The developmental potential of mature oocytes derived from rescue in vitro maturation

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Objective: To examine the developmental competence of immature oocytes in stimulated cycles, that matured after rescue in vitro maturation (IVM) compared with their sibling in vivo matured oocytes.
Design: Retrospective cohort study.
Setting: IVF clinic.
Patients: A total of 182 patients underwent 200 controlled ovarian stimulation cycles with intracytoplasmic sperm injection cycles in which immature oocytes were retrieved and at least one mature oocyte was obtained through rescue IVM.
Intervention: In vitro culture of immature germinal vesicle (GV) and metaphase I (MI) oocytes, retrieved in stimulated cycles.
Main Outcome Measures: Fertilization rate, cleavage rate, blastulation rate, ploidy of embryos evaluated using preimplantation genetic testing for aneuploidy, morphokinetic parameters and pregnancy outcomes.
Results: In total, 2,288 oocytes were retrieved from 200 cycles. After denudation, 1,056 of the oocytes (46% ± 16%) were classified as metaphase II (MII). A total of 333/375 (89%) of MI oocytes and 292/540 (54%) of GV oocytes matured overnight and underwent intracytoplasmic sperm injection. The fertilization rates of matured oocytes from MI rescue IVM (R-MI) and from GV rescue IVM (R-GV) were comparable with those of their sibling MII oocytes (71% vs. 66%; 66% vs. 63%, respectively). Early cleavage rates (80% ± 35% vs. 92% ± 20%; 80% ± 42% vs. 95% ± 28%, respectively) and blastulation rates (32 ± 40% vs. 62 ± 33%; 24 ± 37% vs. 60 ± 35%, respectively) were significantly decreased in rescue IVM matured oocytes (R-oocytes)-derived zygotes, but the blastocyst (BL) euploidy rate and "good quality" BL rate were comparable with those of MII sibling-derived embryos. In addition, rescue IVM embryos showed significantly higher levels of multinucleation at the 2- and 4-cell stages, as well as higher rates of zygote direct cleavage from one to 3 to 4 cells. Overall, 21 transfers of rescue IVM embryos resulted in 3 healthy live births.
Conclusions: For patients with a low maturation rate and/or low numbers of mature oocytes at retrieval, rescue IVM may contribute more competent oocytes and additional viable BLs for transfer from the same stimulation cycle, maximizing the chances for pregnancy and live birth. (Fertil Steril® 2023;120:860–9. ©2023 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Immature oocyte, rescue in vitro maturation, morphokinetics, PGT-A, stimulated cycle

Achieving mature oocytes during assisted reproductive technology treatments is one of the key components predicting treatment success (1). In current clinical practice, the evaluation of oocyte maturity status, performed after denudation of the cumulus-oophorous complex (COC), exclusively relies on nuclear maturation assessment. Therefore, mature oocytes are defined as metaphase II (MII) oocytes, which completed the first meiotic division and extruded the first polar body (PB). Because for immature oocytes, the definition includes both germinal vesicle (GV) stages, characterized by a distinctly large nucleus, arrested in prophase I of the first meiotic division, and metaphase I (MI) oocytes after GV breakdown (GVBD), recognized by the absence of a visible GV and the first PB (2).

The process of cytoplasmic maturation, which includes an extensive range of metabolic and structural modifications, is as essential as nuclear maturation for attaining oocyte competence, but the mechanisms underlying this

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process are not well understood. Moreover, no single morphological characteristic defines its completion (3–5).

After controlled ovarian hyperstimulation (COS), not all oocytes reach the desired maturation state at the time of oocyte retrieval (6). The proportion of immature oocytes at denudation generally ranges between 15% and 20% of aspirated oocytes, but it can vary greatly (7, 8). Several factors related to patient and cycle characteristics have been shown to influence the rate of immaturity within the retrieved oocyte cohort (9–13).

Immature oocytes after COS are usually considered unsuitable for reproductive purposes and are simply discarded in many in vitro fertilization (IVF) programs. Nevertheless, in vitro maturation (IVM) of those oocytes has been reported by many groups previously, using varying culture conditions and culture durations (3, 11, 14–17). The most common term for this process is "rescue in vitro maturation" (18).

The reproductive potential of "rescued" in vitro matured oocytes (R-oocytes) remains unclear. Although some studies report significantly reduced fertilization rates and poorer embryonic development compared with MII oocytes at retrieval (7, 19, 20), others show equivalent rates (21, 22). Moreover, concern has been raised regarding the safety of using R-oocytes because of evidence of an association between delayed maturation and an increased risk of aneuploidy (7, 23, 24), as well as an apparent diminished clinical reproductive outcome (14, 21, 25, 26).

In women with low mature oocyte yield at retrieval, every additional oocyte and embryo might increase the potential for pregnancy. Therefore, for these patients, immature oocytes could be an important source of desired additional embryos (15).

The purpose of the present study was to examine the developmental competence of R-oocytes from cycles with a low maturation rate and/or low mature oocyte MII yield by assessing cleavage and blastulation rates, blastocyst (BL) morphologic quality, and the chromosomal constitution of resultant BLs compared with sibling in vivo matured oocytes from the same cohort. Morphokinetic variables and clinical outcomes were also assessed.

MATERIALS AND METHODS

This retrospective cohort study had research ethics board approval (approval no. 2602) and included patients who underwent an IVF cycle at the CReATe Fertility Centre (Toronto, Canada) between January 2019 and April 2021, meeting the inclusion criteria described below.

