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Late maturing oocyte rescue in poor-prognosis patients: delayed intracytoplasmic sperm injection results in more viable embryos

BIOGRAPHY

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KEY MESSAGE

Metaphase I and germinal vesicle oocytes can mature *in vitro* and produce blastocysts as chromosomally normal as those from metaphase II oocytes, with similar live birth rates. With no differences in birth outcomes, and potential for increased assisted reproductive technology cycle success, delayed intracytoplasmic sperm injection is clinically valuable.

ABSTRACT

Research question: Can delayed intracytoplasmic sperm injection (DICSI) in late maturing oocytes improve fertilization, blastocyst formation, pregnancy and live birth rates for poor-prognosis patients?

Design: Retrospective analysis of 2243 oocytes from 250 poor-prognosis patients who underwent 311 assisted reproductive technology (ART) cycles. Patients were offered DICSI to increase the number of embryos available for testing when over 50% of oocytes collected were immature on day 0, less than 50% of the injected oocytes were fertilized on day 1 or when patients were undergoing preimplantation genetic testing for aneuploidy.

Results: Fertilization and blastulation rates differed depending on the original assessment of the oocyte maturation stage. Euploidy rate did not differ between blastocysts derived from fertilized oocytes originally assessed as metaphase I (MI) or metaphase II (MII). A transferred blastocyst derived from a matured oocyte originally assessed as MI was as likely to result in a live birth as one from a MII oocyte. No differences in delivery method, gestation period or birth weight were found between intracytoplasmic sperm injection and DICSI. As a result of DICSI, at least 27 cycles (8.7%), which would have otherwise been unproductive, resulted in live births, with five ongoing pregnancies.

Conclusions: Both MI and germinal vesicle oocytes can complete maturation *in vitro*. Blastocysts produced from these are likely to be chromosomally normal compared with oocytes originally assessed as MII, and result in similar live birth rates. With no differences in birth outcomes, and DICSI increasing overall ART cycle success, this approach has value for poor-prognosis patients.

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INTRODUCTION

Ovarian stimulation for IVF treatment aims to safely stimulate the growth of multiple ovarian follicles to allow the retrieval of mature, competent oocytes that have progressed to the metaphase II (MII) stage of meiosis that culminates in the extrusion of the first polar body. Oocyte maturity is a key determinant of competency (*Keefe* et al., *2015*), with the use of competent oocytes being crucial for supporting fertilization and subsequent embryonic development (*Esbert* et al., *2024*).

Oocyte competency for successful fertilization requires the synchronous completion of both nuclear and cytoplasmic maturation (*Cha and Chian, 1998*). Immature oocytes are naturally arrested in the ovary at the prophase I stage of meiosis, and characterized by the presence of a germinal vesicle, thereby classifying them as germinal vesicle oocytes. As they mature, these oocytes reach the metaphase I (MI) stage where no germinal vesicle is observable and the first polar body is yet to be extruded. Thereafter, MII oocytes are identified by the presence of a polar body, with the extrusion of the first polar body being documented to be a good indicator of completed nuclear maturation (*Conti and Franciosi, 2018*). Cytoplasmic oocyte maturation is the process by which proteins that will be used by the embryo during early development are synthesized from both mitochondrial and nuclear transcripts. This is not morphologically observable but has been shown to contribute notably to ultimate oocyte competency (*Chian* et al., *2004*).

In standard assisted reproductive technology (ART) laboratory procedures, the minimum follicle size for administering the trigger injection is about 18 mm. Oocyte maturation is expected to occur within 40 h of the trigger, with oocyte retrieval typically taking place 36 h after the injection, followed by an additional 4 h of in-vitro culture. The proportion of oocytes that fail to reach the MII stage varies between patients and cycles, and may be associated with maternal age (*Havrljenko* et al., *2023*), ovarian reserve (*Lorusso* et al., *2007*) and hormonal profile (*Sarhan* et al., *2017*; *Nakhuda* et al., *2023*). After the 4 h incubation period, between 5 and 7% of retrieved oocytes may remain at the germinal vesicle stage (*De Vos* et al., *1999*), and, in general, between 15 and 30% of collected oocytes are still immature after the 4-h incubation (*Jie* et al., *2022*). Given that the number of mature oocytes available for fertilization is critical to the overall success of IVF and intracytoplasmic sperm injection (ICSI) cycles (*Chian* et al., *2004*), this loss of collected oocytes limits the potential of IVF and ICSI. Although adaptations to stimulation protocols (*Anderiesz* et al., *2000*) and tailoring the length of time between oocyte retrieval and ICSI (*Chen* et al., *2023*) may improve oocyte maturation rates, the clinical efficacy of such strategies remains uncertain. Additionally, preimplantation genetic testing for aneuploidy (PGT-A) may be offered to some patients (*Ao* et al., *2006*; *Gorodeckaja* et al., *2020*), e.g. those that have experienced miscarriage, implantation failure through ART or those that are of an advanced maternal age. In these cases, despite PGT-A being intended to shorten the time to pregnancy, it nonetheless reduces the number of embryos that are available for transfer. This effect is compounded by factors such as low oocyte numbers or low oocyte maturity in some patients, further reducing the number of embryos available for transfer.

