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Effect of single versus grouped culture of human cumulus–oocyte complexes in PCOS women treated with biphasic in vitro maturation: A sibling oocyte pilot study

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Abstract

Purpose: This study investigated the differences in the maturation rate of single versus grouped cumulus–oocyte complexes (COCs) culture methods for capacitation in vitro maturation (CAPA-IVM) in women with polycystic ovary syndrome (PCOS).

Methods: This study was performed at My Duc Phu Nhuan Hospital, Vietnam from October 1, 2020 to October 24, 2021. Women aged 18–37 years with a diagnosis of PCOS were recruited. COCs from each woman were randomly divided into two groups: single or grouped culture during CAPA-IVM culture. The primary outcome was the maturation rate.

Results: A total of 322 COCs from 15 eligible women included were randomly assigned to the two study groups. The maturation rate was comparable between the single and grouped culture groups (61.3% vs. 64.8%; $p=0.56$). There were no significant differences in the number of 2-pronuclei fertilized oocytes, number of day-3 embryos, and number of good-quality embryos in the two culture method groups. In the single culture group, COCs morphology was associated with the day-3 embryo formation rate but not the maturation rate.

Conclusions: Comparable oocyte maturation and embryology outcomes between single and grouped COCs culture utilizing sibling COCs derived from women with PCOS suggest the feasibility of both methods for CAPA-IVM culture.

KEYWORDS
biphasic in vitro maturation, cumulus–oocytes complexes, in vitro maturation, single culture

1 | INTRODUCTION

Oocyte in vitro maturation (IVM) is an alternative approach to assisted reproductive technology (ART) that has the advantage of minimal stimulation, resulting in reduced hormone-related side effects and risks, especially in women with polycystic ovary syndrome (PCOS).[1] However, despite these benefits, the utilization of IVM is not widespread due to a perceived efficiency gap compared with conventional ART methods.

Oocytes retrieved for IVM procedures are derived from a diverse pool of follicles with an average diameter of between 2 and 10 mm and are characterized by variable cellular and molecular attributes that

indicate their immature status.[2] Therefore, the development of an IVM culture system that could enable and enhance the acquisition and synchronization of meiotic and developmental competence prior to the meiotic resumption is essential for optimizing human IVM protocols.

A biphasic IVM with a pre-maturation step, known as capacitation-IVM (CAPA-IVM), has been shown to improve the competence of human oocytes matured in vitro and result in live births.[3–5] The pre-maturation culture step of CAPA-IVM utilizes C-type natriuretic peptide (CNP), and maturation takes place in the presence of amphiregulin (AREG), both of which are physiological compounds that have been shown to prevent spontaneous meiotic resumption of oocytes (CNP) and enhance oocyte competence (AREG) during IVM.[6–9]

To date, the results of pilot studies have shown that CAPA-IVM increases the rates of oocyte maturation, good-quality embryos on day 3 and good-quality blastocysts compared with standard IVM.[3,6] Furthermore, CAPA-IVM blastocyst analysis indicated similar methylation and gene expression rates at genomic differentially methylated regions to conventionally matured oocytes, with a comparable expression of major epigenetic regulators.[10] Additionally, the reported cumulative live birth rate after the use of CAPA-IVM and its non-inferiority to the cumulative live birth rate with standard in vitro fertilization highlight the clinical utility and potential of this approach.[5,11]

So far, all CAPA-IVM studies have been completed using a grouped cumulus–oocyte complexes (COCs) culture approach.[3–5] On the other hand, monitoring the development of each oocyte individually throughout the culture in relation to the embryological outcomes would be highly beneficial in identifying the markers for oocytes competence and adapting the strategies to improve the CAPA-IVM culture system. Therefore, this pilot study investigated the feasibility of applying a single COC culture strategy during CAPA-IVM in women with polycystic ovary syndrome (PCOS) through comparing it with the standard grouped COCs culture approach.

2 | MATERIALS AND METHODS

2.1 | Study design

This prospective sibling oocyte pilot study (NCT04562883) was conducted at My Duc Phu Nhuan Hospital, Ho Chi Minh City, Vietnam from October 1, 2020 to October 24, 2021. The study was approved by the Institutional Ethics Committee at My Duc Hospital, Ho Chi Minh City, Vietnam (13/20/DD-BVMD, dated September 3, 2020) and conducted according to Good Clinical Practice and Declaration of Helsinki 2002 principles. All participants provided written informed consent.

2.2 | Study population

This study recruited women aged 18–37 years with polycystic ovarian morphology, defined as having ≥25 follicles of 2–9 mm in diameter throughout the ovary and/or increased ovarian volume (>10 mL),[12] who had ≥15 COCs collected during oocyte pick-up (OPU). Exclusion criteria were high grade endometriosis (above grade 2) and cases with extremely poor sperm (concentration <1 million, mobility <10%, and sperms retrieved after percutaneous epididymal sperm aspiration/testicular sperm extraction [TESE]/microTESE). On the first visit, women were provided with information about the study and those who agreed to participate signed the consent form. After OPU, the collected COCs from each patient were randomly divided into two groups: single COC culture and grouped COCs culture during the CAPA-IVM steps.

