



Could fertility clinics offer a sizable improvement of live birth rates by maturing post-GVBD oocytes in vitro?

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Abstract

Purpose The objective of this study is to analyze the potential of immature, denuded, post-GVBD (germinal vesicle breakdown) oocytes (including prometaphase I, metaphase I, and prometaphase II stages) to result live birth after in vitro maturation. Furthermore, we compared two culture media to identify which of them provides better reproductive outcomes when used for in vitro maturation.

Methods We performed a retrospective cohort study including 4022 IVF-ICSI (in vitro fertilization-intracytoplasmic sperm injection) cycles between 2011 and 2015. A total of 4450 immature post-GVBD oocytes from 1442 cycles were cultured in vitro; of these, 2364 oocytes reached MII (metaphase II) stage (IVMC oocytes, in vitro meiotic completion) and were fertilized. Overall, 3933 embryo transfers were performed: 3579 were embryos derived from MII oocytes (ET-MII); 264 were embryos derived from MII + IVMC oocytes (ET-MIX), and 90 embryos from IVMC oocytes (ET-IVMC). In total, 399 IVMC embryos were transferred.

Results Maturation rate for immature post-GVBD oocytes was 54.1%. G-2TMPLUS (Vitrolife) medium provided significantly higher maturation rate ($p < 0.001$) than G-IVFTMPLUS (Vitrolife) (65.7% vs. 42.5%, $p < 0.001$). Embryos in ET-IVMC in cleavage stage had an average morphological score of 6.8/10 (7.7 in ET-MII; $p < 0.001$). Regarding reproductive outcomes, ET-IVMC gave 11.1% biochemical pregnancy rate, 10.0% clinical pregnancy rate, 7.8% ongoing pregnancy rate, and 5.6% live birth rate.

Conclusions Embryos arising from IVMC oocytes resulted in a live birth rate of 5.6%. We suggest that in vitro maturation of denuded immature post-GVBD oocytes should be performed at the very least when few MII are collected, and likely in all patients, as they provide acceptable maturation and fertilization rates, and a sizeable increase in live birth.

Keywords IVMC oocyte · In vitro maturation · Live birth · Immature post-GVBD oocyte · Culture medium

Introduction

During each menstrual cycle, a cohort of follicles start a process of growth and maturation during which the oocytes inside the follicles undergo nuclear and cytoplasmic maturation to reach their full developmental competence, i.e., the ability to support early embryonic development. Nuclear maturation requires the progression from prophase I (germinal vesicles

(GV)) to metaphase II (MII) of meiosis, signaled by the extrusion of the first polar body (PB). Nevertheless, no visible morphological changes take place when the oocyte completes cytoplasmic maturation, essential for fertilization and early embryonic development [1].

Frequently, a portion of the oocytes is still immature at the time of oocyte pick-up (OPU) [2–4]; about 4% are in metaphase I (MI) and 11% at the GV stage [4], although these rates may differ slightly across studies depending on stimulation protocols and patient demographics. Oocytes might be immature at OPU for several reasons: the follicular stage when the hormonal stimulation was initiated, aspiration of smaller follicles during OPU, patient age, or poor response to stimulation [5]. Within ART patients, the use of in vitro meiotic completion (IVMC), i.e., the culture of immature oocytes for a few hours to allow for the extrusion of the first PB, might be

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worthwhile [5]. A higher number of mature oocytes could increase the number of oocytes available for fertilization, resulting in a higher number of embryos obtained in each cycle and therefore potentially increasing the chances of pregnancy and live birth [3, 6]. However, immature oocytes at OPU present lower fertilization rates after IVMC [3–6], present higher rates of abnormal fertilization [6, 7], worse embryo development [5–7], diminished implantation capacity [5], and lower pregnancy rates compared with in vivo matured MII oocytes [5, 8].

Culture conditions are crucial for a correct in vitro maturation [1, 9, 10]. Two different aspects should be considered regarding oocyte maturation: the environmental maturation conditions [1] and the incubation time in the media of choice [7]. Regarding the first aspect, a specific medium for the in vitro maturation of denuded immature oocytes at OPU has not been developed so far. Regarding the second aspect, a minimum of 3 h of culture after nuclear maturation and before ICSI has been reported as optimal to reach reasonable fertilization rates in in vitro matured oocytes [7].