Some immature oocytes have been observed to complete maturation successfully *in vitro* up to 24 h after oocyte retrieval (*Kim* et al., *2000*). It is still not fully understood, however, why some oocytes fail to mature (*Ozturk, 2022*). Late matured oocytes, derived from either germinal vesicle or MI, present potential avenues for clinical use, particularly in patients who respond poorly to stimulation, or have low rates of oocyte maturity (*Vanhoutte* et al., *2005*; *Vellez* et al., *2020*). The use of such 'rescued' oocytes after in-vitro maturation (IVM), however, presents challenges. Several studies have reported live births from the fertilization of germinal vesicle, and post-germinal vesicle breakdown oocytes, or MI oocytes that matured *in vitro* (*Farsi* et al., *2011*; *Martin-Palomino Olid* et al., *2019*; *Shani* et al., *2023*); however, others have reported lower fertilization (*Moon* et al., *2023*), blastocyst formation, implantation and pregnancy rates (*Reichman* et al., *2010*) compared with those that matured immediately after retrieval (*Ko* et al., *2015*).

Taken together, despite the potential of oocyte rescue, the value of this approach in routine clinical practice remains unclear. In the present study, the effect on fertilization, blastocyst formation, pregnancy and live birth rates of an oocyte rescue programme is assessed using delayed intracytoplasmic sperm injection (DICSI) of late-matured germinal vesicle and MI oocytes that was introduced at a single IVF centre for poor-prognosis patients.

MATERIALS AND METHODS

This retrospective cohort analysis included 2243 oocytes collected from 250 patients who underwent 311 ART cycles between November 2016 and December 2023. Patients with poor prognosis were offered DICSI: oocytes remained immature on day 0 after 4 h of in-vitro culture, when more than 50% of the oocytes collected were immature on day 0, or if less than 50% of the injected oocytes were fertilized on day 1. Patients undergoing PGT-A were also offered DICSI, with the aim of increasing the number of embryos available for testing. In all these cases, immature oocytes were cultured for 16–18 h to allow them to reach the MII stage. The MI and germinal vesicle (GV) oocytes were kept separately to ensure traceability. Cycles were excluded from the analysis if more than 50% oocyte maturity was achieved, over 50% of mature oocytes fertilized or if the cycle involved the use of testicular sperm aspiration derived spermatozoa, or if IVF was used as the method of fertilization. As most (250/311 [81.0%]) of the cycles included in this study contained mature and immature oocytes that were cultured for an additional period, no separate control group of cycles involving the use of only mature oocytes was included. This means that an invidual patient may have contributed immature oocytes assessed as either GV, MI or both, and may have also contiributed MII oocytes. The use of anonymized patient data in this analysis was approved by the University of Kent Research Ethics Advisory Group (approval number CREAG042-02-24, 5 March, 2024).

Ovarian stimulation, fertilization and embryo culture

All the patients included in the present study underwent ovarian stimulation using urinary FSH, recombinant FSH or both, with gonadotrophin releasing hormone antagonist co-treatment used to prevent premature luteinization. A trigger injection of either a gonadotrophin releasing hormone agonist or HCG was administered when the leading follicle

reached a diameter greater than 18 mm, and oocytes were retrieved 35–37 h thereafter. Oocytes were denuded within 4 h of retrieval using hyaluronidase media, followed by ICSI, which was indicated for these patients because of either previous fertilization failures using IVF or male factor infertility. Oocytes classified as being at the germinal vesicle or MI stages were cultured overnight, and their maturity was checked the next morning. Sperm samples were kept at 37°C overnight, and their viability was checked the next morning. All sperm samples used in this study retained their viability. After appropriate patient counselling, DICSI was carried out on the resulting oocytes that matured to the MII stage. After DICSI, oocytes were cultured in an Embryoscope time-lapse incubator (Vitrolife, Viby, Denmark), with fertilization initially checked after 4 h and verified the following day. All embryos were incubated for 7 days or until blastocysts had formed and expanded in continuous single culture medium (Vitrolife, Västra Frölunda, Sweden) in an Embryoscope incubator.