2.3 | Patient preparation

On the second day of the menstrual cycle, women had an ultrasound scan and levels of the following hormones were determined: anti-Müllerian hormone, sex hormone-binding globulin, testosterone, thyroid-stimulating hormone, 17-hydroxyprogesterone, and dehydroepiandrosterone sulfate. Women were treated with 2 days of gonadotropins (Menopur®, Ferring Pharmaceuticals, Switzerland) 150 IU/day starting on day 2 of a spontaneous menstrual cycle. OPU was performed 42 h after the last injection of gonadotropins. Before OPU, women had the last ultrasound scan to determine follicle count. The OPU procedure was the same for all women, as described previously.[5] After aspiration, follicular fluid was transferred to the laboratory, where COCs were isolated using the sliding technique under a stereomicroscope.

2.4 | IVM protocol

After OPU, all COCs were washed in a retrieval medium (global® Collect®, Life Global, USA).[3,4] If the total number of COCs met the study criteria (≥15) after completion of the aspiration procedure, they were included in the study and divided into the single or grouped culture group for the subsequent CAPA-IVM step. COCs morphology after OPU was considered so that each group contained an equal number of completely covered COCs and partially covered COCs, as described in Figure 1. If there was an odd number of COCs, the odd COC was assigned to grouped culture. Oocytes without any surrounding corona-cumulus were not considered for further culture.

In the pre-maturation step, COCs were cultured in a pre-maturation medium, including IVM medium (MediCult IVM System, ORIGIO, Denmark), supplemented with recombinant follicle-stimulating hormone (rFSH) 1 mIU/mL (Puregon, MSD, Australia), insulin 5 ng/mL (Insulin human, Sigma-Aldrich, Germany), estradiol 10 nmol/L (Estradiol, Sigma-Aldrich, Germany), human serum albumin 10 mg/mL (Human Serum Albumin, SAGE, Denmark), and CNP-22 25 nmol/L (C-type natriuretic peptide 1–22, Tocris, UK) – for 24 h at 37°C, 6% carbon dioxide and 20% oxygen, under oil.

For the single COC culture group, 30 µL of individual droplets of CAPA media were prepared in 60 mm Petri dishes (Nunc™, ThermoFisher Scientific, USA) to culture one COC per droplet. In the grouped COCs culture group, 500 µL CAPA media was added to one well of a 4-well dish (Nunc™, ThermoFisher Scientific, USA)

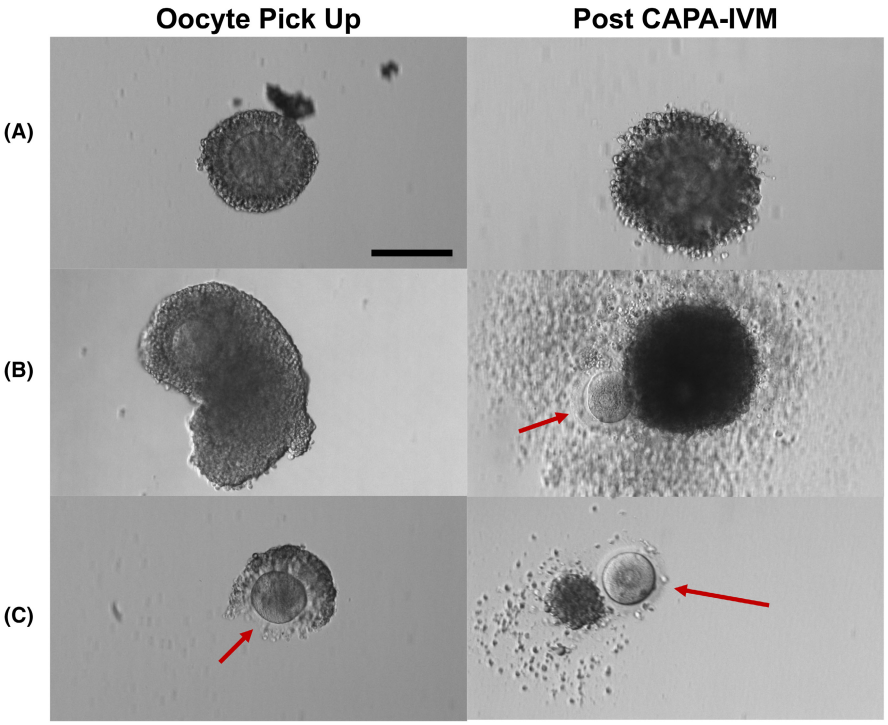

FIGURE 1 Representative examples from the single cumulus–oocyte complexes (COCs) culture group after oocyte pick-up (left panel) with an oocyte that is only partially surrounded by cumulus cells (arrow in C), and the morphology of each COC after capacitation in vitro maturation (CAPA-IVM) culture for 54 h (right panel). Representative examples are shown of one oocyte that was fully surrounded by cumulus cells (A) and two oocytes that were totally disconnected from their cumulus cells after 54 h culture (arrows in B and C). Scale bar 200 μm

for culturing 5–10 COCs, as previously described.[3,5] In both groups, media were overlaid with oil (Liquid Paraffin, ORIGIO, Denmark) and equilibrated overnight at 37°C, 6% carbon dioxide and 20% oxygen.

After pre-maturation culture, COCs were washed and transferred into a maturation medium including IVM medium, supplemented with rFSH 100 mIU/mL, insulin 5 ng/mL, estradiol 10 nmol/L, and human recombinant AREG 100 ng/mL (Recombinant Mouse Amphiregulin Protein, R&D Systems, USA) and incubated for a further 30 h at 37°C, 6% carbon dioxide and 20% oxygen, under oil. The preparation of the IVM step for the two groups was similar to the CAPA step mentioned above. Hence, 30 μL per droplet of IVM media per one COC in single COC culture group and 5–10 COCs per 500 μL IVM media in grouped COC culture group were applied.

