JOURNAL OF EXPERIMENTAL ZOOLOGY 289:322–329 (2001)

Mouse-Rabbit Germinal Vesicle Transfer Reveals That Factors Regulating Oocyte Meiotic Progression Are Not Species-Specific in Mammals

GUANG-PENG LI,[1] DA-YUAN CHEN,[1]* LI LIAN,[1] QING-YUAN SUN,[1,3] MIN-KANG WANG,[1] XIANG-FEN SONG,[1] LI MENG,[2] AND HEIDE SCHATTEN[3]

[1]*State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100080 China*

[2]*IVF Laboratory, Women and Infants Hospital of Rhode Island, Brown University School of Medicine, Providence, Rhode Island 02905*

[3]*Department of Veterinary Pathobiology, University of Missouri-Columbia, Columbia, Missouri 62511*

ABSTRACT A series of experiments were designed to evaluate the meiotic competence of mouse oocyte germinal vesicle (GV) in rabbit ooplasm. In experiment 1, an isolated mouse GV was transferred into rabbit GV-stage cytoplast by electrofusion. It was shown that 71.8% and 63.3% of the reconstructed oocytes completed the first meiosis as indicated by the first polar body (PB1) emission when cultured in M199 and M199 + PMSG, respectively. Chromosomal analysis showed that 75% of matured oocytes contained the normal 20 mouse chromosomes. When mouse spermatozoa were microinjected into the cytoplasm of oocytes matured in M199 + PMSG and M199, as many as 59.4% and 48% finished the second meiosis as revealed by the second polar body (PB2) emission and a few fertilized eggs developed to the eight-cell stage. In experiment 2, a mouse GV was transferred into rabbit MII-stage cytoplast. Only 13.0–14.3% of the reconstructed oocytes underwent germinal vesicle breakdown (GVBD) and none proceeded past the MI stage. When two mouse GVs were transferred into an enucleated rabbit oocyte, only 8.7% went through GVBD. In experiment 3, a whole zona-free mouse GV oocyte was fused with a rabbit MII cytoplast. The GVBD rates were increased to 51.2% and 49.4% when cultured in M199 + PMSG and M199, respectively, but none reached the MII stage. In experiment 4, a mouse GV was transferred into a partial cytoplasm-removed rabbit MII oocyte in which the second meiotic apparatus was still present. GVBD occurred in nearly all the reconstructed oocytes when one or two GVs were transferred and two or three metaphase plates were observed in ooplasm after culturing in M199 + PMSG for 8 hr. These data suggest that cytoplasmic factors regulating the progression of the first and the second meioses are not species-specific in mammalian oocytes and that these factors are located in the meiotic apparatus and/or its surrounding cytoplasm at MII stage. *J. Exp. Zool. 289:322–329, 2001.*

Mammalian oocytes are arrested at the diplotene stage of the first meiotic division. It is generally accepted that maturation promoting factor (MPF) plays an important role in the G2/M transition (Taieb et al., '97). This is well demonstrated by cytoplasm transfer experiments. When a small amount of cytoplasm is removed from a maturing amphibian oocyte and injected into an immature amphibian oocyte, its maturation is attained even in the presence of the protein synthesis inhibitor—cycloheximide (CX) (Wassermann and Masui, '75). This phenomenon occurs by means of autocatalytic amplification, in which active MPF introduced by microinjection triggers the activation of inactive MPF (pre-MPF). In pig, when a zona-free MII oocyte is fused with a freshly isolated zona-free GV oocyte, GVBD is greatly accelerated and two metaphase plates are clearly visible in each mega-oocyte (Mattioli et al., '91). This phe-

Grant sponsor: Rockefeller Foundation; Grant number: PF94025 #30; Grant sponsor: The "973" Project; Grant number: G1999055902; Grant sponsor: Knowledge Innovation Program of the Chinese Academy of Sciences; Grant number: 39770098; Grant sponsor: National Natural Science Foundation of China; Grant number: 39970093.

G.P. Li and D.Y. Chen contributed equally to this work.

