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Reconstruction of enucleated mouse germinal vesicle oocytes with blastomere nuclei

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Summary

We have investigated the possibility that mitotic nuclei originating from preimplantation stage embryos and placed in the oocyte cytoplasm can undergo remodelling that allows them to undergo meiosis in the mouse. To address this question, we have used enucleated germinal vesicle (GV) ooplasts as recipients and blastomeres from the 2-, 4- or 8-cell stage as nuclear donors. We employed two methods to obtain ooplasts from GV oocytes: cutting and enucleation. Although efficiency of the reconstruction process was higher after enucleation than after cutting (90% and 70% respectively), the developmental potential of the oocytes was independent of how they had been produced. Nuclei from the 2-, 4-, or 8-cell stage embryos supported maturation in about 35%, 55% and 60% of cases, respectively. The time between nuclear envelope breakdown and the first meiotic division was shortened by up to 5 h in reconstructed oocytes, a period equivalent to the mitotic division of control blastomeres. About one-third of oocytes reconstituted with blastomere nuclei divided symmetrically instead of extruding a polar body; however, in the majority of them metaphase plates were found, suggesting that reconstructed oocytes (cybrids) underwent a meiotic rather than mitotic division. The highest percentage of asymmetric divisions accompanied by metaphase plates was found in cybrids with 8-cell-stage blastomere nuclei, suggesting that the nuclei from this stage appear to conform best to the cytoplasmic environment of GV ooplasts. Our results indicate that the oocyte cytoplasm is capable of remodelling blastomere nuclei, allowing them to follow the path of the meiotic cell cycle.

Keywords: Blastomeres, GV oocytes, Meiotic maturation, Mouse, Remodelling

Introduction

The ability of anucleate germinal vesicle (GV)-stage oocytes reconstructed by nuclear transfer of auto- and heterologous germinal vesicles to undergo proper maturation *in vitro* has been shown previously in mouse (Liu *et al*., 1999, 2000; Takeuchi *et al*., 1999; Li *et al*., 2001*a*), cow (Bao *et al*., 2003), rabbit (Li *et al*., 2001*b*) and human (Zhang *et al*., 1999). Such reconstituted oocytes are capable of forming a first meiotic spindle, subsequent division with the extrusion of the first polar body and arrest at metaphase of the second meiotic division (MII). Moreover, reconstructed GV oocytes that have undergone *in vitro* maturation can then be fertilized or parthenogenetically activated, resulting in completion of meiosis and further development (Liu *et al*., 2000, 2003; Li *et al*., 2001*c*; Bao *et al*., 2003). It has also been shown that the factors regulating meiotic progression are not species-specific (Li *et al*., 2001*b*).

Bałakier & Czołowska (1977) showed that the anucleate half of a GV oocyte fused to a 2-cell-stage blastomere extruded a small cytoplasmic fragment containing a nucleus, and formed the metaphase spindle near the surface of the larger cell. Later it was shown that the nucleus from a 2-cell-stage blastomere participated in formation of the metaphase I plate a few hours after being introduced into a maturing oocyte, even

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if meiotic bivalents of the oocyte were present at the same metaphase plate (Tarkowski & Bałakier, 1980). Similarly, mouse zygotes fused to maturing oocytes were shown to contribute mitotic chromosomes to meiotic metaphase I (Grabarek & Zernicka-Goetz, 2000). Thus, it is likely that a blastomere-derived nucleus that normally participates in mitotic cycles can adjust to the meiotic programme when placed in the cytoplasm of a maturing oocyte. We have addressed this possibility using mouse oocytes reconstituted from GV ooplasts and nuclei derived from 2-, 4- and 8-cell-stage mouse blastomeres.

Materials and methods

Collection of oocytes and embryos

Oocytes were collected from 6- to 8-week-old F1 female mice (C57BL/10 × CBA/H). Fully grown oocytes at the GV stage were isolated by puncturing ovarian follicles in M2 medium (Whittingham, 1971; Fulton & Whittingham, 1978) supplemented with 0.2 mM dibutyryl cyclic adenosine 3′,5′-monophosphate (dbcAMP; Cho *et al.*, 1974) to prevent spontaneous GV breakdown. Follicular cells were removed by repeated pipetting of the oocytes in the above medium.