2.5 | Assessment of COCs morphology

The assessment of COCs morphology was performed after OPU and after CAPA-IVM culture, as previously described.[3] Briefly, oocytes characterized by a minimum of three layers of cumulus cells fully enveloping them were categorized as "fully surrounded COCs", whereas those with only partial cumulus cell coverage were designated as "partially surrounded COCs". Oocytes that completely disconnected from cumulus cells after incubation in CAPA-IVM were classified as "released oocyte COCs". The COCs morphologies, and the maturation and embryology formation rate, within the single COCs culture group were based on following classification (Figure 1). The subgroup (A) included oocytes with fully surrounded COCs at OPU and CAPA-IVM culture; (B) with fully surrounded COCs at OPU and released oocytes COCs after CAPA-IVM culture; and (C) with partially surrounded COCs at OPU and released oocytes COCs after CAPA-IVM culture.

2.6 | Insemination and embryo culture

After 30 h of IVM culture, oocytes were denudated and oocyte maturation was assessed under the inverted microscope. Oocytes were classified as maturating to the metaphase II (MII) stage by the presence of the first polar body. MII oocytes were fertilized using intracytoplasmic sperm injection (ICSI) and cultured in an incubator at 37°C, 6% carbon dioxide and 5% oxygen, under oil. For both groups, 20 μL medium (global® total® LP, Life Global, USA) per droplet was placed onto 12 wells of a GPS dish (μDrop GPS, LifeGlobal, USA) for embryo culture. Embryos were individually cultured using a time-lapse incubator (CCM-iBIS, Astec, Japan).

Fertilization check was performed 16–18 h after insemination. Day-3 embryo evaluation was performed at 66 ± 2 h after ICSI, based on the Istanbul consensus.[13] Day-3 embryos were vitrified with a maximum of two embryos per cryotec (Cryotec, Cryotech, Japan) based on the quality of embryos and each couple's preference.

2.7 | Cumulus cell collection and gene expression analysis

The expression of five target genes was determined and compared between the single and grouped COCs culture strategy groups: *amphiregulin* (*AREG*), *hyaluronan synthase 2* (*HAS2*), *transient receptor potential cation channel subfamily M Member 7* (*TRPM7*), *gremlin 1* (*GREM1*) and *cell division cycle 42* (*CDC42*). The procedures for cumulus cell collection and gene expression analysis have been described previously.[9] Primer sequences (Integrated DNA Technologies, Singapore) were designed in-house for five target genes and two housekeeping

genes (Table S1). The quantitative-polymerase chain reaction (qPCR) protocol was setup using the same process as previously.[9]

2.8 | Study outcomes

The primary outcome was oocyte maturation rate (defined as the ratio of MII oocytes to the total oocyte count). Secondary outcomes were the rates of 2-pronuclei fertilized (2PN) oocytes, day-3 embryos, and good-quality embryos (defined as day-3 embryos with a minimum of six blastomeres and <25% fragmentation). The correlation between COCs morphology after OPU and CAPA-IVM culture and their respective maturation statuses and embryology outcome was also analyzed.

2.9 | Statistical analysis

Baseline data are presented using descriptive statistics (mean and standard deviation for normally distributed variables, or median and interquartile range for skewed variables). Categorical data are presented as number (%). Between-group differences in primary and secondary outcomes were analyzed using nonparametric methods for paired samples or Fisher's exact test (categorical variables), reported as relative risk (RR) and 95% confidence interval (CI) values. The differences between COCs morphology subgroups and maturation and embryo formation rate were assessed using Fisher's Exact test. Log_2 fold change was used for gene expression analysis, and differences between-group differences were determined using the bootstrap method. A p-value of <0.05 was considered statistically significant for all tests. Statistical analyzes were performed using GraphPad Prism (GraphPad Software, USA, www.graphpad.com) or R statistical program version 3.5.0.

3 | RESULTS

3.1 | Study population

A total of 15 women with PCOS were enrolled (mean age 29.0 ± 3.2 years, mean body mass index 21.6 ± 2.6 kg/m^2), and 322 COCs from these participants were randomly divided into the study groups (160 and 162 in the single and grouped culture groups, respectively) (Figure 2). Mean duration of infertility was 4 years, and half of the study population had primary infertility (Table 1).

3.2 | Maturation and embryology outcomes

The maturation rates were comparable between the single and grouped COCs culture groups (61.3% vs. 64.8%; 95% CI 0.95 [0.8–1.12], respectively; $p = 0.56$) (Table 2). The numbers of 2PN oocytes, day-3 embryos, and frozen embryos were also similar in the two groups (Table 2).