The objective of this study is to analyze the potential of immature, denuded, post-GVBD (germinal vesicle breakdown) oocytes to produce a live birth once matured in vitro. Furthermore, we evaluate two culture media to identify which provides better reproductive outcomes when used for in vitro culture.

Materials and methods

Study population and ethical approval

This is a retrospective study including data from 4022 consecutive IVF-ICSI cycles, corresponding to 3339 patients between January 2011 and December 2015. Ethical approval for performing this study was obtained from the institutional Ethics Committee for Clinical Research.

In vitro oocyte maturation and laboratory procedures

Patients underwent controlled ovarian stimulation (COS) with follitropin alfa, follitropin beta, or highly purified urinary hMG (from 150 to 300 IU/day). Pituitary suppression was obtained by either a long protocol of GnRH agonist (triptorelin 0.1 mg, beginning the 21st day of the menstrual cycle previous to the IVF cycle, decreased to 0.05 mg when starting stimulation) or a GnRH antagonist protocol (cetrotorelix 0.25 mg/day, beginning when estradiol raised ≥ 400 pg/ml or one follicle reached 14 mm of diameter). When at least one follicle reached 17 mm of diameter, final oocyte maturation was triggered with 250 μ g (6500 IU) of choriogonadotropin alfa. Oocyte retrieval was performed strictly 36 h after triggering by ultrasound-guided transvaginal OPU. A strict control of

laboratory times was guaranteed by an automated electromagnetic tracking system (RI Witness® System, Origio, Denmark). Cumulus oophorus complexes (COCs) were placed in a dish containing 1.5 ml of G-MOPSTM PLUS (Vitrolife, Sweden) medium covered with 1.5 ml of IRVINE Scientific® oil, where they were kept until the end of the follicular aspiration procedure. COCs were then washed twice in 1.5 ml of G-IVFTMPLUS medium (Vitrolife, Sweden) covered with 1.5 ml of OVOILTTM (Vitrolife, Sweden) and incubated for 30 min at 37 °C and 6% of CO₂. Cumulus cells were then mechanically removed in hyaluronidase (HYASE 10+TM, Vitrolife, Sweden) 1:10 diluted with G-MOPSTMPLUS. Oocytes were then classified as MII, immature post-GVBD oocytes (including prometaphase I, metaphase I, and prometaphase II stages), or GV. Immature post-GVBD oocytes were washed in 25 μ l of equilibrated G-IVFTMPLUS medium and placed for maturation in a droplet of 25 μ l of the appropriate medium for further maturation.

Two culture media for final meiotic maturation were used: G-IVFTMPLUS and G-2TMPLUS (Vitrolife, Sweden). Both are bicarbonate-buffered media containing human serum albumin and gentamicin as an antibacterial agent. G-IVFTMPLUS was used from January 2011 to October 2013, after which medium was switched to G-2TMPLUS. Usually, ICSI was performed approximately 2–4 h after oocyte pick-up. Before starting ICSI, post-GVBD oocytes were observed to evaluate maturity. If post-GVBD oocytes had extruded the PB, they were injected alongside MII stage oocytes; if not, they were cultured further and checked every 2 h for PB extrusion. Upon PB extrusion, they were injected. Monitoring of the exact incubation time from OPU to ICSI once PB is extruded was carried out for each patient using an automated electromagnetic tracking system (RI Witness® System).

After ICSI, oocytes were washed in a droplet of 15 μ l G-1TMPLUS (Vitrolife, Sweden) and placed individually in droplets of 15 μ l of G-1TMPLUS. Oocytes were observed for fertilization results 16–18 h after ICSI. Only embryos with 2PN were kept in culture. Embryo evaluation was performed on the second, third, or fifth day of embryo development (D2, D3, D5), depending on the day of embryo transfer (ET). The score was assessed using a modified scale system initially proposed by Coroleu and colleagues [11], which takes into account number of blastomeres, symmetry of blastomeres, and percentage of fragmentation, giving a maximum score of 10. As no numerical score is assigned to the embryos transferred on D5, the score used for these embryos for statistical analysis corresponds to the numerical score they had on D3. The highest scoring embryos were chosen for ET and the remaining good-quality embryos were cryopreserved. For statistical analysis, only fresh ETs were taken into account. Most ETs were done with embryos originated from MII oocytes. When no embryos from MII oocytes were available, embryos originated from IVMC oocytes were transferred instead. After

ET, a protocol for luteal phase support (400 mg of progesterone by vaginal administration for the first 8 weeks of pregnancy) was prescribed to patients.

