Rescue of late maturing oocytes in poor prognosis patients: delayed intracytoplasmic sperm injection (DICSI) results in more viable embryos

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Structured abstract

Research question: Does the application of delayed intracytoplasmic sperm injection (DICSI) in late maturing oocytes have the potential to improve fertilisation, blastocyst formation, pregnancy, and live birth rates for poor prognosis patients?

Design: Retrospective analysis of 2,243 oocytes collected from 250 poor prognosis patients that underwent 311 ART cycles. Patients were offered DICSI when: over 50% of oocytes collected were immature on Day 0, fewer than 50% of the injected oocytes were fertilised on Day 1 or when patients were undergoing PGT-A with a view to increase the number of embryos available for testing.

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

Conclusions: MI and GV oocytes can both complete maturation *in vitro*. The blastocysts produced from fertilisation of these oocytes appear to be as likely to be chromosomally normal as their counterparts and to result in similar live birth rates as blastocysts derived from oocytes originally assessed as MII. With no evident differences in birth outcomes and DICSI apparently increasing overall ART cycle success, this approach is shown to have value for poor prognosis patients.

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Keywords: DICSI; *in vitro* maturation; maternal age; oocyte; poor prognosis.

Introduction

Controlled ovarian hyperstimulation for *in vitro* fertilisation (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 fertilisation and subsequent embryonic development (Esbert et al., 2024).

Oocyte competency for successful fertilisation 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, characterized the presence of a germinal vesicle (GV), thereby classifying them as GV oocytes. As they mature, these oocytes reach the metaphase I (MI) stage where no GV 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 synthesised from both mitochondrial and nuclear transcripts. This is not morphologically observable but has been shown to significantly contribute 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 *c.*18 mm. Oocyte maturation is expected to occur

within 40 hours of the trigger, with oocyte retrieval typically taking place 36 hours after the injection, followed by an additional four hours 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 (Nakhuda et al., 2023; Sarhan et al., 2017). After the four-hour incubation period, between five and seven percent of retrieved oocytes may remain at the GV stage (De Vos et al., 1999), and in general, between 15 and 30% of collected oocytes still appear immature after the four-hour incubation (Jie et al., 2022). Given that the number of mature oocytes available for fertilisation 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 both IVF and ICSI. Whilst 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, pre-implantation genetic testing for aneuploidy (PGT-A) may be offered to some patients (Ao et al., 2006; Gorodeckaja et al., 2020), for example 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 hours post oocyte retrieval (Kim et al., 2000). Whilst it is still not fully understood why some

oocytes fail to mature (Ozturk, 2022), late matured oocytes, derived from either GV or MI, present potential avenues for clinical use, particularly in cases when patients 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 post *in vitro* maturation (IVM) however, presents challenges. While several studies have reported live births from the fertilisation of GV, and post germinal vesicle breakdown (GVBD) oocytes, or MI oocytes that matured *in vitro* (Farsi et al., 2011; Martin-Palomino Olid et al., 2019; Shani et al., 2023), others report lower fertilisation (Moon et al., 2023; Yang et al., 2021), blastocyst formation, implantation and pregnancy rates (Reichman et al., 2010) when compared to 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. Here, we assess the impact on fertilisation, blastocyst formation, pregnancy and live birth rates of an oocyte rescue program using delayed intracytoplasmic sperm injection (DICSI) of late-matured GV and MI oocytes that was introduced at a single IVF centre for poor prognosis patients.

Materials and methods

This is retrospective cohort analysis of 2,243 oocytes collected from 250 patients that underwent 311 ART cycles between November 2016 and December 2023. DICSI was offered to poor prognosis patients when: oocytes remained immature on Day 0 following four hours of *in vitro* culture, when more than 50% of the oocytes collected were immature on day 0, or if fewer than 50% of the injected oocytes were fertilised on Day 1. DICSI was also offered to PGT-A patients

with the aim being to increase the number of embryos available for testing. In these cases, immature oocytes were cultured for 16-18 hours to allow them to reach the MII stage. MI and 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 fertilised, or if the cycle involved the use of testicular sperm aspiration (TESA) derived sperm, or if IVF was used the method of fertilisation. As the majority (81.0%, 252/311) of cycles included in this study contained both 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 anonymised patient data in this analysis was approved by the University of Kent Research Ethics Advisory Group (approval number CREAG042-02-24).