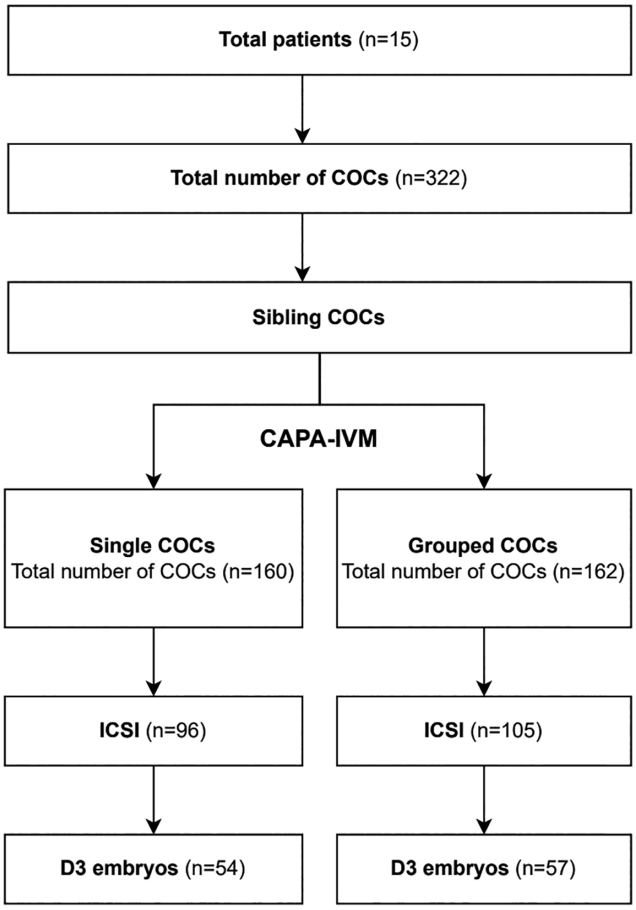

FIGURE 2 Study flowchart. CAPA-IVM, capacitation in vitro maturation; COCs, cumulus–oocyte complexes; D3, day 3; ICSI, intracytoplasmic sperm injection; IVM, in vitro maturation.

The mean numbers of COCs per patient were 10 in each group (Table 3). The median numbers of matured oocytes, day-3 embryos and good-quality day-3 embryos did not differ significantly between groups (Table 3). Although all patients had at least one embryo available from the oocytes in the grouped culture group, one patient had no embryos from the oocytes in the single culture group. The numbers of partially surrounded COCs after OPU were similar between the two groups (15 per group).

3.3 | COCs morphology and embryology outcomes

There was no significant difference in the oocyte maturation rate between the three subgroups based on COCs morphology, but the day-3 embryo formation rate was significantly different among subgroups (Table 4).

3.4 | Cumulus cell gene expression analysis

Of all the cumulus cell samples, 70 from the single culture group and 12 from the grouped culture group were successfully amplified. After the exclusion of samples with low RNA concentration,

gene expression results were available from a total of 41 samples (Table S2). There were no significant differences in the expression of the five evaluated genes between the single and grouped culture groups (Figure 3).

TABLE 1 Patient characteristics and cycle parameters at baseline.

Characteristic	Participants ($n = 15$)
Age, years	29.0 ± 3.2
Body mass index, kg/m^2	21.6 ± 2.6
Anti-Müllerian hormone, ng/mL	9.0 ± 3.0
Duration of infertility, years	4 [2; 5]
Type of infertility, *n* (%)	
Primary	7 (46.7)
Secondary	8 (53.3)
Hormonal profile at first visit	
Testosterone, nmol/L	1.4 [1.1; 2.0]
Sex hormone binding globulin, nmol/L	32.1 [18.8; 42.3]
Thyroid-stimulating hormone, μIU/mL	2.2 [1.7; 3.6]
Dehydroepiandrosterone sulfate, μg/mL	2.4 [2.0; 2.9]
17-Hydroxyprogesterone, ng/mL	1.1 [1.0; 1.2]
Number of follicles at last ultrasound	47 [36; 70]

Note: Values are mean ± standard deviation, median [quartile 1; quartile 3], or number of patients (%).

4 | DISCUSSION

This prospective pilot study showed no significant difference in the maturation rate and embryology results between single versus grouped culture of COCs during CAPA-IVM in women with PCOS. These findings suggested that both culture methods could effectively support oocyte maturation and subsequent embryo development during CAPA-IVM. Moreover, the results provided valuable insights into the feasibility of implementing the CAPA-IVM system using different cultural approaches. This is important because the time-lapse incubators offer added value to investigations but require single unit culture setup.

The comparable embryology results in the current study are consistent with previous investigations into the efficacy of the CAPA-IVM system with respect to embryo development and pregnancy outcomes.[3,5,11] Day-3 good-quality embryo rates per COCs in the current study (24.4% and 22.8% in the single and grouped culture groups, respectively) were also similar to the 24% rate reported by Sanchez et al.[3] Numbers of day-3 embryos and frozen embryos were similar to those in our previous sibling oocyte study,[9] at approximately four per group. Furthermore, the maturation rate was consistent across our current study and four previous studies, ranging from 62% to 64.3%.[3,5,9,11]

To the best of our knowledge, this is the first study to compare the effectiveness of a single versus grouped culture strategy in CAPA-IVM. Until now, there was only one study that investigated whether single COCs culture influenced the quality of mice oocytes after one-step IVM.[14] In that study, the authors used three experimental groups: control (control: 20 COCs per 100 μL medium), culture drop (CD-1: one COC per 100 μL medium), and hanging drop

TABLE 2 Embryology outcomes per cumulus–oocyte complex.

	Single COC culture ($n = 160$)	Grouped COCs culture ($n = 162$)	RR (95% CI)	*p*-Value
MII oocytes, *n* (%)	98 (61.3)	105 (64.8)	0.95 (0.8–1.12)	0.56
2-pronuclei fertilized oocytes, *n* (%)	70 (43.8)	81 (50.0)	0.88 (0.69–1.11)	0.27
Day-3 embryos, *n* (%)	54 (33.8)	57 (35.2)	0.96 (0.71–1.3)	0.82
Good-quality embryos, *n* (%)	39 (24.4)	37 (22.8)	1.07 (0.72–1.58)	0.79
Frozen embryos, *n* (%)	52 (32.5)	52 (32.1)	1.01 (0.74–1.39)	0.95

Abbreviations: CI, confidence interval; COC(s), cumulus–oocyte complex(es); MII, metaphase II; RR, relative risk.

