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Time Course of Meiotic Progression After Transferring Primary Spermatocyte Into Ooplasm at Different Stages

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ABSTRACT This study attempted to investigate the time course of meiotic progression after transferring primary spermatocyte (PS) into ooplasm at different maturing stages. In present experiments, PSs were introduced into maturing ooplasts or oocytes by electrofusion. Higher fusion rate was obtained by phytohemagglutinin (PHA) agglutination than by perivitelline space (PVS) insertion. When the ooplasms prepared at 0, 2, 5, and 8.5 hr of in vitro maturation (IVM) were used as recipients and PSs were used as donors, the reconstructed cells extruded the first polar body (PB1) approximately 8.5, 7, 5.5, and 3 hr after electrofusion, respectively. Especially, when ooplasm cultured for 8.5 hr in vitro after GV removal was fused with PS, the PB1 was emitted 7–11 hr after electrofusion. Additionally, the PB1 extrusions of GV and pro-MI oocytes fertilized with PSs were 2.5 hr earlier than control oocytes. The results suggest that (1) PSs undergo the first meiosis in different time courses when introduced into ooplasm at different maturing stages; (2) GV material plays an important role in determining the timing of PB1 extrusion; and (3) first meiotic division of GV and pro-MI oocytes can be accelerated by introducing PS. *Mol. Reprod. Dev. 74: 1072–1080, 2007.* © 2007 Wiley-Liss, Inc.

Key Words: meiosis; oocyte; primary spermatocyte; nuclear transfer; germinal vesicle

INTRODUCTION

Meiosis inducing capacity of ooplasm is one of the fundamental questions in the field of reproductive biology. To date, successful haploidization has been achieved after introducing 2N (either 2C or 4C) cells into ooplasm. When a mouse secondary spermatocyte (2N and 2C) was injected into an MII oocyte (Kimura and Yanagimachi, 1995) or a primary spermatocyte (PS) (2N and 4C) was injected into a pro-MI oocyte (Ogura et al., 1998; Sasagawa et al., 1998), artificially haploid (1N) was generated, and such a cell was able to fertilize a oocyte and achieve full-term development. When a G0/G1 somatic cell (2N and 2C) was introduced into an MII oocyte (Lacham-Kaplan et al., 2001), and a G2/M phase arrested somatic cell (2N and 4C) was introduced into an enucleated GV oocyte (Chang et al., 2004), somatic nucleus appeared to undergo one or two rounds of meiotic divisions.

Interactions between the nucleus and ooplasm have been studied. Isolated GV karyoplasts undergo germinal vesicle breakdown (GVBD) but failed to extrude the first polar body (PB1) and the larger size karyoplast underwent GVBD earlier with PB1 emission (Takeuchi et al., 1999). Synchronous GV transfer in human has been proposed as a means to reduce the incidence of aneuploidy (Zhang et al., 1999) originating from the first meiotic division (Hassold and Hunt, 2001). Successful GV transfer has also been reported by us and others in mouse (Liu et al., 1999) and rabbit (Li et al., 2001a). We further showed that interspecific GV transfer oocytes could complete the first meiosis (Li et al., 2001b). In addition, we showed that when transferring karyoplasts from mouse GV, MI, and MII oocytes into the cytoplasts at asynchronous developmental stages, the cytoplasm determined the developmental fate of the introduced nucleus (Cheng et al., 2003). When a zona-free MII oocyte was fused with a GV oocyte, GVBD was greatly accelerated and two spindles were visible in the manipulated oocyte (Mattioli et al., 1991). In contrast, progression of mouse oocytes from MI to MII could be inhibited by fusion with G2 zygotes (Grabarek and Zernicka-Goetz, 2000). When GV oocytes were fused with 2-, 4-, or 8-cell stage embryo blastomeres the nuclei underwent meiosis, but the PB1 extrusion was decreased and shortened (Grabarek et al., 2004). These reports indicate that the development of nucleus is regulated by the surrounding cytoplasmic environment, and tempo of meiosis is influenced by donor cell nucleus.

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The aims of this study is to investigate the meiosis progression after transferring PS into immature ooplasm at different maturation stages. Changes in the timing of PB1 extrusion are recorded, and possible effects of GV and PS materials on meiosis are discussed.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this experiment were purchased from Sigma Chemical Company (St. Louis, MO) except for those specifically noted.

Collection of Oocytes

Female Kunming mice, 5–6 weeks old, were used in this experiment. For collection of GV oocytes, Kunming mice ovaries stimulated with 10 IU pregnant mare's serum gonadotropin (PMSG) for 48 hr were removed. GV-stage oocytes surrounded by several layers of cumulus cells were released from large antral follicles by puncturing with a fine sharp needle, and then collected in M2 medium containing 0.2 mM 3-isobutyl-1-methylxanthine (IBMX) to inhibit GVBD. The GV oocytes were alternatively precultured in human tubal fluid (HTF) containing 10% FCS and IBMX for 2 hr to increase perivitelline space (PVS), or cultured in HTF without IBMX for 2, 5, and 8.5 hr to allow GVBD and maturation. Cumulus cells were removed by repeated pipetting, and cumulus-free oocytes were selected for micromanipulation.

