Zygote

cambridge.org/zyg

A case report of a successful pregnancy after intracytoplasmic sperm injection when all oocytes contained abnormal inclusions in the perivitelline space

Juan Peng, Wenda Zou, Liyu Zhu, Xinlin Guo, Juan Zhang and Hui Li

Zhuzhou Hospital Affiliated to Xiangya School of Medicine, Central South University, Zhuzhou, HN, China

Research Article

Cite this article: Peng J *et al.* (2025). A case report of a successful pregnancy after intracytoplasmic sperm injection when all oocytes contained abnormal inclusions in the perivitelline space. *Zygote*. page 1 of 6. doi: 10.1017/S096719942500005X

Received: 5 February 2024
Revised: 30 December 2024
Accepted: 26 January 2025

Keywords:
case report; debris; embryo; perivitelline space; oocyte

Corresponding author:
Hui Li; Email: lihui_1973@126.com

Abstract

Background: The relationship between oocyte morphology and developmental potential has been a hot research topic in assisted reproductive technology (ART). Whether inclusions in the perivitelline space (PVS) affect ART outcomes remains controversial.

Case Presentation: We present a case report of a 34-year-old G3P1A2 woman who sought ART treatment because of sequelae of pelvic disease. As her husband had severe oligospermia due to the stress on the day of oocyte retrieval, intracytoplasmic sperm injection (ICSI) was performed. After denudation, varying degrees of debris were found in the PVS, but all the oocytes were subjected to ICSI. Among the eleven retrieved oocytes, eight were fertilized. The morphology of the embryos was scored on Days 2 and 3. Five embryos were frozen on Day 3, and two best-quality embryos were subsequently transferred via frozen embryo transfer.

Conclusion: Severe debris in the PVS seems to affect embryo quality but not fertilization. Mild debris in the PVS may have little effect on the outcome of ART treatment. In our patient, after two embryos that were derived from oocytes with relatively few debris in the PVS were transferred, a successful live birth occurred.

Introduction

Oocyte quality is a pivotal factor that directly affects the outcome of the ART cycle (Ferrarini Zanetti *et al.*, 2018). The identification of markers that can be used to evaluate oocyte quality remains a major issue in ART (Minasi *et al.*, 2023). To date, the evaluation of oocyte quality relies mainly on morphological observations, which are easy, safe and fast. Oocyte morphology generally includes the size, shape, cytoplasm, perivitelline space (PVS), polar body and zona pellucida (ZP) (Bartolacci *et al.*, 2022).

The PVS is the space between the oolemma and the ZP and provides a specific extracytoplasmic environment for the development of oocytes and embryos before they hatch out (Talbot & Dandekar, 2003). It is generally accepted that good-quality oocytes should not contain any impurities or granules in the PVS (Guimarães *et al.*, 2021). Previous studies have reported conflicting results concerning whether inclusions in the PVS affect the quality of oocytes (Bartolacci *et al.*, 2022; Nikiforov *et al.*, 2022; Farhi *et al.*, 2002; Yu *et al.*, 2021). Herein, we describe a rare case of a woman with different amounts of debris in the PVSs of all her oocytes. After ICSI and frozen embryo transfer treatment, the woman achieved a successful pregnancy and gave birth to a healthy child.

Case report

A 34-year-old woman with secondary infertility came to our clinic seeking ART treatment in September 2020 because of sequelae of pelvic disease. She gave birth to a healthy baby in 2012 by way of natural conception. Two miscarriages subsequently occurred. The last pregnancy was in 2016. She has not been pregnant since stopping contraception in 2019 and has had a normal sex life. She had regular, 30–33-day menstrual periods. Her hormone profile on Day 2 of the menstruation cycle was as follows: anti-Müllerian hormone, 1.76 ng/mL; follicle-stimulating hormone (FSH), 5.10 mIU/mL; serum luteinizing hormone (LH), 7.15 mIU/mL; oestradiol (E2), 75.99 pg/ml; and progesterone (P), 23.29 ng/ml. The antral follicle counts on Day 3 of the cycle were as follows: right ovary, 3; left ovary, 5. Her 37-year-old partner had normal sperm parameters and routine serology tests. The patient underwent progesterone-primed stimulation protocols. Briefly, 225 IU of urofollitropin (u-FSH) (Lizhu Pharmaceutical Factory, China) daily and 10 mg of medroxyprogesterone acetate (MPA) tablet (Zhejiang Xianju Pharmaceutical Co., China) daily were administered for 13 days starting from the 2nd day of menses and continuing until the day of the ovulation trigger. The total gonadotrophin dose was 3550 IU. When three