The procedures described above remained identical during all the study period, limiting potential bias.

Statistical analysis

Laboratory outcomes (in vitro maturation rate and fertilization rate) were analyzed according to the database of oocytes available for ICSI (MII oocytes retrieved at OPU and matured IVMC). The 4022 ICSI cycles of this database were classified according to oocyte maturation: the “ICSI-MII” group, comprising cycles with only MII oocytes; the “ICSI-MIX” group, comprising cycles with both MII and IVMC oocytes; and the “ICSI-IVMC” group, comprising cycles with only IVMC oocytes (Fig. 1).

Reproductive outcomes (biochemical pregnancy: a positive beta-HCG level in serum 14 days after embryo transfer; clinical pregnancy: one or more intrauterine sacs visible by ultrasound scan at 7 weeks of pregnancy; ongoing pregnancy: pregnancy confirmed at the week 12; and live birth) were analyzed according to the database of embryos available for ET. The 3933 ET cycles of this database

were classified according to oocyte maturation in the following: “ET-MII” group, comprising ET cycles of embryos originating only from MII oocytes; the “ET-MIX” group, comprising ET cycles of embryos originating from both MII and IVMC oocytes; and the “ET-IVMC” group, comprising ET cycles of embryos originating only from IVMC oocytes.

Univariable analyses with parametric tests (ANOVA) were performed to evaluate the differences in laboratory outcomes, while non-parametric tests (Pearson’s χ^2) were used for reproductive outcomes among the groups defined by oocyte maturation, as well as between culture media. Post hoc pairwise comparisons with Bonferroni’s correction were further applied to identify statistically significant differences compared with the ICSI-MII or the ET-MII groups.

To calculate the pregnancy probability reduction (relative risk) of transferring IVMC and mixed vs. MII embryos adjusted for potential confounders (age, BMI, and number of transferred embryos), a general linear model procedure was performed (Poisson family, robust error variance calculation, log link function), according to the methodology proposed by Zhou et al. [12].

Additionally, the effect of the time elapsed between OPU and ICSI on maturation rate was evaluated by means of locally