Preparation of Primary Spermatocytes

To collect spermatogenic cells, the seminiferous tubules of a 2- to 3-month-old Kunming male mouse were minced, spermatogenic cells were released from the tubular fragments by repeated pipettings as described previously (Ogura et al., 1993; Liu et al., 1997; Sasagawa et al., 1998) and then kept in HEPES-buffered CZB medium (HCZB) (Chatot et al., 1989). The majority of these cells were PSs, round and elongating spermatids, and spermatozoa. PSs selected in this study were in the pachytene to diplotene stages of the meiotic prophase. They were characterized by their large size (16–18 μm) with a distinct nuclear membrane, and they were readily differentiated from other smaller spermatogenic cells (Bellve et al., 1977; Ogura et al., 1998) (Fig. 1).

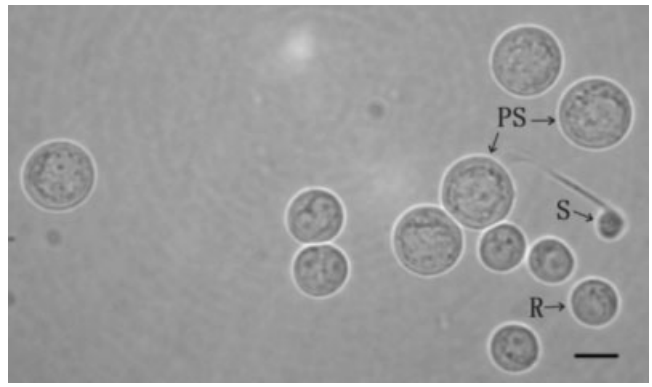

Fig. 1. Isolated mouse PSs. Only those with a distinct nuclear membrane were used in this study. PS, primary spermatocyte; R, round spermatid; S, spermatozoon. Bar = 10 μm.

Reconstruction of Primary Spermatocyte-Ooplast Pairs

Oocytes at GV stage with a visible PVS after preculture and immature oocytes after 2, 5, and 8.5 hr in vitro maturation (IVM) were used for cell reconstruction. GV oocytes cultured in vitro were randomly selected as controls. GV oocytes were placed in a microdroplet of M2 containing cytochalasin B (CB, 10 μg/ml) and IBMX (0.2 mM) at room temperature, and ooplasts at other stages were placed in M2 medium with 5 μg/ml CB during micromanipulation. A slit was made through the zona pellucida (ZP) of each oocyte with a sharp needle. Then an enucleation pipette with an inner diameter of 17.5 μm was used to go through the same slit on the ZP (Fig. 2A, C, E, and G) and remove GV or nuclei with a minimal volume of cytoplasm (Fig. 2B, D, F, and H). After insertion of a PS into the PVS, PS–ooplast complexes were placed into electrofusion medium (0.28 M mannitol, 0.1 mM $MgSO_4$, 0.1 mg/ml PVA, and 3 mg/ml BSA) and subjected to one pulse of 1.6 kV/cm direct current (BTX ECM2001, BTX Inc., San Diego, CA) for 10 μsec. The PS–ooplast complexes were then cultured in HTF and examined for fusion 30 min later.

For recombination of GV oocyte cytoplast with PS, we also used method of ZP-free agglutination. ZPs were removed from mouse GV oocytes by treatment with 0.5% pronase (Calbiochem, San Diego, CA) for 5 min. ZP-free oocytes were washed five times and placed in M2 medium containing 5 μg/ml CB and 200 μg/ml of phytohemagglutinin (PHA) (Fig. 3A). Separated PSs were placed in another droplet of M2 medium without PHA. Germinal vesicle with a minimal volume of cytoplasm was inspired into an enucleation pipette (Fig. 3B) and drawn out from the oocyte (Fig. 3C,D). One PS was agglutinated to an enucleated GV oocyte by gently pressing the two cells together with enucleation pipette (Fig. 3E,F). Constructed PS–ooplast complexes were subjected to electrofusion described above.

Microfertilization With Primary Spermatocytes

GV-stage oocytes cultured for 2 hr in HTF medium with IBMX to inhibit GVBD or without IBMX to allow for GVBD were used for microfertilization with PSs. Micromanipulation procedure was similar to PVS insertion method as described above except that no enucleation was performed. Fusion was achieved with an electrical pulse of the same parameters as mentioned above.

Experimental Design

Experiment 1. GV-stage ooplasms, immature ooplasts from oocytes cultured for 2, 5, 8.5 hr IVM and ooplasts cultured for 8.5 hr after GV removal were