TABLE 3 Embryology outcomes per patient.

	Single COC culture ($n = 15$)	Grouped COCs culture ($n = 15$)	*p*-Value
COCs, *n*	10 [8; 11]	10 [8; 12]	0.83
MII oocytes, *n*	6 [5; 8]	7 [5; 8]	0.51
2-pronuclei fertilized oocytes, *n*	5 [3; 6]	5 [4; 6]	0.1
Day-3 embryos, *n*	3 [2; 5]	4 [3; 5]	0.78
Good-quality embryos, *n*	2 [1;4]	3 [1;3]	0.82
Frozen embryos, *n*	3 [2;4]	4 [3;5]	0.08
No embryo, *n* (%)	1 (6.7)	0	–

Note: Values are median [quartile 1; quartile 3] or number of patients (%).

Abbreviations: COC(s), cumulus–oocyte complex(es); MII, metaphase II.

TABLE 4 Embryology outcomes in subgroups based on cumulus–oocyte complex morphology (single culture group only).

COC morphology subgroups	A (118 COCs)	B (27 COCs)	C (15 COCs)	*p*-Value A vs B	*p*-Value A vs C	*p*-Value B vs C
Maturation rate, *n* (%)	77 (65.3)	14 (51.9)	7 (46.7)	0.270	0.169	0.950
Day-3 embryos, *n* (%)	47 (39.8)	5 (18.5)	2 (13.3)	0.045	0.050	0.950

Note: *p*-Value was calculated using a post-hoc test for Fisher's exact test. A, Fully surrounded cumulus–oocyte complexes (COCs) at oocyte pick-up (OPU) and after capacitation in-vitro maturation (CAPA-IVM) culture; B, fully surrounded COCs at OPU but oocyte extruded from COC by end of CAPA-IVM culture; C, partially surrounded COCs at OPU and oocyte extruded from COC by end of IVM culture (as described in Figure 1).
Abbreviation: COC(s), cumulus–oocyte complex(es).

(HD-1: one COC per 10 μL medium), and the results showed that the maturation rate for COCs in the CD-1 and HD-1 groups was lower than that in the control group. However, an improvement in blastocyst quality was observed with the HD-1 method. In contrast, our study did not show significant differences in either maturation or embryo formation rates between single and grouped COCs cultures.

Another mouse study found no significant difference in developmental competence and quality between the group that used 20 COCs per 100 μL and the group that used five COCs per 200 μL.[15] Interestingly, the findings did show that five or fewer COCs in the IVM culture could produce blastocysts.[15] In the grouped culture group of our study, there were about 5–10 COCs per 500 μL medium for each CAPA-IVM step and one COC per 30 μL medium in the single culture group, both of which provided appropriate culture conditions for the development of the COCs, as shown by the comparable embryology outcomes in the two groups.

Grouping of COCs culture during IVM technique allows for paracrine between COCs, which can influence maturation and developmental outcomes.[16] The exchange of paracrine signals between cumulus cells, and the release of endocrine substances into the maturation medium, can provide crucial regulatory signals for follicular development and oocyte maturation.[16] However, multiple COCs within the medium may result in competition for resources and nutrients. Additionally, the variability in the developmental stage and quality of COCs within a grouped culture setting may affect the overall outcomes because some COCs may be more advanced or of better quality than others. However, culturing a single COC in its own droplet, provides several exciting possibilities in IVM. In natural follicular growth, each developing oocyte is encapsulated within a follicle, which provides a nurturing environment and facilitates communication between the oocyte and its surrounding cumulus cells.[17] This microenvironment plays a crucial role in oocyte maturation, ensuring proper gene expression, metabolic support, and regulatory signaling. Our single culture approach could allow for more precise evaluation and manipulation of individual oocytes, offering the potential for non-invasive molecular analysis of cells and conditioned medium and having a precise link with embryo assessment and pregnancy outcome.

In the context of CAPA-IVM, it is crucial for oocyte growth and maturation that the aspiration procedure preserves the integrity of cumulus–oocyte connections.[3] Central to this process is the role of CNP as a vital component in the capacitation culture medium. The CNP/NPR2 (natriuretic peptide receptor 2) system, which involves cumulus cells generating cyclic guanosine monophosphate (cGMP) and its subsequent diffusion into the oocyte through gap junctions, is essential for maintaining oocytes in a state of meiotic arrest.[18] A previous study reported a small fraction of COCs that were only partially surrounded by cumulus cells at OPU.[3] The authors noted that these partial cumulus oocytes typically disconnected from their cumulus cells but, notably, this occurred only after the IVM step. They deduced that oocytes with limited cumulus cell coverage probably lacked sufficient cGMP to effectively sustain meiotic arrest during pre-IVM culture, which may explain their release from the COC during IVM culture. Our findings were consistent with this, with a small number of COCs having partially surrounded morphology at OPU (30/322; 15 per culture group).

We also investigated the effect of COCs morphology after CAPA-IVM on the maturation ability and embryology outcome of individual oocytes in the single culture group. Approximately 26% of COCs (42/160) showed the phenomenon of released oocytes. Interestingly, COCs morphology before and after CAPA-IVM culture was associated with the day-3 embryo rate but not the maturation rate. These results support the hypothesis proposed earlier[3] that the rapid dissociation of the oocyte from its somatic cells during the maturation process is inherently non-physiological and is expected to have detrimental effects on subsequent embryonic development. Our study could not investigate the potential impacts of group COCs culture on these parameters due to the co-culturing of partially surrounded COCs with fully surrounded COCs. Furthermore, it was challenging to determine the morphology of the released oocyte COCs because they could be obscured by the expansion of larger COCs after the IVM step. Therefore, it was not possible to determine whether the group culture strategy could be beneficial for these partially surrounded COCs.

