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Laboratory and clinical outcomes of oocytes matured from metaphase I to metaphase II on the day of oocyte retrieval in intracytoplasmic sperm injection cycles

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Abstract: The fertilization outcomes of belatedly matured metaphase II (MII) oocytes that extrude the first polar body a few hours after cumulus cell removal have been investigated. However, the influence of using these oocytes on the outcomes of intracytoplasmic sperm injection (ICSI) remains unclear. Here, we investigated the laboratory and clinical outcomes of ICSI using belatedly matured oocytes on the day of retrieval. The laboratory and clinical outcomes of 10,605 ICSI cycles performed using accurately timed MII oocytes (normal MII group) and belatedly matured oocytes (delayed MII group) between January 2010 and December 2018 were retrospectively investigated. The fertilization rate, proportion of morphologically good-quality embryos on day 3, and proportion of embryos that developed into morphologically good-quality blastocysts by day 5 were significantly lower in the delayed MII group than in the normal MII group. However, no significant differences in clinical outcomes (clinical pregnancy rate, miscarriage rate, live birth rate) were observed for thawed blastocyst transfers between groups the two group. Oocytes that extruded the first polar body a few hours after cumulus cell removal and develop ed into morphologically good-quality blastocysts did not have an adverse effect on the clinical outcomes of ICSI embryos.

Key words: Blastocyst development, Immature oocyte, Intracytoplasmic sperm injection, *In vitro* maturation, Metaphase I

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Introduction

During controlled ovarian stimulation for *in vitro* fertilization (IVF), multiple oocytes are often collected as a mixture of large and small follicles because of differences in the responsiveness to the stimulus. Consequently, both mature and immature oocytes may be collected.

Typically, cumulus cells are removed by hyaluronidase treatment before intracytoplasmic sperm injection (ICSI) and only the metaphase II (MII) oocytes containing the first polar body are used for ICSI. However, as described in previous studies, approximately 15–20% oocytes fail to resume maturation or reach the mature stage (MII) [1, 2], and some immature oocytes in metaphase I (MI) or the germinal vesicle (GV) stage may be collected. Nevertheless most immature oocytes (mainly MI) can reach the MII stage during the day of oocyte retrieval or during the next day.

Some studies have shown that oocytes that extrude the first polar body a few hours after cumulus cell removal exhibit low fertilization and embryonic development rates; however, pregnancy has been confirmed with these oocytes [1, 3–4]. Contrastingly, oocytes that fail to reach the mature stage during the day of oocyte retrieval exhibit much lower fertilization and embryonic development rates than those of oocytes that mature during the day of oocyte retrieval [5].

Recent investigations have demonstrated that after cumulus cell removal, immature oocytes cultured in IVM [6] or blastocyst medium improved laboratory outcomes [7]; however, the method has not been established yet.

Moreover, *in vitro* matured oocytes have high probabili-

ties of chromosomal abnormality [8], cytoplasmic maturation failure [9], and spindle abnormality [10, 11]. Therefore, the feasibility of these oocytes remains unclear.

Therefore, in the present study, we analyzed the laboratory and clinical outcomes of oocytes which matured on the day of retrieval at our clinic and investigated the feasibility of using MII oocytes with delayed first polar body extrusion in ICSI.

Materials and Methods

Samples and experimental design

This study was conducted using oocytes obtained from 10,605 ICSI cycles performed between January 2010 and December 2018. The oocytes with the first polar body following cumulus cell removal were classified as the normal MII group, whereas oocytes which extruded the first polar body a few hours after cumulus cell removal were classified as the delayed MII group. The laboratory and clinical outcomes of the two groups were retrospectively reviewed are compared using medical records.