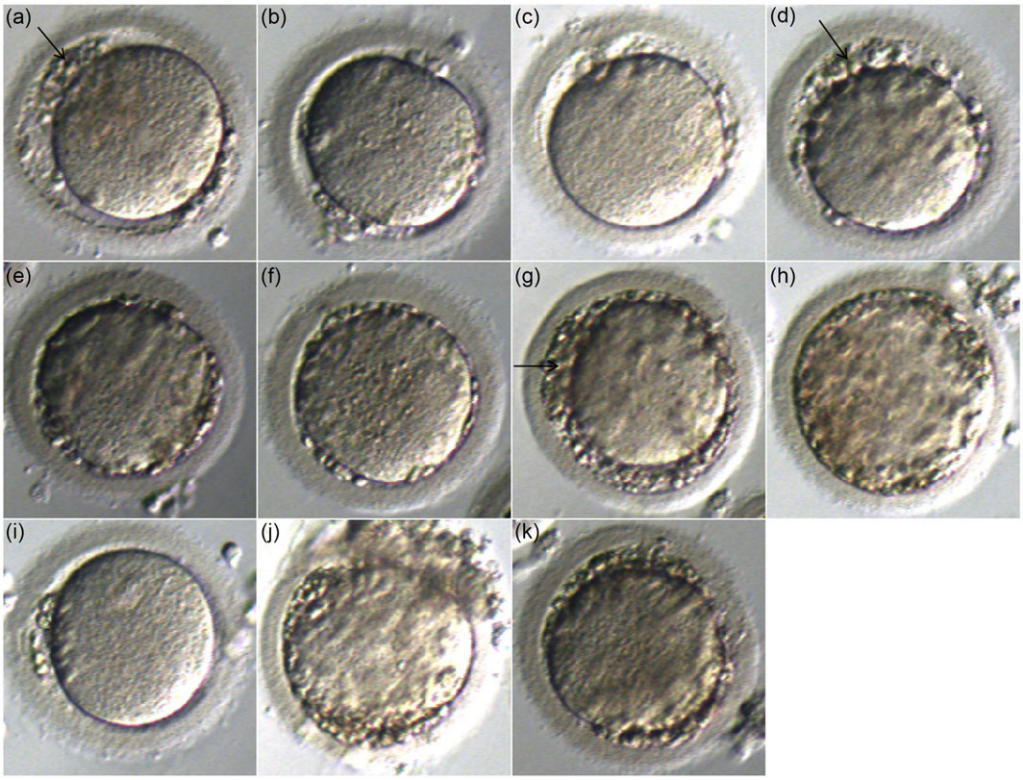

Figure 1. Oocytes exhibiting coarse granular (arrows) in the perivitelline space after the stripping procedure.

dominant follicles reached 18 mm, oocyte maturation waxs triggered with 10000 IU of HCG (Lizhu Pharmaceutical Factory, China) subcutaneously. On this day, her oestradiol level was 2367 pg/ml, and her progesterone level was 1.2 ng/ml, with 3 follicles ≥ 18 mm, 5 follicles 14–18 mm. Ova harvest was carried out 36 h later. Eleven oocytes were retrieved. Among the 11 oocytes retrieved, eight were derived from follicles larger than 14 mm, and three were derived from 12–14 mm follicles. Owing to the stress on the day of oocyte retrieval, the semen volume that the husband extracted was only approximately 0.1 ml, and the sperm concentration was 20×10^6/ml. As the husband was not willing to perform a second semen extraction, the total progressively motile sperm count was not sufficient for conventional IVF after semen optimization, and the couple was counselled to pursue ICSI. Four hours later, the oocytes were denuded with hyaluronidase by using micropipettes.

