

Effect of pre-IVF incubation in maturation medium on oocyte maturity, fertilization, embryonic development, and clinical outcomes following embryo transfer

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Abstract

Objective: Collected human cumulus-oocyte complexes (COCs) are usually inseminated after 4 to 6 hours in *in vitro* fertilization (IVF) laboratories. The purpose of this study was to determine the effect of short-term pre-IVF incubation in culture medium on subsequent oocyte maturation, fertilization, and embryonic development, as well as clinical outcomes.

Methods: Sixty patients were divided randomly into 2 groups, pre-IVF incubation for 5 hours: 1) with (+) the designed oocyte maturation medium; 2) without (–) the designed oocyte maturation medium (transferred directly to fertilization medium for 5 hours before insemination). Oocyte maturation and fertilization were assessed, and the rate of cleavage and good quality embryos were evaluated between the 2 groups on days 2 and 3, respectively. Blastocyst development was based on the remaining number of embryos on day 3, continuously cultured to day 5 after embryo transfer or frozen on day 3, and was compared between the 2 groups. Clinical pregnancy, implantation, and miscarriage rates were also compared.

Results: Oocyte maturation rates did not differ between groups ($85.8 \pm 14.1\%$ vs. $90.7 \pm 9.1\%$). However, the range of oocyte maturation rates ($58.3\%–100.0\%$) for each patient was significantly higher in the (–) group than in the (+) pre-incubation group ($71.4\%–100.0\%$). There were no differences in fertilization rates ($89.9 \pm 10.0\%$ vs. $86.5 \pm 12.2\%$) and good quality embryos ($70.8 \pm 19.1\%$ vs. $62.1 \pm 23.7\%$) between groups; however, the blastocyst development rates were significantly different between groups ($73.1 \pm 20.1\%$ vs. $58.8 \pm 18.2\%$, $P < 0.05$). Nevertheless, clinical pregnancy (62.5% vs. 61.1%) and implantation (46.9% vs. 47.2%) rates did not differ between groups.

Conclusions: These results indicate that a short pre-IVF incubation time in the designed culture medium promotes oocyte maturation and embryonic development, suggesting that short pre-IVF incubation of COCs in the designed culture medium may be important for subsequent final oocyte maturation and early embryonic development.

Keywords: Pre-IVF incubation, Oocyte, Maturation, Fertilization, Embryonic development, Clinical outcomes

Introduction

Today, assisted reproductive technology (ART) consists of many techniques, such as the acquisition of gametes (stimulation with

gonadotropins and retrievals), *in vitro* fertilization (IVF), insemination by intra-cytoplasmic sperm injection (ICSI), embryo transfer (ET), freezing of gametes and embryos, biopsy embryos for preimplantation genetic diagnosis, and cytoplasmic transfer. Recently, stem cell differentiation *in vitro* and in artificial gametes has also shown the capacity to produce new life.

ART has helped millions of women with infertility overcome childlessness worldwide. Multiple factors can affect successful pregnancy in infertile women. In reproductive medicine, when using ART to treat infertile women, the woman's age is considered a key factor correlated with the success rate of pregnancy^[1,2]. More specifically, the quality of oocytes produced by women becomes poorer after a certain age (>35 years)^[3]. Therefore, oocyte quality is an important factor for successful ART treatment. The key issue in infertility treatment with ART in the field of reproductive medicine is the process of obtaining high-quality oocytes for infertility treatment.

Oocyte quality may be affected by the woman's age as well as by stimulation protocols^[4,5]. Pioneering work on ovulation induced by human chorionic gonadotropin (hCG) injection is important because it is difficult to predict the exact time of ovulation in women. It is known that an hCG injection induces ovulation in domestic animals, notably in mice^[6–8], which makes it

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possible to collect *in vivo*-matured oocytes before ovulation^[9]. In the 1970s, laparoscopy was introduced to collect mature human oocytes from pre-ovulatory follicles^[10], resulting in the first reported case of *in vivo*-matured oocytes for IVF^[11,12]. Although pregnancies have been reported^[13,14], the first live birth resulting from human IVF was in 1978^[15]. The first IVF baby was born using *in vivo*-matured oocytes collected before ovulation and 36 hours after hCG injection, without gonadotropin stimulation of the natural cycle.