Ovarian stimulation

Ovulation was induced by mild stimulation from day 3 of menstruation by oral administration of clomifene (Clomid®, Fuji Pharma Co., Ltd., Toyama, Japan) and subcutaneous or intramuscular administration of follicle-stimulating hormone (Follistim®, MSD K.K. a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA or Gonal F®, Merck KGaA, Darmstadt, Germany) or human menopausal gonadotropin (HMG Fuji®, Fuji Pharma Co., Ltd. or HMG Ferring®, Ferring Pharmaceuticals Co., Ltd., Tokyo, Japan). When necessary, a gonadotropin-releasing hormone antagonist (Cetrotide®, Merck KGaA or Ganirest®, MSD K.K.) was also administered. When the mean diameter of two axes of the dominant follicle reached at least 18 mm, a human chorionic gonadotropin formulation (hCG Fuji®, Fuji Pharma Co., Ltd.) or gonadotropin-releasing hormone agonist (Buserecur®, Fuji Pharma Co., Ltd) was administered, and oocytes were collected 34–36 h later.

Oocyte and embryo culture

Oocytes were pre-cultured for 5–6 h after retrieval and cumulus cells were removed using hyaluronidase (ICSI cumulase®; LIFE GLOBAL/CooperSurgical, MÅløv, Denmark). ICSI was conducted using only oocytes that had extruded the first polar body.

After ICSI, embryo culture was carried out in an atmosphere of 6% carbon dioxide, 5% oxygen, and 89% nitrogen in Universal IVF Medium (LIFE GLOBAL/CooperSurgical) for fertilization, and then switched to Global Medium (LIFE GLOBAL/CooperSurgical) for culture from the zygote to blastocyst, or Sydney IVF cleavage medium (Cook Medical, Bloomington, IN, USA) for culture from the zygote to cleavage-stage embryo, and Sydney IVF blastocyst medium (Cook Medical) for culture from the cleavage-stage embryo to blastocyst, after confirming that fertilization had occurred. Two types of culture media were used in our study; however, the laboratory outcomes of using global medium or cleavage medium and blastocyst medium were not significantly different. Fertilization was confirmed 16–18 h after ICSI by examining the ova using a stereomicroscope to detect pronuclei and polar bodies.

All embryos analyzed in this study had Gardner grades of 3BB or higher when cryopreserved on day 5–7. Cryopreservation was carried out using the vitrification method with vitrification medium (Kitazato BioPharma, Shizuoka, Japan), and thawing was carried out using thawing medium (Kitazato BioPharma) [12]. After thawing, assisted hatching was conducted for all embryos by partial zona dissection.

Embryo transfer

Before transferring the thawed embryos during the natural cycle, either natural or induced ovulation was confirmed. Five days later, frozen-thawed single-blastocyst transfer was performed. To transfer thawed embryos during the hormone-replacement cycle, estradiol (Estrana®, Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan) was administered from approximately day 3 of menstruation. After confirming the endometrial thickness was at least 8 mm, progesterone (vaginal suppository) was administered. Frozen-thawed single-blastocyst transfer was carried out on day 6 after initiating progesterone administration using an IVF catheter (Fuji Systems Corporation, Tokyo, Japan) or an ET catheter (Kitazato BioPharma) with ultrasound guidance. Subjects were considered clinically pregnant upon confirmation of the presence of a gestation sac by transvaginal ultrasonography. Informed consent was obtained from all the subjects in accordance with the Denentoshi Ladies Clinic code of ethics approved by the Institutional Review Board of our clinic before participation in the study.

Outcomes

The laboratory outcomes evaluated were fertilization rate, proportion of morphologically good-quality embryos on day 3, and proportion of embryos that developed into morphologically good-quality blastocysts by day 5. Morphologically good-quality embryos on day 3 contained

7–10 cells of grade 1 or 2 according to the Veeck classification [13]. Embryos that developed into morphologically good-quality blastocysts by day 5 were those with a grade of 3BB or higher according to the Gardner classification [14]. Clinical outcomes included the pregnancy rate, miscarriage rate, and live birth rate after thawed blastocyst transfer.