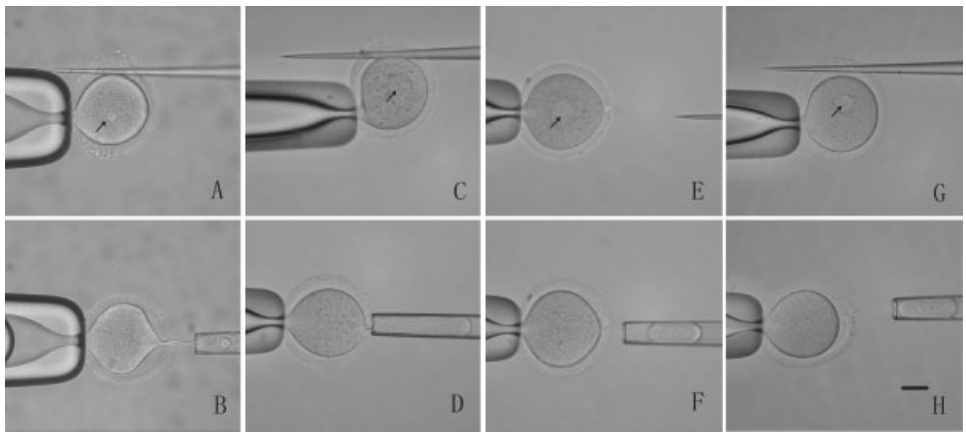

Fig. 2. Microsurgical enucleation of oocytes at GV-stage and after 2, 5, 8.5 hr IVM. **A**: ZP was penetrated by pressing a glass needle and then zona was cut. GV was marked with an arrow. **B**: GV was removed with an enucleation pipette. **C**: ZP of oocyte after 2 hr IVM was penetrated by pressing a glass microneedle. **D**: Spindle with surrounding cytoplasm was aspirated into enucleation pipette. **E**: A slit was made by a glass needle in ZP of the oocyte after 5 hr IVM. **F**: Spindle was removed by enucleation pipette. **G**: ZP of oocyte after 8.5 hr IVM was pierced by a glass needle. **H**: Spindle was removed. Spindle marked with an arrow was visible in (C), (E), and (G). Bar = 20 μm.

electrofused with PSs. Generally, for GV oocytes, enlargement of PVS needs 2 hr of culture. Enucleation, insertion, and fusion for GV oocytes and other stage immature oocytes were completed within 2 hr. The PS–oocyte complexes were immediately transferred into HTF medium after electrical pulse, and this point was designated as 0 hr. Extrusions of the PB1 in all these reconstructed cells were examined at an interval of 30 min by a Nikon invert microscope.

Experiment 2. GV oocytes and GV-removed oocytes were electrofused with PSs after 2 hr preculture to allow PVS enlargement. Micromanipulation and fusion were completed within 2 hr. The time point of transfer of fused oocytes into HTF was designated as 0 hr. Extrusion of the PB1 was recorded.

Control groups were designed as follows:

Control A: In this group, GV oocytes were tested with the same procedures as in experimental groups, that is, exposed to IBMX in 37°C incubator for 2 hr, incubated at room temperature in the same medium as manipulated oocytes for 2 hr and even given the same electrical pulse, but micromanipulation was not performed.

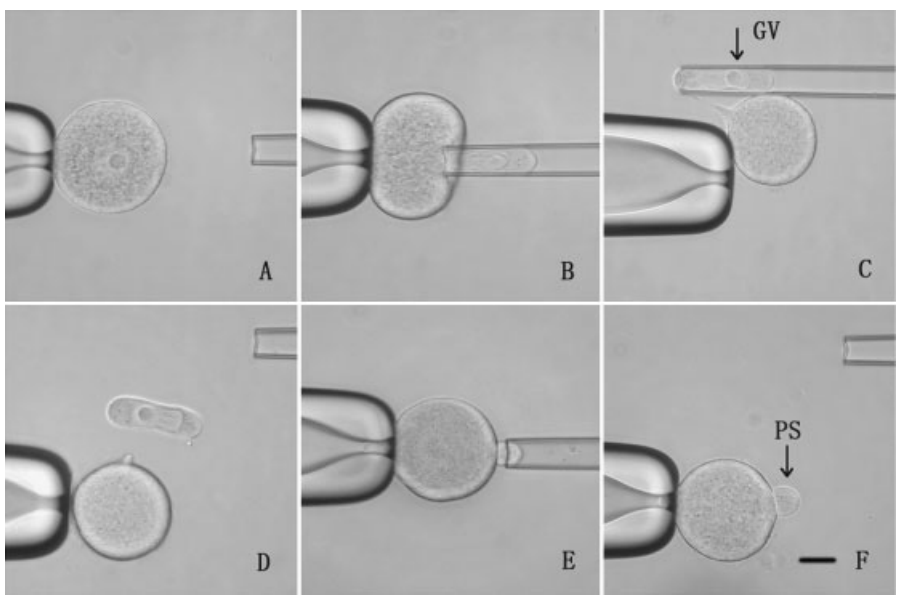

Fig. 3. Enucleation of GV oocytes and reconstruction of cells by fusing GV ooplast with PS using PHA agglutination. GV was marked with an arrow. **A**: ZP-free GV oocyte prior to enucleation. **B**: GV was aspirated into a blunt enucleation pipette. **C**: GV was pulled out from oocyte. **D**: GV with a small amount of surrounding cytoplasm. **E**: PS was placed onto the membrane of an enucleated GV oocyte. **F**: Enucleated GV ooplast attached with a PS. PS, primary spermatocyte. Bar = 20 μm.

Control B: GV oocytes were exposed to IBMX for 4 hr and then matured in HTF till extrusion of the PB1.

Control C: GV oocytes were matured in HTF medium till extrusion of the PB1.

Experiment 3. In this part, pro-MI oocytes were microfertilized with PSs. Two hours after IVM culture, GVBD oocytes were used for microfertilization, which took 1 hr. Time of the PB1 emission was recorded.

Control groups were designed as follows:

Control C: GV oocytes were matured in HTF medium till extrusion of the PB1.

Control D: Oocytes were treated with the same procedures as operated oocytes, that is, oocytes were precultured in 37°C incubator for 2 hr, incubated at room temperature in the same medium for 1 hr, and given the same electrical pulse as electrofusion, but micromanipulation was not conducted.