The procedure to obtain mature oocytes from the ovaries of women in natural and stimulated cycles is an important factor in infertility treatment^[16]. With the development of ovarian stimulation protocols, gonadotropins are combined with gonadotropin-releasing hormone (GnRH) agonists during stimulation to obtain more mature oocytes per retrieval cycle from each woman^[17–19]. The final stage of ovulation induction (i.e., final oocyte maturation) can also be induced by endogenous luteinizing hormone (LH) released by the administration of LH-releasing hormone (LHRH) agonist after follicular stimulation for IVF treatment^[18]. This indicates that GnRH agonists can be used for the final oocyte maturation in GnRH antagonist protocols and reduce the risk of ovarian hyperstimulation syndrome^[20].

Without an *in vivo* LH surge, oocytes collected from the leading or dominant follicles are immature, regardless of natural or stimulated cycles. In most cases, oocyte retrieval is performed immediately before ovulation to obtain mature oocytes. In practice, oocyte maturation or ovulation induction is triggered when one leading follicle reaches 18 mm and/or 2 leading follicles reach 16 mm in diameter during stimulated IVF cycles^[21,22]. Following oocyte collection, most retrieved oocytes were mature from large follicles. However, the maturity of the collected oocytes may be different (described as pre-ovulatory, very mature, luteinizing, and atretic oocytes)^[23], and at times, 10% to 30% of the retrieved oocytes can be at immature (germinal vesicle and metaphase-I) stages, even from the stimulated cycles^[24,25].

At the end of the oocyte retrieval procedure, the collected cumulus-oocyte complexes (COCs) were inseminated after 4 to 6 hours pre-IVF incubation (39–41 hours post-hCG injection) in fertilization medium in IVF laboratories, with an assessment of oocyte quality and maturity to optimize time with insemination by IVF or ICSI^[21,26–28]. However, the composition of fertilization media is designed for sperm capacitation and fertilization and is not optimal for final oocyte maturation. Therefore, we hypothesized that COCs should be pre-incubated in optimal oocyte maturation medium to promote final oocyte maturation. This study aimed at determining the effect of pre-IVF incubation 5 hours after oocyte retrieval in designed maturation medium on oocyte maturity, fertilization, embryonic development, and clinical outcomes following fresh ET.

Materials and methods

Patients

Sixty oocyte pickup (OPU) cycles were performed in 60 patients in this study. Patients with at least 2 years of infertility were enrolled in this study from September 2021 to February 2022. All women had normal ovaries, uteri, and regular menstrual cycles. The patients were diagnosed with polycystic ovary syndrome, and male factors were excluded from this study. Women with ovaries that were difficult to visualize and puncture under ultrasound guidance (uterine fibroids, endometriotic cysts, and/

or extensive pelvic adhesions) were also excluded. The patients were aged <35 years. The study was approved by the Institutional Review Board of the Seventh Medical Center of the PLA General Hospital (No. 2021-51) and was carried out in accordance with the principles of the *Declaration of Helsinki*. Written informed consent was obtained from all patients enrolled in the study.