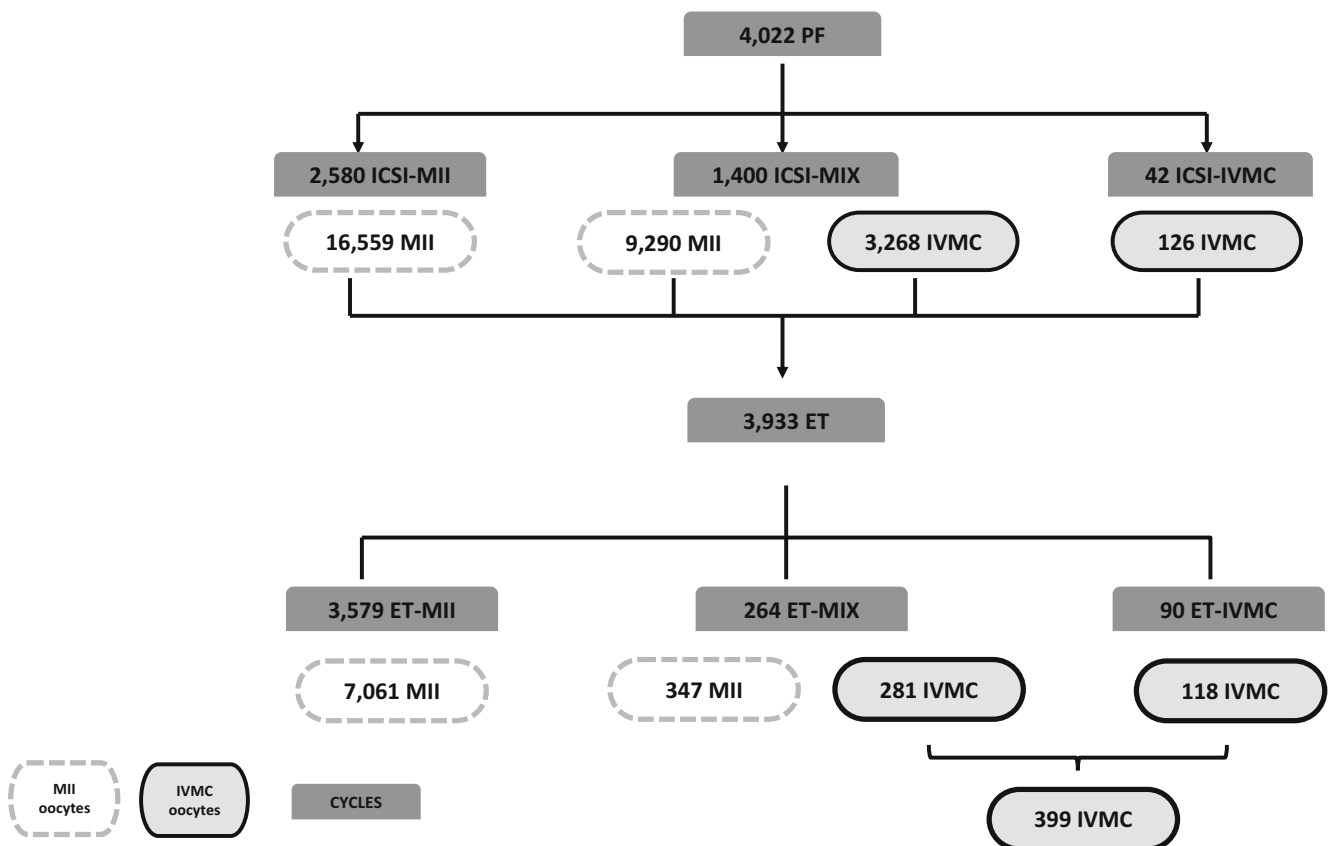


Fig. 1 Study groups analyzed based on the oocytes available for ICSI and the origin of the oocytes that generated the transferred embryos

weighted scatterplot smoothing (LOWESS) regression, with a fit to 50% of the points and an Epanechnikov weight function.

Analyses were performed using SPSS version 22.0 and Stata version 13.0. A p value < 0.05 was set as statistically significant.

Results

Baseline characteristics at times of ICSI and ET

Overall, the mean age of the patients included in the study was 37.7 years old (SD 4.8) and the mean BMI was 23.7 kg/m² (SD 4.1). Women in the ICSI-IVMC group were significantly older and presented a higher BMI than women in ICSI-MII and ICSI-MIX groups. The mean number of cumulus oocyte complexes (COCs) retrieved was significantly higher for the ICSI-MIX group. The longest mean incubation time for the ICSI-IVMC group was 6.6 h (SD 1.7 h). The baseline characteristics at time of ICSI by the study group are presented in Table 1. Of the 4022 ICSI cycles evaluated, 2580 included only MII oocytes (ICSI-MII; $n = 16,559$ MII oocytes); 1400 included a mixture of MII and IVMC oocytes (ICSI-MIX; $n = 9290$ MII and 3268 IVMC oocytes); and 42 included only IVMC oocytes (ICSI-IVMC; $n = 91$ IVMC oocytes) (Fig. 1).

Of the 3933 ET performed, 3579 included only embryos developed from MII oocytes (ET-MII; $n = 7061$ embryos), 264 ET were mixed transfers (ET-MIX; $n = 347$ MII-embryos and $n = 281$ IVMC embryos), and 90 ET included only IVMC embryos (ET-IVMC; $n = 118$ embryos) (Fig. 1). Some of the ET-IVMC embryos came from IVMC oocytes included in the ICSI-MIX group. In total, 399 IVMC embryos

were transferred fresh. Double ET was common in ET-MII and ET-MIX (61.0% and 62.1% respectively), whereas single ET was most frequent in the ET-IVMC group (72.2%; $p < 0.001$). Other ET characteristics are presented in Table 2.

In vitro maturation, fertilization rates, and embryo quality

Overall, the in vitro maturation rate was 54.1%. As expected, an increase in in vitro maturation rates was observed as incubation time increased, reaching a plateau after 8 h (Fig. 2).