Debris was present in the PVS of all the eggs to varying degrees (Figure 1). Five of the oocytes had severe debris (> 40% of the PVS) (Figure 1 e, g, h, j, k), two oocytes had moderate debris (> 20% to 40% of the PVS) (Figure 1 d, f), and four oocytes had mild debris (< 20% of the PVS) (Figure 1 a, b, c, i). As it was impossible to distinguish the first polar body from the inclusions for some of the oocytes, all the oocytes were used for ICSI immediately after denudation. Then, the oocytes were washed and cultured immediately in individual 30-µl droplets of the cleavage medium. Fertilization was examined 18 h later. Six out of the 11 eggs presented normal fertilization with 2 pronuclei (PNs). Two eggs (Figure 2 c, f) showed 1PN fertilization. Three eggs were degenerated (Figure 2 e, h and k). On Day 2 (42 h after ICSI), 3 embryos were graded as Grade 1 (Figure 3 a, d, i), and five embryos were graded as Grade 2 (Figure 3 b, c, f, g, j). On Day 3 (66 h after ICSI), the cleavage-stage embryos were graded as 8-cell Grade 1, 13-cell Grade 1 (Figure 4 d, a, respectively), 6-cell Grade 2 (Figure 4 f, g, i), and 6-cell, 5-cell, or 4-cell Grade 3 (Figure 4 j, b, c). Embryology grading was performed according to the Alpha Scientists in Reproductive Medicine and the European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group of Embryology (Alpha Scientists In Reproductive Medicine & Eshre Special Interest Group Embryology, 2011). The oocytes shown in Figure 1 to Figure 4 were present in a continuous sequence throughout the culture. For protocol reasons, all available embryos (Figure 4 a, d, g, i) were frozen by vitrification on Day 3 as the endometrium became out of phase.

Eight weeks later, hormone replacement therapy was performed for frozen-thawed embryo transfer. Oestradiol valerate tablets (Bayer Vital GmbH) were administered beginning on Day 3 of the patient's cycle, when the E2 and P levels were 29 pmol/ml and 0.20 ng/ml, respectively. On Day 16, with the E2 and P levels of 350 pg/ml and 0.30 ng/ml, respectively, progesterone was added. To understand the developmental potential of the embryos, the 8-cell Grade 1 and 13-cell Grade 1 embryos were thawed 18 h before embryo transplantation. On the day of transfer, they grew into an early blastocyst and an 8-cell Grade 2 embryo (Figure 5), both of which were transferred. Laser-assisted hatching was performed on the embryos before transfer. The β-hCG level was 299.97 mIU/ml at 9 days posttransfer. Ultrasound examination revealed a good foetal heart at 7 weeks and 2 days. At 41 weeks of pregnancy, a healthy live birth was attained, with the baby weighing 3350 g.

Discussion

Oocyte quality plays a major role not only in fertilization but also in subsequent embryo development (Rienzi *et al.*, 2011). An important non-invasive evaluation of oocyte quality is based on morphological assessment, which is widely used in embryology laboratories. According to previous studies, cytoplasmic vacuoles,

https://doi.org/10.1017/S096719942500005X Published online by Cambridge University Press

