Zygote

cambridge.org/zyg

Research Article

Cite this article: Aizer A *et al*. (2022) Embryos derived from delayed mature oocyte should be cryopreserved and are favourable to transfer in a following endometrium synchronize frozen–thawed cycle. *Zygote*. **30**: 689–694. doi: 10.1017/S0967199422000168

Received: 24 August 2021
Accepted: 18 March 2022
First published online: 8 June 2022

Keywords:
Delayed mature oocyte; Fertilization; Germinal vesicle; Immature oocytes; Intracytoplasmic sperm injection; Metaphase I

Author for correspondence:
Adva Aizer. Infertility and IVF Unit, Department of Obstetrics and Gynecology, Sheba Medical Center, Ramat Gan 52621, Israel.
E-mail: adva.aizer@sheba.health.gov.il

Embryos derived from delayed mature oocyte should be cryopreserved and are favourable to transfer in a following endometrium synchronize frozen–thawed cycle

Adva Aizer[1,2], Meirav Noach-Hirsh[1], Hila Gitman[1], Olga Dratviman-Storobinsky[1], Keren Kaner Slabodnik[1], Ravit Nahum[1,2], Jigal Haas[1,2] and Raoul Orvieto[1,2,3]

[1]Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan, Israel; [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel and [3]The Tarnesby-Tarnowski Chair for Family Planning and Fertility Regulation, at the Sackler Faculty of Medicine, Tel Aviv University, Israel

Summary

Oocytes eligible for intracytoplasmic sperm injection (ICSI) are those that have progressed through meiosis to metaphase 2 (MII). The remaining delayed mature oocytes can be injected, aiming to achieve more embryos and a better chance to conceive. We aimed to assess the outcome of delayed matured oocytes, derived from either germinal vesicles or metaphase 1 (MI), that reached maturity (MII) 24 h following retrieval. The study population consisted of 362 women who underwent 476 IVF cycles. While fertilization rates were comparable between the sibling delayed mature oocyte group compared with injection on day 0 group (58.4% vs 62%, respectively, $P = 0.07$), the top-quality embryo rate per injected MII day 0 oocyte was significantly higher compared with day 1 injected oocyte (57.5% vs 43.9% respectively, $P < 0.001$). Moreover, following fresh transfer of embryos derived from delayed mature oocytes, implantation rate and the clinical pregnancy (CPR) and live-birth rates (LBR) per transfer were 3.9%, 3.3% and 1.6% respectively. When considering the following thawed embryo transfer cycles, implantation, pregnancy and LBR were non-significantly higher (10%, 8.3% and 8.3%, respectively). Although clinical outcomes are significantly lower when using embryos derived from delayed mature oocyte to mature day 0 oocytes, the additional embryos derived from delayed mature oocytes might contribute to the embryo cohort and increase the cumulative live-birth rate per retrieval. Moreover, the embryos derived from delayed mature oocyte favour a transfer in a frozen–thawed cycle rather than in a fresh cycle.

Introduction

Ovarian stimulation (OS) during *in vitro* fertilization (IVF) treatment cycle aims to generate multiple oocytes per single aspiration to maximize its success (Trounson *et al.*, 1981). During the IVF treatment cycle, resumption of meiosis and final oocyte maturation of the prophase-arrested oocytes is induced by the administration of hCG or GnRH agonist prior to follicle aspiration (Zhang *et al.*, 2009). Following aspiration, embryologists examined oocyte morphological appearance and classified their maturity as follows: germinal vesicle (GV), metaphase 1 (MI), or metaphase 2 (MII) (Ebner *et al.*, 2003). Within 38 h after triggering final follicular maturation 85% of the retrieved oocytes are expected to be classified as MII, whereas 10% will present as GV, and 5% of the retrieved oocytes appear as MI oocytes (Rienzi *et al.*, 2008).