Vitrification and warming procedures

All blastocysts produced by DICSI (germinal vesivcle/MI oocytes), and a proportion of those derived from ICSI (MII oocytes) that were not used in transfers, were frozen using the Irvine Scientific® (USA) vitrification protocol. Blastocysts were transferred into a freezing dish after double witnessing and equilibrated for 12 min in a medium containing 20% dextran serum supplement and 7.5% each of dimethyl sulfoxide and ethylene glycol. The blastocysts were then moved to a vitrification solution containing 20% dextran serum supplement, 15% each of dimethyl sulfoxide and ethylene glycol, and 0.5 M sucrose. Finally, the blastocysts were vitrified in Cryotop® devices (Kitazato BioPharma Co. Ltd., Fuji, Shizuoka, Japan) with minimal freezing media by plunging them into liquid nitrogen for storage.

Between 2 h and 5 h before transfer, warming commenced by submerging the Cryotop® device in pre-equilibrated (37°C) warming solution (Irvine Scientific, Santa Ana, CA, USA) for 60 s, before transfer to a solution of a lower sucrose concentration for 3 min. After this, blastocysts were transferred to a sucrose-free solution for 5 min. After warming, embryos were cultured in an Embryoscope incubator (Vitrolife, Viby, Denmark) in embryo culture media (Vitrolife, Göteborg Sweden).

Embryo biopsy and preimplantation genetic testing for aneuploidy procedures

Of the 408 embryos included in this study, 131 underwent PGT-A as part of the patient's treatment plan. Embryos were cultured to the blastocyst stage and biopsied on days 5, 6 or 7. The procedure involved a trophectoderm biopsy under sterile conditions and after double witnessing. Five to seven cells were removed, washed, and then placed in sterile Eppendorf tubes supplied by CooperSurgical, Inc. (Trumbull, CT, USA). Subsequently, the biopsy samples were sent for genetic testing to determine their ploidy status.

Frozen and fresh embryo transfers and the establishment of pregnancy

In the present study, 62% (101/164) of patients underwent frozen embryo transfer after endometrial preparation with hormone replacement therapy, 34% (56/164) had fresh embryos transferred in a natural cycle and 4% (7/164) underwent frozen embryo transfer in a natural cycle. For patients undergoing hormone replacement therapy, treatment began with a baseline scan in the early follicular phase. If the scan was normal, patients commenced 6–10 mg/day of oestradiol valerate (Progynova) (Bayer, Leverkusen, Germany) to stimulate endometrial proliferation. The oestradiol treatment lasted between 10 and 17 days, with an aim of reaching an endometrial thickness of more than 7 mm. Once this was achieved, luteal support was started with vaginal progesterone pessaries (Cyclogest 400 mg) (L.D. Collins and Co. Ltd, Hemel Hempstead, UK) taken three times daily. After 5 days of progesterone, a single embryo was warmed, cultured for at least 2 h to ensure viability and re-expansion, and then transferred between 2- and 5-h after warming. For patients undergoing fresh embryo transfers, embryos were assessed morphokinetically on day 5 of culture and transferred on the same day. These patients also began using Cyclogest 400 mg pessaries three times daily starting on the day of oocyte retrieval. Luteal support continued for both fresh and frozen transfers until pregnancy was confirmed with an HCG blood test showing levels above 100 mIU/ml, and then maintained until at least 8 weeks of gestation. An ultrasound scan was carried out between 6 and 8 weeks of gestation to verify the presence of a viable intrauterine pregnancy. The miscarriage rate was defined as the spontaneous loss of a confirmed pregnancy before 6-weeks' gestation.

Data analysis

The study groups analysed the numbers of oocytes in each group, and the numbers that proceeded to ICSI or DICSI are presented in FIGURE 1. Data were analysed in R version 4.2.2 (*R Core Team, 2022*), using RStudio (*RStudio Team, 2020*). Quantitative variables were analysed using Kruskal–Wallis tests with, where appropriate, Dunn tests used for post-hoc testing. Qualitative variables were analysed by two-proportion Z tests or Pearson's chi-squared tests. Unless otherwise noted, data are reported as mean ± SD.