Ovarian stimulation

The treatment procedure for ovarian stimulation involved mild stimulation. Briefly, a baseline transvaginal ultrasound scan was initiated on day 2 or 3 of the menstrual cycle. Controlled ovarian stimulation was initiated by the administration of 150 IU/day of human follicle-stimulating hormone (FSH) for 5 days, and combined with human menopausal gonadotropin (hMG, 150–225 IU/day) (Menotrophin for Injection; Livzon Pharmaceutical Group Inc., Zhuhai, China) until the day of triggering. Follicular development was monitored using transvaginal ultrasound scans on days 7 to 9, and gonadotropins were continuously administered for a few more days. When 1 leading follicle reached 18 mm and/or 2 leading follicles reached 16 mm in diameter, the patients were intramuscularly injected with 5000 IU hCG (Choragon, Ferring Pharmaceuticals, Mexico) to induce final follicular maturation. After 36 hours post-hCG injection, oocyte retrieval was guided by transvaginal ultrasound with an 18-gauge oocyte aspiration needle (COOK, Eight Mile Plains, Queensland, Australia). The aspirated follicular fluid was collected in 10 mL tubes containing G-MOPS PLUS (Vitrolife, Gothenburg, Sweden) with 2 units/mL of sodium heparin. Follicular aspirates were poured into 60 mm Petri dishes for observation of COCs under a dissecting microscope at the warming stage. The collected COCs were washed 3 times with G-MOPS PLUS and randomly transferred to 2 groups of culture media for pre-IVF incubation.

Culture media

Three kinds of culture media were used in this study: 1) oocyte maturation medium, for pre-IVF incubation for COCs immediately after oocyte retrieval; 2) fertilization medium, for oocyte insemination; and 3) embryo development medium, for zygote development from day 1 to 5, and change in medium on day 3 for further continuous culture to day 5. The compositions of the 3 culture media are listed in Table 1.

Sperm preparation and IVF

Ejaculated semen samples were collected from the patient's husband after 45 to 60 minutes of liquification at 37°C. The liquified semen samples were centrifuged (300×g) for 10 minutes with 2 layers of gradients (40% and 80%; SAGE In-Vitro Fertilization Inc., Trumbull, USA). The supernatant was removed from the sperm pellet after centrifugation, resuspended in 3 to 5 mL sperm-washing medium, and centrifuged for 5 minutes (200×g). The pellet was carefully removed from the bottom of the tube, and the sperm pellet was gently transferred into the bottom of another small 5 mL tube containing 0.5 to 1.0 mL fertilization medium for swim-up, for up to 45 minutes. The supernatant was carefully removed from the pellet and placed in a new tube, and motile sperm was adjusted to the desired density. The cap of the sperm sample tube remained untightened and was kept in a 5% CO₂-gassed incubator until insemination.

Table 1
Compositions of 3 culture media.

Components	Oocyte maturation medium	Fertilization medium	Embryo development medium
Sodium chloride	✓	✓	✓
Potassium chloride	✓	✓	✓
Magnesium sulfate heptahydrate	✓	✓	
Magnesium chloride hexahydrate			✓
Sodium phosphate monobasic monohydrate	✓	✓	✓
Sodium bicarbonate	✓	✓	✓
D-(+)-glucose	✓	✓	✓
Sodium pyruvate	✓	✓	✓
Calcium chloride dihydrate	✓	✓	✓
Sodium-DL-Lactate	✓	✓	✓
EDTA tetrasodium salt dihydrate			✓
Alanine-glutamine	✓		✓
L-Asparagine	✓		✓
L-Aspartic acid	✓		✓
Glycine	✓		✓
L-Proline	✓		✓
L-Serine	✓		✓
L-Arginine HCl	✓		✓
L-Cystine dihydrochloride	✓		✓
L-Cysteine			✓
L-Histidine hydrochloride monohydrate	✓		✓
L-Isoleucine	✓		✓
L-Leucine	✓		✓
L-Lysine hydrochloride	✓		✓
L-Methionine	✓		✓
L-Phenylalanine	✓		✓
L-Threonine	✓		✓
L-Tryptophan	✓		✓
L-Tyrosine	✓		✓
L-Valine	✓		✓
D-Calcium pantothenate	✓		
Choline chloride	✓		
Folic acid	✓		
i-Inositol	✓		
Nicotinamide	✓		
Pyridoxine HCl	✓		
Riboflavin	✓		
Thiamine HCl	✓		
Gentamicin	✓	✓	✓
Human serum albumin (HSA)	✓	✓	✓