Study Population and Eligibility Criteria

In vitro fertilization cycles in which rescue IVM was performed were identified using an electronic database query. All autologous oocyte IVF cycles in women aged 18–45 years, in which at least one mature oocyte was obtained through rescue IVM and was subsequently used for intracytoplasmic sperm injection (ICSI), were included. During the study period, rescue IVM was performed in cases where the fraction of MII oocytes at cumulus striping was $\leq$50% and/or the total number of retrieved MII oocytes was $\leq$6 and/or in cases with a borderline maturation rate (50%–70%) and a history of low maturation and/or low fertilization rates. To enable a comparison of developmental competence between MII oocytes and rescue IVM oocytes from the same cohort, we excluded all cycles with "complete rescue IVM failure" (cycles in which none of the immature oocytes subjected to rescue IVM had matured). Cycles of patients using donor oocytes and those using surgically retrieved sperm were excluded as well.

Ovarian Stimulation Protocol, Trigger, and Oocyte Retrieval

All patients underwent COS with human menopausal gonadotropin alone (Menopur, Ferring, Canada) or in combination with recombinant follicle-stimulating hormone (Gonal F, Merck, or Puregon, MSD). Either a gonadotropin-releasing hormone agonist (leuprolide) or antagonist (Cetrotide, Merck, or Orgalutran, MSD) was used for pituitary suppression. Final maturation was triggered on the day when at least 2 leading follicles with a diameter $\geq$18 mm were detected using ultrasound, using either human chorionic gonadotropin (2,500/5,000 or 10,000 IU) alone, leuprolide (2 mg, 0.4 cc) alone, or a combination of both (dual trigger). The trigger type was determined by the treating physician, taking into consideration patients' histories and cycle parameters, with the goal of achieving optimal maturation although maintaining patient safety. Oocyte retrieval was conducted 36 hours post-trigger under transvaginal ultrasound guidance. All follicles $\geq$12 mm in diameter visible on ultrasound were aspirated.

In vitro Fertilization Laboratory Protocol

Approximately 1–3 hours after oocyte retrieval, COC were denuded through exposure to 80 IU/mL hyaluronidase solution (HYASE-10x, VitroLife, Sweden) and the oocytes were immediately evaluated for nuclear maturation status. Oocytes classified as MII were subjected to ICSI. Their immature sibling oocytes, at GV and MI stages, were placed in Sage 1-stepTM medium (Copper Surgical, USA) supplemented with 75 IU/mL follicle-stimulating hormone and 75 IU/mL luteinizing hormone (Menopur, Ferring) for overnight incubation. Germinal vesicle and MI oocytes that have reached the MII stage within 17–24 hours from the time of retrieval were defined as oocytes from GV rescue IVM (R-GV) and MI rescue IVM (R-MI) oocytes, respectively, and were injected with the partner's or donor's sperm. All injected oocytes were individually cultured in 25 μL droplets in a trigas incubator (K-Systems or time-lapse Embryo Scope; Cooper Surgical, Vitrolife) at 5% O_2, 5.5% CO_2, and 89.5% N_2 using paraffin oil (OVOILTM, VitroLife, USA).

Fertilization was assessed 16–18 hours after ICSI. Normal fertilization was confirmed by the detection of 2 PB and 2 pronuclei (2PN), whereas the detection of one pronucleus or 3 pronuclei was classified as abnormal fertilization. Embryo cleavage was evaluated 48 hours after the detection of the pronuclei.

Most 2PN zygotes were cultured to the BL stage (86% from 172 of 200 cycles). The decision for extended culture was predetermined by the treating physician ("intention to

grow to blast" cycles). In a small number of cases when an extremely small number of oocytes were retrieved and/or the embryos demonstrated compromised quality, embryos were cryopreserved or transferred at cleavage stage on day 3.

Blastocyst morphology was evaluated on days 5–6, according to a previously described grading system (27). Blastocysts were defined as "good quality", when the inner cell mass and trophectoderm (TE) grades were AA, AB, BA, and BB. The rest were defined as "poor quality" (BC, CB, and CC). The level of BL expansion (no.1: expanded BL; no. 2: expanding; no. 3: early BL with a very small cavity) was assigned to each embryo but was not included in our grading (28). Blastocysts were cryopreserved using vitrification, according to the Irvine vitrification protocol (Somagen Diagnostics Inc., Canada).

MORPHOKINETICS

A total of 117/172 (68%) of "intention to grow to blast" cycles of embryos were cultured to the BL stage in a time-lapse EmbryoScope incubator (Unisense FertiliTech, Denmark), although the remainders were cultured in K-System incubators. The incubator type choice was made on the basis of physician recommendation and/or patient request. Embryo images (EmbryoScope only) were recorded every 10 minutes in 7 different focal planes, and the embryo score was determined using the numerical algorithm model described previously (28). The following morphokinetic parameters were recorded: time of pronuclear fading (tPNf), when both pronuclei were no longer visible; cleavage time to the 2-, 3-, 4-, and 5-cell stages (t2, t3, t4, and t5, respectively); abnormal zygote cleavage (AZC) (1–3) cells at the first mitotic division; time to start blastulation (tSB), the first sign of a visible blastocoele cavity; and time to full BL formation (tB). Calculated morphokinetic features included the length of the first cleavage (t2–tPNf), the second (t3–t2), and the third cell cycle (t5–t3), as well as the synchronicity of cell division from the 3- to 4-cell stage (t4–t3). All embryos were also examined for the presence of multinucleated (MN) blastomeres at 2- and 4-cell stages (MN2, MN4).