Statistical analysis

The average ages of the recipients of the normal MII and delayed MII group were compared using the t-test. The laboratory and clinical outcomes of the two groups were compared using the χ^2 test. A *P* value <0.05 was considered to indicate statistical significance.

Results

There were no significant differences in the average ages at the time of oocyte retrieval and freezing of the blastocysts (Table 1). As shown in Fig. 1, all laboratory outcomes (fertilization rate, proportion of morphologically good-quality embryos on day 3, and proportion of embryos that developed into morphologically good-quality blastocysts by day 5) of the delayed MII group (56.0%: 362/647, 42.3%: 153/362, 28.6%: 101/353) were significantly poorer than those of the normal MII group (81.6%: 22,776/27,919, 62.6%: 14,248/22,776, 43.5%: 9,318/21,417). However, the clinical outcomes (clinical pregnancy rate, miscarriage rate, and live birth rate) did not significantly differ between the normal MII group (38.9%: 3,672/9,432, 26.5%: 974/3,672, 21.5%: 2,026/9,432) and the delayed MII group (33.9%: 21/62, 14.3%: 3/21, 21.0%: 13/62) (Fig. 2).

Discussion

Different institutions have different protocols for handling oocytes that are immature at the time of retrieval for ICSI, and studies of the laboratory and clinical outcomes of oocytes that mature from MI to MII on the day of oocyte retrieval have reported conflicting results. Some studies demonstrated significantly reduced fertilization and embryonic development rates [3, 4, 15–17], whereas another study indicated a reduced fertilization rate but no adverse effect on subsequent embryonic development and clinical outcomes [1]. Moreover, other studies revealed no significant differences in the fertilization rate, embryonic development rate [18, 19], or clinical outcomes [20].

Our results indicate that the laboratory outcomes for belatedly matured MII oocytes are significantly poorer than those of MII oocytes when the first polar body is present at the time of oocyte retrieval. Because the properties of morphological maturation such as the extrusion

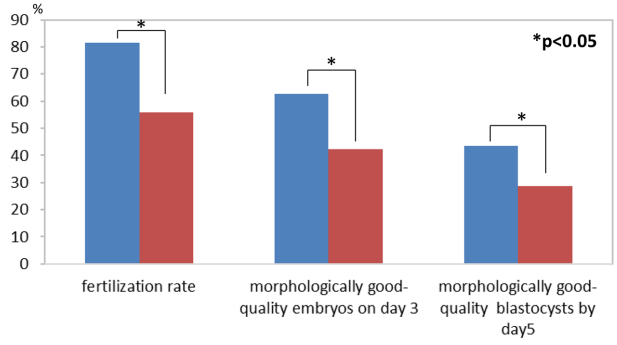

Fig. 1. Laboratory outcomes of embryo culture (fertilization rate, proportion of morphologically good-quality embryos on day 3, and proportion of embryos that developed into morphologically good-quality blastocysts by day 5) of the normal MII (blue) and delayed MII (red) groups. *$P < 0.05$.

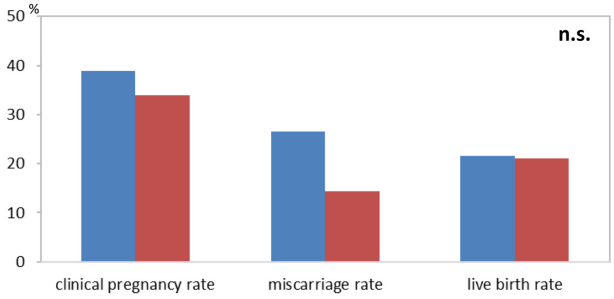

Fig. 2. Clinical outcomes of thawed blastocyst transfer (clinical pregnancy rate, miscarriage rate, live birth rate) of the normal MII (blue) and delayed MII (red) groups.