The COCs were inseminated 5 hours after collection with a 2-well organ culture dish in an inner well containing 1.0 mL fertilization medium with a final concentration of 1.0×10^5 /mL motile sperm. They were cultured at 37°C in a tri-gas incubator (5% CO₂, 5% O₂, and 90% N₂ with high humidity). After 5 hours of pre-IVF incubation in the maturation medium (Fig. 1, group 1), the COCs were transferred to the fertilization medium for insemination. Briefly, the COCs were washed twice in pre-warmed fertilization medium and transferred to a fertilization dish in an inner well containing 1.0 mL of fertilization medium. Post 16 to 18 hours of insemination, fertilization was confirmed by the appearance of 2 distinct pronuclei and 2 polar bodies. The individual fertilized zygotes were transferred to 10 µL droplets of embryo development medium covered with paraffin oil and cultured to day 3 to assess the quality of the embryos, followed by ET or freezing. The remaining embryos were further cultured in fresh embryo development medium until day 5 for blastocyst development. The developed blastocysts were frozen using the vitrification method according to standard procedures.

Endometrium preparation and ET

Fresh ET was performed on day 3 after insemination using an Ultrasoft Frydman catheter set (Laboratoire CCD, France) with an echogenic guide. Endometrial preparation began with the administration of 6 mg estradiol valerate (Delpharm Lille SAS, Rue de Toufflers, France) once daily from the date of oocyte retrieval. Luteal support was initiated with 100 mg of progesterone in oil (Solvay Pharmaceuticals B.V., CJ van Houtelaan 36, Weesp, Netherlands) daily on the day of insemination. Following ET (day 14), the level of serum β-hCG was tested to determine pregnancy, and 6 weeks after ET, clinical pregnancy was confirmed by the appearance of a gestational sac and fetal heartbeat on ultrasound scan.

If the patients did not become pregnant following ET, FET was planned in future cycles depending on the patient's situation with regard to standard procedures. FET results were not included in this study.

Study design

Patients were randomly divided into 2 groups containing 30 patients each. The mean age of the patients, basal hormone levels, and body mass index of the 2 groups are shown in Table 2. As shown in Fig. 1, group 1 included pre-IVF incubation with (+) maturation medium. Following oocyte retrieval, the COCs were pre-IVF incubated with oocyte maturation medium (2-well organ culture dish, inner well containing 1.0 mL for a maximum of 15 COCs, and outer well containing 2.0 mL for washing of the COCs before inner well) supplemented with 7.5 IU/mL hMG for 5 hours before transfer to fertilization medium. Group 2 included pre-IVF incubation without (−) oocyte maturation medium. Following oocyte retrieval, the COCs were transferred to fertilization medium (Two Well Organ Culture Dish, inner well containing 1.0 mL for a maximum of 15 COCs, and outer well containing 2.0 mL for washing of COCs before inner well), and incubated until insemination. Following insemination for 16 to 18 hours, oocyte maturation and fertilization were assessed and compared between the 2 groups. Mature oocytes included unfertilized metaphase-II (M-II) and fertilized oocytes. Briefly, after denuding cumulus cells and sperm from the oocyte, M-II was identified via extrusion of the first polar body or by observing more than one pronucleus in the oocyte cytoplasm and second polar body in the perivitelline space. The rates of normal fertilized zygote cleavage and good quality embryos were compared between the 2 groups on days 2 and 3, respectively. Blastocyst development calculation was based on the remaining number of day 3 embryos that were continuously cultured until day 5 after ET or frozen on day 3, and the 2 groups were compared. Clinical pregnancy, implantation, and miscarriage rates were also compared between the 2 groups following ET.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Comparisons of frequency data between the groups, such as oocyte maturation, fertilization, cleavage, good-quality embryo, clinical pregnancy, implantation, and miscarriage rates, were performed using the Chi-squared test. The non-paired *t* test and Mann–Whitney test were used to compare the mean. Analysis of variance was used for other quantitative comparisons. Statistical significance was set at *P* < 0.05.