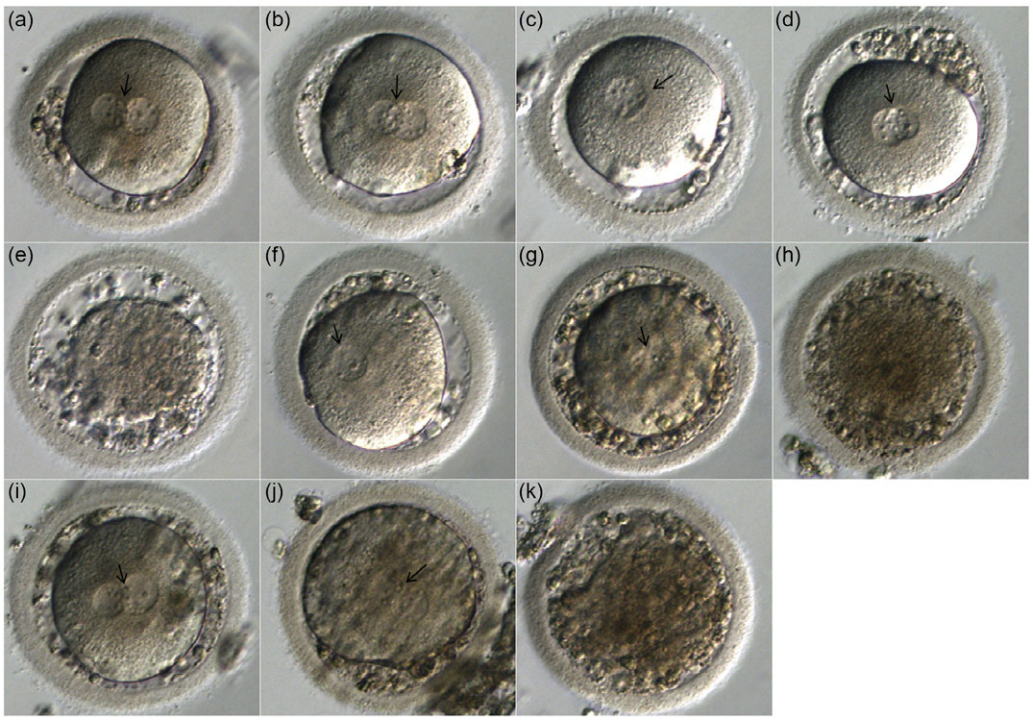

Figure 2. Embryonic development of oocytes at the pronuclear stage. The arrows point to the pronucleus.

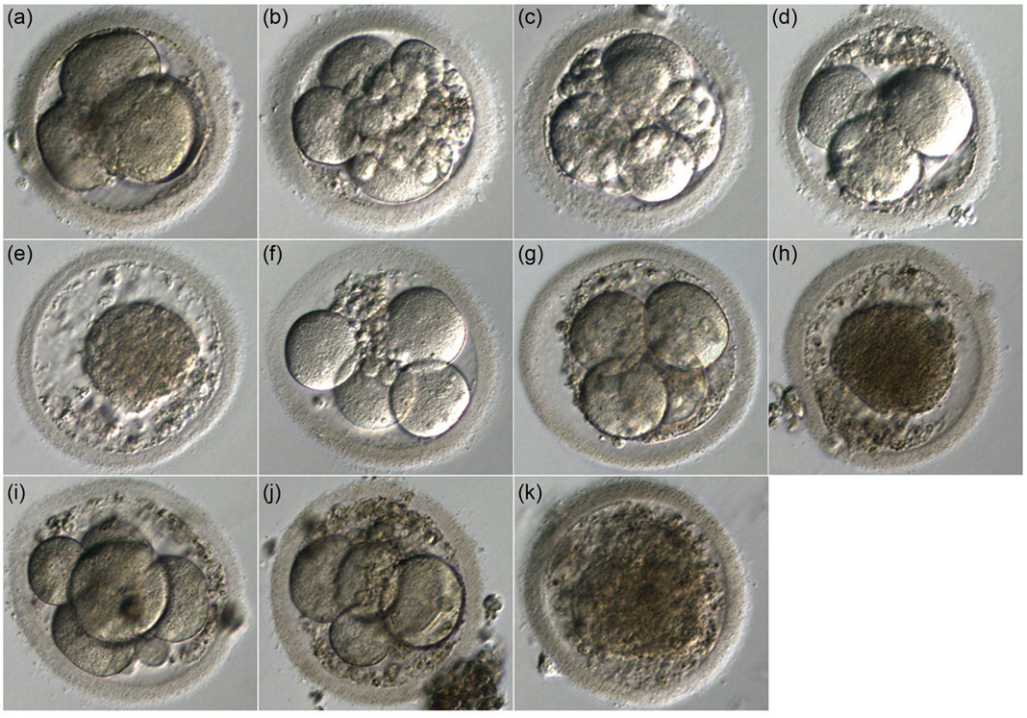

Figure 3. Embryonic development of oocytes at the second day.

centrally located cytoplasmic granularity and clusters of the smooth endoplasmic reticulum were suggested as indicators of low oocyte quality, with a high proportion of aneuploidy (Nikiforov *et al.*, 2022; Van Blerkom & Henry, 1992). However, the influence of certain types of dysmorphic oocytes on embryo quality, implantation rates and pregnancy rates remains unclear (Yu *et al.*, 2015), and there are no clear or well-defined criteria for evaluating oocyte morphology.