The mean fertilization rates were 68.1% and 47.9% for the ICSI-MII and ICSI-IVMC groups respectively ($p < 0.001$). The number of non-fertilized oocytes was higher for IVMC oocytes than for MII oocytes: 35.7% vs. 16.1% ($p < 0.001$). However, we observed similar results for abnormal fertilization (6.4% in the ICSI-MII group vs. 6.8% in the ICSI-IVMC group, $p = 0.48$), as well as percentage of degenerated oocytes (9.4% in the ICSI-MII group vs. 9.6% in the ICSI-IVMC group, $p = 0.79$). Embryos in the ET-IVMC group presented lower morphological scores (6.8, SD 1.2) compared with those included in the ET-MII group (7.7, SD 1.3) ($p < 0.001$).

Reproductive outcomes

Reproductive outcomes by the study group (ET-MII, ET-MIX, ET-IVMC) are presented in Table 3. As expected, all reproductive outcomes were significantly higher in the ET-MII group, although they were also sizeable for the ET-IVMC group: biochemical pregnancy rate was 11.1%, clinical pregnancy rate 10.0%, ongoing pregnancy rate 7.8%, and live birth

Table 1 Baseline characteristics of patients at time of ICSI by the study group (ICSI-MII, ICSI-MIX, ICSI-IVMC)

	Overall ($n = 4022$)	ICSI-MII ($n = 2580$)	ICSI-MIX ($n = 1400$)	ICSI-IVMC ($n = 42$)	p -value ¹
Age (years); mean (SD)	37.7 (4.8)	37.8 (4.8)	37.2 (4.7)*	38.8 (5.3)	0.001
BMI (kg/m ²); mean (SD)	23.6 (4.1)	23.6 (4.1)	23.7 (4.1)	25.9 (5.5)*	0.001
Oocyte recovered at OPU; mean (SD)					
COC	9.2 (6.0)	8.4 (5.9)	10.9 (5.8)*	4.6 (3.8)*	< 0.001
MII	6.4 (4.4)	6.4 (4.5)	6.6 (4.1)	0*	< 0.001
MI	1.1 (1.6)	0.4 (0.9)	2.3 (1.8)*	3.0 (2.1)*	< 0.001
GV	1.1 (1.8)	0.9 (1.6)	1.4 (2.0)*	0.4 (0.9)	< 0.001
IVMC	0.6 (1.0)	0	1.6 (1.0)*	2.2 (1.3)*	< 0.001
Timeframe between OPU and ICSI (h); mean (SD)	4.6 (1.8)	4.2 (1.5)	5.3 (2.1)*	6.6 (1.7)*	< 0.001

COC cumulus-oophorus complex, GV germinal vesicle, ICSI = intracytoplasmic injection, IVMC in vitro meiotic completion, MI metaphase I oocytes (immature post-GVBD oocytes), MII metaphase II oocytes (mature oocytes), OPU oocytes pick-up, SD standard deviation

*Statistically significant difference after pairwise comparison with Bonferroni's correction between this group and ICSI-MII

¹ One-way ANOVA or Pearson's Chi² test, as appropriate

Table 2 Embryo transfer (ET) characteristics by the study group (ET-MII, ET-MIX, ET-IVMC)

	ET-MII (n = 3579)	ET-MIX (n = 264)	ET-IVMC (n = 90)	p-value ¹
Origin of sperm; n (%)				
Partner	2002 (55.9)	133 (50.4)	60 (66.7)	0.018
Donor	1577 (43.7)	131 (49.6)	30 (33.3)	
Status of sperm; n (%)				
Fresh	1347 (37.7)	90 (34.1)	35 (38.9)	0.48
Frozen	2228 (62.3)	174 (65.9)	55 (61.1)	
ET day; n (%)				
D2–4	3442 (96.2)	258 (97.7)	89 (98.9)	0.18
D5	137 (3.8)	6 (2.3)	1 (1.1)	
Transferred embryos; n (%)				
1	747 (20.9)	0 (0.0)*	65 (72.2)*	< 0.001
2	2182 (61.0)	164 (62.1)	22 (24.4)*	
3	650 (18.2)	100 (37.9)*	3 (3.3)*	
Mean embryo quality at D2–D3 of transferred embryos; mean (SD)	7.7 (1.3)	7.6 (1.3)	6.8 (1.2)*	< 0.001

COC cumulus-oophorus complex, GV germinal vesicle, ICSI intracytoplasmic injection, IVMC in vitro meiotic completion, MI metaphase I oocytes (immature post-GVBD oocytes), MII metaphase II oocytes (mature oocytes), n number of cycles, OPU oocyte pick-up, SD standard deviation

*Statistically significant difference after pairwise comparison with Bonferroni’s correction between this group and ICSI-MII

¹ One-way ANOVA or Pearson’s Chi² test, as appropriate

5.6%. Supplementary table 1 provides the main characteristics of the five ET-IVMC cycles resulting in live birth.

