This abnormality was reported as an indication of cytoplasmic immaturity and appeared to be more frequent among patients diagnosed with endometriosis[24] Pronuclear morphology was affected by a centrally located granular area[25] Azita Faramarzi, et al. analyzed the outcomes of 478 MII oocytes retrieved from 52 women. They reported that on assessment of normal fertilized oocytes, 284 (77.17%) had at least one abnormal morphological characteristic.[26] The previous two studies have demonstrated that the similar presence of some abnormalities in mature oocytes may affect further development. Among abnormal features of mature oocytes, diffused or central granulation of cytoplasm and oocyte shape may impair subsequent development [27,28]

Most previous studies were performed based on static conventional observation. In recent years, continuous monitoring of embryo development until the blastocyst stage has been possible using time-lapse tracking. We assess oocyte morphology and its influence on the embryo morphokinetic parameters using time-lapse monitoring. In a previous study, researchers revealed no effect of oocyte morphology on the morphokinetic time points of derived embryos, except for the second polar body extrusion time (ESPB).[26] In our study, two morphokinetic variables in the abnormal oocyte group (time to 5 cells and time to 7 cells) were significantly longer than the standard oocyte group. Prolonged cell cycles in the human preimplantation embryo are likely associated with activated DNA repair processes, incorrect attachment of chromosomes to the spindle, or failure to complete previous phases of the cell cycle appropriately.[29] The relationship between embryo morphokinetic variables and ICSI outcomes has been discussed previously. In these studies, tPNf was associated with live birth and t5 and t7 were associated with implantation and blastocyst formation.[30, 31] In this study, embryo quality was not affected by oocyte morphological abnormalities. We preferred to consider all the oocyte abnormalities listed in the AOQI scores and not focus on only some. The average oocyte quality index negatively correlated with several good embryos in our study.

Our study has two main limitations. The first limitation is the small number of participants. The sample size was relatively small, which may limit the generalizability of the findings to the broader infertile patients. Second, we have not included information such as blastocyst rate, implantation, and pregnancy outcome, which are crucial in IVF treatment. Because of these reasons, future studies are needed to validate the findings in the present study. A larger sample size would have allowed for examining subgroups within the patients of IVF treatment, which may be necessary for identifying potential differences in the relationships between variables. Another possible direction for future research is to explore using time-lapse technology-based interventions for abnormal embryo divisions and abnormal fertilization. These interventions may benefit advanced-aged patients with few retrieved oocytes and a high risk of chromosomal abnormalities.

Conclusion

To conclude, we have shown that some abnormalities of oocyte morphology predict not only fertilization but also morphogenetic parameters. We believe that oocyte morphological examination should be introduced into IVF laboratory initial practice. All morphological abnormalities, especially oocytes with organelle clusters and abnormal shapes, need to be assessed carefully.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

The authors acknowledge the Creation and Love Women's Hospital's colleagues and all the participants.