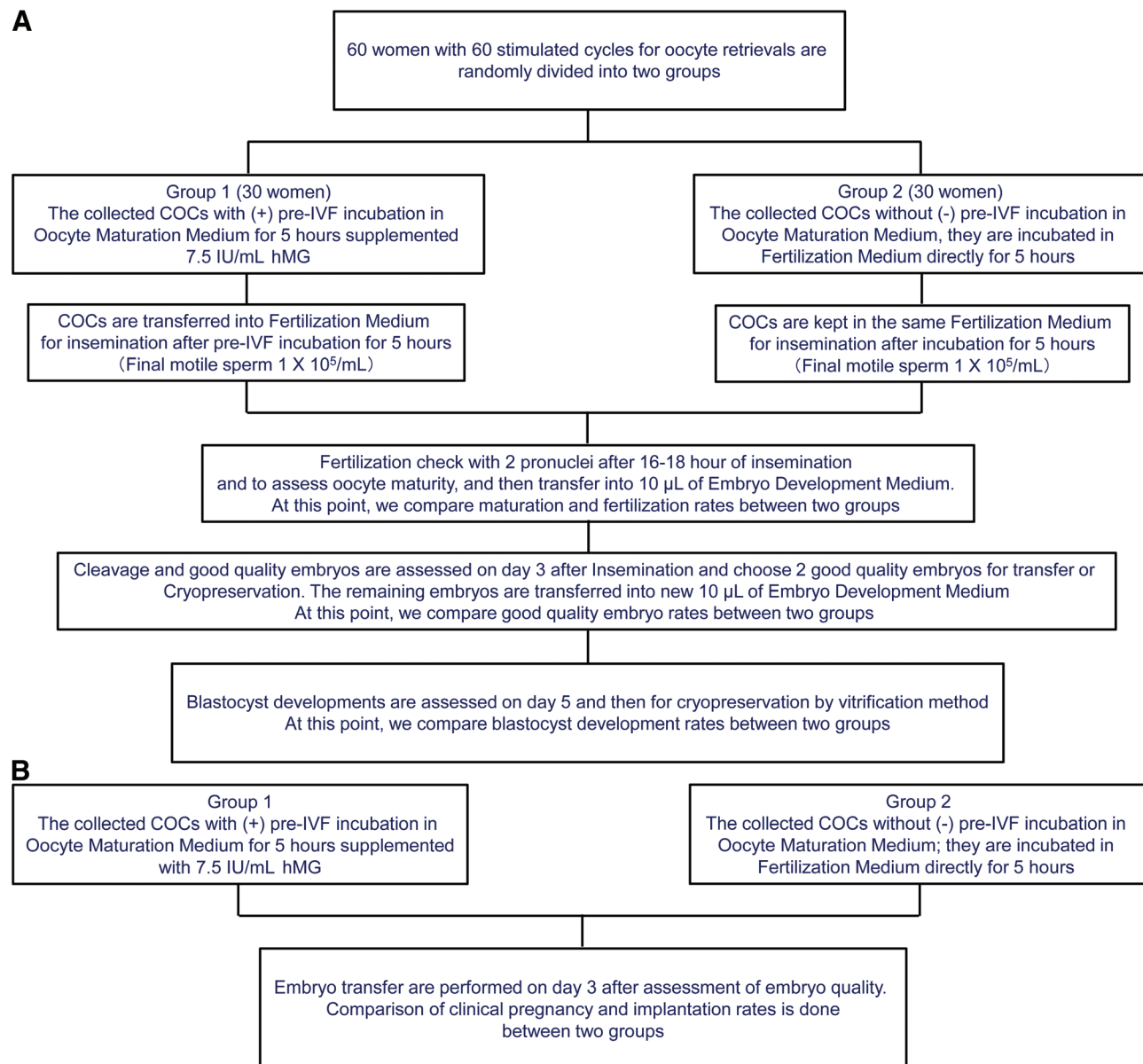


Fig. 1. Flowchart of 2 groups with (+) and without (-) pre-IVF incubation for 5 hours in oocyte maturation medium after oocyte retrieval and before insemination. (A) Comparison of oocyte maturation, fertilization, good embryo quality, and blastocyst development rates. (B) Comparison of clinical pregnancy, implantation, and miscarriage rates.