The use of cumulus biomarkers, specifically gene expression analysis, provides valuable insights into the underlying molecular processes associated with oocyte maturation and subsequent embryo development.[9] This study analyzed the expression levels of five target genes *AREG*, *HAS2*, *TRPM7*, *GREM1*, and *CDC42*. These genes were selected based on their known roles in cumulus cell health and/or relation to oocyte quality.[19–21] Our results showed no significant differences in the expression of these five genes between the single and grouped COCs culture methods (Figure 3).

AREG is a critical growth factor that mediates the luteinizing hormone signal driving cumulus expansion and oocyte maturation.[22–24] Our previous study showed that the presence of AREG in the IVM medium positively affects oocyte maturation in the CAPA-IVM system.[9] *HAS2*, located downstream of *AREG*, is a regulator of hyaluronic acid synthesis, which is important for cumulus

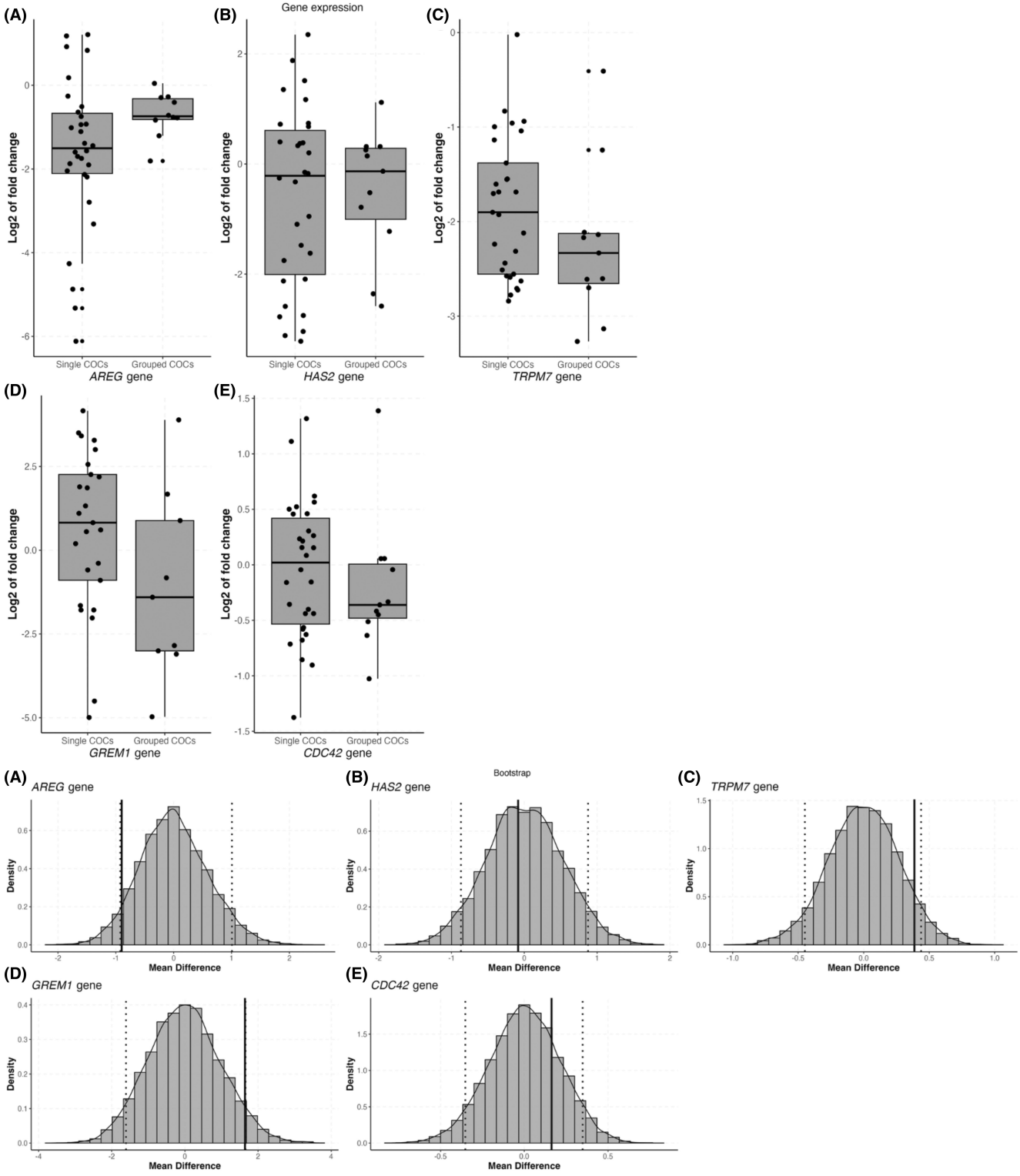

FIGURE 3 Cumulus cell gene expression analysis. The analysis was performed on the $\log_2$ fold change analysis (top panels A–E) and the mean differences between the two groups were analyzed using the bootstrap method (lower panels A–E), where the solid vertical line indicates the observed mean difference and the dotted vertical line indicates the 5% and 95% quartiles of the mean difference. There were no significant differences in the expression of the five evaluated genes between the single and grouped culture groups. AREG, amphiregulin; CDC42, cell division cycle 42; COC, cumulus–oocytes complex; GREM1, gremlin 1; HAS2, hyaluronan synthase 2; TRPM7, transient receptor potential cation channel subfamily M member 7.