Ovarian stimulation, fertilisation, and embryo culture

All the patients included in this study underwent ovarian stimulation using either urinary or recombinant follicle-stimulating hormone (FSH), or both, with GnRH antagonist co-treatment used to prevent premature luteinization. A trigger injection of either a GnRH agonist or hCG was administered when the leading follicle reached a diameter greater than 18 mm, and oocytes were retrieved 35 to 37 hours thereafter. Oocytes were denuded within four hours of retrieval using hyaluronidase media, followed by ICSI. ICSI was indicated for these patients due to either previous fertilisation failures using IVF or male factor infertility. Oocytes classified as being at the GV 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 performed on the resulting oocytes that matured to the MII stage. Post-DICSI, oocytes were cultured in an Embryoscope time-lapse incubator (Vitrolife, Denmark), with fertilisation initially checked after four hours and verified the following day. All embryos were incubated for seven days or until blastocysts had formed and expanded in continuous single culture medium (Vitrolife, Sweden) in an Embryoscope incubator.

Vitrification and warming procedures

All blastocysts produced by DICSI (GV/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 minutes in a medium containing 20% dextran serum supplement (DSS) and 7.5% each of dimethyl sulfoxide (DMSO) and ethylene glycol. The blastocysts were then moved to a vitrification solution containing 20% DSS, 15% each of DMSO 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 two and five hours prior to transfer, warming commenced by submerging the Cryotop® device in pre-equilibrated (37°C) warming solution (Irvine Scientific, USA) for 60 seconds, before transfer to a solution of a lower sucrose concentration for three minutes. After this, blastocysts

were transferred to a sucrose-free solution for five minutes. Following warming, embryos were cultured in an Embryoscope incubator (Vitrolife, Denmark) in embryo culture media (Vitrolife, Sweden).

Embryo biopsy and Preimplantation Genetic Testing for Aneuploidy (PGT-A) procedures

Out 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 post double witnessing. Five to seven cells were removed, washed, and then placed in sterile Eppendorf tubes supplied by CooperSurgical, Inc. (Trumbull, CT). 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 this study, 62% (101/164) of patients underwent frozen embryo transfer (FET) following endometrial preparation with hormone replacement therapy (HRT), 34% (56/164) had fresh embryos transferred in a natural cycle and 4% (7/164) underwent FET in a natural cycle. For HRT patients, 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) to stimulate endometrial proliferation. The oestradiol treatment lasted between 10 and 17 days, with an aim of reaching an endometrial thickness of more than seven mm. Once this was achieved, luteal support was started with vaginal progesterone pessaries (Cyclogest 400 mg, L.D. Collins and Co. Ltd) taken three times daily. After five days of progesterone, a single embryo was warmed,

cultured for at least two hours to ensure viability and re-expansion, and then transferred between two- and five-hours post-warming. For patients undergoing fresh embryo transfers, embryos were assessed morphokinetically on day five 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 eight weeks of gestation. An ultrasound scan was performed between six and eight 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 six 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 shown 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 means plus and minus the standard deviation (SD).

Results

In the 311 cycles analysed here, 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), with an average 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 an average 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, $X^2 = 10.34$, df = 4, $p = 0.04$, with a Holm corrected Dunn test identifying no significant pairwise differences, for details see Supplementary Table 1). That is, the variation in individual rates of MII recovery seen here are poorly explained by differences in maternal age.

Of the 1,322 immature oocytes identified in these 311 cycles (with an average 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 GV stage. A total of 1,709 oocytes therefore underwent ICSI or DICSI, with both the fertilisation 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 fertilised, but the overall blastocyst rate did not ultimately differ between MI and MII oocytes. Conversely, a similar fertilisation rate was achieved for oocytes originally assessed as GV and MII, but a lower blastocyst rate was observed for oocytes originally assessed as GV.