Results

As shown in Fig. 2, oocyte maturation rates ranged from 71.4% to 100.0% for each patient in pre-IVF incubation with (+) oocyte maturation medium, and from 58.3% to 100.0% in pre-IVF incubation without (-) oocyte maturation medium. However, the average oocyte maturation rates were not statistically different between the 2 groups ($90.7 \pm 9.1\%$ vs. $85.8 \pm 14.1\%$), and fertilization rates and good quality embryos on day 1 and 3 were not significantly different between the groups. However, blastocyst development rates on day 5, following *in vitro* culture, were significantly higher in pre-IVF incubation with (+) than without (-) oocyte maturation medium (Table 3).

As shown in Table 4, the clinical pregnancy and implantation rates were 62.5% (10/16) vs. 61.1% (11/18) and 46.9% (15/32) vs. 47.2% (17/36) in the 2 pre-IVF incubation groups with (+) and without (-) oocyte maturation medium, respectively; there were no significant differences between the 2 groups.

Discussion

In the present study, we demonstrated that a short period (5 hours) of pre-IVF incubation in the designed culture medium for COCs, as opposed to regular fertilization medium after oocyte retrieval, promotes final oocyte maturation and subsequent embryonic development. This indicates that a short pre-IVF incubation time of COCs in the designed culture medium may be important for final oocyte maturation and early embryonic development following IVF.

In addition to the age of women, stimulation protocols also affect the quality of retrieved oocytes. With gold-standard stimulation protocols and laboratory procedures, oocyte retrieval is performed 36 hours after triggering with hCG injection^[15], and insemination occurs with IVF or ICSI at 4 to 6 hours after hCG administration^[21,23,26–28]. Ovarian stimulation protocols usually result in asynchronous follicular development in the ovary, even though most oocytes collected may be mature at oocyte retrieval

Table 2**Basal characteristics in 2 groups.**

	Pre-IVF incubation with (+) oocyte maturation medium	Pre-IVF incubation without (–) oocyte maturation medium	P value
No. of patients	30	30	
Age (mean ± SD) (years)	32.1 ± 2.7	31.4 ± 3.1	0.254
Body mass index (kg/m ²)	23.1 ± 3.5	23.0 ± 3.5	0.929
Duration of infertility (years)	4.0 ± 2.7	4.2 ± 2.4	0.606
Basal hormone levels			
FSH (mIU/mL)	6.34 ± 2.31	6.19 ± 2.60	0.805
LH (mIU/mL)	5.16 ± 2.49	4.99 ± 2.85	0.806
Estradiol (pg/mL)	35.73 ± 18.10	35.24 ± 19.72	0.920
Progesterone (ng/mL)	0.23 ± 0.19	0.28 ± 0.18	0.141

Values shown are mean ± SD unless otherwise indicated. No.: number; FSH: follicle-stimulating hormone; LH: luteinizing hormone.