*Correspondence to Da-Yuan Chen, State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China. E-mail: chendy@panda.ioz.ac.cn

Received 30 May 2000; Accepted 23 October 2000

nomenon is also observed in bovine oocytes (Tatemoto and Horiuchi, '95).

Recently, studies have shown that GVBD occurred and a normal first polar body was emitted when a GV was removed from one oocyte and transferred by electrofusion into the enucleated cytoplasm of another GV-stage oocyte in the mouse (Liu et al., '99; Takeuchi et al., '99) and human (Zhang et al., '99). This approach provides a useful tool for studying nucleus–cytoplasm interactions. This article describes experimental designs that have made it possible to study interspecific nucleus–cytoplasm interactions by transferring a GV from one kind of animal to the ooplasm of another kind. Because both mouse and rabbit oocyte GVs are easy to remove due to the transparency of their cytoplasm and because rabbit oocytes are resistant to micromanipulation, we transferred mouse oocyte GVs into rabbit ooplasm in order to observe the influence of rabbit ooplasmic factors on the behavior of the mouse oocyte nucleus.

MATERIALS AND METHODS

Animals

ICR mice were housed in a temperature- and light-controlled room on a 14 hr light: 10 hr dark photoperiod. Six- to eight-week-old females were used as germinal vesicle donors and 8–12-week-old males were used as sperm supply. Six- to twelve-month-old female Japanese Big Ear rabbits were used for collecting the germinal vesicle (GV) and MII-stage oocytes. The animals used in these experiments were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Science.

Collection of oocytes

Mouse

Female mice were injected intraperitoneally with 10 IU pregnant mare serum gonadotropin (PMSG). Immature GV-stage oocytes were collected by puncturing the ovarian follicles at 40–44 hr post-injection.

Rabbit

Female rabbits were injected intraperitoneally with 120–150 IU PMSG and killed 72 hr after injection. Ovaries were excised and punctured to collect the GV-stage oocytes. The other group was injected with 80–100 IU hCG (Institute of Zoology, Academia Sinica) at 72 hr following PMSG priming and killed 15–16 hr later to collect MII-stage oocytes.

Cumulus cells of all oocytes were removed by exposure to M2 medium containing 500 IU/ml hyaluronidase (Sigma). The GV-stage oocytes were cultured in M199 medium (Sigma) supplemented with 10% fetal calf serum (FCS, Sigma) and 20 μg/ml 3-isobutyl-1-methylxanthine (IBMX, Sigma) for 2 hr to prevent spontaneous GVBD and to develop a perivitelline space.

Preparation of mouse GV-karyoplasts

GV-stage oocytes were cultured in 50 μl M2 medium droplets with 10% FCS, 7.5 μg/ml cytochalasin B, and 20 μg/ml IBMX for 30 min at room temperature before GV aspiration. GV removal followed the methods described by Takeuchi et al. ('99) and Liu et al. ('99). Briefly, the zona pellucida was penetrated by pressing a glass microneedle tangentially into the perivitelline space against the holding pipette, and the GV surrounded by a small amount of cytoplasm (GV karyoplast) was removed with a pipette of an inner diameter of 15 μm. The diameter of the GV karyoplast was about 15–20 μm. In experiment 2, a whole mouse zona-free GV-oocyte, which was obtained via removal of zona pellucida digested in M199 medium containing 0.3% pronase for 3–4 min, was used as a GV donor. Zona-free intact GV oocytes, 80–90 μm in diameter, were maintained in IBMX-containing medium until injection.

Removal of nucleus from rabbit oocyte

The rabbit GV enucleation was achieved by increasing the pressure inside a holding pipette to repel a GV karyoplast through a slit made in the zona as described by Meng et al. ('96). Enucleation of MII rabbit oocytes was carried out as described by Chen et al. ('99). The enucleated eggs were stained with 10 μg/ml Hoechst 33342 (Sigma Co.) for 8–10 sec and examined under a fluorescent microscope. Eggs from which all the chromosomes were removed were used for GV transfer.