Immunofluorescence Staining

After removal of zona pellucida in acidified Tyrode's solution (pH 2.5), oocytes at the desired stages were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min and permeabilized for 30 min in the incubation buffer (0.5% Triton X-100 in 20 mM HEPES, pH 7.4, 3 mM $MgCl_2$, 50 mM NaCl, 300 mM sucrose, 0.02% NaN_3), then washed in PBS with 0.1% Tween 20 three times and incubated with fluorescein isothiocyanate (FITC)-labeled anti α-tubulin diluted 1:75 for 1 hr. After being washed three times in PBS buffer, DNA was stained with 10 μg/ml propidium iodide for 10 min. Finally, the oocytes were mounted on glass slides and examined using a TCS-4D laser scanning confocal microscope (Leica Microsystems, Bensheim, Germany).

Statistical Analysis

Percentages were analyzed by the Chi-square analysis. Significant difference was determined at $P < 0.05$.

RESULTS

Comparison of Two Methods Used for GV Reconstruction

Oocytes at GV stage were subjected to enucleation directly after removal of cumulus cells or after additional digestion of the ZP by treatment with 0.5% pronase. In the PHA agglutination method, a single PS cell was attached to a GV-removed oocyte in M2 medium containing PHA and then subjected to electrofusion. Alternatively, one PS cell was inserted into the PVS of enucleated oocytes retaining the zona, and an electrical pulse was given for fusion. The former method was more efficient than the latter regarding fusion, leading to about 38% fusion rate, but no differences were found between the two groups in proportions of survival after enucleation and polar body extrusion after culture (Table 1).

First Polar Body Extrusion of Reconstructed Immature Oocytes

We transferred PSs into GV-removed ooplasts, which were prepared after 0, 2, 5, 8.5 hr of IVM, and ooplasts prepared from GV oocytes and cultured 8.5 hr after GV removal. We compared the timing of the PB1 extrusion in reconstructed oocytes originating from ooplasts at different maturation stages. Differences in the timing of the PB1 extrusion were observed. GV-removed oocytes, which reconstructed with PS immediately after enucleation, extruded the PB1 at 7.5–9.5 hr after culture in HTF medium, and oocytes reconstructed by fusion of the PS with ooplasts prepared at 2, 5, and 8.5 hr of IVM extruded the PB1 at 6–8, 4.5–6, and 2.5–3.5 hr, respectively. With the increase of maturation degree (0, 2, 5, and 8.5 hr IVM), time of the PB1 extrusion in reconstructed oocytes becomes shorter (about 8.5, 7, 5.5, and 3 hr correspondingly). However, in oocytes reconstructed by fusion of the 8.5 hr culture ooplasts prepared from GV oocytes with the PS, extrusions of the PB1 occurred 7–11 hr after reconstruction (Fig. 4).

Over half of the manipulated oocytes fused successfully extruded the PB1. However, large discrepancies in the size of the PB1 were observed in reconstructed oocytes. Both normal-size PB1 (similar size of control oocytes) and large-size PB1 (up to four times the size of controls) were found in reconstructed oocytes (Fig. 5A,B). Symmetrical cell division, which resulted in a large-size PB1 equal to the oocyte size, was also seen in control oocytes cultured in vitro, but rate was much lower (3.7%). As much as 35.9% (14/39) of GV ooplast-PS reconstructed cells and 34.1% (15/44) of 2 hr IVM ooplast-PS reconstructed cells had shown equal division after 13 hr culture postreconstruction. And significantly lower symmetrical cell division occurred in cells

TABLE 1. Comparison of the Survival, Fusion, and Polar Body Extrusion of Reconstructed Oocytes Fused by PHA Agglutination or PVS Insertion

Method	Number of survival of GV ooplasts after enucleation (%)	Number of oocytes fused (%)	Number of oocytes extruded polar body (%)
PHA agglutination	63/66 (95.5)[a]	24/63 (38.1)[a]	14/24 (58.3)[a]
PVS insertion	159/172 (92.4)[a]	39/159 (24.5)[b]	24/39 (61.5)[a]

Values with different superscripts within each column are significantly different ($P < 0.05$).