PVS abnormalities are the most commonly observed aberrations of extracytoplasmic components (Hassa *et al.*, 2014), and the PVS size and PVS granularity have already been used as parameters to predict embryo quality via artificial intelligence techniques (Lazzaroni-Tealdi *et al.*, 2015; Xia, 1997). However, the fate of embryos with these anomalies remains unclear, leading to contradictory findings in previous studies. Earlier studies revealed that the debris in the PVS negatively affected fertilization and

https://doi.org/10.1017/S096719942500005X Published online by Cambridge University Press

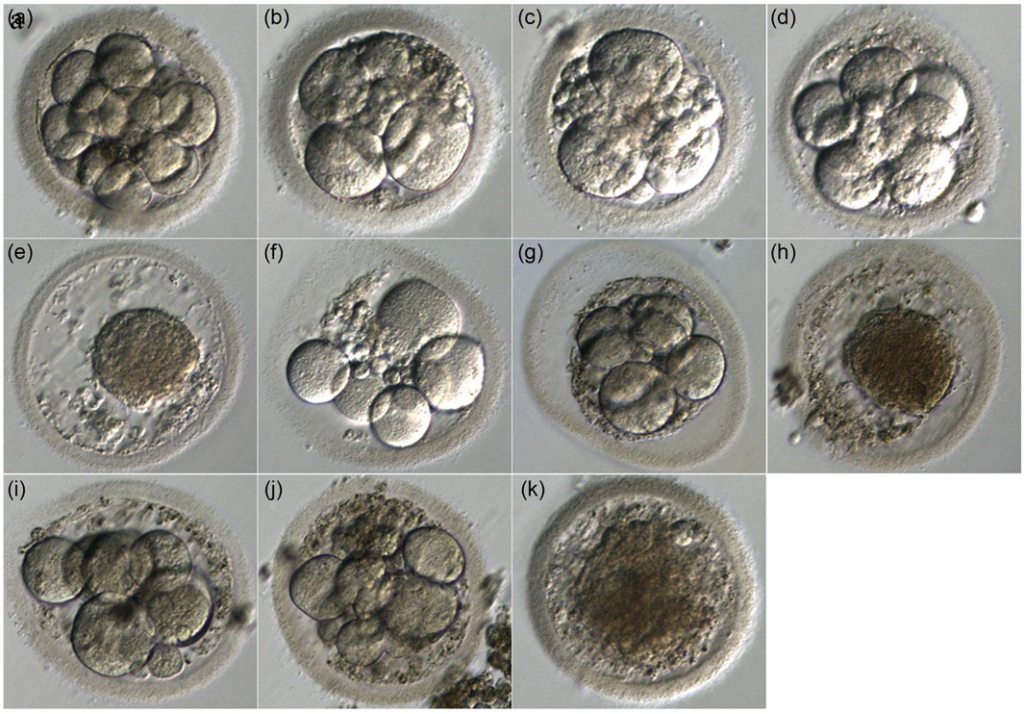

Figure 4. Embryonic development of oocytes at the third day.

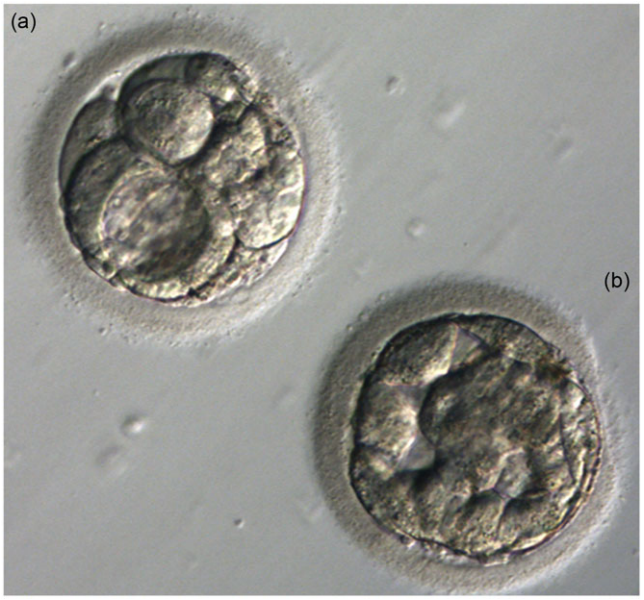

Figure 5. The two transplanted embryos cultured in vitro for 18 h after thawing. (a) 8 cell grade II, (b) Early blastula.