RESULTS

In the 311 cycles analysed, 47 cycles were included as they involved PGT-A, with the remainder assessed as poor prognosis. Patients varied in age between 25 and 45 years (mean 38.4 ± 4.0 years), with a mean number of 7.2 (± 5.5) oocytes collected per cycle. Just over two-fifths of the recovered oocytes were at the MII stage (911/2243 [40.6%]), with a mean of 2.9 (± 2.8) MII oocytes identified per cycle. These oocytes underwent ICSI as per patient treatment plans. Analysis of the oocyte recovery data by age showed only limited differences in the proportion of MII oocytes recovered from each individual (FIGURE 2), chi-squared = 10.34, df = 4, $P = 0.04$, with a Holm corrected Dunn test identifying no significant pairwise differences (Supplementary Table 1). The variation in individual rates of MII recovery seen here are poorly explained by differences in maternal age.

Of the 1322 immature oocytes identified in these 311 cycles (with a mean number of 4.2 ± 4.0 immature oocytes identified per cycle), additional incubation time resulted in a total of 798 oocytes that were, by morphology, assessed as mature and subsequently underwent DICSI. Of these, 628 were derived from oocytes originally assessed as MI and 170 were from oocytes originally assessed to be at the germinal vesicle stage. A total of 1709 oocytes, therefore, underwent ICSI or DICSI, with both the fertilization and blastocyst rates observed differing depending on the original assessment of the oocyte maturation stage (TABLE 1). Specifically, a greater proportion of oocytes originally assessed as MI were successfully fertilized ($P < 0.001$), but the overall blastocyst rate

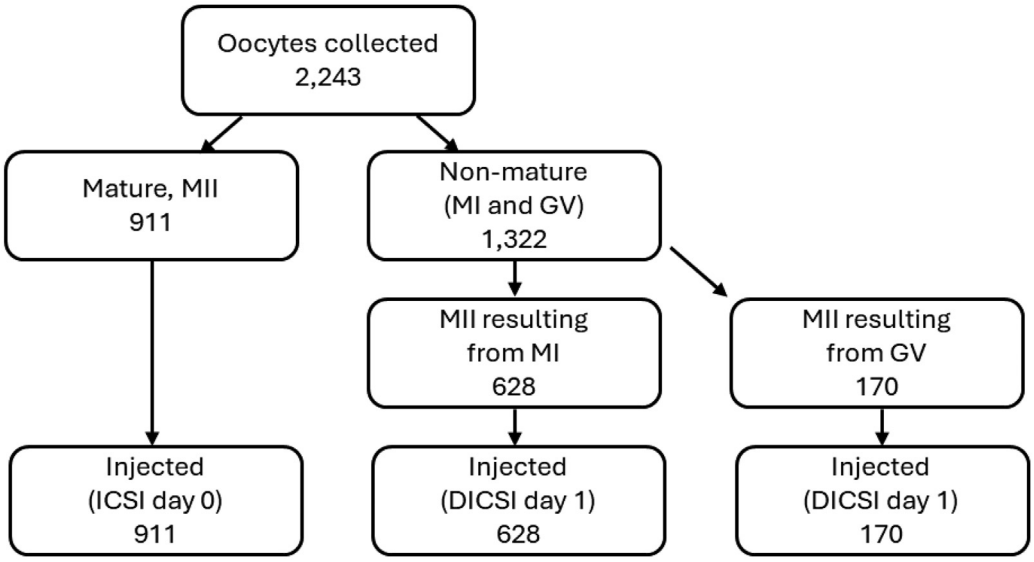

FIGURE 1 Study groups investigated, and the numbers of oocytes that proceeded to intracytoplasmic sperm injection (ICSI) (metaphase II [MII] oocytes), or delayed intracytoplasmic sperm injection (ICSI) (originally non-mature metaphase I [MI] and germinal vesicle [GV] oocytes). Note that 10 collected oocytes could not be conclusively staged and, therefore, were not taken further.

did not ultimately differ between MI and MII oocytes ($P = 0.62$). Conversely, a similar fertilization rate was achieved for oocytes originally assessed as GV and MII ($P = 0.30$), but a lower blastocyst rate was observed for oocytes originally assessed as GV ($P = 0.002$).