Double ET gave an average implantation rate of 13.1% and 20.2% in the ET-MIX and ET-MII groups respectively (p = 0.002). Implantation rates for single

ET were significantly lower in the ET-IVMC group compared with the ET-MII group (6.2% vs. 14.2% (p = 0.09)).

Supplementary table 2 shows the pregnancy probability reduction when IVMC-derived embryos or mixed embryos

Fig. 2 In vitro maturation rate across the time elapsed between the oocyte pick-up (OPU) and ICSI by means of locally weighted scatterplot smoothing (LOWESS) regression

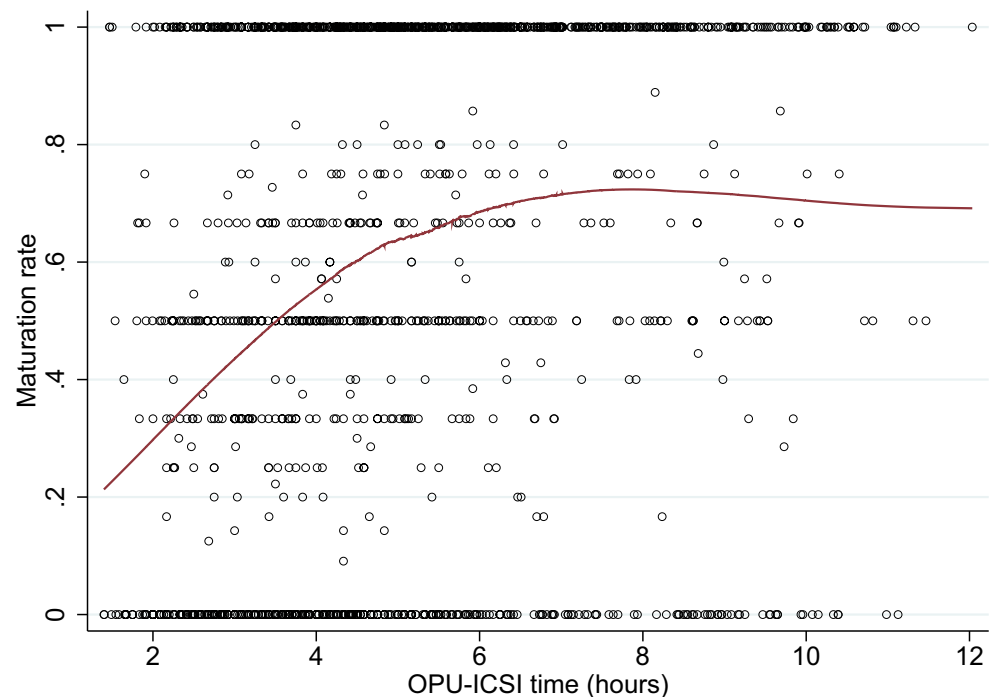


Table 3 Reproductive outcomes by the study group (ET-MII, ET-MIX, ET-IVMC)

	ET-MII (<i>n</i> = 3581)	ET-MIX (<i>n</i> = 264)	ET-IVMC (<i>n</i> = 90)	<i>p</i> -value ¹
Biochemical pregnancy, <i>n</i> (%)	1319/3577 (36.9)	84/264 (31.8)	10/90 (11.1)*	< 0.001
Implantation rate at 7 weeks, sacs/transferred embryos (%)	1174/7061 (16.6)	68/628 (10.8)	9/118 (7.6)*	< 0.001
Clinical pregnancy, <i>n</i> (%)	1061/3520 (30.1)	65/264 (24.6)	9/90 (10.0)*	< 0.001
Ongoing pregnancy, <i>n</i> (%)	858/3519 (24.4)	49/263 (18.7)	7/90 (7.8)*	< 0.001
Live birth, <i>n</i> (%)	632/3348 (18.9)	39/255 (15.3)	5/89 (5.6)*	0.003

ET embryo transfer, IVMC in vitro meiotic completion, *n* number of cycles, MII metaphase II

*Statistically significant difference after pairwise comparison with Bonferroni's correction between this group and ICSI-MII

¹ Pearson's Chi² test

were transferred instead of MII-derived embryos, after adjustment for potential confounders. To note, the probability of live birth was reduced by 53% (95% CI 80% reduction, 11% increase; $p = 0.08$) and by 19% (95% CI 40% reduction, 9% increase; $p = 0.17$), for IVMC-derived embryos and mixed embryos, respectively. Nevertheless, the chances of pregnancy with IVMC-derived embryos are not negligible.