Analysis of blastocyst rates by age showed, in contrast to what was observed for the rates of MII recovery, that there was a significant age effect for the oocytes that were originally assessed as MI (Figure 3, $X^2 = 24.50$, 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, $X^2 = 5.47$, df = 4, $p = 0.24$ and $X^2 = 6.28$, df = 4, $p = 0.18$, respectively for GV and MII oocytes). Here, *post hoc* analysis of the MI oocytes, via a Holm corrected Dunn test, showed that the blastocyst rates were significantly higher ($p < 0.05$) in the 18-34 group than in all of the other age groups except the 38-39 group, and that the blastocyst rates were higher in the 40-42-year-old group than in the 43-50 group ($p < 0.05$) (for results of all pairwise comparisons, see Supplementary Table 2).

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 timeframe, but given that these data are from a heterogeneous group of patients and hence 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, indicates that the proportion of live births did not differ for the groups ($p = 0.46$, Fisher's exact test comparing totals for all oocytes originally assessed as GV, MI and MII). *i.e.*, a transferred blastocyst derived from a matured oocyte

253 originally assessed as MI is as likely to result in a live birth as one derived from an MII oocyte that
254 developed within the standard timeframe. For the births recorded to date, there was no
255 difference between groups in maternal age and no differences were observed in delivery
256 method, gestation period or birth weight (Table 3). A higher proportion of births were male in
257 transfers of embryos derived from oocytes originally assessed as MI than MII (Table 3). The
258 limited numbers of total births to date precludes any more complex analysis of these birth
259 outcomes.
260
261 Considered in terms of the success of ART cycles, these data indicate that, for the predominantly
262 poor prognosis patients analysed here, the majority (209/311 cycles) produced no transferable
263 blastocysts from oocytes originally identified as MII (for a full breakdown of these data see
264 Supplementary Figure 1). In contrast, for those cycles with transferable blastocysts from oocytes
265 originally identified as MII, reasonable success rates were achieved (29 live births and six ongoing
266 pregnancies from 96 transfers). The continued culture of MI and GV oocytes and the subsequent
267 transfer of blastocysts derived from matured oocytes originally assessed as MI or GV also
268 increased overall success rates. Specifically, 18 live births and three ongoing pregnancies resulted
269 from DICSI in cycles where no transferable blastocysts had been produced from oocytes originally
270 assessed as MII. Additionally, in cycles where transfers of blastocysts derived from oocytes
271 originally assessed as MII had been unsuccessful, the subsequent transfer of blastocysts derived
272 from DICSI resulted in nine live births and a further two ongoing pregnancies. DICSI has therefore,
273 in the cycles analysed here, resulted in a minimum of 27 cycles (8.7%) that would otherwise have
274 been unsuccessful resulting in a live birth. Further to this, there are nine more cycles that have

no live birth or ongoing pregnancy to date, but where all remaining frozen embryos are DICSI derived. 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 fertilised oocytes originally assessed as both MI and GV; these results are in line with previous studies (Shani et al., 2023) and as recently reviewed (Jie et al., 2022). We observed that a higher proportion of MI oocytes that were matured *in vitro* were successfully fertilised when compared with MII oocytes that matured *in vivo*. This may indicate that, in these poor prognosis patients, oocytes that appear to be at the MII stage at the initial check may be cytoplasmically immature, *i.e.*, there is a mismatch between nuclear and cytoplasmic maturation in these oocytes. There is some evidence to suggest that this may be the case, particularly in cases where a high proportion of immature oocytes are retrieved (Neri et al., 2014; Parrella et al., 2019). Despite the higher fertilisation 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 timeframe, as expected given their more delayed development, but the retention of both GV and MI oocytes did increase 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 either being at either the GV or MII stage, there was no evident effect of maternal age on blastocyst rates. Given however the limited number of GV oocytes that were included in this study, 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 impact many aspects of ART (Ubaldi et al., 2019; Wennberg et al., 2016). 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 to those matured *in vivo* remain scarce, with some identifying additional euploid embryos and others not (Sam et al., 2022; Shani et al., 2023; Vellez et al., 2020). 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 timeframe. 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 did result in additional live births. Our findings are therefore in concordance with others that report live births from GV oocytes. Whilst such studies