Analysis of blastocyst rates by age showed, in contrast to what was observed for the rates of MII recovery, a significant age effect for the oocytes that were originally assessed as MI (FIGURE 3), chi-squared = 24.50, df = 4, $P < 0.001$), but not for oocytes that were originally assessed as either being at either the GV stage or MII (FIGURE 3), chi-squared = 5.47, df = 4, $P = 0.24$ and chi-squared = 6.28, df = 4, $P = 0.18$, respectively for GV and MII oocytes). In the present study, post-hoc analysis of the MI oocytes, via a Holm corrected Dunn test, showed that the blastocyst rates were significantly higher in patients aged 18–34 years compared with other age groups, except patients aged 38–39 years (18–34 years versus 35–37 years, $P = 0.02$; 18–34 years versus 38–39 years, $P = 0.10$, 18–34 years versus 40–42 years, $P = 0.03$ and 18–34 versus 43–50 years, $P < 0.001$), and that the blastocyst rates were higher in the group aged 40–42 years than in the group aged 43–50 years ($P = 0.04$) (for results of all pairwise comparisons see Supplementary Table 2).

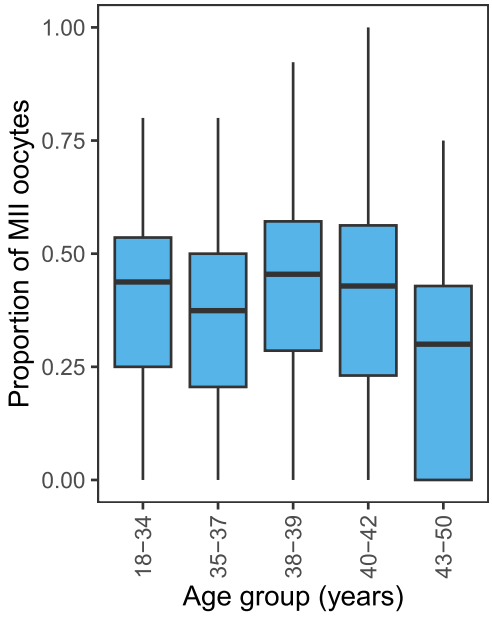

FIGURE 2 Metaphase II (MII) oocyte proportion recovered per cycle as a function of maternal age. Minimal variation between age groups was observed. Pairwise comparison results are presented in Supplementary Table 1.

Of the blastocysts reported here, a total of 131 underwent PGT-A analysis. None of the oocytes originally assessed as GV were found to be euploid (0/3). The euploidy rate for oocytes originally assessed as MI was similar to that for MII oocytes at 25.9% (7/27) and 26.7% (27/101), respectively. This suggests that late developing oocytes are no more likely to be chromosomally abnormal than those that develop within the standard time frame, but, given that these data are from a heterogeneous group of patients, firm conclusions cannot be drawn.

At the time of writing, 164 of the blastocysts obtained have been transferred (TABLE 2), with 56 (34.1%) of these resulting in a live birth. Analysis of transfer outcomes, excluding the ongoing pregnancies as final outcomes, are not known. This indicates that the proportion of live births did not differ significantly for the groups ($P = 0.46$, Fisher's exact test

TABLE 1 FERTILIZATION AND BLASTOCYST RATES FOR MATURE OOCYTES ORIGINALLY ASSESSED AS GERMINAL VESICLE, OR METAPHASE I OR METAPHASE II THAT UNDERWENT INTRACYTOPLASMIC SPERM INJECTION OR DELAYED INTRACYTOPLASMIC SPERM INJECTION

Original assessment of oocyte maturation stage	Mature oocytes that underwent ICSI or DICSI, *n*	Zygotes,[a] *n* (%)	*P*-value	Blastocysts on day 5-7,[a] *n* (%)	*P-value*
Germinal vesicle	170 (DICSI)	92 (54.1)	0.30	24 (14.1)	0.002
Metaphase I	628 (DICSI)	422 (67.2)	<0.001	152 (24.2)	0.62
Metaphase II	911 (ICSI)	450 (49.4)	N/A	232 (25.5)	N/A

P-values derived from two-proportion Z-test comparisons between proportions observed in either metaphase II or germinal vesicle oocytes and those observed in metaphase II oocytes.

[a] Percentage of oocytes that underwent ICSI or DICSI.