cell expansion during ovulation.[25] We have also shown that *TRPM7*, as a cumulus cell biomarker in IVM, was positively correlated with rates of total embryos and good-quality embryos per mature oocytes.[9] This finding is supported by the results of the current study, with comparable good-quality embryo rates between the two study groups. Cumulus cell health is crucial for establishing

oocyte competence due to the essential bi-directional communication between oocytes and cumulus cells. However, the results showed no difference in embryo outcomes between the two culture methods. Moreover, comparable levels of apoptotic regulatory gene *CDC42* indicate that cumulus cell health during single COCs culture is not affected by the lack of paracrine signals that are present during grouped COCs culture.[26] *GREM1* expression in cumulus cells is positively correlated with oocyte maturation, fertilization rates, embryo development, and pregnancy success in women undergoing IVF.[27–29] However, comparable clinical outcomes between the two study groups suggest an additional role for *GREM1* within the oocyte maturation context.

Some points limited the gene expression analysis in this study and need to be mentioned. First, our selection of cumulus cell samples exclusively from MII oocytes in the single COC culture group for comparison with oocytes from the grouped COCs culture group may appear imbalanced, given that cumulus cell samples were drawn from groups comprising MII, metaphase I (MI), and germinal vesical (GV) oocytes. However, it was not feasible to isolate GV and MI oocytes for cumulus cell collection within this group. Moreover, it is important to note that group culture of COCs is a standard practice in our CAPA-IVM protocol. Additionally, the objective was to compare the gene expression of mature oocytes in the single culture, which may better represent in vivo oocyte development, with our routine practice of group culture strategy. Second, it is possible that the limited number of cells collected or the collection method prevented us from analyzing all cumulus cell samples acquired in this study. Therefore, we were not able to include an analysis of the correlation between gene expression levels and embryological outcomes. Future studies could include additional molecular analyzes in a larger sample to assess the specific regulatory mechanisms and pathways involved in signaling the expression of these genes during single COCs culture.

There are also several other limitations of the current study that should be acknowledged. First, the sample size was relatively small and included individuals from Vietnam only, which may limit the generalizability of the findings. Second, the study focused on women with PCOS, and therefore caution should be exercised when extrapolating the findings to other patient populations. Furthermore, there was a lack of data on clinical outcomes after transferring of embryos from the study. Finally, the study did not include the analysis of gene expression from individual cumulus cells in the COC and its association with oocyte maturation and subsequent embryo outcomes, and future research including this analysis is needed to facilitate better understand of gene expression in individual cumulus cells on oocyte maturation and embryology outcomes.

In conclusion, data from this preliminary study showed no significant differences in the maturation and embryology outcomes between single and grouped COCs culture utilizing sibling COCs derived from women with PCOS. These findings indicate that both methods could be effectively utilized for CAPA-IVM culture. This opens up the possibility of studying the relationship between modulation of the culture environment of the COC retrieved from a small follicle and the quality of the resulting blastocyst using time lapse monitoring. The potential implications of gene expression analysis for adapting the culture milieu of the COC, and for helping the embryologist to determine embryo quality remains to be explored.

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CONFLICT OF INTEREST STATEMENT

LNV has received speaker and conference fees from Merck; and grant, speaker, and conference fees from Merck Sharpe & Dohme and Ferring. TMH has received speaker fees from Merck, Merck Sharp & Dohme, and Ferring. JS reports lecture fees from Ferring Pharmaceuticals, BioMerieux, Besins Female Healthcare and Merck, grants from Fund for Research Flanders (FWO), and is a co-inventor on granted patents on CAPA-IVM methodology in the USA (US10392601B2) and Europe (EP3234112B1). JS is currently Corporate Science Officer (CSO) of Lavima Inc, a spinoff company of the Free University Brussels (VUB) that aims to commercialize in vitro maturation media for oocytes. Other authors have no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee at My Duc Hospital, Ho Chi Minh City, Vietnam (13/20/DD-BVMD, dated September 3rd, 2020) and conducted according to Good Clinical Practice and Declaration of Helsinki 2002 principles.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

All participants were provided with comprehensive study information and those willing to choose to participate then signed their names on the consent form provided.