Oocytes eligible for intracytoplasmic sperm injection (ICSI) are those that have progressed through meiosis to MII, while the remaining immature oocytes are usually discarded (Coetzee and Windt, 1996). Although the injection of immature oocytes has been reported to result in a low fertilization rate (De Vos *et al.*, 1999; Shu *et al.*, 2007; Li *et al.*, 2011; Álvarez *et al.*, 2013; De Vincentiis *et al.*, 2013; Ko *et al.*, 2015; Margalit *et al.*, 2019), with the potential for abnormal embryonic development due to chromosomal abnormalities and defective cytoplasmic maturation (Racowsky and Kaufman, 1992; Smith, 2001), it is usually adopted in an attempt to increase the number of embryos achieved to enhance the chance of pregnancy (Vanhoutte *et al.*, 2005).

Sachdev *et al.* (2016) demonstrated that embryos derived from immature oocytes that were injected (ICSI) following a delay *in vitro* maturation within 1 day after aspiration, showed equivalent fertilization and blastocyst formation rates. However, these showed increased aneuploidy rates, as demonstrated by preimplantation genetic testing for aneuploidy (PGT-A) prior to embryo transfer (Sachdev *et al.*, 2016). Moreover, embryos derived from delayed mature oocytes may also show a different morphokinetic profile from their sibling oocytes aspirated at the MII stage (Margalit *et al.*, 2019).

© The Author(s), 2022. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

ICSI for delayed immature oocytes has been described in the published literature as having lower outcomes compared with mature oocytes on the day of ovum pick up (De Vos *et al.*, 1999; Shu *et al.*, 2007; De Vincentiis *et al.*, 2013; Ko *et al.*, 2015; Margalit *et al.*, 2019). However, delayed mature oocyte injection increased the number of embryos achieved to enhance the chance of pregnancy (Vanhoutte *et al.*, 2005). Still, when considering patients with very poor ovarian reserve, or those undergoing fertility preservation for different medical backgrounds, any additional embryo might possess a crucial clinical value, including those achieved from the rescue of delayed matured oocytes.

While most of the aforementioned studies have focused on oocytes that reached maturity a few hours after oocyte retrieval or specifically MI delayed oocyte (De Vincentiis *et al.*, 2013; De Vos *et al.*, 1999; Ko *et al.*, 2015; Margalit *et al.*, 2019; Shu *et al.*, 2007), in the present study, we aimed to assess the outcome of all *in vitro* matured oocytes, either derived from GV or MI, that reached maturity (MII) and injected (ICSI) 24 h following retrieval. Although it is reasonable to perform ICSI for delayed immature oocytes on the day after ovum pick up in cases in which the fertilization rate is low, in our centre the delayed ICSI was performed in all cases in which it was possible in terms of the workload in the laboratory.

Of notice, Ming *et al.* (2012) analyzed the outcome of fresh versus frozen–thawed embryo transfer (ET) following rescue ICSI cycles performed in cases of total fertilization failure in conventional IVF cycles. Significantly higher clinical pregnancy and implantation rates were achieved following frozen–thawed compared with fresh ET. They therefore recommended that embryos derived from rescue ICSI cycles should be cryopreserved and subsequently used in frozen–thawed cycles. Prompted by this information, and due to the asynchrony between the endometrium and embryonic development stage of those derived from delayed mature oocytes, we aimed to further evaluate the ET timing, either in the index fresh cycle or during a subsequent frozen–thawed ET.

Materials and methods

Study population

We reviewed the computerized files of all consecutive patients admitted to our IVF programme at Sheba medical centre from September 2016 to December 2020. For the purpose of this study, we included only patients undergoing IVF who had at least one injected day 1 delayed mature oocyte. The decision whether to inject a delayed mature oocyte was according to the workflow in the IVF laboratory and the fertilization oocyte status on day 1.

The study was approved by the Institutional Review Board of the Sheba Medical Center ethical committee.

Ovarian stimulation

The decision as to which type of OS protocol to use was made by the treating physician. Most of the patients underwent OS using the GnRH antagonist protocol and the remaining used the long GnRH agonist protocol. Gonadotropins were administered in variable doses, depending on patient age and/or ovarian responsiveness in previous cycles. These doses were further adjusted according to serum oestradiol (E2) levels and vaginal ultrasound measurements of follicular diameter obtained every 2 or 3 days. Oocyte retrieval (OPU) was undertaken ~35–38 h post hCG trigger. Endometrial preparation, ET, and other procedures, were performed as previously described (Mohr-Sasson *et al.*, 2020).