DICSI, delayed intracytoplasmic sperm injection; ICSI, intracytoplasmic sperm injection.

comparing totals for all oocytes originally assessed as GV, MI and MII). Therefore, a transferred blastocyst derived from a matured oocyte originally assessed as MI is as likely to result in a live birth as one derived from an MII oocyte that developed within the standard time frame. For the births recorded to date, no significant difference was found between groups in maternal age, and no significant differences were observed in delivery method, gestation period or birth weight (TABLE 3). More births were male in transfers of embryos derived from oocytes originally assessed as MI compared with MII (TABLE 3). The limited numbers of total births to date precludes any more complex analysis of these birth outcomes.

Considered in terms of the success of ART cycles, these data indicate that, for the predominantly poor-prognosis patients analysed here, most (209/311 cycles) produced no transferable blastocysts from oocytes originally identified as MII (Supplementary Figure 1). In contrast, for those cycles with transferable blastocysts from oocytes originally identified as MII, reasonable success rates were achieved (29 live births and six ongoing pregnancies from 96 transfers). The continued culture of MI and GV oocytes, and the subsequent transfer of blastocysts derived from matured oocytes originally assessed as MI or GV, also increased overall success rates. Specifically, 18 live births and three ongoing pregnancies resulted from DICSI in cycles in which no transferable blastocysts had been produced from oocytes originally assessed as MII. Additionally, in cycles in which transfers of blastocysts derived from oocytes originally

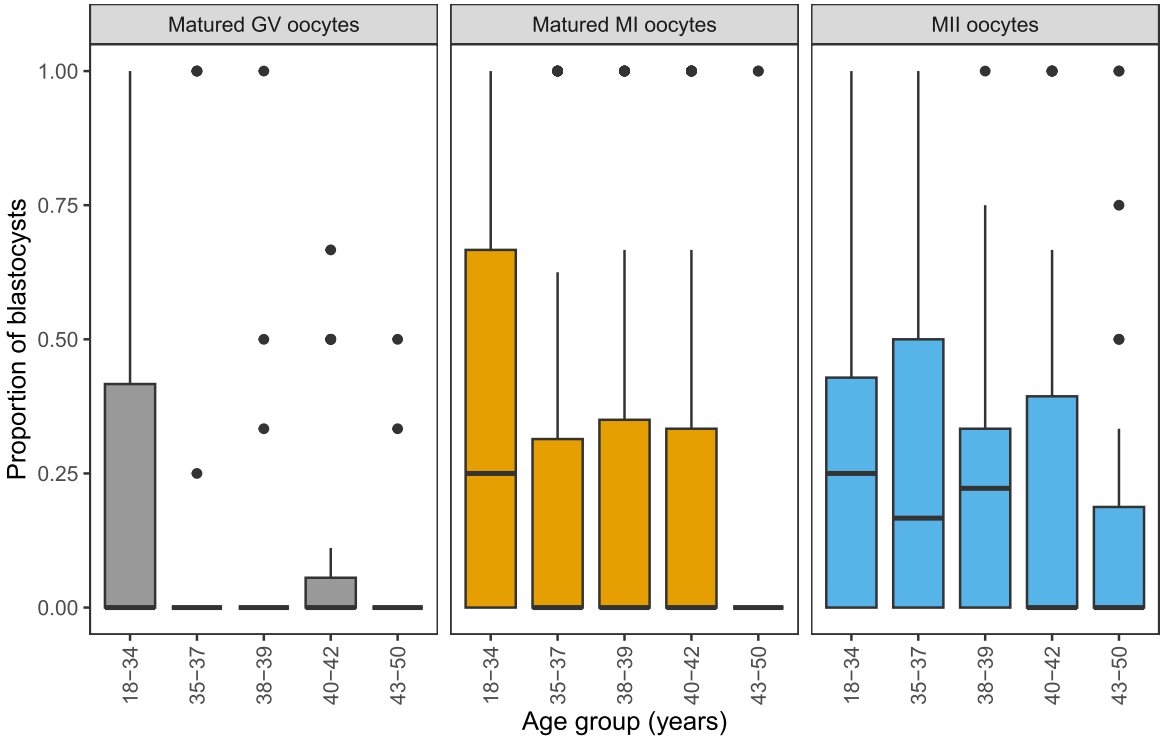

FIGURE 3 Blastocyst proportions that developed from mature oocytes originally isolated at the germinal vesicle stage, and metaphases I (MI) and metaphase II (MII) across the five age groups. An age effect is only seen for matured MI oocytes ($P < 0.001$). Pairwise comparison results are presented in Supplementary Table 2.