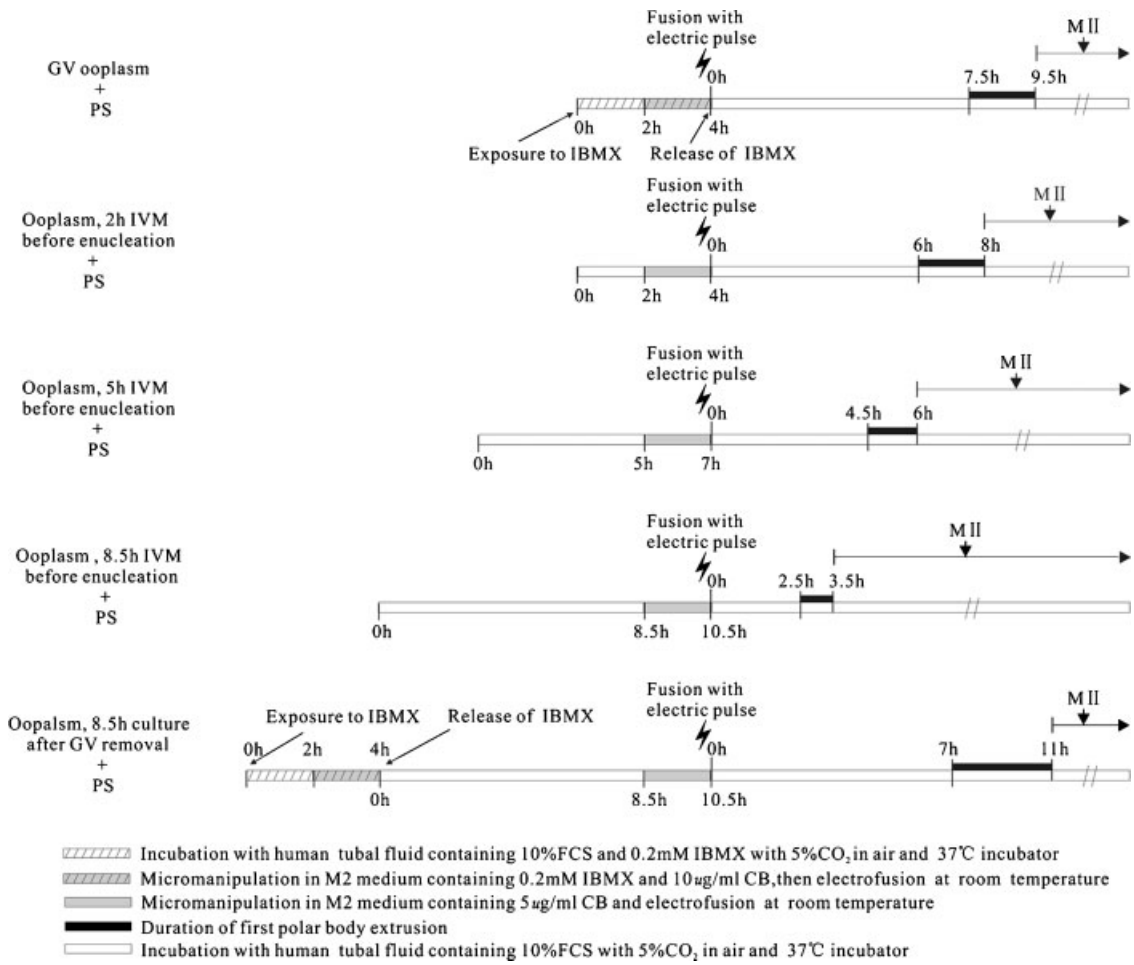

Fig. 4. Schematic summary of first meiosis timing of cells reconstructed by fusing GV ooplasm, 2 hr IVM ooplasm, 5 hr IVM ooplasm, and 8.5 hr IVM ooplasm or 8.5 hr culture ooplasm prepared from GV oocyte with PS. The black boxes represent the durations of polar body extrusion. PS, primary spermatocyte.

reconstructed after 5 and 8.5 hr IVM, and proportions were 15.0% (6/40) and 0% (0/32), respectively. The majority of cells reconstructed after 5 and 8.5 hr IVM had a normal-size PB1 (60.0% and 78.1%, respectively), while the proportions of normal-size PB1 in GV ooplasts-PS reconstructed cells and 2 hr IVM ooplasts-PS reconstructed cells were 25.6% (10/39) and 31.8% (14/44), respectively (Table 2).

First Polar Body Extrusion of Immature Oocytes Fertilized With Primary Spermatocytes

To determine whether the timing of first meiosis in mouse oocyte was influenced after fertilization with spermatogenic cells, PS was introduced into immature oocyte by electrofusion. We compared the timing of PB1 extrusion in fertilized GV oocytes and in oocytes

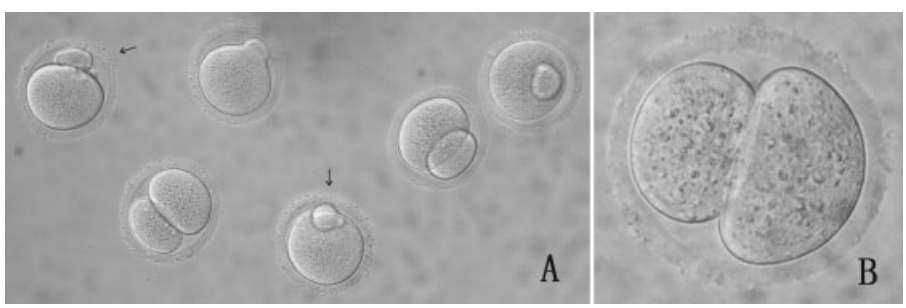

Fig. 5. Reconstructed GV oocytes after 9 hr IVM. **A**: Reconstructed oocyte marked with an arrow had a normal-size PB1. **B**: Oocyte with a large polar body.