Embryo culture, assessment and vitrification

Cumulus–oocyte complexes were isolated during follicle aspiration/ovum pick up (OPU) and then held in equilibrated SAGE fertilization medium + protein (Quinn's advantage protein plus fertilization, SAGE, Cooper Surgical) under SAGE mineral oil (oil for tissue culture, SAGE, Cooper Surgical) in a 37°C 5% O_2 and 5.5% CO_2 atmosphere incubator (benchtop G210 K-system) for 2–4 h. Oocyte denuding was performed ~38 h post final follicular maturation triggering and re-examined 2–3 h later for ICSI. Only MII oocytes were eligible for ICSI and the remaining oocytes were cultured for re-evaluation during fertilization evaluation. Spermatozoa collection for ICSI was performed using ICSI dishes prepared by adding multipurpose handling medium (MHM; FUJIFILM Irvine Scientific, USA) drops covered with paraffin oil (SAGE, Cooper Surgical, Inc., USA). Semen were kept at room temperature for an additional 24 h.

Following ICSI, oocytes were divided into two groups, in the same dish and culture medium: half were incubated in equilibrated Global Total medium (Global Total, Life Global, Cooper Surgical), and the remaining were incubated in equilibrated continuous single culture medium (FUJIFILM Irvine Scientific). The medium drops were covered with SAGE mineral oil. At ~16–20 h later, oocytes were examined for the presence of pronuclei.

Embryos were assessed on day 3 (68 h ± 1 h post-ICSI) based on the individual embryo scoring parameters according to the Istanbul consensus (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). A top-quality embryo (TQE) was defined as having seven or eight blastomeres on day 3, having equally sized blastomeres, and up to 10% fragmentation with no vacuoles or multinucleation. For cycles in which embryos were vitrified or transferred on day 2, four blastomere embryos with up to 10% fragmentation, with equally sized blastomeres and with no vacuoles or multinucleation were also considered to be top-quality embryos. Embryo transfer had been performed mostly on cleavage-stage embryos. According to our laboratory policy, embryos not eligible for transfer/cryopreservation at the cleavage stage are cultured up to days 5–6. Only those that developed to good quality blastocysts were transferred/cryopreserved. Grading of blastocysts was according to the Gardner method (Gardner *et al.*, 2000, 2002), based on the assessment of the inner cell mass and trophectoderm appearance. Only type A and type B blastocysts were transferred or vitrified.

The vitrification–warming methods were carried as previously described (Aizer *et al.*, 2021).

Fertilization, clinical pregnancy, and live-birth rate

For fertilization analysis, sibling oocytes were divided into two groups: Mature oocytes were those documented as MII on day 0 post follicle aspiration and delayed mature oocytes were those documented as MII on day 1 post follicle aspiration (and were defined as MI or GV on day 0). Fertilization rate was defined as the presence of two pronuclear (2PN) divided to the total MII oocyte.

Clinical pregnancy was defined as visualization of a gestational sac and fetal cardiac activity on transvaginal ultrasound. Implantation rate was defined as the number of gestational sacs observed, divided by the number of embryos transferred. The live-birth rate was defined as the number of live offspring delivered, divided by the number of ETs.

https://doi.org/10.1017/S0967199422000168 Published online by Cambridge University Press

Table 1. Patients' baseline clinical characteristics

Number of patients	362
Number of cycles	476
Maternal age, years (mean ± SD)	35.4 ± 5.9
Maternal body mass index (BMI), kg/m^2 (mean ± SD)	24.9 ± 6.1
Smoking (%)	54/362 (14.9)
Type of infertility	
Male (%)	132/362 (36.5)
Tubal (%)	25/362 (6.9)
Endometriosis (%)	17/362 (4.7)
Unexplained infertility (%)	100/362 (27.6)
Ovulatory disorder (%)	16/362 (44.2)
Uterine factor (%)	2/362 (0.6)
Others (%)	131/362 (36.2)

Clinical pregnancy and live-birth rate were calculated for embryos obtained from delayed mature oocytes that were transferred in the fresh or adjacent frozen–thawed cycles.