318 are scarce and report low success rates when using such an approach (for example a 5.6% live
319 birth rate was reported in (Martin-Palomino Olid et al., 2019)), these findings nonetheless
320 suggest that the use of GV oocytes could offer a viable option for infertility treatment, particularly
321 for patients that have a poor prognosis. In 2014 Alcoba *et al.*, highlighted that developing ways
322 to distinguish between GV oocytes that have the potential to mature and fertilise and those that
323 do not, would be useful to maximise the efficiency of their use (Alcoba et al., 2014). Such
324 technologies did not exist a decade ago, and observations such as the ones made here suggest
325 that the development of these would still be beneficial.
326
327 Despite the relatively small number of embryos included in this study, and the fact that immature
328 oocytes were not monitored in a time-lapse incubator, this study supports the argument that
329 late-maturing oocytes can develop into genetically normal blastocysts that lead to successful live
330 births. The continuous culture of immature oocytes would nonetheless be valuable as the optimal
331 timing for DICSI after polar body extrusion could potentially be determined and cytoplasmic
332 maturation could be observed.
333
334 Whilst progress in IVM methods and the identification of competent MII oocytes that result from
335 IVM is being made (Madkour et al., 2018), robust methods are yet to exist. Ensuring the correct
336 time that DICSI is performed is likely to be a critical factor for success, given the complexity seen
337 in maturation when the morphokinetics of maturing GV oocytes is considered (Escrich et al.,
338 2012; Yang et al., 2021). Taken together, these findings underscore the importance of rescuing
339 late maturing GV and MI oocytes for poor prognosis patients. We show that DICSI can increase

the number of blastocysts suitable for transfer for these patients; in this case we observe an increase in the number of cycles that resulted in a live birth of almost 10%. DICSI might also be of value in other patient groups, but a recent systematic review and meta-analysis indicates 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

The data underlying this article cannot be shared publicly due to privacy reasons.

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Conflict of interest

The authors have no conflicts of interest to declare in relation to this work.

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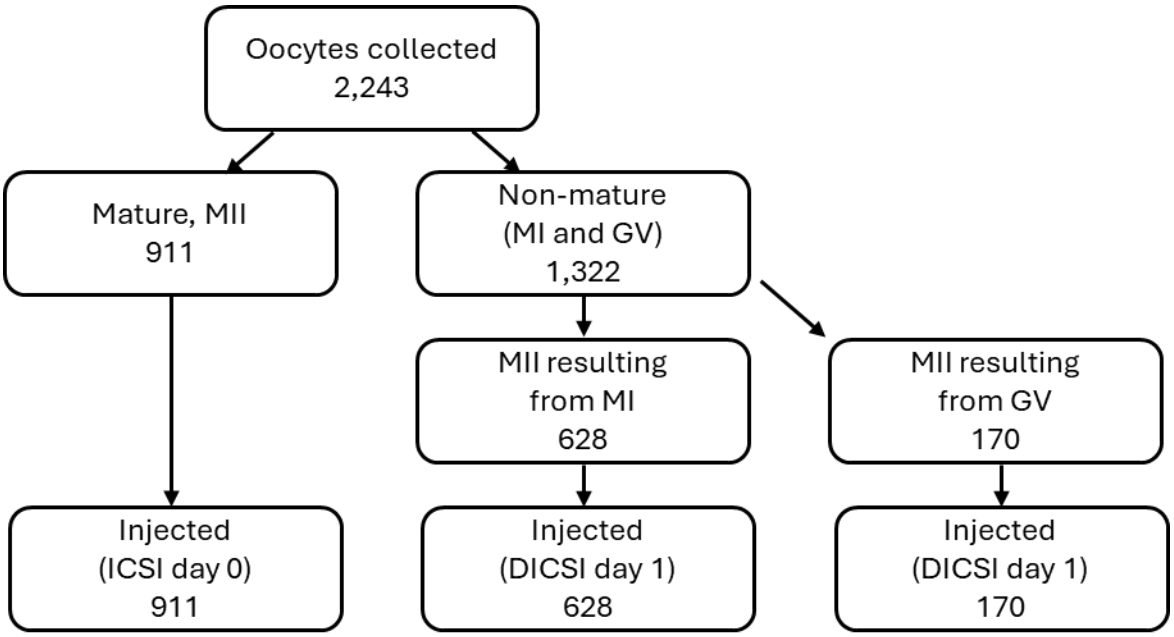