Table 1. Ages of the women (mean ± standard deviation) at the times of oocyte retrieval and frozen of freeze-thawed blastocysts

	Age at the time of oocyte retrieval (years)	Age at the time of freezing (years)
MII group	38.4 ± 4.0 (n=27,919)	37.5 ± 4.1 (n=9,432)
MI–MII group	38.1 ± 4.1 (n=647)	37.4 ± 4.3 (n=62)

of the first polar body do not necessarily reflect cytoplasmic and nuclear maturation [9, 21], conducting ICSI before cytoplasmic maturation may help to improve these laboratory outcomes. Previous studies reported that most belatedly matured MII oocytes injected soon after first polar body extrusion remained unfertilized [22, 23]. For 40–60 min no spindle was detectable, superseded by the formation of the metaphase II meiotic spindle, which typically appeared under the first polar body approximately 115–150 min after extrusion of the first polar body [24].

Other studies have reported that 87% of belatedly matured MII oocytes developed a spindle signal after 2 h incubation, and that the fertilization rates of detectable spindle oocytes were significantly higher than those of nondetectable spindle oocytes [25]. Therefore, detecting the spindle with a spindle visualization system is important for conducting ICSI for belatedly matured MII oocytes. However, in the absence of a visualization system, ICSI needs to be conducted at least 2–3 h after first polar body extrusion, as performed in our study.

Several studies have shown that the belatedly matured MII oocytes exhibit higher rates of chromosomal abnormalities [8, 26] and induce abnormal fertilization owing to the inability of these oocytes to extrude the second polar body and distribute chromosomes [9]. Therefore, using this protocol of conducting ICSI at least 2–3 h after first polar body extrusion, alone did not lead to improve laboratory outcomes.

The use of IVM [6] or blastocyst medium [7], or addition of EGF-like factor [27] or dbc-AMP [28] to improve the laboratory outcomes of belatedly matured MII oocytes has been reported by several recent studies. Therefore, the culture medium may improve fertilization and embryo development rates.

However, in the present study, there were no significant differences in the clinical pregnancy rates, miscarriage rates, or live birth rates between the normal MII and delayed MII groups. The birth prognoses were also favorable, suggesting that embryos which develop into blastocysts of a grade for which fertilization and freezing are possible have the same fertility as those developed from normally maturing oocytes.

There are some concerns that *in vitro*-cultured oocytes have higher rates of chromosomal abnormalities [5, 8, 29–31], and that oocyte aging can influence oocyte quality when oocytes are cultured *in vitro* until polar body extrusion after cumulus cell removal. However, other studies have reported that *in vitro* culture within 5–8 h does not influence laboratory or clinical outcomes [1, 3, 15, 23]. Moreover, given that the clinical outcomes of the delayed MII group, which developed into morphologically good-quality blastocysts in this study, were not significantly different from those of the normal MII group, it is unlikely that short-term *in vitro* culture has a substantial effect on fertility. Overall, our study suggests that although oocytes with delayed polar body extrusion have relatively poor laboratory outcomes, the same success rate can be achieved as when using accurately timed MII oocytes if they develop into morphologically good-quality blastocysts. Thus, the use of oocytes that extrude the first polar body belatedly is a safe option and this may be particularly important in the treatment of patients with low oocyte counts.

Disclosures

Conflict of interest: The authors declare no conflict of interest.

Human/Animal Rights statement and informed consent: This study was approved by an institutional ethics committee. This article does not cite any studies with human or animal subjects performed by any of the authors. Informed consent was obtained from all patients included in the study.