Embryos were collected from female F1 (C57BL/10 × CBA/H) or Swiss albino mice superovulated by injections of 10 IU of pregnant mare serum gonadotrophin (PMSG; Folligon, Intervet) followed by 10 IU of human chorionic gonadotrophin (hCG; Chorulon, Intervet) 48–56 h later and mated with F1 males. Two-cell, 4-cell and 8-cell-stage embryos were obtained 44–46 h, 56–58 h and 68–70 h after hCG injection, respectively, at times corresponding to the G2 stage of the cell cycle.

Obtaining GV ooplasts by cutting and reconstruction of oocytes

Zonae pellucidae were removed from GV oocytes and embryos by treatment with 0.5% pronase in phosphate-buffered saline (PBS) for 3–5 min. Zona-free oocytes were placed in M2 medium supplemented with dbcAMP, cytochalasin D (CD; 1 μg/ml) and nocodazole (0.25 μg/ml) and left at 5 °C for 20 min. After that time the oocytes were cut with a glass needle on an agar-coated Petri dish (Tarkowski, 1977) in M2 medium supplemented with dbcAMP, CD and nocodazole. The diameter of anucleate fragments (GV ooplasts) comprised approximately three-quarters that of the oocyte.

To obtain oocytes reconstructed from GV ooplasts, zona-free embryos were placed in PBS containing hyaluronidase (300 IU/ml) and disaggregated into separate blastomeres by vigorous pipetting. GV ooplasts were then agglutinated with single blastomeres in phytohaemagglutinin (PHA, 300 μg/ml) in bovine serum albumin (BSA)-free M2 supplemented with dbcAMP. The ooplast × blastomere pairs were washed in PBS and placed in an electrofusion chamber filled with fresh PBS. Fusion was induced with two series of electric pulses, each composed of three pulses of 110 V for 35 μs. Electric pulses were delivered by a 2001 Electro Cell Manipulator (BTX, San Diego, CA). Fusion usually occurred within 10–15 min after application of electric pulses.

Obtaining GV ooplasts using enucleation technique and reconstruction of oocytes

Enucleation pipettes (15 μm diameter) were prepared on a Narishige vertical puller and bevelled on an EG-4 Narishige grinding wheel. The enucleation pipette was then sharpened as described by Latham & Solter (1993). Micromanipulations were performed using a Leitz micromanipulator, under a Leitz inverted microscope with Nomarski optics (DIC).

Prior to enucleation, oocytes were incubated for 30–40 min in enucleation solution (M2 supplemented with 16 mM glucose, and with dbcAMP, CD and nocodazole). After preincubation, oocytes were examined under an inverted microscope. They responded to the enucleation solution by showing deformations in cell shape. One to five small depressions in the oocyte surface were present (Fig. 1A). These indentations persisted until glucose, CD and nocodazole withdrawal, after which they disappeared within 15 min. Only oocytes with indentations were used for the experiments. They were placed in a small drop of enucleation solution under paraffin oil in a micromanipulation chamber. Oocytes were secured with a holding pipette and an enucleation pipette was carefully inserted into the perivitelline space through the zona pellucida in the area of the depression in the surface of the oocyte (Fig. 1B). The GV with a small amount of surrounding cytoplasm was removed by smooth suction (Fig. 1C, D). Karyoplasts containing a GV vesicle were then removed from the enucleation pipette or used for reconstruction of GV oocytes. The diameter of GV ooplasts comprised approximately three-quarters that of the oocyte.

For reconstruction of GV oocytes with blastomere nuclei, 2-, 4- or 8-cell-stage embryos were preincubated in M2 medium supplemented with CD for 30–40 min, then placed in a small drop of enucleation solution under paraffin oil in a micromanipulation chamber, next to the GV ooplasts. The embryos were secured with a holding pipette and the enucleation pipette was carefully inserted into the perivitelline space through the zona pellucida (Fig. 1E). Blastomere nuclei with a