TABLE 2. First Polar Body Extrusion of Cells Reconstructed by Fusing 0, 2, 5, or 8.5 hr IVM Ooplasts With PSs

	Number of reconstructed oocytes (%)			
Oocyte type reconstructed	Without first polar body	With normal-size first polar body	With large-size first polar body	Degenerated
GV ooplasm + PS	12/39 (30.8)	10/39 (25.6)[a]	14/39 (35.9)[a]	3/39 (7.7)
2 hr IVM ooplasm + PS	13/44 (29.6)	14/44 (31.8)[a]	15/44 (34.1)[a]	2/44 (4.5)
5 hr IVM ooplasm + PS	10/40 (25.0)	24/40 (60.0)[b]	6/40 (15.0)[b]	0/40 (0)
8.5 hr IVM ooplasm + PS	6/32 (18.8)	25/32 (78.1)[c]	0/32 (0)[b]	1/32 (3.1)
Control (intact GV oocyte)	16/108 (14.8)	87/108 (80.6)	4/108 (3.7)	1/108 (0.9)

Values with different superscripts within each column are significantly different ($P < 0.05$).

reconstructed by fusion GV ooplasts with PS. The PB1 extrusions of both fertilized GV and GV-removed oocytes occurred at 7.5–9.5 hr after electrofusion. But Control A oocytes, which were treated under the same conditions including medium, temperature, and even electrical pulse except micromanipulation, extruded PB1 at 10–12 hr after electric pulse. For the Control B and C, GV oocytes without any operation, extrusion of the PB1 occurred 10–12 hr after the release from IBMX (Fig. 6).

Similar to GV oocytes, pro-MI (shortly after GVBD at 2 hr of IVM) oocytes underwent polar body extrusion at 5.5–7.5 hr postfusion, and total time requirement for PB1 extrusion was 7.5–9.5 hr if 2 hr IVM for GVBD

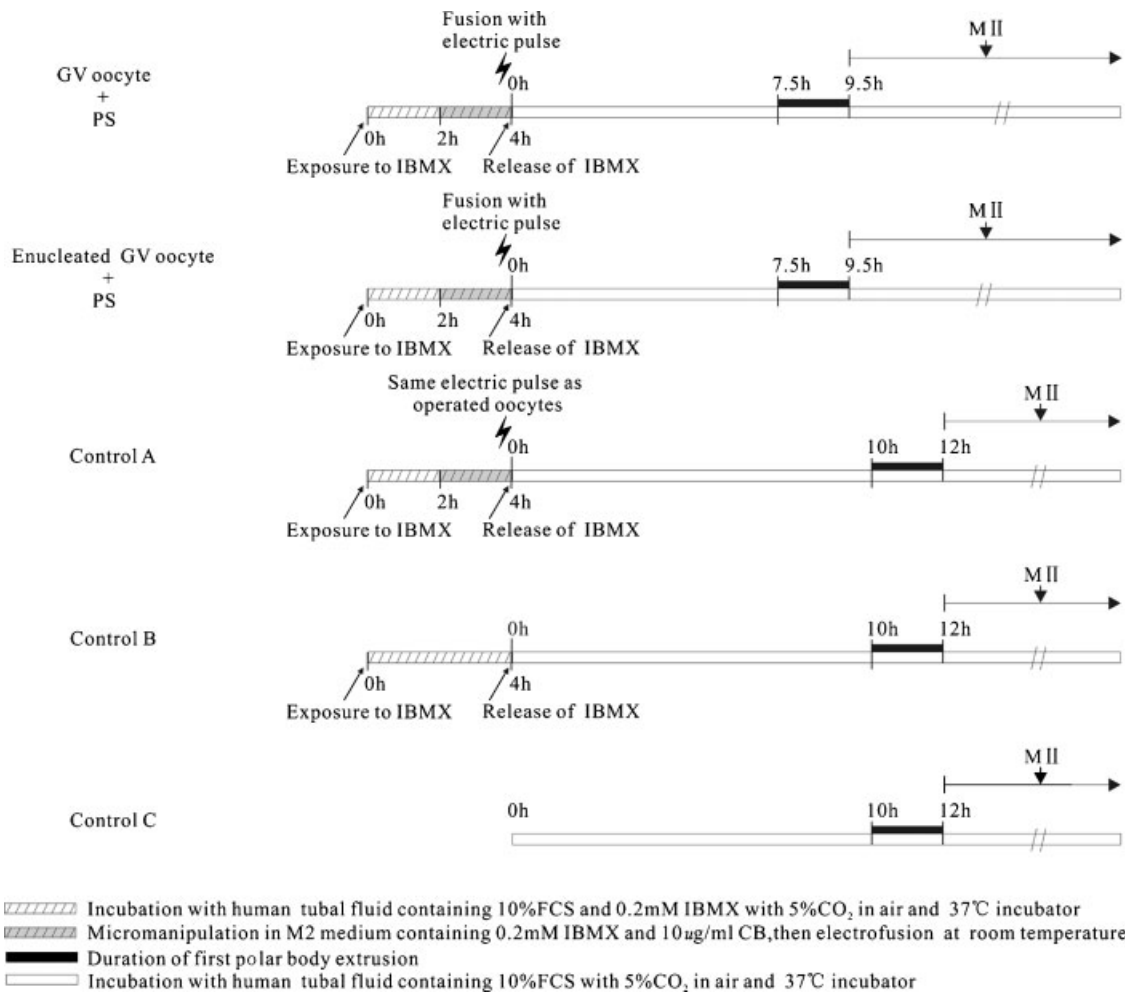

Fig. 6. Schematic summary of first meiosis timing of reconstructed and fertilized GV oocytes. The black boxes represent the durations of polar body extrusion. PS, primary spermatocyte.