Statistical analysis

Statistical analysis was performed using Student's *t*-test and chi-squared test, as appropriate. Continuous variables are presented as means ± standard deviations (SD). The significance threshold was set as $P < 0.05$.

Results

The study population consisted of 362 women who underwent 476 IVF cycles, with at least one injected day 1 delayed mature oocyte. The clinical characteristics of the study group are presented in Table 1.

Table 2 presents the laboratory and embryological variables of the corresponding ICSI cycles. In total, 4023 oocytes were aspirated with a mean of 8.5 ± 5.8 oocytes per OPU; 2150 oocytes were mature (MII) on day 0 and 851 of the remaining immature oocytes were injected on day 1. The mean time interval between OPU and injection was 4.5 ± 0.9 and 23.6 ± 3.7 h for mature and delayed mature oocytes, respectively. Figure 1 describes the pronuclear (PN) status on day 2 post OPU during fertilization check. In total, 68/851 (8%) were degenerative, 33/851 (3.9%) contained multi-PN, 24/851 (2.8%) were mono-PN (1PN), 497/851 (58.4%) were 2PN, and 67 had undocumented fertilization. Fertilization rates per injected mature oocytes were comparable between the delayed mature oocyte group compared with injection in the day 0 group (58.4% vs 62%, respectively; $P = 0.07$) (Table 2).

In contrast, the TQE rates per injected MII day 0 oocyte were significantly higher compared with day 1 injected oocytes (57.5% vs 44.4%, respectively; $P < 0.001$) (Table 2).

Clinical outcomes are presented in Table 3. Following fresh embryo transfer, implantation rate and the clinical pregnancy and live-birth rates (LBR) per transfer were 3.9%, 3.3% and 1.6% respectively (Table 3). When considering the following thawed ET cycles, implantation, pregnancy and LBRs were non-significantly higher (10%, 8.3% and 8.3%, respectively) (Table 3).

Table 2. IVF cycles' characteristics

	Total		
Total no. of oocytes retrieved	4023		
Mean oocytes/OPU (mean ± SD)	8.5 ± 5.8		
No. of oocyte denuded for ICSI	3586		
No. of mature oocytes on day 0 (ICSI)	2150		
No. of immature oocytes (MI/GV) on day 0	1288		
No. of degenerative/Z0 oocyte on day 0	148		
No. of delayed mature oocytes on day 1	851		
	Mature oocytes	Delayed mature oocytes	*P*-value
Mean time interval between OPU and injection, h (mean ± SD)	4.5 ± 0.9	23.6 ± 3.7	
No. of fertilized oocytes	1334	564	
Fertilization rate (%)	1334/2150 (62)	497/851 (58.4)	0.07
TQE rate (%)	767/1334 (57.5)	221/497 (44.4)	<0.001

Discussion

Ovarian hyperstimulation is a fundamental step in the success of IVF, enabling the recruitment of multiple oocytes. However, due to the extreme heterogeneity in ovarian response to OS, some patients may yield only a few mature follicles, if any (Ben-Rafael *et al.*, 1994). The follicular cohort might consist of small-sized follicles, yielding immature oocytes that did not complete cytoplasmic maturation (Ectors *et al.*, 1997; Triwitayakorn *et al.*, 2003). The presence of immature oocytes among the cohort of the retrieved oocytes is a common feature that results from heterogeneous, asynchronous follicles at different developmental stages (Smitz and Cortvrindt, 1999) following OS.

Our strategy to perform rescue of delayed mature oocyte was intended for women with zero fertilized oocytes on the next day of OPU, but not only this. It was suitable for patients with low ovarian reserve, patients undergoing preimplantation genetic diagnosis (PGT), in which there were few embryos suitable for ET, and patients undergoing fertility preservation for medical reasons, etc. (Table 1).