Comparison between incubation media

The incubation medium for immature post-GVBD oocytes was either G-IVFTMPLUS ($n = 2421$ oocytes) or G-2TMPLUS ($n = 2029$ oocytes). A significantly higher in vitro maturation rate was registered when using G-2TMPLUS vs. G-IVFTMPLUS (65.7% vs. 42.5%, $p < 0.001$). Similarly, higher fertilization rates were observed with G-2TMPLUS than with G-IVFTMPLUS (54.5% vs. 40.7% ($p < 0.001$)). Abnormal fertilization rate was, however, comparable between the two media (G-IVFTMPLUS, 7.2% vs. G-2TMPLUS, 6.7%; $p = 0.76$), whereas a higher number of non-fertilized oocytes were observed with G-IVFTMPLUS (44.5% vs. 27.6%, $p < 0.001$). The average morphological score for embryos originated from IVMC matured in G-2TMPLUS medium (6.3, SD 1.6) was similar to that of embryos matured in G-IVFTMPLUS (6.4, SD 1.5) ($p = 0.45$).

Table 4 Reproductive outcomes by maturation media

<i>n</i> = number of cycles	G-IVF TM PLUS (Vitrolife) (<i>n</i> = 32)	G-2 TM Plus (Vitrolife) (<i>n</i> = 58)	<i>p</i> -value ¹
Biochemical, <i>n</i> (%)	2 (6.3)	8 (13.8)	0.28
Clinical, <i>n</i> (%)	2 (6.3)	7 (12.1)	0.38
Ongoing, <i>n</i> (%)	2 (6.3)	5 (8.6)	0.69
Live birth, <i>n</i> (%)	2 (6.3)	3 (5.3)	0.85

¹ Pearson's Chi² test

When analyzing reproductive outcomes by maturation media, oocytes in ET-IVMC seemed to give a higher clinical pregnancy rate when matured in G-2TMPLUS compared with G-IVFTM PLUS (12.1% vs. 6.3%), although this difference was not statistically significant ($p = 0.38$). The size of the groups was too small to draw conclusions: 7/58 vs. 2/32 positive clinical pregnancies, respectively (Table 4).

Discussion

The clinical use of immature post-GVBD oocytes might be worthwhile in order to increase the number of transferrable embryos in a patient, hence increasing cycle efficiency and pregnancy rates. However, several studies have shown that IVMC oocytes have a compromised developmental competence [5–7].

Nevertheless, these studies either date back several years ago (i.e., were performed using laboratory techniques and equipment currently superseded in the ART field [4]) or had small sample sizes in terms of ET [3]; in addition, IVMC oocyte developmental competence was not scored. Moreover, since there are no commercial media designed to support the final stages of meiotic maturation, there is no current guidance on the type of medium that might preserve their ability to develop into viable pregnancies. In the present study, a total of 2364 IVMC-fertilized oocytes were analyzed; importantly, 354 ET cycles included at least one embryo derived from IVMC, making it one of the largest studies of IVMC outcomes to date.

Both nuclear and cytoplasmic maturation are required for immature post-GVBD oocytes in order to gain developmental competence [7]. In our study, overall, 54.1% of immature post-GVBD oocytes retrieved at OPU extruded the first PB after in vitro culture. Similar maturation rates had been previously reported: 43.0% when cultured in Sydney IVF Fertilization Medium (Cook, Ltd., Ireland) or Early Cleavage Medium (Irvine Scientific, Belgium) from 2 to 11 h after OPU and 45.1% when cultured in G1.2 (Vitrolife, Sweden) from 1.5 to 4.5 h after OPU [5, 6]. In our study, a

significant higher maturation rate was observed when using G-2TMPLUS rather than G-IVFTMPLUS.