TABLE 2 EMBRYO TRANSFER OUTCOMES FOR BLASTOCYSTS DERIVED FROM MATURE OOCYTES ORIGINALLY ASSESSED AS GERMINAL VESICAL, OR METAPHASE I OR METAPHASE II, THAT UNDERWENT INTRACYTOPLASMIVC SPERM INJECTION OR DELAYED INTRACYTOPLASMIC SPERM INJECTION

Original assessment of oocyte maturation stage	PGT-A	Frozen	Cycle type	Transferred, *n*	Live births, %	Ongoing pregnancies, %	Miscarriages, %	Biochemical pregnancies, %	Negative β-HCG test, %
Germinal vesicle total				7	2 (28.6)	1 (14.3)	1 (14.3)	1 (14.3)	2 (28.6)
Germinal vesicle	No	Yes	Modified	7	2	1	1	1	2
Metaphase I total				61	25 (41.0)	3 (4.9)	9 (14.8)	2 (3.3)	21 (34.4)
Metaphase I	Yes	Yes	Modified	3	1	0	2	0	0
Metaphase I	No	Yes	Modified	53	22	3	7	2	18
Metaphase I	No	Yes	Natural	5	2	0	0	0	3
Metaphase II total				96	29 (30.2)	6 (6.3)	4 (4.2)	0 (0)	57 (59.4)
Metaphase II	Yes	No	Natural	2	2	0	0	0	0
Metaphase II	Yes	Yes	Modified	14	8	3	0	0	3
Metaphase II	No	No	Natural	54	12	1	2	0	39
Metaphase II	No	Yes	Modified	24	6	2	2	0	14
Metaphase II	No	Yes	Natural	2	1	0	0	0	1

Data are shown aggregated as the total for each group of oocytes and stratified based on preimplantation genetic testing for aneuploidy, vitrification (frozen) and cycle type, i.e. modified or natural.

β-HCG, beta HCG; PGT-A, preimplantation genetic testing.

assessed as MII had been unsuccessful, the subsequent transfer of blastocysts derived from DICSI resulted in nine live births and a further two ongoing pregnancies. In the cycles analysed, DICSI resulted in a minimum of 27 cycles (8.7%) that would otherwise have been unsuccessful in achieving a live birth. Further to this, nine further cycles, in which all remaining frozen embryos were derived from DICSI, have not achieved a live birth or ongoing pregnancy to date. These have the potential to increase the success rate of DICSI.

DISCUSSION

This study indicates that, even though not all oocytes collected in ART are mature, a proportion of these can complete maturation if they are incubated for longer. The subsequent DICSI of these late matured oocytes resulted in the production of blastocysts from fertilized oocytes originally assessed as both MI and GV; these results are in line with previous studies, such as those reported by Shani et al. (2023) and as recently reviewed by Jie et al. (2022). We observed that a higher proportion of MI oocytes that were matured *in vitro* were successfully fertilized compared with MII oocytes that matured *in vivo*. This may indicate that, in these poor-prognosis patients, oocytes that seem to be at the MII stage at the initial check may be cytoplasmically immature, i.e. a mismatch occurs between nuclear and cytoplasmic maturation in these oocytes. Some evidence suggests that this may be the case, particularly in cases in which a high proportion of immature oocytes are retrieved (Neri et al., 2014; Parrella et al., 2019). Despite the higher fertilization rates, the proportion of blastocysts derived from oocytes originally assessed as MI was similar to that for the MII oocytes matured *in vivo*. The blastocyst formation rate for oocytes originally assessed as GV was lower than that seen in oocytes that matured in the normal time frame, as expected given their more delayed development, but the retention of both GV and MI oocytes increased the total number of blastocysts available for transfer.