In the present study, the fertilization rate of delayed mature oocytes was comparable with mature oocytes after injection on day 0, in which TQE rate was significantly lower. Moreover, following fresh ET, implantation rate and the clinical pregnancy and LBRs per transfer were non-significantly lower compared with the following thawed ET cycles. These observations were in accordance with Ming *et al.* (2012), who emphasized the importance of synchronization between embryo development and the endometrium. Therefore, for good quality embryos derived from delayed mature oocytes, we recommend that the ongoing embryo should be frozen and transferred in a subsequent frozen–thawed cycle. According to our findings, although the clinical outcomes

https://doi.org/10.1017/S0967199422000168 Published online by Cambridge University Press

Table 3. Reproductive outcomes stratified according to fresh vs frozen cycles of delayed mature oocyte-derived embryos

	Fresh	Frozen	*P*-value
No. of ET	61	48	
No. of embryos transferred	84	50	
No. of embryos vitrified	137		
No. of embryos thawed		71	
Survival rate (%)		66/71 (92.3)	
No. of positive β-HCG	3	4	
No. of clinical pregnancy	2	4	
Clinical pregnancy rate/per transfer (%)	2/61 (3.3)	4/48 (8.3)	0.27
No. of sacs observed	3	5	
Implantation rate (%)	3/84 (3.9)	5/50 (10)	0.17
No. of live births	1	4	
Live-birth rate/per transfer (%)	1/61 (1.6)	4/48 (8.3)	0.12

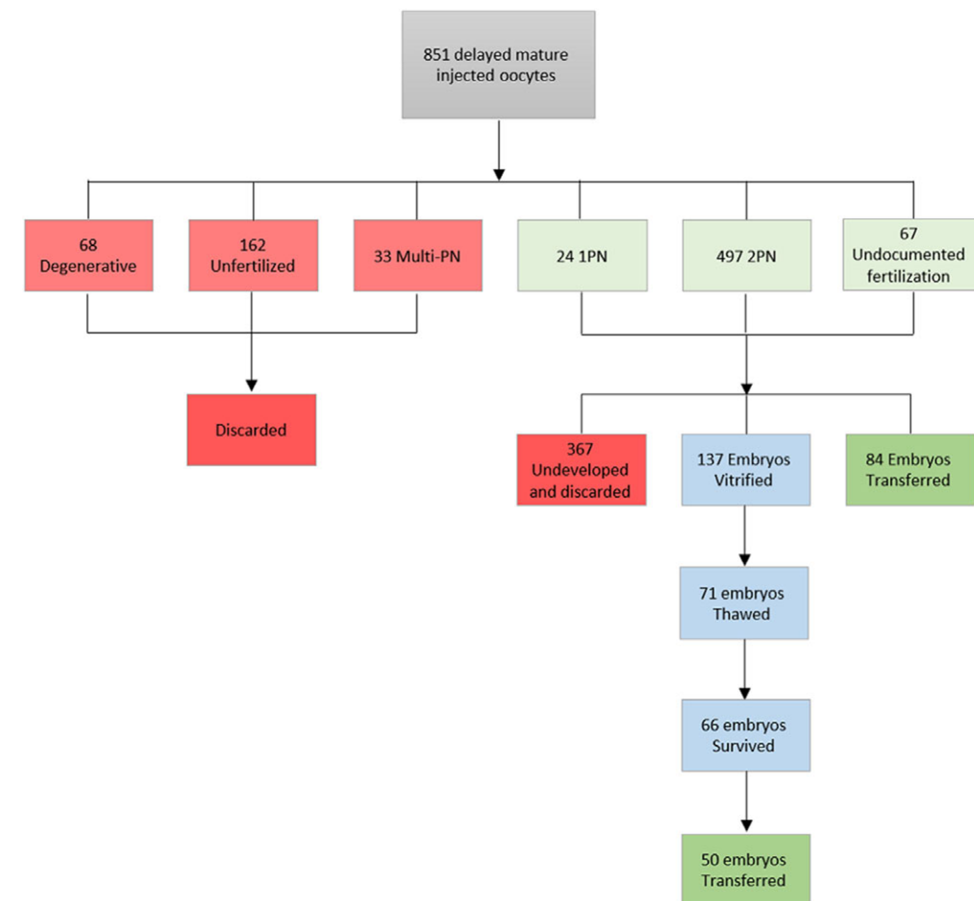

Figure 1. Allocation of delayed mature oocytes according to fertilization outcomes.