GV transfer and electrofusion

The mouse GV karyoplast was microinjected into the perivitelline space of a rabbit oocyte with a pipette of 25 μm inner diameter (Fig. 1A,B). The reconstructed oocytes were equilibrated in M199 + 10% FCS medium for 30 min and then transferred into a drop of fusion medium (0.3 M mannitol, 0.1 mM $CaCl_2$ and 0.05 mM $MgSO_4$ in M2 medium). Electrofusion was stimulated with three electrical pulses (180 V/mm DC for 80 μs) delivered by a Kefa Electro Cell manipulator (Aca-

demia Sinica). The fusion was examined 30 min later (Fig. 1D).

ICSI Experimental design

Experiment 1

Mouse GVs were transferred into GV-removed rabbit oocytes. The reconstructed oocytes were randomly cultured in M199 or M199 + PMSG for 24 hr. Cumulus-free rabbit GV oocytes were cultured for 24 hr; oocytes with the first PB were used for ICSI with rabbit sperm as control.

Experiment 2

One or two mouse GV-karyoplast (s) was (were) fused with enucleated rabbit MII oocytes, and cultured in M199 + PMSG for up to 24 hr.

Experiment 3

An intact zona-free mouse GV-stage oocyte was fused with an enucleated rabbit MII oocyte and cultured for 24 hr.

Experiment 4

One or two mouse GVs was (were) transferred into a partial cytoplasm-removed rabbit MII oocytes (Fig. 1C), and then cultured in M199 + PMSG for 8 hr to observe GVBD.

Evaluation of maturation of the reconstructed oocytes

After incubation, the oocytes were mounted on glass slides, fixed for 48 hr in methanol:acetic acid (3:1), stained with 1% orcein in 45% acetic acid for 3–5 min, and examined under a phase-contrast microscope. The stages of the nuclear progression were classified as (1) GV, (2) GVBD as defined by the absence of visible nuclear membrane, (3) metaphase I, and (4) metaphase II (Hunter and Polge, '66).

Cytogenetic analysis

Oocytes that had extruded the first polar body (PB1) were prepared for chromosomal analysis as previously described (Tarkowski, '66). Briefly, oocytes were treated in 1% hypotonic trisodium citrate solution for 10 min. The swollen ones were transferred individually onto a clean glass slide and fixed by serial fixation: fixative I (methanol: acetic acid, 3:1) for 2 min, fixative II (methanol: acetic acid, 1:1) for 1 min and finally fixative III (methanol:acetic acid, 1:3). Fixed oocytes were then stained with 5% Giemsa for chromosome counting (Li et al., '98).

Intracytoplasmic sperm injection (ICSI) and in vitro culture of the injected oocytes

Oocytes with extruded PB1 were used for ICSI with mouse sperm (Fig. 1G) that were collected from epididymis or vas deferens duct, and treated as described by Li et al. ('99). Briefly, a single spermatozoon was immobilized by striking the tail with the pipette and aspirated tail first into the tip of the injection pipette. Oocytes were hold on the holding pipette, with the polar body at the 6- or 12-o'-clock position. The microinjection pipette was pushed through the zona pellucida and into the cytoplasm of the oocyte at the 3-o'-clock position. The spermatozoon was deposited with a minimum amount of 10% PVP-containing medium. Following this, the injection pipette was withdrawn and the oocyte was released. After ICSI, the oocytes were cultured in RD (RPMI 1640:M199 = 1:1) medium (Li et al., '93) with 10% FCS at 37.5°C, 5% CO_2.

Statistical analysis

Data were analyzed by using the χ^2 test. Significant difference was determined at $P < 0.05$.

RESULTS

Rabbit GV-stage ooplasm supports the first and the second meioses of mouse oocyte GV

Oocytes were reconstructed by fusing mouse GV-stage karyoplasts with rabbit GV-stage cytoplasts. Of the 165 reconstructed karyoplast-cytoplast pairs, 131 (79.4%) were fused. The maturation rates as revealed by the PB1 emission were 71.8% and 63.3% 24 hr after culture in M199 + PMSG and M199, respectively. This was not significantly different from that of the control mouse oocytes (69.5–75%), but lower than that of control rabbit oocytes cultured in M199 + PMSG (92.7%) (Table 1).