Preimplantation Genetic Testing for Aneuploidy (PGT-A): Biopsy and Genetic Analysis

The decision to perform a TE biopsy for PGT-A analysis was predetermined on the basis of the advice of the treating physician and the patient's request. All viable BLs of good, fair, or poor static morphology (AA–CC) were biopsied. Laser-assisted TE biopsy was performed at the expanded BL stage (days 5–6) as previously described (28). Preimplantation genetic testing for aneuploidy analysis using next-generation sequencing (NGS) was conducted at the CReATe Fertility Centre Genetics laboratory. Briefly, DNA from all samples was amplified using the SurePlex kit (Illumina), according to the manufacturer's instructions, and quantified using a Qubit3.0 fluorimeter (Thermo Fisher Scientific). Amplified DNA was assessed for whole and segmental chromosome aneuploidy screening with a previously validated VeriSeq preimplantation genetic screening kit on the MiSeq system (Illumina). Preimplantation genetic testing for aneuploidy results were classified as euploid, aneuploid, or mosaic (10 Mb resolution; 30%–70% mosaicism).

Embryo Transfer (ET)

Embryo selection criteria were on the basis of morphology (inner cell mass and TE quality) and PGT-A results, when applicable (29). Embryo transfers on day 3 were performed during fresh or frozen cycles. All BLs were vitrified and transferred at a subsequent frozen-thawed embryo transfer cycle (FET). A FET was performed either using a medicated or a natural cycle protocol. An endometrial thickness assessment was performed before the ET. The ET was performed under ultrasound guidance. Luteal support was provided using progesterone in oil, daily injections, or suppositories. Pregnancy was confirmed using serum β-human chorionic gonadotropin levels 12 days after the ET.

Outcome Measures

Main outcomes: fertilization rate (number of 2PN fertilizations out of total matured oocytes); cleavage rate (number of cleaved embryos out of total 2PN fertilizations); blastulation rate, which was calculated per mature oocyte (number of BLs out of mature and matured oocytes) and per zygote (number of BLs out of 2PN fertilizations); and for biopsied BL, euploid, aneuploid, and mosaic rates (number of euploid, aneuploid, or mosaic embryos out of the total number of biopsied BLs).

Secondary outcomes were "good quality" BL rate (number of "good quality" BLs out of the total number of BLs) and morphokinetic parameters.

Embryos derived from R-oocytes were deprioritized for transfer and were transferred only when no MII sibling oocytes-derived embryos were available, hence comparisons of clinical outcomes were not suitable. Nevertheless, we have reported clinical pregnancies (CP), ongoing pregnancies (OP), and live births (LB) derived from R-oocytes.

Statistical Analysis

Distributions of variables were represented using the mean $\pm$ standard deviation (SD) for normally distributed variables and the median with an interquartile range for nonnormally distributed variables. Normality was assessed through skew and kurtosis, and outliers were identified by examining standardized residuals.

The study design was on the basis of paired analysis. In each cycle, R-oocytes (R-GV or R-MI) were compared with their corresponding "in vivo" matured (MII) sibling oocytes (used as a control group). The analyses were performed separately for each type of R-oocyte vs. MII control. When comparing MII and R-GV oocytes, only cycles with both MII and R-GV oocytes ($n = 133$ cycles) were included. Comparably, comparing R-MI to MII oocytes, only cycles with both MII and R-MI oocytes were included ($n = 150$ cycles). Cycles with "complete maturation failure" (no MII at retrieval) were excluded from the paired analysis.

Continuous variables such as rates were compared using paired tests whenever appropriate and when sample size allowed (30). Parametric (t or paired t) or nonparametric (Mann-Whitney U or Wilcoxin) tests were used as appropriate.

RESULTS

Overall, 2,288 oocytes, retrieved from 182 patients who underwent a total of 200 COS cycles, met the inclusion criteria. The mean (± SD) age of patients in the study group was 37 ± 4 (25–45) years. The main indications for treatment were diminished ovarian reserve (25.8%) and male factor (17.6%) as a single diagnosis. Thirty-five patients had a combination of at least 2 diagnoses. Most (69.5%) patients received a dual trigger for final maturation. On the day of trigger, the average number of follicles measuring 15 mm or greater was 9.4 ± 5.9 (confidence interval 95%: 8.5–10.2). Patient demographics and cycle characteristics are presented in Supplemental Table 1 (available online).

Maturation Status

After COC denudation, 1,056 of the oocytes (46% ± 16%, average 5.7 ± 1.6 per cycle) were classified as MII (MII sibling oocytes). This rate was expected because a low maturation rate of 50% or lower was the main indication for rescue IVM in the study population.

In total, 375 oocytes were immature at the MI stage and 688 were at the GV stage (16%; 1.88 ± 1.69 per cycle and 30%; 3.44 ± 3.2 per cycle, respectively). A total of 169 oocytes were degenerated and postmatured and were discarded. All MI oocytes retrieved were subjected to overnight incubation. Germinal vesicle oocytes were not subjected to rescue IVM when high numbers of MII and MI oocytes were achieved at retrieval. From the 688 GV oocytes retrieved, only 540 oocytes were cultured for rescue IVM. A total of 333/375 (89%) of MI oocytes and 292/540 (54%) of GV oocytes matured overnight and underwent ICSI.