of pregnancy and live-birth rate are relatively low, when comparing fresh versus frozen–thawed ET of delayed mature oocyte, the frozen–thawed cycle revealed a better outcome as reflected by non-significantly higher implantation and clinical pregnancy and LBRs.

Whereas delayed mature oocyte may resume meiosis and reach the MII stage, it has lower developmental competence. This could be observed by comparing high-quality embryos derived from sibling oocytes of matured versus delayed mature oocytes. We found that delayed mature oocytes produced significantly lower numbers of high-quality embryos. This observation was in agreement with other studies, demonstrating more cleavage-stage arrest (Chen *et al.*, 2000; Wang and Keefe, 2002), higher numbers of multinucleated blastomeres (Chen *et al.*, 2000) and a reduced development to the blastocyst stage (Li *et al.*, 2006). Generally,

https://doi.org/10.1017/S0967199422000168 Published online by Cambridge University Press

the aforementioned outcomes were in accordance with evidence indicating that delayed matured oocytes had an increased incidence of spindle abnormalities and disrupted chromosomal alignments compared with mature oocytes on day of OPU (Balakier *et al.*, 2004; Strassburger *et al.*, 2004).

The limitations of the present study are its retrospective nature, which limits the ability to control for confounding factors, and its limited sample size. Although CPR and LBR are significantly higher when using embryos derived from mature oocyte compared with delayed mature oocyte, additional embryos derived from delayed mature oocytes might contribute to the embryo cohort and increase the cumulative live-birth rate per retrieval. Moreover, the embryos derived from delayed mature oocyte favoured a transfer in a frozen–thawed cycle rather than in a fresh cycle.

Acknowledgements. The authors would like to thank Moran Madari for her contributions to data collection and secretarial assistance.

Financial support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest. The authors report no financial or commercial conflicts of interest.

Ethical standards. The authors assert that all procedures contributing to this work complied 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.

References

Aizer, A., Noach-Hirsh, M., Dratviman-Storobinsky, O., Haas, J. and Orvieto, R. (2021). Does the number of embryos loaded on a single cryo-carrier affect post-vitrification survival rate? *Zygote*, **29**(1), 87–91. doi: 10.1017/S0967199420000453.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. (2011). The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. *Proceedings of the an Expert Meeting. Human Reproduction*, **26**(6), 1270–1283. doi: 10.1093/humrep/der037

Álvarez, C., García-Garrido, C., Taronger, R. and González de Merlo, G. (2013) *In vitro* maturation, fertilization, embryo development & clinical outcome of human metaphase-I oocytes retrieved from stimulated intracytoplasmic sperm injection cycles. *Indian Journal of Medical Research*, **137**(2), 331–338.

Balakier, H., Sojecki, A., Motamedi, G. and Librach, C. (2004). Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. *Human Reproduction*, **19**(4), 982–987. doi: 10.1093/humrep/deh158.

Ben-Rafael, Z., Orvieto, R. and Feldberg, D. (1994). The poor-responder patient in an *in vitro* fertilization-embryo transfer (IVF-ET) programme. *Gynecological Endocrinology*, **8**(4), 277–286. doi: 10.3109/09513599409023632

Chen, S. U., Chen, H. F., Lien, Y. R., Ho, H. N., Chang, H. C. and Yang, Y. S. (2000). Schedule to inject *in vitro* matured oocytes may increase pregnancy after intracytoplasmic sperm injection. *Archives of Andrology*, **44**(3), 197–205. doi: 10.1080/014850100262173