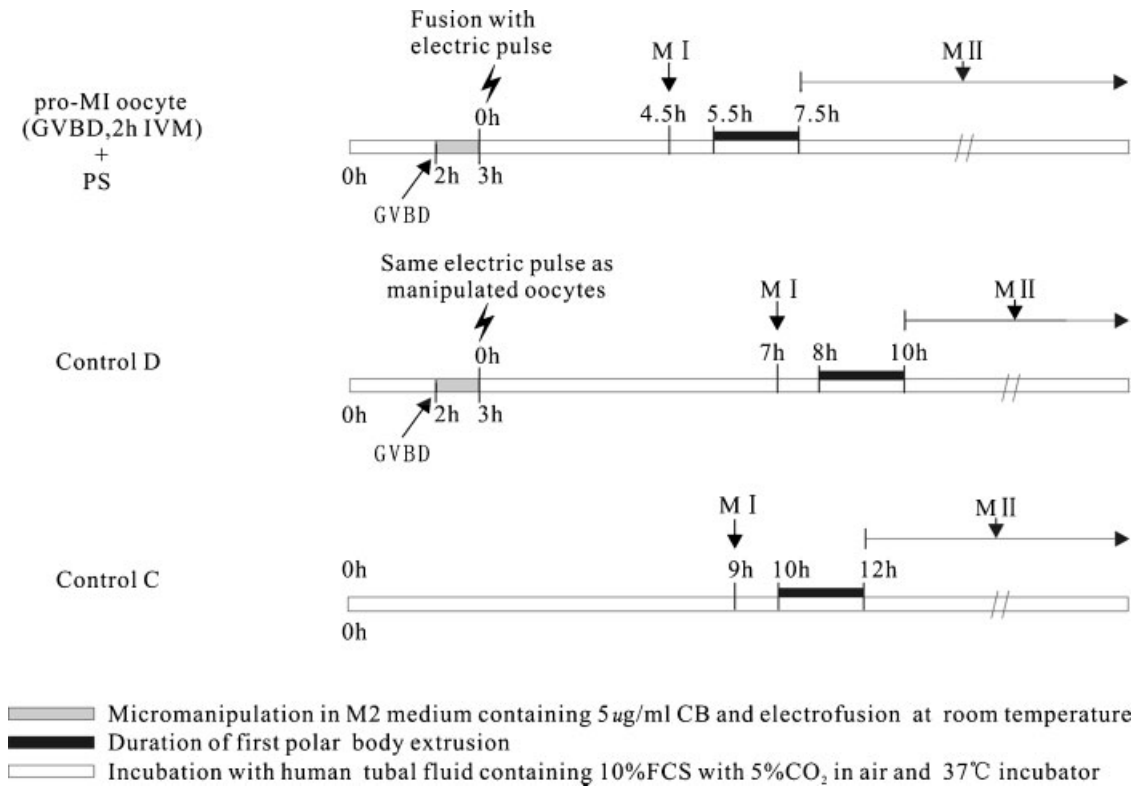

Fig. 7. Schematic summary of first meiosis timing of fertilized pro-MI oocytes. The black boxes represent the durations of polar body extrusion. PS, primary spermatocyte.

was taken into account (Fig. 7). However, for Control D oocytes with same treatments as manipulated oocytes or Control C oocytes without any manipulation, cell division occurred after 10–12 hr IVM in both groups. In summary, timing of PB1 extrusion in immature oocytes was accelerated after fertilization with PS, which was approximately 2.5 hr earlier than controls.

Immature oocytes fertilized with PS were observed with confocal microscopy. We checked spindle morphology at expected MI stage. One or two spindles formed in oocytes fertilized at GV or pro-MI stages. Two sets of metaphase chromosomes in oocytes fertilized at GV-stage showed a nearly coincident development at 7.5 hr after electrofusion (Fig. 8A–C). The double spindle oocytes extruded two first polar bodies when observed at 17 hr after electrofusion, one was derived from the oocyte and the other from the PS. No more than 1 hr interval between extrusion of two first polar bodies was observed.

DISCUSSION

By far, most researchers use direct PVS insertion and fusion to obtain reconstructed oocytes. GV oocytes freshly collected from ovaries have no PVS between plasma membrane and zona, so for the classical GV removal method, GV oocytes must be cultured in a medium containing IBMX to inhibit GVBD and allow for

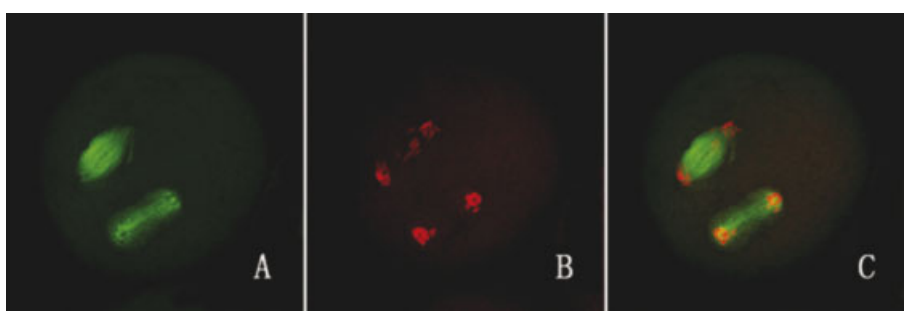

Fig. 8. Laser scanning confocal microscopy images of spindle organization in reconstructed GV oocyte after 7.5 hr of in vitro culture. **A**: Immunostaining of microtubles with FITC-anti-α-tubulin. **B**: Nucleus stained by propidium iodide. **C**: Overlapping of (A) and (B). Green, microtubule; red, nucleus. [See color version online at www.interscience.wiley.com.]