Culture conditions have a significant influence on the ability of oocytes to complete meiosis in vitro [1, 9]. In the absence of a specifically formulated medium for in vitro maturation, G-IVFTMPLUS and G-2TMPLUS were used in our laboratories for this purpose. Unfortunately, detailed official compositions of these media are not available, although a summary of their components is given by the manufacturer. However, the G-2TMPLUS protein content has been analyzed independently [13]. The presence of hyaluronan in G-2TMPLUS represents the main difference compared with G-IVFTMPLUS. The ability of granulosa cells to synthesize hyaluronan, essential for oocyte maturation and further embryo development, is well known [14]. While culture with cumulus cells did not seem to alter significantly the maturation rate of MI to MII stage oocytes [15], a considerable but not significant improvement in the maturation rates of GV cultured with granulosa cells was reported [16]. Nevertheless, well-designed comparative studies carried out with media specially designed for in vitro maturation in the future would be helpful to identifying a medium ideal for maturation.

In vitro incubation time is another crucial factor [7]. Most immature post-GVBD oocytes extruded the first PB between 2 and 8 h of in vitro culture. The maturation rate did not increase significantly after 8 h, indicating that oocytes with the ability to complete meiosis are already relatively close to doing so when collected from the follicle; in other words, removal of the oocyte from the follicular environment too early after GVBD does not seem to allow for efficient completion of meiosis. Up to 4 h is the most often cited incubation time for maturing oocytes [3, 6], as it seems to balance the time needed for immature post-GVBD oocytes to reach MII stage and post-maturation oocyte aging of the rest of the cohort [6]. The effect of oocyte aging on its developmental competence seems to be mediated by the woman's age, with little or no clinical effect in younger women [17], and a significant decrease in viability in older women [18]. Time-lapse systems may be optimal to minimize disturbance to oocyte culture during assessment of oocyte maturation in further studies and to record data in a more precise way.

Even though immature post-GVBD oocytes could reach the MII stage, their fertilization rate remains lower compared with MII stage oocytes at OPU (47.9% vs. 68.1%; $p < 0.001$). Our results are in agreement with those previously reported: 52.0% IVMC vs. 68.0% MII in oocytes cultured in Sydney IVF Fertilization Medium or Early Cleavage Medium [5] and 52.7% IVMC vs. 70.8% MII in oocytes cultured in B2 medium [4]. Currently, oocyte maturity is assessed morphologically by the extrusion of the first PB, i.e., nuclear maturation. An additional time for cytoplasmic maturation after first PB extrusion might be necessary to obtain higher fertilization rates [7].

Embryo developmental competence of IVCM oocytes also appears to be reduced [5–7]; a potentially higher rate of aneuploid blastomeres has been observed in embryos developed from IVCM oocytes [19], as well as abnormalities in embryo development linked to incomplete cytoplasm maturation [20]. Nevertheless, the crucial issue about IVMC oocytes is their capacity to give rise to healthy pregnancies, which is clearly reduced compared with MII oocytes at OPU [4–7]. In our study, a clinical pregnancy rate of 10.0% was measured in IVMC-derived embryos. Our results are in concordance with previously reported results of 7.7% in Sydney IVF Fertilization Medium or Early Cleavage Medium [5]. An overall live birth rate of 5.6% was observed in the ET-IVMC group. We recognize three main limitations to our study. First, the fact that the vast majority of embryos originated from IVMC oocytes were transferred together with one or two embryos developed from a MII oocyte, making it impossible to monitor all pregnancies obtained by an IVMC oocyte. Second, the impossibility to record the exact timing between PB extrusion and ICSI. Third, each culture medium for oocyte maturation was used in a different period of time. Nevertheless, other laboratory and medical procedures remained identical during all the study period, limiting potential bias.

In conclusion, IVMC oocytes should not be discarded, as they offer a sizable improvement in the live birth rate per cycle at no extra cost to the patient. Until a culture medium specifically designed for meiosis completion in absence of cumulus cells is developed, culturing immature post-GVBD oocytes in G-2TMPLUS is an acceptable strategy to preserve their limited developmental potential.

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Authors' roles N.M.: involved in study design, data analysis, and manuscript preparation. D.G.: involved in study design, data analysis, statistical analysis, and manuscript preparation. R.V. and A.R.: involved in study design, implementation, supervision, expert knowledge, and manuscript preparation.

Compliance with ethical standards

Ethical approval for performing this study was obtained from the institutional Ethics Committee for Clinical Research.

Conflict of interest The authors declare that they have no conflict of interest.

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