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REFERENCES

1. Delvigne A. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update. 2002;8:559–77.
2. Sanchez F, Romero S, De Vos M, Verheyen G, Smitz J. Human cumulus-enclosed germinal vesicle oocytes from early antral follicles reveal heterogeneous cellular and molecular features associated with in vitro maturation capacity. Hum Reprod. 2015;30:1396–409.
3. Sanchez F, Le AH, Ho VNA, Romero S, Van Ranst H, De Vos M, et al. Biphasic in vitro maturation (CAPA-IVM) specifically improves the developmental capacity of oocytes from small antral follicles. J Assist Reprod Genet. 2019;36:2135–44.
4. Vuong LN, Le AH, Ho VNA, Pham TD, Sanchez F, Romero S, et al. Live births after oocyte in vitro maturation with a prematuration step in women with polycystic ovary syndrome. J Assist Reprod Genet. 2020;37:347–57.
5. Vuong LN, Ho VNA, Ho TM, Dang VQ, Phung TH, Giang NH, et al. In-vitro maturation of oocytes versus conventional IVF in women with infertility and a high antral follicle count: a randomized non-inferiority controlled trial. Hum Reprod. 2020;35:2537–47.
6. Sánchez F, Lolicato F, Romero S, De Vos M, Van Ranst H, Verheyen G, et al. An improved IVM method for cumulus-oocyte complexes from small follicles in polycystic ovary syndrome patients enhances oocyte competence and embryo yield. Hum Reprod. 2017;32:2056–68.
7. Vaccari S, Weeks JL, Hsieh M, Menniti FS, Conti M. Cyclic GMP signaling is involved in the luteinizing hormone-dependent meiotic maturation of mouse oocytes. Biol Reprod. 2009;81:595–604.
8. Romero S, Sanchez F, Lolicato F, Van Ranst H, Smitz J. Immature oocytes from unprimed juvenile mice become a valuable source for embryo production when using C-type natriuretic peptide as essential component of culture medium. Biol Reprod. 2016;95:64.
9. Akin N, Le AH, Ha UDT, Romero S, Sanchez F, Pham TD, et al. Positive effects of amphiregulin on human oocyte maturation and its molecular drivers in patients with polycystic ovary syndrome. Hum Reprod. 2021;37:30–43.
10. Saenz-de-Juano MD, Ivanova E, Romero S, Lolicato F, Sánchez F, Van Ranst H, et al. DNA methylation and mRNA expression of imprinted genes in blastocysts derived from an improved in vitro maturation method for oocytes from small antral follicles in polycystic ovary syndrome patients. Hum Reprod. 2019;34:1640–9.
11. Pham HH, Le AH, Nguyen AM, Ha UD, Nguyen TC, Pham TD, et al. Cumulative live birth rate after oocyte in vitro maturation with a pre-maturation step in women with polycystic ovary syndrome or high antral follicle count. J Assist Reprod Genet. 2023;23:1–9.
12. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41–7.
13. Alpha Scientists in Reproductive Medicine, ESHRE Special Interest Group Embryology. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod Biomed Online. 2011;22:632–46.
14. Nishio M, Hoshino Y, Tanemura K, Sato E. Effect of single-oocyte culture system on in vitro maturation and developmental competence in mice. Reprod Med Biol. 2014;13:153–9.
15. Nishio M, Hoshino Y, Sato E. Effect of droplet size and number of oocytes examined on mouse oocyte quality in in vitro maturation. J Mamm Ova Res. 2011;28:53–60.
16. Eppig J. Oocyte control of ovarian follicular development and function in mammals. Reproduction. 2001;122:829–38.
17. Shah JS, Sabouni R, Cayton Vaught KC, Owen CM, Albertini DF, Segars JH. Biomechanics and mechanical signaling in the ovary: a systematic review. J Assist Reprod Genet. 2018;35:1135–48.
18. Zhang M, Su Y-Q, Sugiura K, Xia G, Eppig JJ. Granulosa cell ligand NPPC and its receptor NPR2 maintain meiotic arrest in mouse oocytes. Science. 2010;330:366–9.
19. McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, et al. Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Hum Reprod. 2004;19:2869–74.
20. Wathlet S, Adriaenssens T, Segers I, Verheyen G, Van de Velde H, Coucke W, et al. Cumulus cell gene expression predicts better cleavage-stage embryo or blastocyst development and pregnancy for ICSI patients. Hum Reprod. 2011;26:1035–51.
21. Van Vaerenbergh I, Adriaenssens T, Coucke W, Van Landuyt L, Verheyen G, De Brucker M, et al. Improved clinical outcomes after non-invasive oocyte selection and day 3 eSET in ICSI patients. Reprod Biol Endocrinol. 2021;19:26.
22. Feuerstein P, Cadoret V, Dalbies-Tran R, Guerif F, Bidault R, Royere D. Gene expression in human cumulus cells: one approach to oocyte competence. Hum Reprod. 2007;22:3069–77.
23. Freimann S, Ben-Ami I, Dantes A, Ron-El R, Amsterdam A. EGF-like factor epiregulin and amphiregulin expression is regulated by gonadotropins/cAMP in human ovarian follicular cells. Biochem Biophys Res Commun. 2004;324:829–34.
24. Ouandaogo ZG, Frydman N, Hesters L, Assou S, Haouzi D, Dechaud H, et al. Differences in transcriptomic profiles of human cumulus cells isolated from oocytes at GV, MI and MII stages after in vivo and in vitro oocyte maturation. Hum Reprod. 2012;27:2438–47.
25. Sugimura S, Ritter LJ, Rose RD, Thompson JG, Smitz J, Mottershead DG, et al. Promotion of EGF receptor signaling improves the quality of low developmental competence oocytes. Dev Biol. 2015;403:139–49.
26. Tu S, Cerione RA. Cdc42 is a substrate for caspases and influences Fas-induced apoptosis. J Biol Chem. 2001;276:19656–63.
27. Adriaenssens T, Segers I, Wathlet S, Smitz J. The cumulus cell gene expression profile of oocytes with different nuclear maturity and potential for blastocyst formation. J Assist Reprod Genet. 2011;28:31–40.
28. Adriaenssens T, Wathlet S, Segers I, Verheyen G, De Vos A, Van der Elst J, et al. Cumulus cell gene expression is associated with oocyte developmental quality and influenced by patient and treatment characteristics. Hum Reprod. 2010;25:1259–70.
29. Jindal S, Greenseid K, Berger D, Santoro N, Pal L. Impaired gremlin 1 (GREM1) expression in cumulus cells in young women with diminished ovarian reserve (DOR). J Assist Reprod Genet. 2012;29:159–62.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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