In 10 cycles of "complete maturation failure," there were no MII at retrieval. Those cycles were not included in the paired analyses.

Fertilization and Embryonic Development

Oocytes that matured in vitro (from either R-GV or R-MI) had a similar fertilization (2PN) rate as their MII sibling oocytes (71% vs. 66%; P=0.23 and 66% versus 63%; $P=.52$, respectively). Furthermore, no significant difference was observed between the different oocyte types with regards to abnormal fertilization (one pronucleus and 3 pronuclei) rates ($P>.05$). The cleavage rates were significantly lower for R-GV and R-MI zygotes than for MII sibling zygotes (80% ± 35% vs. 92% ± 20%; $P<.0001$ and 80% ± 42% vs. 95% ± 28%; $P=.002$, respectively) (Table 1).

Overall, 172/200 (86%) of the cycles were cultured to BL and yielded 52 R-GV BL (2 from "no MII" cycles) from 32 cycles (range 1–5 BL per cycle), 66 R-MI BL (1 from "no MII" cycles) from 53 cycles (range 1–3 BL per cycle), and 382 MII sibling BL.

In 42 of 172 cycles, there were no BL from MII siblings, and in 17 of those cycles, BL from R-oocytes were the only BL available for biopsy or transfer.

Calculating Blastulation Rates

Blastulation rates were significantly lower for R-GV and for R-MI compared with their MII siblings, respectively, in both types of analyses: per mature oocyte (17 ± 31% vs. 38% ± 30%; $P<.0001$; 22 ± 33% vs. 42 ± 30%; $P<.0001$; respectively) and per zygote (24 ± 37% vs. 60 ± 35%; $P<.0001$; 32 ± 40% vs. 62 ± 33%; $P<.0001$; respectively) (Fig. 1). Nevertheless, R-oocyte BLs added an average of 0.5 ± 0.87 BLs per cycle from R-GV zygotes and 0.53 ± 0.73 BLs per cycle from R-MI zygotes. This meaningful blastulation rate difference between the oocyte types was kept in all age groups when stratified using age (using 3 groups aged <35, 35–39, ≥40 years) (Supplemental Table 2, available online).

Although the rates of "good quality" BLs derived from both R-GV (32 ± 42%) and R-MI (41 ± 47%) oocytes were lower compared with those originating from MII sibling oocytes (about 61%), the difference was not statistically significant ($P<.09$, $P<.32$, respectively). Most BLs were biopsied on day 5 or day 6 (74.6% MII-siblings and 72% rescue IVM), and their expansion ranged from no. 1 to no. 2. Only a few had a very small cavity and were evaluated as level no. 3. (Fig. 1).

Preimplantation Genetic Testing for Aneuploidy Results

A total of 285 BLs from MII sibling BLs and 86 R-oocyte-BLs from 112 cycles underwent TE biopsy and PGT-A analysis. The average age of patients in this group was comparable to that of the entire study population (36.93 ± 3.68). In total, 38% (15/39) of R-GV BLs and 42.5% (20/47) of R-MI BLs tested for PGT-A were euploid. There was no significant difference in euploidy rate (44 ± 33% vs. 36 ± 44%; $P=.35$; 44 ± 47% vs. 43 ± 35%; $P=.92$) as well as in aneuploidy rate (44 ± 35% vs. 46 ± 47%; $P=.83$; 41 ± 47% vs. 44 ± 37%; $P=.73$) between either R-GV or R-MI BLs vs. MII sibling BLs, respectively (Fig. 2).

Morphokinetic Parameters

The morphokinetic parameters of 95 (out of 169) R-GV zygotes, 126 (out of 207) R-MI zygotes, and 277 of their MII sibling zygotes are presented in Supplemental Table 3, available online. In general, the timing of early developmental parameters in R-oocyte embryos was significantly altered compared with MII sibling embryos. A significantly prolonged duration of the first mitotic division (t2–tPNf) (3.7 ± 1.7 and 3.6 ± 1.7 vs. 2.6 ± 0.7; $P<.001$, respectively) and the second mitotic division (t3–t2) (11.0 ± 4.3 and 10.6 ± 2.9 vs 11.4 ± 1.7; $P=.003$, respectively) was observed for R-oocytes.

Substantial delay in synchrony of blastomere cleavage during the second cycle (t4–t3) was recorded also for embryos

TABLE 1

Fertilization rates and early embryonic development of R-oocytes and MII sibling oocytes.

	MII siblings for GV	R-GV	*P* value[b] n = number of cycles	MII siblings for MI	R-MI	*P* value[b] n = number of cycles
ICSI of matured oocytes (N)	759	285		881	323	
Fertilization rate 2PN Rate ± SD (N)	0.63 ± 0.29 (469)	0.66 ± 0.38 (188)	*P*=52 n = 133	0.66 ± 0.28 (564)	0.71 ± 0.37 (226)	*P*=.231 n = 150
Abnormal fertilization rate (1PN + 3PN) Rate ± SD (N)	0.09 ±0.18 (70)	0.06 ±0.18 (21)	*P*=.16 n = 133	0.08 ±0.18 (85)	0.10 ± 0.25 (34)	*P*=.50 n = 150
D3 (>4 cells) rate[a] Rate ± SD (N)	0.95 ±0.28 (432)	0.8 ± 0.42 (149)	*P*=.002 n = 103	0.92 ± 0.195 (466)	0.80 ± 0.35 (171)	*P*<.0001 n = 126

GV = germinal vesides; ICSI = intracytoplasmic sperm injection; 1PN = one pronucleus; 3PN = 3 pronuclei; MI = metaphase I; MII = metaphase II; SD = standard deviation; R-GV = GV oocytes rescued by IVM; R-MI = MI oocytes rescued by IVM; R-oocytes= either GV and/or MI oocytes rescued by IVM.