embryo quality (Balaban *et al.*, 1998; Sutter *et al.*, 1996; Meriano *et al.*, 2001). However, Farhi et al. reported that implantation and pregnancy rates were significantly reduced for embryos derived from oocytes with coarse granules in the PVS during IVF–ICSI cycles, but the fertilization rate and embryo quality were not affected (Farhi *et al.*, 2002). The results of Kuran et al. and Chamayou et al. revealed that PVS granulation and granules in the PVS were not a direct connection between implantation rates and clinical pregnancy rates (Kuran *et al.*, 2023; Chamayou *et al.*, 2006), whereas the miscarriage rate was positively correlated with those (Kuran *et al.*, 2023). Moreover, studies have shown no correlation between the presence of the debris in the PVS and embryo development or blastocyst formation (Ten *et al.*, 2007; Hassan-Ali *et al.*, 1998; Braga *et al.*, 2013). In the present case, the entire oocyte cohort presented with debris in the PVS. Although a healthy baby was born, we found that the presence of severe debris in the PVS had a negative effect on subsequent embryo quality, whereas fertilization was not seemingly affected. These results are similar to those of Kuran et al., who reported that the presence of granules in the PVS was positively correlated with poor embryo quality on Day 3 (Kuran *et al.*, 2023). In addition, as the amount of debris gradually decreased with an increasing embryo culture time, these means that the embryos themself can absorb some of it, so mild debris in the PVS may have had little effect on subsequent embryo development. These could be the reasons for the contradictory conclusions of previous studies.

Oocyte quality is affected by many factors, such as advanced age, genetic mutations, inappropriately controlled ovarian stimulation protocols, and poor lifestyles (Vollenhoven & Hunt, 2018; Figueira *et al.*, 2015; Bosch *et al.*, 2016). Few investigations have focused on the origin of PVS inclusions. Hassan-Ali et al. noted that PVS granularity was likely a physiological phenomenon related to oocyte maturation status and was increased by the use of high doses of Human Menopausal Gonadotropin (HMG) (Hassan-Ali *et al.*, 1998). Similarly, Kuran et al. reported that especially prolonged antagonist induction was associated with the presence of coarse granules in the PVS in their study population (Kuran *et al.*, 2023). However, the study by Farhi et al. did not support this hypothesis, and their results revealed no difference between patients with oocytes that repeatedly exhibited coarse granulation in the PVS and control groups (Farhi *et al.*, 2002). One animal study suggested that coarse granulation in the PVS may result from entrapment of cumulus cells in the PVS due to abnormal development of the ZP during the early stages of folliculogenesis (Rankin *et al.*, 1999). A study by Kuran showed

https://doi.org/10.1017/S096719942500005X Published online by Cambridge University Press

that coarse granules in the PVS had a distinct ultrastructure associated with lipid structures (Kuran *et al.*, 2023). In the present case, debris in the PVS could degrade on its own over time; these inclusions likely represent remnants of transzonal processes and were withdrawn from the oocyte surface at LH-induced meiotic resumption/ovulation, but further research is needed. It is still difficult to analyze the cause of this abnormality. Daniele et al. reported a similar feature of perivitelline space abnormality, but in their case, the PVS inclusions could increase the PVS gradually during the first few hours after the injection, stretching the ZP until it broke (Ferri *et al.*, 2023); this phenomenon was different from our case. These results indicate that the origin and impact of PVS inclusions are not singular. More research is needed to better understand the nature of PVS inclusions, including their impacts on fertilization, embryo development, implantation and survival.

In conclusion, this study presents a successful case of conception following an ICSI procedure with all oocytes presenting debris in the PVS. Although most embryos with debris in the PVS are of poor quality, there is still hope for a successful pregnancy.

Data availability. Not applicable.

Acknowledgements. None.

Author contributions. J Peng and H Li collected and analyzed the case. All authors contributed to the article and approved the submitted version.

Competing interests. The authors declare no competing interests.

Funding. The present study was supported by the Natural Science Foundation of Hunan Province (No.2023JJ50216).