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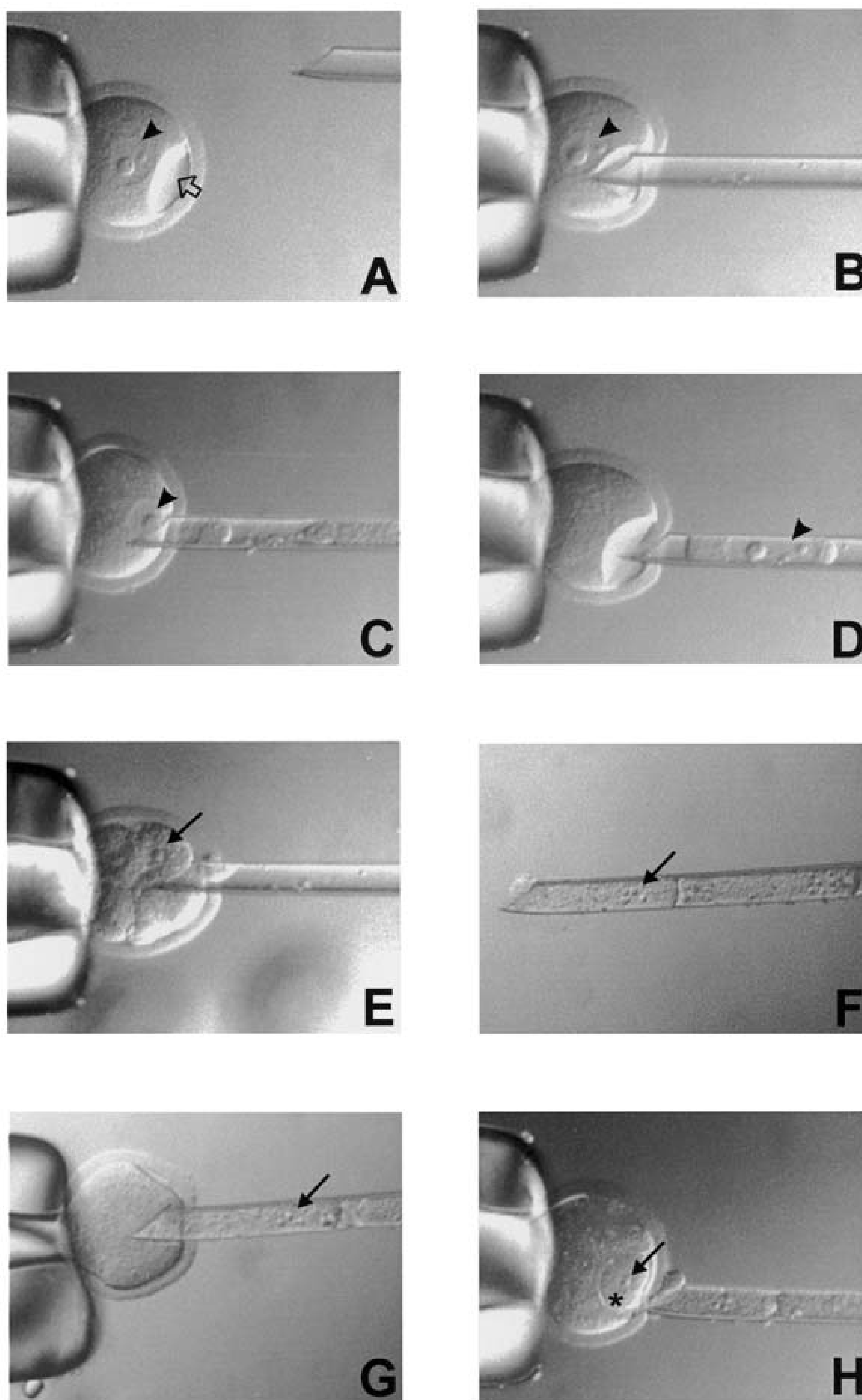

Figure 1 Microsurgical enucleation and reconstruction of germinal vesicle (GV) oocytes with blastomere nuclei. GVs are marked with arrowheads, blastomere nuclei are marked with arrows. (A) GV oocyte prior to enucleation. A single indentation is present in the oocyte surface (thick open arrow). (B) Insertion of the enucleation pipette into the perivitelline space in the area of the indentation. (C) Aspiration of the GV with the enucleation pipette. (D) GV with a small amount of surrounding cytoplasm is present in the enucleation pipette. (E) Aspiration of a blastomere of an 8-cell embryo. (F) Blastomere present in the enucleation pipette. (G) Insertion of the enucleation pipette carrying the blastomere into the perivitelline space of the enucleated GV oocyte through the slit in the zona pellucida. (H) Blastomere (aster) placed under the zona pellucida of an enucleated GV oocyte.