[a] Cleavage rate per number of 2PN

[b] Paired analyses were performed separately for each type of R-oocyte vs. its MII-sibling control.

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derived from R-oocytes vs. those from MII sibling oocytes (3.9 ± 5.0 hours and 2.2 ± 4.0 hours vs. 1.0 ± 1.8 hours; *P*<.001, in the R-GV and R-MI vs. MII embryos, respectively).

Overall, the mean timing of early cleavage divisions up to tSB was accelerated in R-zygotes compared with their sibling controls, with R-GV and R-MI zygotes reaching tSB significantly earlier than their MII sibling zygotes (97.2 ± 6.7, 95.2 ± 18.4 vs. 100.0 ± 13.3; *P*=.25, *P*=.04 for GV, MI vs. MII, respectively). However, no difference was observed between study and control groups when comparing the time for formation of full BLs (tB; 112.0 ± 5.4, 109.1 ± 11.3 vs. 112.1 ± 14.8; *P*=.98; *P*=.37 for R-GV, R-MI vs. MII, respectively).

Interestingly, R-oocyte embryos showed significantly higher levels of blastomere multinucleation at the 2-cell and 4-cell stages, as well as high levels of AZC compared with MII sibling embryos (Fig. 3).

Clinical Outcomes

During the study period, 18 patients had 20 R-oocyte embryos ETs, resulting in 7 pregnancies (5 OPs and 3 LBs). (Supplemental Table 4, available online).

Seven patients had 9 transfers of day 3 R-oocyte embryos (2 R-GV and 5 R-MI transfers), achieving 2 pregnancies (from R-MI embryos). Unfortunately, both were delivered at 21 weeks.

Three patients underwent euploid R-GV BL transfers, and one pregnancy was achieved, resulting in a LB. Five patients underwent 4 euploid and one mosaic R-MI BL SETs, resulting in 3 CPs. Of these 3 pregnancies, one (from an euploid FET) resulted in a spontaneous abortion (SA), although the other 2 (from an euploid and mosaic FET) led to 2 term, healthy LBs. In addition, 3 patients underwent transfers of untested R-oocyte BLs (one from R-GV and 2 from R-MI), resulting in one PR, which ended with a SA.

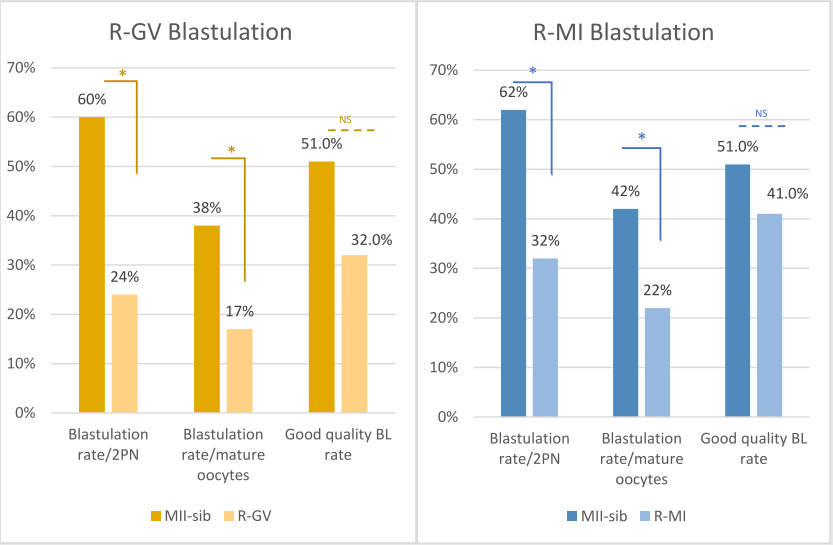
DISCUSSION

Rescue IVM was applied in our center as a strategy for augmenting cycle outcomes for patients with a low yield of mature oocytes at retrieval, especially those with a low maturation rate, striving to increase the number of embryos available for transfer.

To our knowledge, this is the largest series to date of R-oocyte BLs that also includes copy number variation analysis using NGS-based PGT. Our findings confirm that R-oocytes display compromised developmental competence. This was characterized by lower cleavage rates, altered morphokinetic profiles, high blastomere MN, and lower blastulation rates. Nevertheless, R-oocyte BLs showed comparable morphological quality and similar euploidy rates to their MII sibling BLs.