References

1) De Vos, A., Van de Velde, H., Joris, H. and Van Steirteghem, A. (1999): In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. Hum. Reprod., 14, 1859–1863. [Medline] [CrossRef]
2) Cha, K.Y. and Chian, R.C. (1998): Maturation in vitro of immature human oocytes for clinical use. Hum. Reprod. Update, 4, 103–120. [Medline] [CrossRef]
3) Álvarez, C., García-Garrido, C., Taronger, R. and González de Merlo, G. (2013): In vitro maturation, fertilization, embryo development & clinical outcome of human metaphase-I oocytes retrieved from stimulated intracytoplasmic sperm injection cycles. Indian J. Med. Res., 137, 331–338. [Medline]
4) Vanhoutte, L., De Sutter, P., Van der Elst, J. and Dhont, M. (2005): Clinical benefit of metaphase I oocytes. Reprod. Biol. Endocrinol., 3, 71. [Medline] [CrossRef]
5) Son, W.Y., Lee, S.Y. and Lim, J.H. (2005): Fertilization, cleavage and blastocyst development according to the maturation timing of oocytes in in vitro maturation cycles. Hum. Reprod., 20, 3204–3207. [Medline] [CrossRef]
6) Chian, R.C. and Tan, S.L. (2002): Maturational and developmental competence of cumulus-free immature human oocytes derived from stimulated and intracytoplasmic sperm injection cycles. Reprod. Biomed. Online, 5, 125–132. [Medline] [CrossRef]
7) Pongsuthirak, P., Songveeratham, S. and Vutyavanich, T. (2015): Comparison of blastocyst and Sage media for in vitro maturation of human immature oocytes. Reprod. Sci., 22, 343–346. [Medline] [CrossRef]