A total of 16 reconstructed mature oocytes were fixed for cytogenetic study. Of the 12 well-spread samples, 9 had 20 chromosomes, 2 had 19 chromosomes, and 1 had 17 chromosomes. Most of those oocytes that failed to extrude PB1 had bivalent chromosomes (Fig.1-E).

After ICSI, 59.4% (19/32) and 48% (12/25) of the oocytes matured in M199 + PMSG and M199, respectively, emitted the second polar body. Furthermore, 12.5% (4/32) and 6.3% (2/32) of the oocytes matured in M199 + PMSG developed to two-cell and eight-cell stage, respectively (Table 2; Fig. 1-H, I). The oocytes matured in M199, however, failed to cleave after ICSI. The results above show that cy-

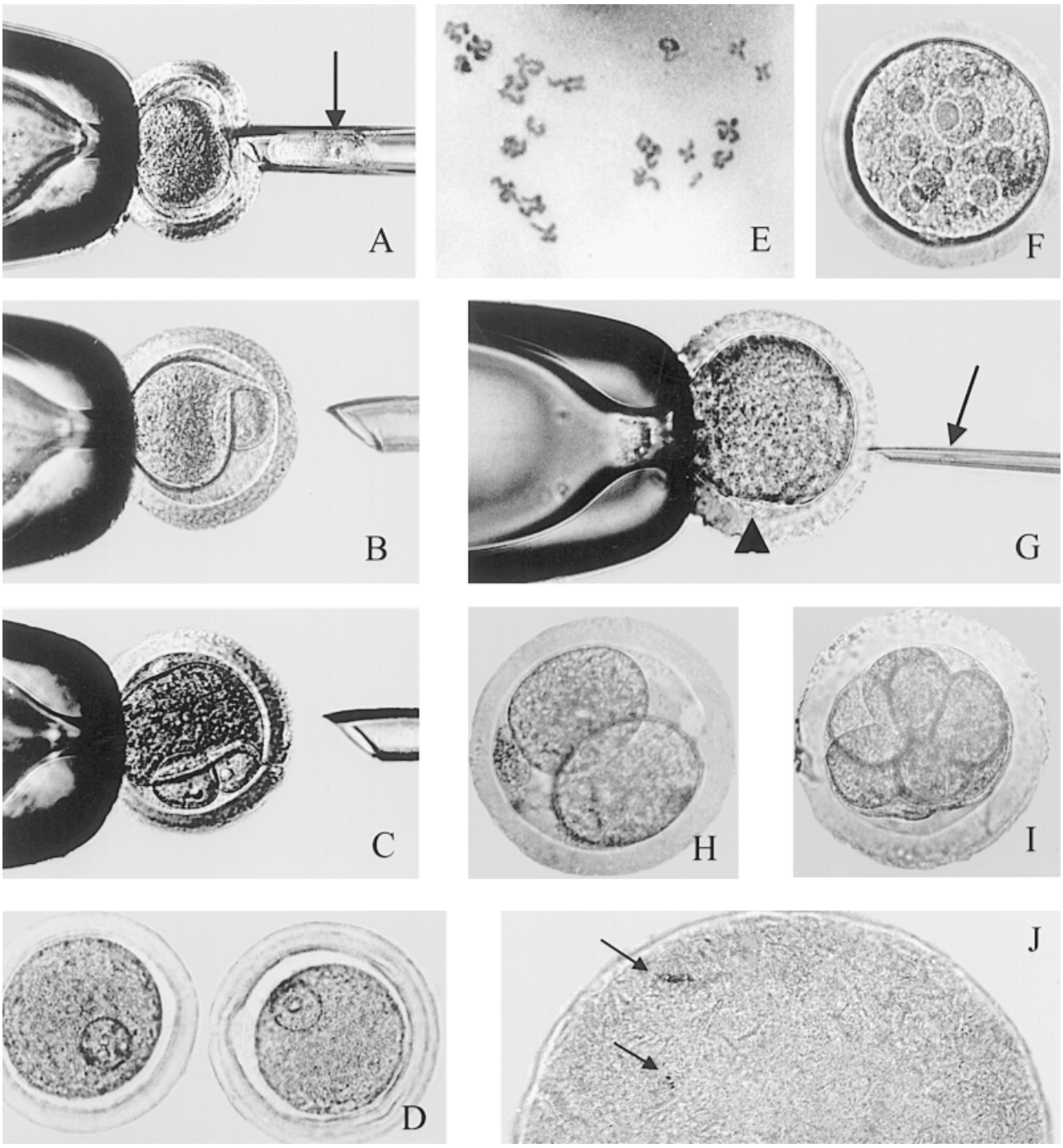

Fig. 1. Transfer of one (**A**, **B**) or two (**C**) mouse GVs into the perivitelline space of an enucleated (A, B) or a part-cytoplasm-removed but nucleus present (C) oocyte (arrow shows the mouse GV); fusion of mouse GVs into rabbit cytoplasm (**D**); chromosome spreads of an oocytes reconstructed by fusing a mouse GV with a rabbit GV-stage cytoplast after maturation culture (without PB1 emission), showing 20 bivalent chromosome (**E**); vacuoles present in the GVBD oocyte reconstructed fusing a mouse GV with an enucleated rabbit MII oocyte (**F**); ICSI of a mature GV-GV reconstructed oocyte (**G**), arrow points at the sperm and arrowhead points at the first polar body; 2-cell (**H**) and 8-cell (**I**) stage embryos obtained by ICSI; two spindles in a mature oocyte reconstructed by fusing a mouse GV with a part-cytoplasm-removed rabbit oocyte (**J**), arrows pointing at the spindles.

TABLE 1. Maturation of oocytes reconstructed by fusing mouse GV-stage karyoplasts with rabbit GV-stage cytoplasts

Groups	Culture media	No. oocytes cultured	Maturation		
			PB1(%)	MI	GV
Reconstructed oocytes	M199+PMSG	71	51(71.8)**	3	17
	M199	60	38(63.3)**	6	16
Control (mouse GV-oocytes)	M199+PMSG	56	42(75)	10	4
	M199	42	29(69.1)**	6	7
Control (rabbit GV-oocytes)	M199+PMSG	41	38(92.7)*	1	2
	M199	38	26(68.4)**	8	4