Release from the follicular suppressive microenvironment and breakdown of communication between the oocyte and cumulus cells result in the resumption of meiosis (31). Hence, immature oocytes retrieved and denuded from COC tend to resume meiosis spontaneously (32, 33). However, "in vivo" maturation and acquisition of oocyte competence are induced not only by the interruption of the inhibitory effect of follicular cells but also by active molecular and cellular events

FIGURE 1



Blastulation rates and blastocysts quality. R-GV and R-MI oocytes blastulation compared with metaphase II (MII) sibling oocytes, respectively. 1 Blastulation rate = number of blastocysts out of 2 pronuclei (2PN). 2 Blastulation rate per mature oocyte = number of blastocysts out of mature oocyte: MII siblings per R-GV per R-MI. 3 Good quality blastocysts (BL) rate = number of good quality BL (inner cell mass and trophectoderm grades of AA, AB, BA, and BB) out of total number of BL. Stage of BL development was not included in our grading. * $P<.0001$ MII- sib = metaphase II sibling; R-GV = GV oocytes rescued by IVM; R-MI = MI oocytes rescued by IVM.

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essential for the successful completion of maturation. Furthermore, before and during maturation, cumulus cells actively produce and deliver molecules that are inefficiently synthesized by the oocyte, such as pyruvate and cholesterol, which are necessary for oocyte development (34). Early interruption of the strong connection between oocytes and their surrounding cumulus cells might lead to inadequate oocyte cytoplasmic maturation as well as a loss of synchrony with nuclear maturation. This could have a major detrimental influence on oocyte developmental competence (5, 35, 36). Unlike oocytes collected from small antral follicles of partially stimulated IVM cycles, immature oocytes retrieved after COS were already exposed to many days of high doses of gonadotropins and subjected to an ovulation trigger before retrieval but failed to mature in vivo (3, 37, 38). Doubts remain as to whether some or all of these oocytes are intrinsically compromised, having a reduced ability to gain full developmental competence in vitro (5), or whether they would be able to complete full maturation given time (16).

In the present study, we aimed to evaluate the developmental potential of those immature oocytes that successfully matured "in vitro" compared with their sibling in vivo-matured MII oocytes.

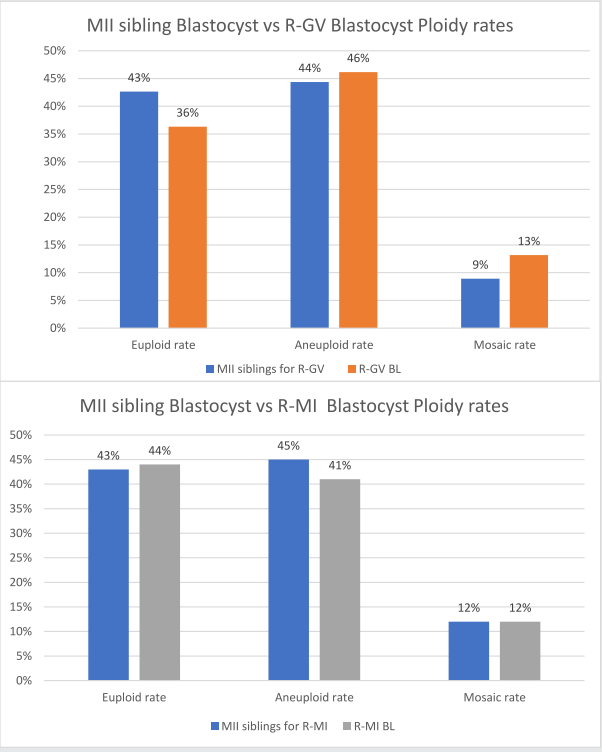
Normal fertilization (2PN) rates of both R-GV and R-MI oocytes were comparable with those of their MII sibling oocytes, which corroborates some previous reports (14, 21, 22, 39). However, several other studies reported decreased fertilization rates from R-oocytes (6, 7, 20). The variation in fertilization rates and further developmental milestones might be explained by the discrepancies in study populations, stimulation protocols, culture conditions, and rescue IVM culture duration (21, 25, 40, 41).

The developmental competence of embryos derived from R-oocytes seems to be reduced compared with MII sibling embryos (16, 20, 21, 25, 26). Embryos derived from R-oocytes demonstrated higher rates of pronuclear stage arrest and failure to undergo the first mitotic division (42) as well as arrest in later cleavage stages (3, 6, 14). Our data showed a significantly higher rate of cleavage arrest in R-oocytes zygotes compared with those originating from their in vivo MII-derived sibling embryos.

Data regarding BL development from R-oocytes are scarce because most early studies reported on the culture of embryos up to day 3 of development only. Although Escrich et al. (22) presented similar blastulation rates for zygotes originating from R-GV oocytes and MII sibling oocytes (44% vs. 65%), 3 other recent studies with relatively small sample sizes showed lower blastulation rates, ranging between 7% and 13% (16, 26, 43). Similarly, we also demonstrated significantly lower blastulation rates of R-GV and R-MI compared with their MII siblings ($17 \pm 31\%$ vs. $38 \pm 30\%$; $P<.0001$; $22 \pm 33\%$ vs. $42 \pm 30\%$; $P<.0001$, respectively; calculated per mature oocyte). On the basis of those rates, 5 R-GV oocytes or 4.5 R-MI oocytes were needed to obtain one BL.

This compromised embryonic development appears to be the consequence of incomplete cytoplasmic maturation. Defective formation of cellular elements essential for cytoskeletal rearrangement can lead to mitotic spindle abnormalities and aberrant chromosome alignment, resulting in nuclear disorganization, multinucleation, abnormal cleavage,

FIGURE 2



Ploidy in rescue in vitro maturation vs. metaphase II (MII)-sibling blastocysts (BLs). Percentage, preimplantation genetic testing for aneuploidy results for BLs from MII sibling vs. R-germinal vesicles BLs (20 cycles) and MII siblings vs. R-metaphase I (MI) BLs (34 cycles). $P<.05$ was considered statistically significant NS for all comparisons. NS = not significant; R-GV = GV oocytes rescued by IVM; R-MI = MI oocytes rescued by IVM.