Figure 1. Flow chart showing the study groups investigated, with the numbers of oocytes that proceeded to ICSI (the MII oocytes), or DICSI (the originally non-mature MI and GV oocytes), shown. MII – metaphase II, MI – metaphase I, GV – germinal vesicle.

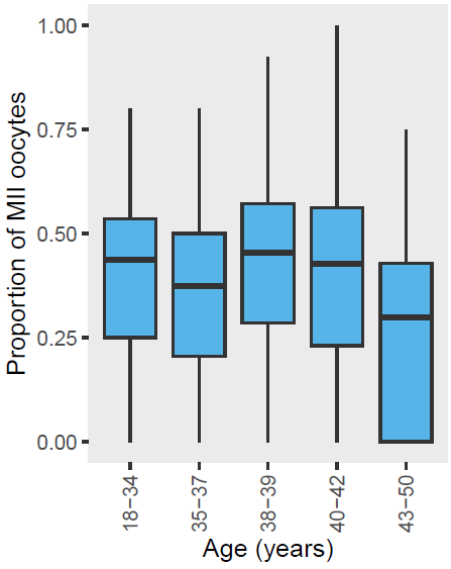


Figure 2. The proportion of MII oocytes shows little variation across five age groups. Shown are box plots of the proportion of MII oocytes recovered per cycle as a function of maternal age.

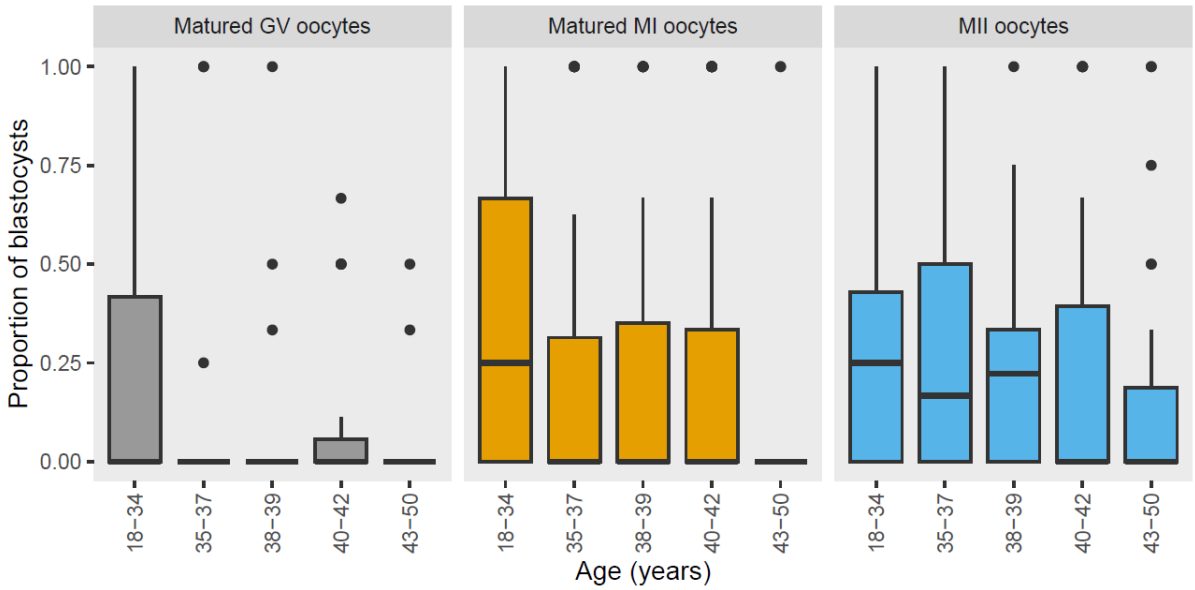


Figure 3. The proportion of blastocysts that developed from mature oocytes that were originally isolated at the GV, MI or MII stage across five age groups.