Reconstructed oocytes and control oocytes were cultured for 24 hr and nuclear progression was evaluated by orcein staining as described in Materials and Methods.
*,** Different superscripts indicate values significantly different ($P < 0.05$).

toplasmic factors in rabbit GV-stage ooplasm support the mouse oocyte GV to undergo two meioses.

Rabbit MII-stage cytoplast fails to induce mouse GV to resume meiosis

Because MPF is thought to play an important role in inducing GVBD and because MPF reaches the peak level in MII oocytes, we expected that rabbit MII cytoplast would induce the mouse GV to break down. Unexpectedly, when a mouse GV-karyoplast was fused with an enucleated rabbit MII oocyte, only 14.3% and 13.0% of the reconstructed oocytes underwent GVBD 24 hr after culture in M199 + PMSG and M199, respectively. No oocytes reached the MI stage. When two GVs were transferred into an enucleated rabbit MII oocyte, only 8.7% underwent GVBD and only one GV broke down in some of the oocytes. Meiosis resumption of the reconstructed oocyte was improved by fusing an intact mouse GV oocyte with a MII rabbit cytoplast. When the whole mouse zona-free GV oocytes were fused with MII rabbit cytoplasts, 51.2% and 49.4% went through GVBD after culturing in M199 + PMSG and M199, respectively. Part of the oocytes (24.4% in M199 + PMSG and 10.0% in M199) could develop but arrested at the MI stage (Table 3). No PB1 emission was observed. Nearly half of the GVBD oocytes contained many (3 to 15) vacuoles in the center of the cell (Fig. 1F).

The existence of meiotic apparatus in rabbit MII ooplasm is important for inducing mouse GVBD

Since MII rabbit cytoplasts did not support mouse GV breakdown, an experiment was designed to evaluate whether the MII apparatus and/ or its surrounding cytoplasmic elements were required for GVBD. One or two GVs were transferred into a partial cytoplasm-removed, nucleus-present rabbit oocyte. GVBD occurred in all the reconstructed oocytes except for those degenerated 8 hr after culture in M199 + PMSG (Table 4). Two (Fig. 1J) or three metaphase plates were observed in these oocytes.