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small amount of surrounding cytoplasm (karyoplasts) were removed by smooth suction. They comprised 15–20% of the volume of the oocyte. The karyoplasts were then introduced into the perivitelline space of enucleated GV oocytes through the slit in the zona pellucida made during enucleation (Fig. 1G, H). The ooplast × blastomere pairs were washed in PBS and placed in an electrofusion chamber. Fusion was induced in the same way as applied to zona-free ooplast–blastomere pairs.

Experimental groups

In the first experiment (Comparison of cutting and enucleation . . .) ooplasts were obtained by cutting or enucleation and fused to 4-cell or 8-cell-stage blastomeres. In the second experiment (Maturation of GV ooplasts . . .) ooplasts obtained exclusively by enucleation were reconstructed by fusion with karyoplasts from 2-cell, 4-cell, or 8-cell-stage blastomeres.

Control groups were as follows:

Control A: zona-free GV oocytes treated the same way as cut oocytes except that they were not cut.

Control B: zona-enclosed oocytes treated the same way as enucleated oocytes except that they were not enucleated.

dbcAMP control: zona-enclosed GV oocytes incubated with dbcAMP until the time of fusion in experimental groups.

Untreated control; zona-enclosed, not preincubated GV oocytes.

O + GV karyoplast control: oocytes reconstituted by autotransfer of a GV nucleus into an anucleate GV oocyte.

Culture and examination of the cells

After fusion, pairs were washed in M2 medium and cultured in KSOM medium (Lawitts & Biggers, 1993) under paraffin oil at 37.5 °C in an atmosphere of 5% CO_2 in air. Reconstructed oocytes were examined under a Leitz inverted microscope with Nomarski optics (DIC) every 10 min during the first hour of culture. After that time, unfused pairs were counted and discarded from further culture. Reconstructed oocytes that degenerated within the first hour of culture were also discarded. Fused pairs, as well as control oocytes, were cultured for up to 24 hours after fusion.

Whole-mount preparations (Tarkowski & Wróblewska, 1967) were made after 4 and 24 h of culture.

Photographs

All photographs were made using a Coolpix 950 digital camera (Nikon, Japan) and processed using Corel PHOTO-PAINT 7.0 for Windows. Contrast was enhanced up to 30% of the original.

All reagents used, unless otherwise stated, were purchased from Sigma.

Results

Comparison of the cutting and enucleation methods for producing GV ooplasts

We used two methods to produce GV ooplasts for reconstruction with blastomere nuclei, namely cutting and enucleation. The latter was more efficient than cutting at any stage of the procedure, leading to more than 90% survival of reconstructed oocytes (Table 1, stage 4). Indeed, three to four times as many oocytes were needed to obtain the same number of surviving reconstructed oocytes after cutting compared with enucleation (Table 1, stage 5).

Efficiency of reconstruction after cutting was much poorer with 8-cell-stage nuclei than 4-cell-stage nuclei (Table 1, stages 3 and 4). Nevertheless, once reconstructed the oocytes underwent nuclear envelope breakdown (NEBD) within 2–3 h of culture, i.e. at the same time as control oocytes, independent of the method used to produce them. When fixed after 4 h of culture, reconstructed oocytes that had undergone NEBD contained condensing chromatin in the centre of the cell, similar to that in control oocytes. In oocytes that had not undergone NEBD or GV breakdown (GVBD), an interphase nucleus was present (Fig. 2).

The majority of reconstructed oocytes (cybrids) in both experimental groups as well as in control groups divided within 24 h of culture. The rate of division was not dependent on the method used (Table 1, stages 6–8) and was higher in the oocytes reconstructed with blastomere nuclei than in controls.

Maturation of GV ooplasts reconstructed with blastomere nuclei

NEBD was observed in 70% of cybrids at 2.5–3 h post-fusion, simultaneously with GVBD in the dbcAMP control and Control B. Anaphase plates were found in cybrids with 1/2- and 1/4-blastomere karyoplasts as early as 4–5 h post-fusion, and telophases as early as 5–6 h post-fusion. In cybrids with 1/8 blastomeres, anaphases did not appear until about 8–9 h post-fusion.