Coetzee, K. and Windt, M. L. (1996). Fertilization and pregnancy using metaphase I oocytes in an intracytoplasmic sperm injection programme. *Journal of Assisted Reproduction and Genetics*, **13**(10), 768–771. doi: 10.1007/BF02066495

De Vincentiis, S., De Martino, E., Buffone, M. G. and Brugo-Olmedo, S. (2013). Use of metaphase I oocytes matured *in vitro* is associated with embryo multinucleation. *Fertility and Sterility*, **99**(2), 414–421.e4. doi: 10.1016/j.fertnstert.2012.10.028

De Vos, A., Van de Velde, H., Joris, H. and Van Steirteghem, A. (1999). In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. *Human Reproduction*, **14**(7), 1859–1863. doi: 10.1093/humrep/14.7.1859

Ebner, T., Moser, M., Sommergruber, M. and Tews, G. (2003). Selection based on morphological assessment of oocytes and embryos at different stages of preimplantation development: A review. *Human Reproduction Update*, **9**(3), 251–262. doi: 10.1093/humupd/dmg021

Ectors, F. J., Vanderzwalmen, P., Van Hoeck, J., Nijs, M., Verhaegen, G., Delvigne, A., Schoysman, R. and Leroy, F. (1997). Relationship of human follicular diameter with oocyte fertilization and development after in-vitro fertilization or intracytoplasmic sperm injection. *Human Reproduction*, **12**(9), 2002–2005

Gardner, D. K., Lane, M., Stevens, J., Schlenker, T. and Schoolcraft, W. B. (2000). Blastocyst score affects implantation and pregnancy outcome: Towards a single blastocyst transfer. *Fertility and Sterility*, **73**(6), 1155–1158. doi: 10.1016/s0015-0282(00)00518-5

Gardner, D. K., Lane, M. and Schoolcraft, W. B. (2002). Physiology and culture of the human blastocyst. *Journal of Reproductive Immunology*, **55**(1–2), 85–100. doi: 10.1016/s0165-0378(01)00136-x

Ko, D. S., Lee, S. H., Park, D. W., Yang, K. M. and Lim, C. K. (2015). Pregnancy and fertilization potential of immature oocytes retrieved in intracytoplasmic sperm injection cycles. *Clinical and Experimental Reproductive Medicine*, **42**(3), 118–125. doi: 10.5653/cerm.2015.42.3.118

Li, Y., Feng, H. L., Cao, Y. J., Zheng, G. J., Yang, Y., Mullen, S., Critser, J. K. and Chen, Z. J. (2006). Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured *in vitro*. *Fertility and Sterility*, **85**(4), 827–832. doi: 10.1016/j.fertnstert.2005.06.064

Li, M., Li, Y., Ma, S. Y., Feng, H. L., Yang, H. J., Wu, K. L., Zhong, W. X., Che, L. and Chen, Z. J. (2011). Evaluation of the developmental potential of metaphase I oocytes from stimulated intracytoplasmic sperm injection cycles. *Reproduction, Fertility, and Development*, **23**(3), 433–437. doi: 10.1071/RD10228

Margalit, T., Ben-Haroush, A., Garor, R., Kotler, N., Shefer, D., Krasilnikov, N., Tzabari, M., Oron, G., Shufaro, Y. and Sapir, O. (2019). Morphokinetic characteristics of embryos derived from in-vitro-matured oocytes and their in-vivo-matured siblings after ovarian stimulation. *Reproductive Biomedicine Online*, **38**(1), 7–11. doi: 10.1016/j.rbmo.2018.10.002.