8) Emery, B.R., Wilcox, A.L., Aoki, V.W., Peterson, C.M. and Carrell, D.T. (2005): In vitro oocyte maturation and subsequent delayed fertilization is associated with increased embryo aneuploidy. Fertil. Steril., 84, 1027–1029. [Medline] [CrossRef]
9) Lin, Y.H. and Hwang, J.L. (2006): In vitro maturation of human oocytes. Taiwan. J. Obstet. Gynecol., 45, 95–99. [Medline] [CrossRef]
10) Windt, M.L., Coetzee, K., Kruger, T.F., Marino, H., Kitshoff, M.S. and Sousa, M. (2001): Ultrastructural evaluation of recurrent and in-vitro maturation resistant metaphase I arrested oocytes. Hum. Reprod., 16, 2394–2398. [Medline] [CrossRef]
11) Combelles, C.M., Albertini, D.F. and Racowsky, C. (2003): Distinct microtubule and chromatin characteristics of human oocytes after failed in-vivo and in-vitro meiotic maturation. Hum. Reprod., 18, 2124–2130. [Medline] [CrossRef]
12) Kuwayama, M., Vajta, G., Ieda, S. and Kato, O. (2005): Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod. Biomed. Online, 11, 608–614. [Medline] [CrossRef]
13) Veeck, L.L. (1988): Oocyte assessment and biological performance. Ann. N. Y. Acad. Sci., 541, 259–274. [Medline] [CrossRef]
14) Gardner, D.K., Lane, M., Stevens, J., Schlenker, T. and Schoolcraft, W.B. (2000): Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil. Steril., 73, 1155–1158. [Medline] [CrossRef]
15) Shin, S.B., Cho, J.W., Lee, S.H., Yang, K.M., Lim, C.K. and Lee, H.S. (2013): Fertilization and pregnancy potential of immature oocytes from stimulated intracytoplasmic sperm injection cycles. Clin. Exp. Reprod. Med., 40, 7–11. [Medline] [CrossRef]
16) Ko, D.S., Lee, S.H., Park, D.W., Yang, K.M. and Lim, C.K. (2015): Pregnancy and fertilization potential of immature oocytes retrieved in intracytoplasmic sperm injection cycles. Clin. Exp. Reprod. Med., 42, 118–125. [Medline] [CrossRef]
17) Kim, B.K., Lee, S.C., Kim, K.J., Han, C.H. and Kim, J.H. (2000): In vitro maturation, fertilization, and development of human germinal vesicle oocytes collected from stimulated cycles. Fertil. Steril., 74, 1153–1158. [Medline] [CrossRef]
18) Chen, S.U., Chen, H.F., Lien, Y.R., Ho, H.N., Chang, H.C. and Yang, Y.S. (2000): Schedule to inject in vitro matured oocytes may increase pregnancy after intracytoplasmic sperm injection. Arch. Androl., 44, 197–205. [Medline] [CrossRef]
19) Shu, Y., Gebhardt, J., Watt, J., Lyon, J., Dasig, D. and Behr, B. (2007): Fertilization, embryo development, and clinical outcome of immature oocytes from stimulated intracytoplasmic sperm injection cycles. Fertil. Steril., 87, 1022–1027. [Medline] [CrossRef]
20) Li, M., Li, Y., Ma, S.Y., Feng, H.L., Yang, H.J., Wu, K.L., Zhong, W.X., Che, L. and Chen, Z.J. (2011): Evaluation of the developmental potential of metaphase I oocytes from stimulated intracytoplasmic sperm injection cycles. Reprod. Fertil. Dev., 23, 433–437. [Medline] [CrossRef]
21) Racowsky, C. and Kaufman, M.L. (1992): Nuclear degeneration and meiotic aberrations observed in human oocytes matured in vitro: analysis by light microscopy. Fertil. Steril., 58, 750–755. [Medline] [CrossRef]
22) Hyun, C.S., Cha, J.H., Son, W.Y., Yoon, S.H., Kim, K.A. and Lim, J.H. (2007): Optimal ICSI timing after the first polar body extrusion in in vitro matured human oocytes. Hum. Reprod., 22, 1991–1995. [Medline] [CrossRef]
23) Balakier, H., Sojecki, A., Motamedi, G. and Librach, C. (2004): Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. Hum. Reprod., 19, 982–987. [Medline] [CrossRef]
24) Montag, M., Schimming, T. and van der Ven, H. (2006): Spindle imaging in human oocytes: the impact of the meiotic cell cycle. Reprod. Biomed. Online, 12, 442–446. [Medline] [CrossRef]
25) Holubcová, Z., Kyjovská, D., Martonová, M., Páralová, D., Klenková, T., Otevřel, P., Štěpánová, R., Kloudová, S. and Hampl, A. (2019): Egg maturity assessment prior to ICSI prevents premature fertilization of late-maturing oocytes. J. Assist. Reprod. Genet., 36, 445–452. [Medline] [CrossRef]
26) Magli, M.C., Ferraretti, A.P., Crippa, A., Lappi, M., Feliciani, E. and Gianaroli, L. (2006): First meiosis errors in immature oocytes generated by stimulated cycles. Fertil. Steril., 86, 629–635. [Medline] [CrossRef]
27) Ben-Ami, I., Komsky, A., Bern, O., Kasterstein, E., Komarovsky, D. and Ron-El, R. (2011): In vitro maturation of human germinal vesicle-stage oocytes: role of epidermal growth factor-like growth factors in the culture medium. Hum. Reprod., 26, 76–81. [Medline] [CrossRef]
28) Amano, N., Kobatake, M., Yoshikawa, K., Takano, T., Saji, F. and Hasegawa, A. (2013): Pre-treatment with dbc-AMP and IBMX improved oocyte maturation in IVM procedure. Fertil. Steril., 100, S227. [CrossRef]
29) Strassburger, D., Goldstein, A., Friedler, S., Raziel, A., Kasterstein, E., Mashevich, M., Schachter, M., Ron-El, R. and Reish, O. (2010): The cytogenetic constitution of embryos derived from immature (metaphase I) oocytes obtained after ovarian hyperstimulation. Fertil. Steril., 94, 971–978. [Medline] [CrossRef]
30) Nogueira, D., Staessen, C., Van de Velde, H. and Van Steirteghem, A. (2000): Nuclear status and cytogenetics of embryos derived from in vitro-matured oocytes. Fertil. Steril., 74, 295–298. [Medline] [CrossRef]
31) Strassburger, D., Friedler, S., Raziel, A., Kasterstein, E., Schachter, M. and Ron-El, R. (2004): The outcome of ICSI of immature MI oocytes and rescued in vitro matured MII oocytes. Hum. Reprod., 19, 1587–1590. [Medline] [CrossRef]