Half the oocytes reconstructed with 1/2- and 1/4-blastomere nuclei divided 4 and 5–6 h post-GVBD (7 and 8–9 h post-fusion) respectively. This timing corresponded to the cleavage completion of control 1/2- and 1/4-blastomeres (Table 2) rather than that of the control oocytes. Oocytes reconstructed with 1/8 blastomere nuclei divided the latest. Half of them

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Table 1 Comparison of the survival of GV ooplasts produced by cutting or enucleation and their maturation after reconstruction with blastomeres from 4- and 8-cell-stage embryos

Stage of experiment	dbcAMP	Cutting			Enucleation		
		Control A	4-cell	8-cell	Control B	4-cell	8-cell
1. Survival of GV ooplasts			64% (725/1132)	64.2% (706/1100)		89.6% (190/212)	90% (180/200)
2. Ooplast–blastomere pairs			69.1% (501/725)	68.8% (486/706)		92.6% (176/190)	93.9% (169/180)
3. Fusion efficiency			61% (306/501)	49.6% (241/486)		91.4% (161/176)	88.2% (149/169)
4. Surviving oocytes[b]			65.4% (187/286[a])	49.8% (110/221[a])		93.6% (146/156[a])	93.8% (121/129[a])
5. Surviving oocytes from surviving ooplasts			25.8% (187/725)	15.6% (110/706)		76.8% (146/190)	67.2% (121/180)
6. NEBD (GVBD)	93% (69/74)	89.3% (291/326)	87.7% (164/187)	100% (110/110)	84.9% (248/292	86.3% (126/146)	100% (121/121)
7. Division	63.8% (44/69)	50.4% (119/236)	67% (110/164)	56.4% (62/110)	51.6% (127/246)	68% (86/126)	58.7% (71/121)
8. Total division	59.5% (44/74)	36.5% (119/326)	58.8% (110/187)	56.4% (62/110)	43.5% (127/292)	58.9% (86/146)	58.7% (71/121)

NEBD, nuclear envelope breakdown; GVBD, germinal vesicle breakdown.
Control A, zona-free GV oocytes treated like the experimental group except for not being cut; Control B, zona-enclosed GV oocytes treated like the experimental group except for not being enucleated; dbcAMP, GV oocytes incubated in M2 + dbcAMP until fusion in experimental groups.
[a] Subtracted are the oocytes that having undergone GVBD degenerated in further culture.
[b] Twenty-four hours post-fusion.

Table 2 Timing of division of oocytes reconstructed by fusing karyoplasts to enucleated GV oocytes

Type of cell	No. (%) of divided cybrids						
	Time of division (h after GVBD[a])						
	4	5	6	7	8	9	24
O + 1/2	6 (54.5)	7 (63.6)	10 (90.9)	11 (100)	11 (100)	11 (100)	11 (100)
O + 1/4	1 (4.3)	10 (43.5)	20 (86.9)	23 (100)	23 (100)	23 (100)	23 (100)
O + 1/8	–	–	9 (47.4)	13 (68.4)	19 (100)	19 (100)	19 (100)
Blastomere 1/2	33 (91.6)	36 (100)	36 (100)	36 (100)	36 (100)	36 (100)	36 (100)
Blastomere 1/4	19 (79.2)	23 (95.8)	24 (100)	24 (100)	24 (100)	24 (100)	24 (100)
Blastomere 1/8	4 (22.2)	14 (77.8)	16 (88.9)	18 (100)	18 (100)	18 (100)	18 (100)
dbcAMP control	–	–	–	–	4 (7.8)	29 (56.9)	51 (100)

O + 1/2 (1/4 and 1/8), cybrids obtained by the introduction into GV ooplasts of karyoplasts from 1/2, 1/4 and 1/8 blastomeres.
[a] GVBD occurred in control oocytes about 3 h post-fusion.

underwent division 6–7 h post-GVBD (9–10 h post-fusion), timing again comparable to that of the cleavage of control 1/8 blastomeres. The first divisions (extrusion of the first polar body) in dbcAMP control oocytes occurred not earlier than 8 h post-GVBD (Table 2).