References

1. Purevtogtokh M, Batmunkh G. Prevalence of infertility in Mongolia. Maternal and Child Health 2017;1:180-182.
2. Negdel T, Enkhbat A, Munkhbayar B, et al. Prevalence and risk factors of infertility in a Mongolian population. *Asian Pacific Journal of Reproduction* 2023;12:109-116. https://doi.org/10.4103/2305-0500.377501
3. Van Der Gaast MH, Eijkemans MJC, Van Der Net JB, et al. The optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online*. 2006;13:476–480. https://doi.org/10.1016/s1472-6483(10)60633-5
4. Cota AMM, Oliveira JBA, Petersen CG, et al. GnRH agonist versus GnRH antagonist in assisted reproduction cycles: Oocyte morphology. *Reprod Biol Endocrinol*. 2012;10:33. https://doi.org/10.1186/1477-7827-10-33
5. Nisekhkhuu A, Baldandorj B, Khasag A, et al. Mild Ovarian Stimulation for Infertility in Women with Polycystic Ovary Syndrome. *Cent Asian J Med Sci*. 2022;8:4-13. https://doi.org/10.24079/CAJMS.2022.03.002.
6. Magli MC, Jones GM, Lundin K, et al. Atlas of human embryology: from oocytes to preimplantation embryos. *Hum reprod*. 2012;27:2-21. https://doi.org/10.1093/humrep/des229
7. Sigala J, Sifer C, Dewailly D, et al. Is polycystic ovarian morphology related to poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. *Fertil Steril.* 2015;103:112–8. https://doi.org/10.1016/j.fertnstert.2014.09.040
8. Apter S, Ebner T, Freour T, et al. Good practice recommendations for the use of time-lapse technology. *Hum Reprod Open*. 2020;2:1–26. https://doi.org/10.1093/hropen/hoaa008
9. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic literature review. *Hum Reprod Update*. 2011;17:34–45. https://doi.org/10.1093/humupd/dmq029
10. Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Hum Reprod Update*. 2008;14:159–177. https://doi.org/10.1093/humupd/dmm040
11. Gardner D, Weissman A, Howles C, et al. *Textbook of Assisted Reproductive Technologies: Laboratory and Clinical Perspectives*, 3rd ed. London, UK: Informa Healthcare. 2009:5–101.
12. Smith GD. In vitro maturation of oocytes. *Curr Womens Health Rep.* 2001;1:143–151.
13. Ebner T, Sommergruber M, Moser M,et al. Basal level of anti-Müllerian hormone is associated with oocyte quality in stimulated cycles. *Hum Reprod*. 2006;21:2022-2026. https://doi.org/10.1093/humrep/del127
14. Balaban B, Urman B. Effect of oocyte morphology on embryo development and implantation. *Reproductive biomedicine online*. 2006;12:608-615. https://doi.org/10.1016/s1472-6483(10)61187-x
15. Swain JE, Pool TB. ART failure: oocyte contributions to unsuccessful fertilization. *Hum Reprod Update*. 2008;14:431-446. https://doi.org/10.1093/humupd/dmn025
16. Braga DPF, Setti AS, De Cássia S, et al. Influence of oocyte dysmorphisms on blastocyst formation and quality. *Fertil Steril.* 2013;100:748–754. https://doi.org/10.1016/j.fertnstert.2013.05.021
17. Ashrafi M, Karimian L, Eftekhari-Yazdi P, et al. Effect of oocyte dysmorphisms on intracytoplasmic sperm injection cycle outcomes in normal ovarian responders. *J Obstet Gynaecol Res*. 2015;41:1912–1920. https://doi.org/10.1111/jog.12818
18. Nikiforov D, Grøndahl ML, Hreinsson J, et al. Human Oocyte Morphology and Outcomes of Infertility Treatment: A Systematic Review. *Reproductive Sciences*. 2022;29:2768–2785. https://doi.org/10.1007/s43032-021-00723-y
19. Yu EJ, Ahn H, Lee JM,et al. Fertilization and embryo quality of mature oocytes with specific morphological abnormalities. *Clin Exp Reprod Med*. 2015;42:156–162. https://doi.org/10.5653/cerm.2015.42.4.156
20. Loutradis D, Drakakis P, Kallianidis K, et al. Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. *Fertil Steril*. 1999;72:240-244. https://doi.org/10.1016/s0015-0282(99)00233-2
21. Marteil G, Richard-Parpaillon L, Kubiak JZ. Role of oocyte quality in meiotic maturation and embryonic development. *Reprod Biol*. 2009;9:203–224. https://doi.org/10.1016/s1642-431x(12)60027-8
22. Setti AS, Figueira RC, Braga DP, et al. Relationship between oocyte abnormal morphology and intracytoplasmic sperm injection outcomes: a meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2011;159:364–70. https://doi.org/10.1016/j.

ejogrb.2011.07.031
23. Kahraman S, Yakin K, Dönmez E, et al. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. *Hum Reprod*. 2000;15:2390-2393. https://doi.org/10.1093/humrep/15.11.2390
24. Borges E, Braga DPF, Setti AS, et al. Endometriosis Affects Oocyte Morphology in Intracytoplasmic Sperm Injection Cycles. *JBRA Assist Reprod*. 2015;19:235-240. https://doi.org/10.5935/1518-0557.20150046
25. Rienzi L, Ubaldi FM, Iacobelli M, et al. Significance of metaphase II human oocyte morphology on ICSI outcome. *Fertil Steril.* 2008;90:1692-1700. https://doi.org/10.1016/j.fertnstert.2007.09.024
26. Faramarzi A, Khalili MA, Ashourzadeh S. Oocyte morphology and embryo morphokinetics in an intra-cytoplasmic sperm injection program. Is there a relationship? *Zygote*. 2017;25:190-196. https://doi.org/10.1017/s0967199417000041
27. Xia P. Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. *Hum Reprod*. 1997;12:1750–1755. https://doi.org/10.1093/humrep/12.8.1750
28. Verlinsky Y, Lerner S, Illkevitch N. Is there any predictive value of first polar body morphology for embryo genotype or developmental potential? *Reprod Biomed Online*. 2003;7:336–341. https://doi.org/10.1016/s1472-6483(10)61874-3
29. Meseguer M, Rubio I, Cruz M, et al. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. *Fertil Steril*. 2012;98:1481–1489. https://doi.org/10.1016/j.fertnstert.2012.08.016
30. Magli MC, Gianaroli L, Ferraretti AP, et al. Embryo morphology and development depend on the chromosomal complement. *Fertil Steri*l. 2007;87:534-541. https://doi.org/10.1016/j.fertnstert.2006.07.1512
31. Mizobe Y, Tokunaga M, Oya N, et al. Synchrony of the first division as an index of the blastocyst formation rate during embryonic development. *Reprod Medicine Biol.* 2018;17:64–70. https://doi.org/10.1002/rmb2.12070