DISCUSSION

GV transfer is an invaluable tool for studying nucleus–cytoplasm interactions during meiosis.

TABLE 2. Development of reconstructed mature oocytes after ICSI with mouse sperm

Groups	Culture media	No. oocytes survived ICSI	Oocytes extruded PB2 (%)	Development after culture (%)				
				8-cell	2-cell	2PB+1PN	2PB+2PN	2PB+0PN
Reconstructed oocytes	M199 +PMSG	32/40	19 (47.5)	2 (10.5)	4 (21.1)	6 (31.6)	5 (26.3)	2 (10.5)
	M199	25/33	12 (36.3)	0	0	3 (25)	5 (41.7)	4 (33.3)
Control (rabbit GV-oocytes)	M199 +PMSG	33/36	26 (72.2)	12 (46.1)	7 (26.9)	3 (11.5)	3 (11.5)	1 (3.8)
	M199	23/24	17 (70.8)	10 (58.8)	5 (29.4)	1 (5.9)	0	1 (5.9)

Oocytes reconstructed by fusing mouse GVs and rabbit GV-stage cytoplasts were matured in vitro and then fertilized with mouse sperm by ICSI.
Control rabbit GV stage oocytes were matured in vitro and then fertilized with rabbit sperm by ICSI.
Fertilization and embryo cleavage were evaluated with a phase contrast microscope.

TABLE 3. Maturation of oocytes reconstructed by fusing mouse GV or intact GV oocytes with enucleated rabbit MII oocytes

Culture media	Types of CVs transferred	No. oocytes used	Oocytes fused (%)	Maturation in vitro (%)			
				MI	Pre-MI	GV	Degenerated
M199+ PMSG	Intact GV-oocyte	45	41(91.1)	10(24.4)	11(26.8)	16(39.0)**	4
	GV-karyoplast	30	21(70)	0	3(14.3)	18(85.7)*	0
	Intact GV-oocyte	110	85(77.2)	9(10.6)	33(38.8)	39(45.9)**	4
M199	GV-karypolast	69	54(78.3)	0	7(13.0)	42(77.8)*	5

Oocytes reconstructed by fusing mouse GVs or intact mouse GV oocytes with enucleated rabbit MII oocytes were cultured in vitro. Nuclear meiotic progression was evaluated by orcein staining 24 hr after culture.
The same column *:**, $P < 0.05$.

We report here that oocytes reconstructed by transferring mouse GVs into rabbit GV-stage cytoplasts can mature to MII stage and can further cleave and develop to eight-cell stage after ICSI. The maturation rate was not significantly different with that of control mouse oocytes or control rabbit oocytes cultured in M199 as shown in Table 1. The addition of PMSG to the maturation medium did not increase the maturation rate, while it improved early cleavage after fertilization. These results indicate that rabbit GV-stage ooplasm can support the first and the second meioses of mouse oocyte GV. Thus, we conclude that cytoplasmic factors that regulate oocyte meiotic cell cycle progression are not species-specific in mammals.

After ICSI of the reconstructed mature oocytes, development occurred to eight-cell stage (6.3%), but not beyond this stage. This finding is similar to the results of inter-species fertilization. Li et al. ('99) reported that the hybrid embryos made by rabbit oocytes and mouse spermatozoa developed to, but not beyond, the eight-cell stage.

Many studies have identified cytoplasmic factors such as MPF that are responsible for inducing nuclear envelope breakdown and chromosome condensation. In both MI and MII metaphase arrests, the MPF activity is maintained at very high levels. MPF activity appears shortly before GVBD, maintains a high level during MI, decreases prior to extrusion of PB1, and rises again throughout the MII stage (Sagata, '96, Takeuchi et al., '99).