Oocytes reconstructed with GV karyoplasts underwent maturation in 42.3% (22/52), indistinguishably from Control B oocytes (43.1%, Table 3). We observed that a small fraction of Control B oocytes (7.6%, Table 4) underwent equal division instead of extruding the first polar body. Together with a relatively low ratio of meiotic maturation success in this group, these observations suggest negative effects of nocodazole and CD on cell division (but not NEBD: see Table 3). Oocytes reconstructed with 1/2-blastomere nuclei matured in 35.8% with about 70% of them undergoing asymmetric divisions (Table 4). Oocytes reconstructed with 1/4- and 1/8-blastomere nuclei matured in 55.7% and 60.3% respectively (Table 3), with asymmetric

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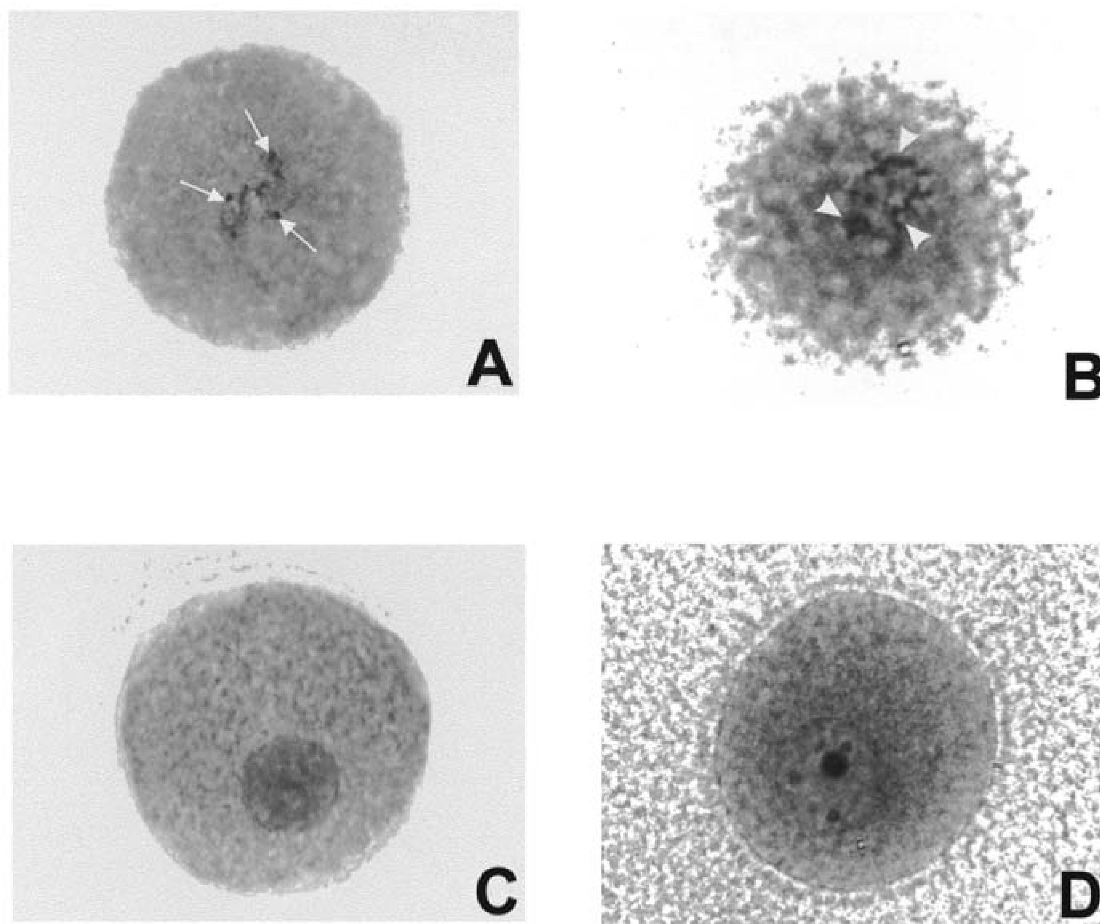

Figure 2 Reconstructed and control oocytes fixed after 4 h of culture. (A) Chromosomes (white arrows) present in the reconstructed oocyte after nuclear envelope breakdown (NEBD). (B) Chromosomes (white arrowheads) present in the control oocyte after germinal vesicle breakdown (GVBD). (C) Blastomere nucleus present in the reconstructed oocyte. (D) Germinal vesicle in a control oocyte.