Table 1: Fertilisation and blastocyst rates for mature oocytes originally assessed as GV, MI or MII that underwent ICSI or DICSI.

Original assessment of oocyte maturation stage	Number of mature oocytes that underwent ICSI/DICSI	Number of zygotes (% of oocytes that underwent ISCI/DICSI)	Number of blastocysts (% of oocytes that underwent ICSI/DICSI)
GV	170 (DICSI)	92 (54.1%), $p = 0.30$	24 (14.1%), $p = 0.002$
MI	628 (DICSI)	422 (67.2%), $p < 0.001$	152 (24.2%), $p = 0.62$
MII	911 (ICSI)	450 (49.4%)	232 (25.5%)

Shown are *p* values from two-proportion z-test comparisons of the proportions observed in either MI or GV oocytes when compared to those observed in MII oocytes.

Table 2: Embryo transfer outcomes.

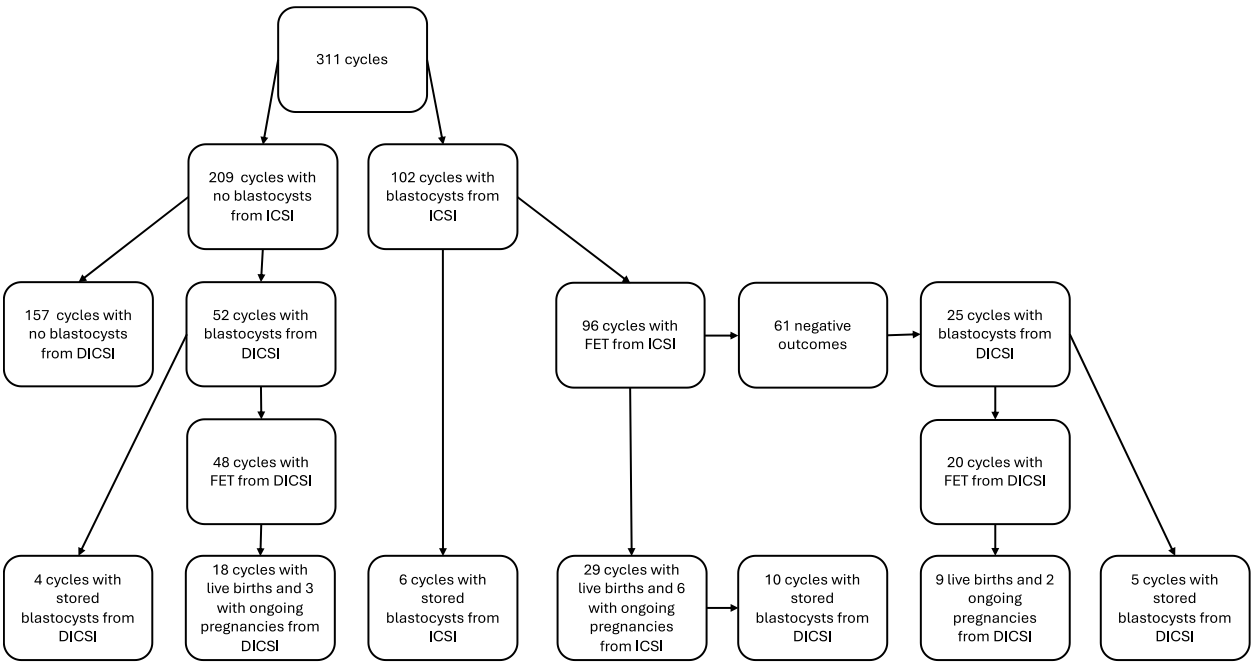
Original assessment of oocyte maturation stage	PGT-A	Frozen	Cycle type	Number transferred	Live births (%)	Ongoing pregnancies (%)	Miscarriages (%)	Biochemical pregnancies (%)	Negative β hCG test (%)
GV total				**7**	**2 (28.6%)**	**1 (14.3%)**	**1 (14.3%)**	**1 (14.3%)**	**2 (28.6%)**
GV	no	yes	mod	7	2	1	1	1	2
MI total				**61**	**25 (41.0%)**	**3 (4.9%)**	**9 (14.8%)**	**2 (3.3%)**	**21 (34.4%)**
MI	yes	yes	mod	3	1	0	2	0	0
MI	no	yes	mod	53	22	3	7	2	18
MI	no	yes	nat	5	2	0	0	0	3
MII total				**96**	**29 (30.2%)**	**6 (6.3%)**	**4 (4.2%)**	**0 (0%)**	**57 (59.4%)**
MII	yes	no	nat	2	2	0	0	0	0
MII	yes	yes	mod	14	8	3	0	0	3
MII	no	no	nat	54	12	1	2	0	39
MII	no	yes	mod	24	6	2	2	0	14
MII	no	yes	nat	2	1	0	0	0	1