Interestingly, when a mouse GV was transferred into a rabbit MII cytoplast, the reconstructed oocyte did not resume meiosis. When the reconstructed oocytes comprised of one or two mouse GV-karyoplasts and an enucleated rabbit cytoplast were cultured for 24 hr, only 12.7% and 8.6% underwent GVBD, respectively. Even when a whole mouse GV was fused with a rabbit MII cytoplast, none developed to the MII stage. The same phenomenon was recently also reported by others in mouse intra-species GV transfer (Liu et al., '99; Takeuchi et al., '99). We propose that rabbit MII-stage ooplasm contains does not contain the necessary factors that are required for inducing the transferred mouse GV to break down. In Experiment 3, about 50 present reconstructed oocyte occurred GVBD when mouse intact zona-free oocytes fused with enucleated rabbit oocytes. It may ex-

TABLE 4. Maturation of oocytes reconstructed by fusing 1 or 2 mouse GVs with partial cytoplasm-removed or enucleated rabbit MII oocytes

Recipients	No. of GV-karyoplasts transferred	Oocytes successfully manipulated	Oocytes fused (%)	Maturation (%)		
				GVBD	GV	Degenerated
Part-cytoplasm -removed MII oocytes	1	46	39 (87.8)	35 (89.7)*	0	4
	2	30	23 (76.6)	22 (95.7)*	0	1
Enculeated MII oocytes	1	39	31 (79.5)	4 (12.9)**	22 (71.0)	5
	2	33	23 (69.7)	2 (8.7)**	19 (82.6)[a]	2

One or two mouse GBs were transferred into a part-cytoplasm-removed or an enucleated rabbit MII oocyte. GVBD was evaluated 8 hr after in vitro culture.
[a]In 6 reconstructed oocytes, one GV underwent GVBD, the other one did not.
*,**, $P < 0.05$.

plained fewer MPF and other factors in mouse GV oocytes induced the GVBD in reconstructed oocytes.

The failure of MII cytoplast to induce GVBD is not because the cytoplasm was partially removed during enucleation. When one or two GVs were transferred into a partial cytoplasm-removed rabbit MII oocyte in which the MII meiotic apparatus still existed, almost all of the reconstructed oocytes underwent GVBD, and two or three metaphase plates were observed in these fused oocytes (Fig 1J). Related intra-species work has been reported previously. Tatemoto and Horiuchi ('95) reported that when one mature oocyte was fused with one GV oocyte in bovine, all the fused oocytes underwent GVBD after culture for 3 hr and 67% of the fused oocytes had two metaphase plates. The same phenomenon was also observed in pig by Mattioli et al. ('91). The authors showed that most of the fused oocytes composed of one MII oocyte and one to three immature GV oocytes went through GVBD after culture for 1 hr. Furthermore, meiotic incompetent small mouse oocytes could be induced to mature when fused with meiotic or mitotic cells (Balakier, '78). The induction of meiotic resumption is triggered by active MPF in MII oocytes (Gerhart et al., '84; Hashimoto and Kishimoto, '88). These reports all indicate that active MPF is present in the MII oocytes and causes resumption of meiotic division of the transferred GV. In Experiment 4, GVBD occurred in all the reconstructed oocytes when one or two mouse GVs were transferred into partial cytoplasm-removed rabbit MII oocytes. Maybe the asynchronouse cytoplasm between GV ooplasm and MII cytoplasm was responsible for the lack of further development after GVBD. Huchon et al. ('93) indicated that MI and MII spindle are not regulated by the same effects.

Our results suggest that the MPF function of inducing meiotic resumption is not species-specific. We also suggest that the presence of MII apparatus and/or its surrounding cytoplasmic elements play a critical role in the meiotic resumption of the transferred GV. A plausible explanation for the failure of rabbit MII cytoplast to induce the mouse GV to undertake GVBD is that MPF is preferentially bound to the meiotic apparatus and enucleation removes active MPF from the MII oocytes. Kubiak et al. ('93) showed that MPF activity is preferentially associated with the spindle.

In conclusion, oocytes reconstructed by fusing mouse GV karyoplasts with rabbit GV-stage cytoplasts can mature to MII stage, and can cleave to eight-cell stage after ICSI with mouse sperm. Enucleated rabbit MII cytoplasm does not support the transferred mouse GV to undergo GVBD, while the existence of MII apparatus enables the fused mouse GV to resume meiosis.