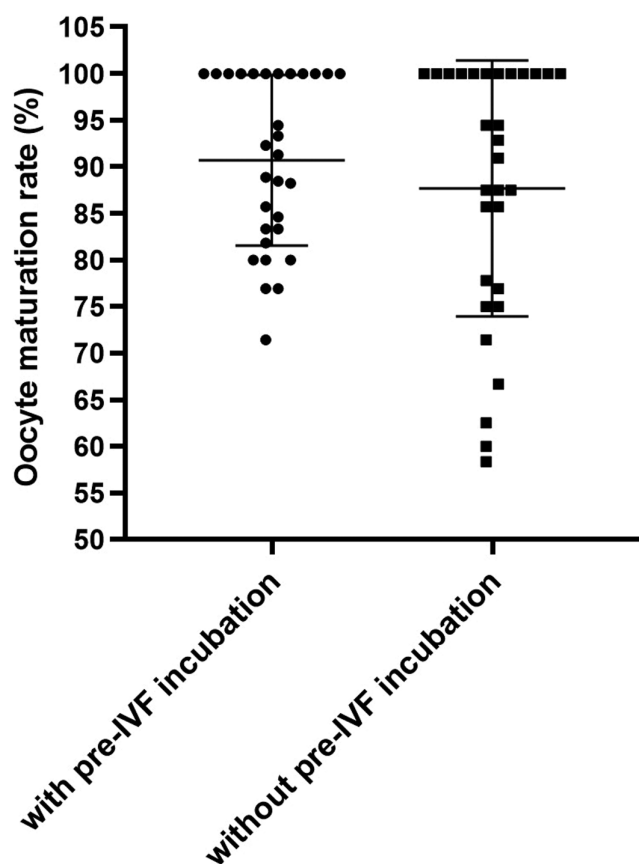


Fig. 2. Comparison of oocyte maturation rates following with (+) and without (–) pre-IVF incubation in oocyte maturation medium evaluated at 16 to 18 hours after insemination. The dots indicate the oocyte maturation rate of each patient. Although there is no statistical difference between the 2 groups, the range of maturation rates for each patient is clearly bigger in the group without (–) pre-IVF incubation compared to the group with (+) pre-IVF incubation.

36 hours after hCG injection. However, 10% to 30% of the retrieved oocytes were immature at metaphase-I and germinal vesicle stages^[24,25,29], and some of them were not fully matured, although they extruded the first polar bodies observed morphologically^[30]. Morphologically mature oocytes may require *in vitro* culture before insemination to achieve complete maturation for fertilization and early embryonic development^[24,25,31]. It is well known that the ability of oocytes to mature and develop

Table 3**Effect of pre-IVF incubation in oocyte maturation medium on maturation, fertilization and embryonic development.***

	Pre-IVF incubation with (+) oocyte maturation medium	Pre-IVF incubation without (–) oocyte maturation medium	P value
No. of patients	30	30	
No. of oocytes retrieved (mean ± SD)	332 (11.1 ± 6.6)	310 (10.3 ± 4.0)	0.853
No. of oocytes matured (%)	296 (90.7 ± 9.1)	272 (85.8 ± 14.1)	0.254
No. of oocytes fertilized (%)	264 (89.9 ± 10.0)	263 (86.5 ± 12.2)	0.252
No. of oocytes polyspermied (%)	12 (2.9 ± 5.3)	5 (1.7 ± 3.8)	0.389
No. of zygotes cleaved (%)	259 (98.6 ± 3.3)	255 (97.9 ± 4.6)	0.653
No. of good quality embryos developed (%)	177 (70.8 ± 19.1)	161 (62.1 ± 23.7)	0.122
No. of blastocysts formed (**)	130 (73.1 ± 20.1)	79 (55.8 ± 18.2)	0.008

*Percentage also appeared with mean ± SD from each patient.

**Indicate significantly different between 2 groups. There were 2 good quality embryos transferred or frozen on day 3, therefore, the base numbers of continuously cultured embryos to day 5 were different from the numbers of fertilized oocytes or cleaved zygotes.

competence is acquired progressively with increasing follicular size. However, it should be mentioned that the development of embryos in cohort follicles from the stimulated cycles appears to be independent of the diameter of the leading follicle at the time of hCG injection^[32,33].

It has been suggested that insemination time should be optimized based on oocyte maturity after oocyte retrieval^[23]; however, this is difficult to follow in practice, especially in busy IVF laboratories. Therefore, it is common practice that insemination by IVF or ICSI is performed 40 to 41 hours post-hCG injection^[22,27,28]. Some studies have observed the effect of ovulation trigger–OPU interval of different ovarian stimulation protocols on oocyte maturation and clinical outcomes^[34–38], indicating that a longer trigger–OPU interval does not result in more mature oocytes and better clinical outcomes. Other studies have reported that the ovulation trigger–OPU time from different stimulation protocols should be gradually prolonged to obtain more mature oocytes as well as a better and cumulative live birth rate^[39–41]. Nevertheless, there is little information available on pre-IVF incubation in specially designed oocyte maturation medium. This study is the first report to compare pre-IVF incubation with (+) and without (–) specifically designed oocyte maturation medium, which we believe is much better than the fertilization medium for oocyte maturation during short pre-IVF incubation.