Ming, L., Liu, P., Qiao, J., Lian, Y., Zheng, X., Ren, X., Huang, J. and Wu, Y. (2012). Synchronization between embryo development and endometrium is a contributing factor for rescue ICSI outcome. *Reproductive Biomedicine Online*, **24**(5), 527–531. doi: 10.1016/j.rbmo.2012.02.001

Mohr-Sasson, A., Orvieto, R., Blumenfeld, S., Axelrod, M., Mor-Hadar, D., Grin, L., Aizer, A. and Haas, J. (2020). The association between follicle size and oocyte development as a function of final follicular maturation triggering. *Reproductive Biomedicine Online*, **40**(6), 887–893. doi: 10.1016/j.rbmo.2020.02.005

Racowsky, C. and Kaufman, M. L. (1992). Nuclear degeneration and meiotic aberrations observed in human oocytes matured *in vitro*: Analysis by light microscopy. *Fertility and Sterility*, **58**(4), 750–755. doi: 10.1016/s0015-0282(16)55323-0

Rienzi, L., Ubaldi, F. M., Iacobelli, M., Minasi, M. G., Romano, S., Ferrero, S., Sapienza, F., Baroni, E., Litwicka, K. and Greco, E. (2008). Significance of metaphase II human oocyte morphology on ICSI outcome. *Fertility and Sterility*, **90**(5), 1692–1700. doi: 10.1016/j.fertnstert.2007.09.024

Sachdev, N. M., Grifo, J. A. and Licciardi, F. (2016). Delayed intracytoplasmic sperm injection (ICSI) with trophectoderm biopsy and preimplantation genetic screening (PGS) show increased aneuploidy rates but can lead to live births with single thawed euploid embryo transfer (STEET). *Journal of Assisted Reproduction and Genetics*, **33**(11), 1501–1505. doi: 10.1007/s10815–016–0743-z

Shu, Y., Gebhardt, J., Watt, J., Lyon, J., Dasig, D. and Behr, B. (2007). Fertilization, embryo development, and clinical outcome of immature oocytes from stimulated intracytoplasmic sperm injection cycles. *Fertility and Sterility*, **87**(5), 1022–1027. doi: 10.1016/j.fertnstert.2006.08.110

Smith, G. D. (2001). *In vitro* maturation of oocytes. *Current Women's Health Reports*, **1**(2), 143–151.

https://doi.org/10.1017/S0967199422000168 Published online by Cambridge University Press

Smitz, J. and Cortvrindt, R. (1999). Oocyte in-vitro maturation and follicle culture: Current clinical achievements and future directions. *Human Reproduction*, **14** Suppl. 1, 145–161. doi: 10.1093/humrep/14.suppl_1.145

Strassburger, D., Friedler, S., Raziel, A., Kasterstein, E., Schachter, M. and Ron-El, R. (2004). The outcome of ICSI of immature MI oocytes and rescued *in vitro* matured MII oocytes. *Human Reproduction*, **19**(7), 1587–1590. doi: 10.1093/humrep/deh236

Triwitayakorn, A., Suwajanakorn, S., Pruksananonda, K., Sereepapong, W. and Ahnonkitpanit, V. (2003). Correlationbetween human follicular diameter and oocyte outcomesin an ICSI program. *Journal of Assisted Reproduction and Genetics*, **20**(4), 143–147. doi: 10.1023/a:1022977002954

Trounson, A. O., Leeton, J. F., Wood, C., Webb, J. and Wood, J. (1981). Pregnancies in humans by fertilization *in vitro* and embryo transfer in the controlled ovulatory cycle. *Science*, **212**(4495), 681–682. doi: 10.1126/science.7221557

Vanhoutte, L., De Sutter, P., Van der Elst, J. and Dhont, M. (2005). Clinical benefit of metaphase I oocytes. *Reproductive Biology and Endocrinology: RB&E*, **3**, 71. doi: 10.1186/1477-7827-3-71

Wang, W. H. and Keefe, D. L. (2002). Prediction of chromosome misalignment among *in vitro* matured human oocytes by spindle imaging with the PolScope. *Fertility and Sterility*, **78**(5), 1077–1081. doi: 10.1016/s0015-0282(02)04196-1

Zhang, M., Ouyang, H. and Xia, G. (2009). The signal pathway of gonadotrophins-induced mammalian oocyte meiotic resumption. *Molecular Human Reproduction*, **15**(7), 399–409. doi: 10.1093/molehr/gap031

https://doi.org/10.1017/S0967199422000168 Published online by Cambridge University Press