the enlargement of the PVS. After 2–5 hr precultre, sensitivity of plasma membrane is decreased and tolerance to manipulation increased. Grabarek et al. (2004) found that GV oocytes show identified depression on the oocyte surface when incubated in M2 supplemented with 16 mM glucose, dbcAMP, cytochalasin D, and nocodazole for 30–40 min. A bevel pipette was used to aspirate GV after making an opening on the zona and donor cell was inserted through the ZP. In this study, zona-free GV oocytes were subjected to GV removal and fused with PS by PHA agglutination. PHA enabled adequate contact between a PS and a recipient oocyte, and thereby achieved a higher rate of fusion. Improved fusion efficiency by PHA is also found in somatic nuclear transfer in cattle (Du et al., 2006). But PHA agglutination method is more complicated than direct PVS insertion because additional zona digestion with pronase and extra attentions on averting self-agglutination between zona-free oocytes are required, so total efficiency is decreased by slow manipulation.

GV ooplasm can support meiotic progression of nuclei blastomeres from the 2-, 4-, and 8-cell stage embryos, but cell division is accelerated. The oocytes reconstructed with 2- and 4-cell blastomere nuclei divided 4 and 5–6 hr post-GVBD, respectively, and oocytes reconstructed with 8-cell blastomere nuclei underwent division 6–7 hr post-GVBD, while extrusion of the PB1 in controls occurred not earlier than 8 hr post-GVBD (Grabarek et al., 2004). A difference in the timing of PB1 extrusion was also observed in oocytes reconstructed with G2/M somatic nuclei. Compared with control GV oocytes, the reconstructed oocytes extruded their first pseudo-PBs 5–7 hr after fusion, which was approximately 3–4 hr earlier than controls (Chang et al., 2004). In this study, ooplasts at various statuses were obtained and PSs in the pachytene to diplotene stages of the meiotic prophase were selected as donor cells. When ooplasms prepared at 0, 2, 5, and 8.5 hr of IVM were fused with PSs, extrusions of the PB1 were observed at 8.5, 7, 5.5, and 3 hr, respectively. This indicates that oocyte maturation degree determines the timing of PB1 extrusion in reconstructed oocytes. However, if GV ooplast was cultured in vitro for 8.5 hr and then fused with PS, extrusion of the PB1 occurred 7–11 hr after electrofusion. These results demonstrate that GV material may be important for ooplasm abilities to accelerate meiotic progression of reconstructed cells, and that if GV material is retained in the ooplasm, the later maturing ooplasm will require a shorter time for PB1 extrusion.

Previous researches by us and others on reconstruction of GV oocytes showed that large polar body was released (Cheng et al., 2003; Chang et al., 2004; Grabarek et al., 2004). And this phenomenon also occurred after fertilization of pro-MI oocyte (Sasagawa et al., 1998). In this study, large first PBs were observed in reconstructed oocytes. GV ooplasm and 2 hr IVM ooplasm produced a higher proportion of large PB1 (35.9% and 34.1%) than the ooplasms prepared from oocytes cultured for a longer time, and 8.5 hr IVM ooplasm did not cause large PB1. Choi et al. (1996) found that Mos/mitogen-activated protein kinase (MAPK) are required for regulating the size and degradation of the PB1, and the first polar bodies can be abnormally large and sometimes undergo an additional cleavage instead of undergoing rapid degeneration in $Mos^{-/-}$ mouse. The spindle formed centrally but did not migrate or the spindle elongated during anaphase, and the pole closest to the cortex moved while the other pole which remained in place is responsible for the equal divisions in these $Mos^{-/-}$ mouse oocytes (Verlhac et al., 2000). However, GV-removed oocytes have normal MAPK activation (Fisher et al., 1998; Sugiura et al., 2001). The reasons of large PB1 formation after transfer of PS into early-stage enucleated oocytes need further clarification.

Introduction of a G2/M cell to an immature oocyte causes chromosome condensation and hybrid arrest at MII. MI oocyte + M-phase zygote hybrids underwent accelerated maturation, but the timing of the PB1 extrusion in MI oocyte + G2 zygote hybrids was comparable with control oocytes (Grabarek and Zernicka-Goetz, 2000). In this study, when PSs were transferred into GV or pro-MI (just after GVBD) oocytes, extrusion of the PB1 was accelerated about 2.5 hr earlier than control oocytes.

Activation of M-phase promoting factor (MPF) leads to resumption of first meiosis as indicated by GVBD in mammals (Jones, 2004). The MPF, which is composed of a catalytic subunit p34cdc2 and a regulatory subunit cyclin B, accumulates during oocyte growth (Mitra and Schultz, 1996; de Vantery et al., 1997). MAPK cascade is another principal regulator that functions in driving the meiotic cell cycle progression of oocytes. After GVBD, MAPK is involved in the regulation of microtubule organization and meiotic spindle assembly (Fan and Sun, 2004). As ooplasms at different maturation stages have different MPF and MAPK kinase activities, nuclei of PS experience different durations to extrude the PB1. Simultaneously, PS material may influence the tempo of first meiotic division of an immature oocyte. Expression of phosphorylated cyclin B1 in PS was detected by confocal microscopy (data not shown) in this study, which may cause a raise or an earlier activation of MPF or MAPK, resulting in earlier PB1 extrusion when the PS was introduced into an immature oocyte.

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