Two decades ago, a new *in vitro* maturation (IVM) medium was designed and shown to be beneficial for nuclear and cytoplasmic maturation of human immature oocytes derived from stimulated IVF cycles^[42]. Complex culture media supplemented with human serum albumin, gonadotropins (FSH and LH), and estradiol have been widely used in research or in the clinical application of human immature oocytes, although the rationale for choosing a specific medium for IVM of immature oocytes appears to stem largely from adapting methods developed from culturing other cell types. Evidence indicates that the newly designed IVM medium is beneficial for clinical application in human immature oocytes and has resulted in several thousands of healthy babies born^[43–47].

As shown in this study, oocyte maturation rate improved in the group with COCs cultured in the designed oocyte maturation medium supplemented with 7.5 IU/mL of hMG for 5 hours pre-IVF incubation compared to that without oocyte maturation medium, where oocyte maturation rates were assessed 16 to 18 hours after insemination because COC maturity was impossible

Table 4**Effect of pre-IVF incubation in oocyte maturation medium on clinical outcomes following day 3 embryo transfer.**

Pre-IVF incubation with (+) or without (–) oocyte maturation medium	No. of patients (n)	Age (mean ± SD)	No. of embryos transferred (mean ± SD)	No. of patients who became pregnant (%)	No. of embryos implanted (%)	No. of Patients who miscarried (%)
+	16	32.8 ± 3.5	32 (2.0 ± 0)	10 (62.5)	15 (46.9)	0
–	18	31.9 ± 3.4	36 (2.0 ± 0)	11 (61.1)	17 (47.2)	1 (8.3)
P value		0.559	1.000	0.951	0.500	0.391

Values shown are mean ± SD or number (percentage) unless otherwise indicated.

to assess at the time of insemination (post 5 hours of pre-IVF incubation) (Fig. 2). Interestingly, our preliminary data indicated that oocyte maturation rates did not differ between pre-IVF incubations with (+) and without (–) oocyte maturation medium for 5 hours at the time of denuding COCs in ICSI treatment cycles (data not shown). In addition to the oocyte maturation medium itself, the medium was supplemented with 7.5 IU/mL hMG. This means that apart from the medium itself, important factors for oocyte maturation may also come from gonadotropins and other factors^[48,49]. Therefore, this short pre-IVF incubation time, apart from optimum to mature oocytes, resulted in a significant increase in blastocyst development rate (Table 3).

As mentioned above, the reported results of the clinical outcomes were different from ovulation trigger–OPU interval of different ovarian stimulation protocols on oocyte maturation and clinical outcomes^[34–37,41]. The results of the present study showed that the clinical pregnancy rate (62.5% *vs.* 61.1%) and implantation rate (46.9% *vs.* 47.2%) did not differ between pre-IVF incubation for 5 hours with (+) and without (–) oocyte maturation medium (Table 4). It is possible that the number of patients for ET was too small to statistically indicate the real results because some of the patients did not perform ET during the treatment cycles. Our preliminary results indicate that clinical outcomes, in terms of clinical pregnancy and implantation rates, are unaffected by pre-IVF incubation for 5 hours with (+) or without (–) oocyte maturation medium. However, clinical trials (CTs) in multiple centers are required to confirm this result.

Conclusions

The results from this study demonstrate that a short pre-IVF incubation time of 5 hours with a specially designed oocyte maturation medium is beneficial for oocyte maturation and subsequent embryonic development following fertilization. For clinical outcomes, a multicenter CT is required to confirm the results.

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Author contributions

R.C. and J.L. conceived the study, participated in its design, and drafted the manuscript. J.W. and S.Z. performed the clinical protocol and operations of the patients. J.L. and Y.B. performed laboratory experiments. T.J., Y.W., and Y.W. participated in data analysis and interpretation. R.C., S.T., and S.Z. supervised and critically revised the manuscript for important intellectual content. All authors contributed to the final manuscript and approved its submission.

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Conflicts of interest

All authors declare no conflict of interest.

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