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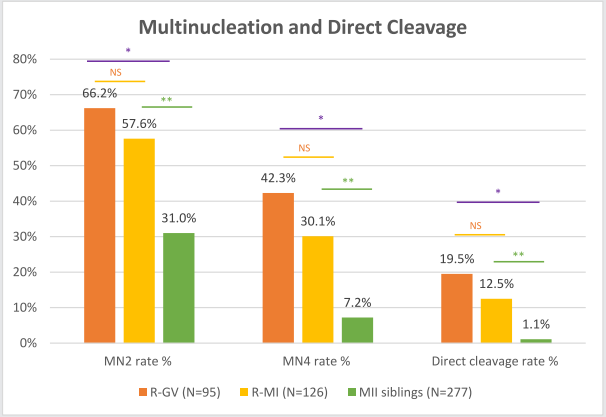
FIGURE 3



Multinucleation and AZC MN2, MN4 = multinucleated blastomeres at 2- and 4-cell stages, respectively; AZC = abnormal zygote cleavage, meaning direct cleavage of zygotes from one to 3 to 4 cells. Percentage of embryo MN and AZC in rescue in vitro maturation and control groups. $P<.05$ was considered statistically significant. Non paired analysis. * R-GV zygotes vs. MII zygotes. $P<.0001$ ** R-MI zygotes vs. MII zygotes. $P<.0001$ NS = not significant; R-GV = GV oocytes rescued by IVM; R-MI = MI oocytes rescued by IVM.

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and chromosomal abnormalities. This might lead to a high ratio of arrested embryos in all cleavage stages (5, 24, 44, 45).

Previous studies have reported either identical or delayed morphokinetic developmental patterns of R-oocyte embryos compared with embryos originating from MII oocytes (20, 22, 46). The present study showed that although the mean times of early cell divisions were mostly accelerated, the morphokinetic parameters seemed to normalize at the BL stage and correlated with those of MII sibling embryos. This observation may suggest heterogeneity in oocyte quality and the existence of active embryonic corrective mechanisms during the cleavage period.

Additional evidence of the compromised potential of R-oocyte embryos in our findings was indicated by high rates of multinucleated R-oocyte embryos at the 2-cell stage (MN2), especially those from GV oocytes (66%, 57.6% vs. 31% for GV, MI vs. MII, respectively; $P<.0001$). This increased abnormality persisted at the 4-cell stage and was associated with high rates of AZC (about 20% vs. 1% in siblings). It is known that MN is related to errors in cell division after malfunction of the first mitotic spindle (47, 48). There is a strong correlation between MN and abnormal cleavage patterns such as AZC (49). This is possibly associated with the formation of a tripolar and multipolar spindle and uneven chromosomal segregation and might explain the correlation with decreased embryonal developmental competence, manifested as reduced BL formation, a higher rate of chromosomal abnormalities, and significantly lower implantation of cleavage-stage embryos (42, 50–52). Nevertheless, Zhan et al. (49) demonstrated that AZC BLs had a comparable euploidy rate as nonAZC, suggesting "self-correction" mechanisms for eliminating abnormal blastomeres in cleavage embryos.

Earlier studies addressing the cytogenetic constitution of R-oocyte embryos compared with that of MII embryos showed higher rates of chromosomal abnormalities, mainly complex chromosomal mosaicism (23, 24, 44). These observations relied on genetic analyses performed on day 3 embryos and on the detection of a limited number of chromosomes using the fluorescence in situ hybridization technique. Such observations may account for the high rates of embryonic arrest and lower blastulation rates in the present study as well as the poor reproductive outcomes of R-oocytes on day 3 ETs in other studies (21, 25). Nevertheless, it appears that R-oocyte BLs have comparable chromosomal abnormality rates as their MII-siblings' BLs. Escrich et al. (22) reported on R-GV BL biopsies analyzed using array comparative genomic hybridization after whole-genome application, demonstrating a median euploidy rate of 50% (median patient age: 33.8 ± 3.1 years old; 7/14 embryos). However, that study's main limitation was the lack of comparison to the PGT-A results of embryos derived from in vivo mature MII oocytes. Moreover, our results using TE biopsy and NGS analysis demonstrate comparable euploidy, aneuploidy, and mosaic rates of R-BLs to those of MII sibling BLs.

Examining day 3 R-oocyte embryo transfers, we had 2 pregnancies achieved from 9 cleavage-stage R-oocyte ETs (both with undesired outcomes). Those results are in line with numerous previous studies, which reported low rates of CP and LB after R-oocytes' day 3 ET (14, 53). Martin-Palomino Olid et al. (25) showed that ET of R-MI yielded 10.0% CP rate, 7.8% OP rate, and 5.6% LB rate, significantly lower than conventional IVF cycles (30%, 24%, and 18.9%). On the other hand, we demonstrated better outcomes for euploid BL FET. We had one pregnancy leading to a LB out of 3 single euploid R-GV BL transfers of a patient that exhausted her MII-BLs, and 3 pregnancies out of 5 single euploid and mosaic R-MI BL transfers, leading to 2 LBs. These results provide evidence that BLs originating from R-oocytes have a capacity for implantation and can yield CPs and LBs. This is of major significance, particularly in our study population, which is characterized by a low number of mature oocytes at retrieval. In this population, rescue IVM might improve the chances of achieving a pregnancy and a LB per stimulation cycle (15, 16, 54).