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Additional Information and Declarations. Written consent was obtained from the patient prior to writing this article.

Code Availability. Not applicable.

References

Alpha Scientists In Reproductive Medicine & Eshre Special Interest Group Embryology. (2011) Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Human Reproduction*, **26**(6), 1270–1283. doi: 10.1093/humrep/der037.

Balaban, B., Urman, A., Sertac, C., Alatas, S., Aksoy, R. (1998) Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection. *Human Reproduction* **13**(12), 3431–3433. doi: 10.1093/humrep/13.12.3431.

Bartolacci A., Intra G., Coticchio G., dell'Aquila M., Patria G., Borini A. (2022) Does morphological assessment predict oocyte developmental competence? A systematic review and proposed score. *Journal of Assisted Reproduction and Genetics* **39**(1), 3–17. doi: 10.1007/s10815-021-02370-3.

Bosch, E., Labarta, E., Kolibianakis, E., Rosen, M., Meldrum, D. (2016) Regimen of ovarian stimulation affects oocyte and therefore embryo quality. *Fertility and Sterility* **105**(3), 560–570. doi: 10.1016/j.fertnstert.2016.01.022.

Braga, D.P.A.F., Setti, A.S., Figueira, R.D.C.S., Machado,R.B., Jr, A.I., Edson Borges Jr, E.B. (2013) Influence of oocyte dysmorphisms on blastocyst formation and quality. *Fertility and Sterility* **100**(3), 748–754. doi: 10.1016/j.fertnstert.2013.05.021.

Chamayou, S., Ragolia, C., Alecci, C., Storaci, G., Guglielmino, A. (2006) Meiotic spindle presence and oocyte morphology do not predict clinical icsi outcomes: a study of 967 transferred embryos. *Reproductive Biomedicine Online* **13**(5), 661–667. doi: 10.1016/s1472-6483(10)60656-6.

Farhi, J., Nahum, H., Weissman, A., Zahalka, N., Glezerman, M., Levran, D. (2002) Coarse granulation in the perivitelline space and ivf-icsi outcome. *Journal of Assisted Reproduction & Genetics* **19**(12), 545–549. doi: 10.1023/a:1021243530358.

Ferrarini Zanetti, B., Paes, D.A.F.B.D., Souza Setti, A., de Cássia Sávio Figueira, R., Iaconelli, A., Borges, E. (2018) Is perivitelline space morphology of the oocyte associated with pregnancy outcome in intracytoplasmic sperm injection cycles?. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **231**, 225–229. doi: 10.1016/j.ejogrb.2018.10.053.

Ferri, D., Baldini, D., Baldini, G.M. (2023) A successful twin healthy live birth achieved from eggs with a rare perivitelline space abnormality: a case report. *International Journal of Reproductive Biomedicine* **21**(3), 263–267. doi: 10.18502/ijrm.v21i3.13202.

Figueira, R.D.C.S., Aoki, T., Borges Junior, E. (2015) Limitações e controvérsias na determinação do valor preditivo de critérios de morfologia oocitária e embrionária. *Revista Brasileira Ginecologia e Obstetricia* **37**(11), 533–546. doi: 10.1590/SO100-720320150005330.

Guimarães, R.M.G.C., Ribeiro, L.M., Sasaki, L.P., Nakagawa, H.M., Cabral, I. O. (2021). Oocyte morphology and reproductive outcomes-case report and literature review. *JBRA Assisted Reproduction* **25**(3), 500–507. doi: 10.5935/1518-0557.20210001.

Hassa, H., Aydin, Y., Taplamacioglu, F. (2014) The role of perivitelline space abnormalities of oocytes in the developmental potential of embryos. *Journal of the Turkish German Gynecological Association* **15**(3), 161–163. doi: 10.5152/jtgga.2014.13091.

Hassan-Ali, H., Hisham-Saleh, A., El-Gezeiry, D., Baghdady, I., Ismaeil,I., Mandelbaum, J. (1998) Perivitelline space granularity: a sign of human menopausal gonadotrophin overdose in intracytoplasmic sperm injection. *Human Reproduction* **13**(12), 3425–3430. doi: 10.1093/humrep/13.12.3425.