Little variation in individual rates of MII recovery was observed across different maternal ages, but this likely reflects the variation in factors that were not controlled for in this study population. For oocytes that were originally assessed as

TABLE 3 CHARACTERISTICS AND OUTCOMES OF LIVE BIRTHS FOR BLASTOCYSTS DERIVED FROM MATURE OOCYTES ORIGINALLY ASSESSED AS GERMINAL VESICAL, OR METAPHASE I OR METAPHASE II

Variable	Germinal vesicle	Metaphase I	Metaphase II	Statistical data
Live births, *n*	2	25	29	–
Maternal age, years	40.0 ± 0	35.8 ± 2.9	36.2 ± 3.5	Chi-squared = 4.43, df = 2, *P* = 0.11
Delivery method, vaginal or caesarean section, *n* (%)	0/2 (0/100)	6/19 (24.0/76.0)	8/21 (27.6/72.4)	*P* = 1.00
Sex, female/male	0/2 (0/100)	6/19 (24/76)	16/13 (55.2/44.8)	*P* = 0.02
Gestation period, weeks	38.1 ± 1.5	39.5 ± 1.0	39.2 ± 1.3	Chi-squared = 2.69, df = 2, *P* = 0.26
Birth weight, kg	3.5 ± 1.0	3.6 ± 0.5	3.5 ± 0.6	Chi-squared = 0.64, df = 2, *P* = 0.73

Values are expressed as number (percentage) or mean ± SD. Quantitative and qualitative variables were analysed using Kruskal–Wallis rank sum test and Fisher's exact test, respectively.

either being at either the GV or MII stage, no effect of maternal age was found on blastocyst rates. Given the limited number of GV oocytes that were included in this study, however, firm conclusions about these oocytes cannot be drawn. For oocytes originally assessed as MI, the blastocyst rate reduced with maternal age; this is unsurprising given that advancing maternal age has been well documented to affect many aspects of ART (*Wennberg* et al., *2016*; *Ubaldi* et al., *2019*). This suggests that DICSI may be more suitable for younger patients.

Studies that have used PGT-A approaches to compare the chromosomal constitution of embryos derived from oocytes matured *in vitro* compared with those matured *in vivo* remain scarce, with some identifying additional euploid embryos and others not (*Vellez* et al., *2020*; *Sam* et al., *2022*; *Shani* et al., *2023*). Only a limited number of the blastocysts analysed here had been tested by PGT-A as part of routine practice, but these data suggest that late developing oocytes are no more likely to be chromosomally abnormal than those developing within the standard time frame. This suggests that PGT-A would not be specifically indicated for the implementation of a wider DICSI treatment option.

Only a limited number of blastocysts derived from oocytes originally assessed as GV were transferred within this study, but these resulted in additional live births. Our findings are, therefore, in concordance with others that report live births from GV oocytes. Although such studies are scarce and report low success rates when using such an approach, e.g. a 5.6% live birth rate was reported in (*Martin-Palomino Olid* et al., *2019*), these findings nonetheless suggest that the use of GV oocytes could offer a viable option for infertility treatment, particularly for patients that have a poor prognosis. In 2014, *Alcoba* et al. *(2014)* highlighted that developing ways to distinguish between GV oocytes that have the potential to mature and fertilize and those that do not would be useful to maximize the efficiency of their use. Such technologies did not exist a decade ago, and observations such as the ones made here suggest that the development of these would still be beneficial.

In conclusion, despite the relatively small number of embryos included in this study, and the fact that immature oocytes were not monitored in a time-lapse incubator, this study supports the argument that late-maturing oocytes can develop into genetically normal blastocysts that lead to successful live births. The continuous culture of immature oocytes would nonetheless be valuable as the optimal timing for DICSI after polar body extrusion could potentially be determined and cytoplasmic maturation could be observed.

Although progress in IVM methods and the identification of competent MII oocytes that result from IVM is being made (*Madkour* et al., *2018*), robust methods need to be developed. Ensuring the correct time that DICSI is carried out is likely to be a critical factor for success, given the complexity seen in maturation when the morphokinetics of maturing GV oocytes is considered (*Escrich* et al., *2012*; *Yang* et al., *2021*). Taken together, these findings underscore the importance of rescuing late maturing GV and MI oocytes for poor-prognosis patients. In the present study, we have shown that DICSI can increase the number of blastocysts suitable for transfer for these patients; in this case, we observed an increase in the number of cycles that resulted in a live birth of almost 10%. Although DICSI might be of value in other patient groups, a recent systematic review and meta-analysis has indicated that rescue IVM can lead to compromised oocyte developmental competence (*Bartolacci* et al., *2024*). Any recommendation for use of DICSI in other patient groups, therefore, requires further investigation.

DATA AVAILABILITY

Data will be made available on request.

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SUPPLEMENTARY MATERIALS

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