A main strength of the present study is the focus on patients with a low maturation rate and low numbers of MII at retrieval, a specific infertility patient population that could most benefit from employing a rescue IVM strategy. Another strength is the study design. Matched pair analysis, using each cycle as a control for itself, gave a more accurate picture of the main outcomes although controlling for patients and cycle parameters. This made better use of the available sample size and enabled a true comparison of the oocytes' competence.

However, several limitations also need to be considered. First, the relatively small sample size limits our ability to stratify the results according to patient or cycle parameters. Second, cycles in which none of the immature oocytes retrieved, matured after overnight incubation were excluded, which compromised our ability to calculate the overall maturation rate after rescue IVM. Additionally, because of the deprioritization of R-oocyte embryos for transfer and the small number of embryos available, we had only a few reports of CP and LB. As such, we were unable to compare the clinical outcomes to those of MII siblings ETs and calculate how many rescue IVM oocytes were needed to achieve one LB.

CONCLUSIONS

Immature oocytes subjected to rescue IVM can complete meiosis and undergo fertilization at comparable rates to sibling MII oocytes. However, the subsequent embryonic development is significantly compromised, probably because of incomplete cytoplasmic maturation. This affects embryonic cell division and leads to a low yield of viable embryos. Nevertheless, R-oocyte embryos that can reach the BL stage appear to have comparable euploidy rates to their MII-derived sibling embryos, and their transfers have the potential to yield CPs and LBs.

Therefore, for poor-prognosis women, especially those with a low yield of mature oocytes at retrieval and/or a low maturation rate, applying the rescue IVM approach improves the efficacy of a stimulation cycle by increasing the availability of developmentally competent and genetically normal BLs. For some patients, using rescue IVM prevents cycle cancellation because of a lack of mature oocytes at retrieval or a lack of embryos suitable for transfer. For others, rescue IVM provides additional BLs available for transfer, therefore potentially enhancing the probability of achieving a pregnancy and a LB.

Declaration of interests: A.K.S. has nothing to disclose. L.M.H. has nothing to disclose. A.K.S. has nothing to disclose. H.B has nothing to disclose. I.K. has nothing to disclose. S.B. has nothing to disclose. E.N.D. has nothing to disclose. C.L.L. has nothing to disclose.

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El potencial de desarrollo de ovocitos maduros derivados de la maduración in vitro de rescate

Objetivo: Examinar la competencia evolutiva de ovocitos inmaduros en ciclos estimulados, que maduraron después de la maduración in vitro de rescate (MIV) en comparación con sus ovocitos hermanos madurados in vivo.

Diseño: Estudio de cohorte retrospectivo.

Entorno: Clínica de FIV.

Pacientes: Un total de 182 pacientes se sometieron a 200 ciclos controlados de estimulación ovárica con ciclos de inyección intracitoplasmática de espermatozoides en los que se recuperaron ovocitos inmaduros y se obtuvo al menos un ovocito maduro mediante MIV de rescate.

Intervención: Cultivo in vitro de ovocitos de vesículas germinales inmaduras (GV) y metafase I (IM), recuperados en ciclos estimulados.

Principales medidas de resultado: tasa de fertilización, tasa de escisión, tasa de blastulación, ploidía de embriones evaluados mediante pruebas genéticas preimplantacionales para aneuploidía, parámetros morfocinéticos y resultados del embarazo.

Resultados: En total, se recuperaron 2.288 ovocitos de 200 ciclos. Después de la denudación, 1.056 de los ovocitos (46% ± 16%) fueron clasificados como metafase II (MII). Un total de 333/375 (89%) de ovocitos IM y 292/540 (54%) de ovocitos GV maduraron durante la noche y se sometieron a inyección intracitoplasmática de esperma. Las tasas de fertilización de los ovocitos maduros de IM de rescate IVM (R-MI) y de GV de rescate IVM (R-GV) fueron comparables con las de sus ovocitos MII hermanos (71% vs. 66%; 66% vs. 63%, respectivamente). Tasas de escisión temprana (80% ± 35% vs. 92% ± 20%; 80% ± 42% vs. 95% ± 28%, respectivamente) y las tasas de blastulación (32 ± 40% vs. 62 ± 33%; 24 ± 37% vs. 60 ± 35%, respectivamente) disminuyeron significativamente en los cigotos derivados de ovocitos maduros con MIV de rescate (ovocitos R), pero la tasa de euploidía de blastocisto (BL) y la tasa de BL de "buena calidad" fue comparable con la de los embriones derivados de hermanos MII. Además, los embriones de MIV de rescate mostraron niveles significativamente más altos de multinucleación en las etapas de 2 y 4 células, así como tasas más altas de escisión directa del cigoto de uno a 3 a 4 celdas. En general, 21 transferencias de embriones de MIV de rescate resultaron en 3 nacidos vivos sanos.

Conclusiones: Para pacientes con baja tasa de maduración y/o bajo número de ovocitos maduros en recuperación, la MIV de rescate puede aportar ovocitos más competentes y BLs viables adicionales para la transferencia desde el mismo ciclo de estimulación, maximizando las posibilidades de embarazo y nacidos vivos.