Kuran, S.B., Altun, A., Karakus, F.N., Kotil, T., Selcuk, B.O. (2023) Ultrastructure of coarse granules in the perivitelline space and association with ovulation induction protocols. *JBRA Assisted Reproduction* **27**(4), 660–667. doi: 10.5935/1518-0557.20230032.

Lazzaroni-Tealdi E, Barad D.H., Albertini D.F., Yu Y., Kushnir V.A., Russell H., Wu Y.G., Gleicher N. (2015) Oocyte scoring enhances embryo-scoring in predicting pregnancy chances with IVF where it counts most. *PLoS One* **10**(12), e0143632. doi: 10.1371/journal.pone.0143632.

Meriano, J.S., Alexis, J., Visram-Zaver, S., Cruz, M., Casper, R. F. (2001) Tracking of oocyte dysmorphisms for ICSI patients may prove relevant to the outcome in subsequent patient cycles. *Human Reproduction* **16**, 2118–2123. doi: 10.1093/humrep/16.10.2118.

Minasi M.G., Anagnostopoulou C., Boitrelle F., Vogiatzi P., Sallam H., Saleh R., Colpi G., Agarwal A. (2023) Oocytes evaluation and in-vitro fertilization/intra cytoplasmic sperm injection outcomes. *Panminerva Medica* **65**(2), 179–187. doi: 10.23736/S0031-0808.23.04838-3.

Nikiforov, D., Grøndahl, M.L., Hreinsson, J., Andersen, C.Y. (2022) Human oocyte morphology and outcomes of infertility treatment: a systematic review. *Reproductive Sciences* **29**(10), 2768–2785. doi: 10.1007/s43032-021-00723-y.

Rankin, T., Talbot, P., Lee, E., Dean, J. (1999) Abnormal zonae pellucidae in mice lacking ZP1 result in early embryonic loss. *Development (Cambridge, England)* **126**(17), 3847–3855. doi: 10.1242/dev.126.17.3847.

Rienzi, L., Vajta, G., Ubaldi, F. (2011) Predictive value of oocyte morphology in human IVF: a systematic review of the literature. *Human Reproduction Update* **17**(1), 34–45. doi: 10.1093/humupd/dmq029.

Sutter, P. D., Dozortsev, D., Qian, C., Dhont, M. (1996) Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. *Human Reproduction* **11**(3), 595–597. doi: 10.1093/humrep/11.3.595.

Talbot, P., Dandekar, P. (2003) Perivitelline space: does it play a role in blocking polyspermy in mammals? *Microscopy Research and Technique* **61**(4), 349–357. doi: 10.1002/jemt.10348.

Ten, J., Mendiola, J., Vioque, J., De Juan, J., Bernabeu, R. (2007) Donor oocyte dysmorphisms and their influence on fertilization and embryo quality. *Reproductive Biomedcine Online* **14**(1), 40–48. doi: 10.1016/s1472-6483(10)60762-6.

Van Blerkom, J., Henry, G. (1992) Oocyte dysmorphism and aneuploidy in meiotically mature human oocytes after ovarian stimulation. *Human Reproduction (Oxford, England)* **7**(3), 379–390. doi: 10.1093/oxfordjournals.humrep.a137655.

Vollenhoven, B., Hunt, S. (2018) Ovarian ageing and the impact on female fertility. *F1000Research* **7**: F1000 Faculty Rev-1835. doi: 10.12688/f1000research.16509.1.

Xia, P. (1997) Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. *Human Reproduction* **12**(8), 1750–1755. doi: 10.1093/humrep/12.8.1750.

Yu, B., Van Tol, H.T.A., Stout, T.A.E., Roelen, B.A.J. (2021) Cellular fragments in the perivitelline space are not a predictor of expanded blastocyst quality. *Frontiers in Cell and Developmental Biology* **8**, 616801. doi: 10.3389/fcell.2020.616801.

Yu, E.J., Ahn, H., Lee, J.M., Jee, B.C., Kim, S.H. (2015) Fertilization and embryo quality of mature oocytes with specific morphological abnormalities. *Clinical and Experimental Reproductive Medicine* **42**(4), 156–162. doi: 10.5653/cerm.2015.42.4.156.

https://doi.org/10.1017/S096719942500005X Published online by Cambridge University Press