Transfer outcomes for blastocysts derived from mature oocytes originally assessed as GV, MI or MII that underwent ICSI or DICSI. mod = modified cycle, nat = natural cycle. Data are shown aggregated as the total for each group of oocytes (in bold) and stratified based on PGT-A testing, vitrification (frozen) and cycle type.

519 Table 3: Characteristics and outcomes of live births.
520

	GV	MI	MII	Test
Number of live births	2	25	29	-
Maternal age	40.0 ± 0	35.8 ± 2.9	36.2 ± 3.5	$X^2 = 4.43$, df = 2, p = 0.11
Delivery method (vaginal / C-section)	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	$X^2 = 2.69$, df = 2, p = 0.26
Birth weight (kg)	3.5 ± 1.0	3.6 ± 0.5	3.5 ± 0.6	$X^2 = 0.64$, df = 2, p = 0.73

521
522 Values are expressed as number (percentage) or mean ± standard deviation of the mean.
523 Quantitative variables were analysed using Kruskal-Wallis rank sum test and qualitative
524 variables were analysed by Fisher's exact test.
525



Supplementary Figure 1. Flow chart showing the outcomes by ART cycle. Shown are the number of cycles with and without transferable blastocysts, the numbers of cycles with stored blastocysts, the number of transfers and the transfer outcomes.

Supplementary Table 1. Pairwise comparison, via Dunn test, of the proportion of MII oocytes recovered per individual by age, as per the categorical variables identified by the Human Fertilisation and Embryo Authority (HFEA) (HFEA, 2023). Shown are the age classes compared, the Z scores, the unadjusted *p* values and the Holm corrected *p* values.

Comparison	Z	*p* unadjusted	*p* corrected
18-34 vs 35-37	1.23	0.22	1.00
18-34 vs 38-39	-0.79	0.43	0.86
35-37 vs 38-39	-1.99	0.05	0.33
18-34 vs 40-42	-0.01	0.99	0.99
35-37 vs 40-42	-1.42	0.15	0.93
38-39 vs 40-42	0.87	0.39	1.00
18-34 vs 43-50	2.12	0.03	0.27
35-37 vs 43-50	1.07	0.28	1.00
38-39 vs 43-50	2.77	0.01	0.06
40-42 vs 43-50	2.37	0.02	0.16

Supplementary Table 2. Pairwise comparison, via Dunn test, of the proportion of blastocysts that developed from oocytes originally assessed as MI per individual by age, as per the categorical variables identified by the Human Fertilisation and Embryo Authority (HFEA) (HFEA, 2023). Shown are the age classes compared, the Z scores, the unadjusted *p* values and the Holm corrected *p* values.

Comparison	Z	*p* unadjusted	*p* corrected
18-34 vs 35-37	3.06	0.00	0.02
18-34 vs 38-39	2.23	0.03	0.10
35-37 vs 38-39	-0.52	0.60	1.00
18-34 vs 40-42	2.90	0.00	0.03
35-37 vs 40-42	-0.37	0.71	1.00
38-39 vs 40-42	0.23	0.82	0.82
18-34 vs 43-50	4.88	0.00	0.00
35-37 vs 43-50	2.35	0.02	0.09
38-39 vs 43-50	2.58	0.01	0.06
40-42 vs 43-50	2.77	0.01	0.04