LITERATURE CITED

Balakier H.1978 Induction of maturation in small oocytes from sexually immature mice by fusion with meiotic or mitotic cells. Exp Cell Res 112:137–141.

Chen DY, Sun QY, Liu JL, Li GP, Lian L, Wang MK, Han ZM, Song XF, Li JS Sun Q, Chen YC, Zhang YP, Ding B.1999. The giant panda *(Ailuropoda melanoleuca)* somatic cell nucleus can dedifferentiate in rabbit ooplasm and support early development of the reconstructed egg. Sci Chi 42:346–353.

Gerhart J, Wu M, Kirschner M. 1984. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in *Xenopus laevis* oocytes and eggs. J Cell Biol 98:1247–1255.

Hashimoto N, Kishimoto T. 1988. Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. Dev Biol 126:242–252.

Huchon D, Rime H, Jessus C, Ozon R. 1993. Control of metaphase I formation in *Xenopus* oocyte: effects of an indestructible cyclin B and of protein synthesis. Biol Cell 77:133–141.

Hunter RHF, Polge C. 1966. Maturation of follicular oocytes in the pig after injection of human chorionic gonadotropin. J Reprod Fert 12:525–531.

Kubiak JZ, Weber M, de Pennart H, Winston NJ, Maro B. 1993. The metaphase II arrest in mouse oocytes is controlled through microtubule-dependent destruction of cyclin B in the presence of CSF. EMBO J 12:3773–3778.

Li GP, Cai SX, Xu LB, Sun XS, Liu ZH, Tan JH. 1998. Studies on the early development of mouse tetraploid embryos produced by electrofusion. Dev Reprod Biol 7:17–23.

Li JM, Foote RH, Simkin M. 1993. Development of zygotes cultured in protein-free medium with catalase, taurine or superoxide dismutase. Biol Reprod 48:33–37.

Li ZY, Tan, JH Zhou Q, Sun XS, Li GP, Liu ZH. 1999. Studies on microinjection of mouse spermatozoa into rabbit oocytes. Acta Exp Biol Sin 32:101–111.

Liu H, Wang CW, Grifo JA, Krey LC, Zhang J. 1999. Reconstruction of mouse oocytes by germinal vesicle transfer: maturity of host oocyte cytoplasm determines meiosis. Hum Reprod 14: 2357–2361.

Mattioli M, Galeati G, Bacci ML, Barboni B. 1991. Changes in maturation-promoting activity in the cytoplasm of pig oocytes throughout maturation. Mol Reprod Dev 30:119–125.

Meng L, Rutledge J, Kidder G, Khamsi F, Armstrong DT. 1996. Influence of germinal vesicle on the variance of patterns of protein synthesis of rat oocytes during maturation in vitro. Mol Reprod Dev 43: 407–411.

Sagata N. 1996. Meiotic metaphase arrest in animal oocytes: its mechanisms and biological significance. Trends Cell Biol.6:22–28.

Taieb F, Thibier C, Jessus C. 1997. On cyclins, oocytes, and eggs. Mol Reprod Dev 48: 397–411.

Takeuchi T, Ergun B, Huang HT, Rosenwakes Z, Paleimo GD.1999. A reliable technique of nuclear transplantation for mammalian oocytes. Hum Reprod 14:1312–1317.

Tarkowski AK. 1966. An air-drying method for chromosome preparations from mouse eggs. Cytogenetics 5:394–400.

Tatemoto H, Horiuchi T. 1995. Requirement for protein synthesis during the onset of meiosis in bovine oocytes and its involvement in the autocatalytic amplification of maturation-promoting factor. Mol Reprod Dev 41: 47–53.

Wassermann WJ, Masui Y. 1975. Effects of cycloheximide on a cytoplasmic factor initiating meiotic maturation in *Xenopus* oocytes. Exp Cell Res 91:381–388.

Zhang J, Wang CW, Krey L, Liu H, Meng L, Blaszczyk A, Adler A, Grifo J. 1999. In vitro maturation of human preovulatory oocytes reconstructed by germinal vesicle transfer. Fertil Steril 71:726–731.