Table 3 Maturation of enucleated GV oocytes reconstructed with various karyoplasts or blastomeres

	No enucleation			Enucleation				
Maturation	Untreated control	dbcAMP control	Control B	GV karyoplasts	O + GV karyoplasts	O + 1/2 karyoplasts	O + 1/4 karyoplasts	O + 1/8 karyoplast
GVBD/NEBD	75.7% (305/403)	93% (69/74)	83% (454/547)	77% (100/130)	67.3% (35/52)	84.5% (191/226)	88.6% (78/88)	100% (73/73)
1 polar body/ equal division	77.7% (237/305)	63.8% (44/69)	52% (236/454)	70% (70/100)	62.8% (22/35)	42.4% (81/191)	62.8% (49/78)	60.3% (44/73)
Total	58.8% (237/403)	57.4% (44/74)	43.1% (236/547)	53.8% (70/130)	42.3% (22/52)	35.8% (81/226)	55.7% (49/88)	60.3% (44/73)

GVBD, germinal vesicle breakdown; NEBD, nuclear envelope breakdown; O + GV (1/2, 1/4, 1/8), GV ooplast fused to GV karyoplasts (karyoplasts from 1/2 blastomere, 1/4 blastomere, 1/8 blastomere).
Untreated control, GV oocytes not preincubated; dbcAMP control, GV oocytes preincubated in dbcAMP-containing medium; Control B, GV oocytes treated like the experimental group except for not being enucleated.

divisions observed respectively for 65.6% and 68.0% in these groups of cybrids (Table 4).

When examined after fixation at 24 h of culture, metaphase plates were present in the majority of divided oocytes (Table 5), including those that divided equally (Fig. 3). These observations indicate a metaphase II-like arrest in the cell cycle, and thus a 'meiotic' type of behaviour.

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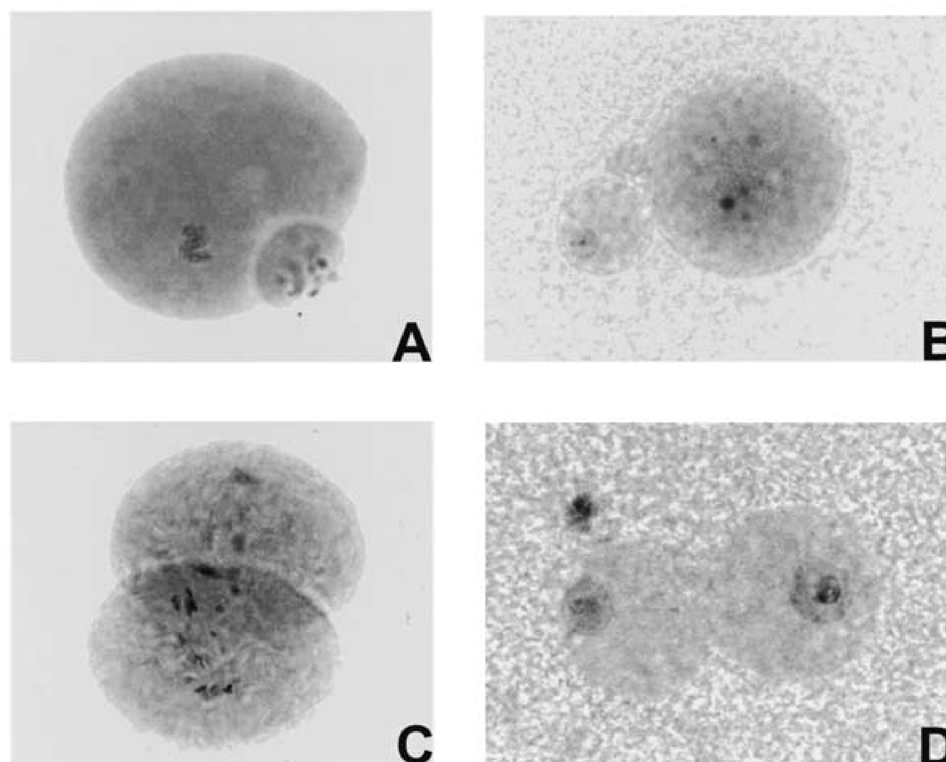

Figure 3 Reconstructed divided oocytes fixed after 24 h of culture. (A) Asymmetrically divided oocyte; chromosomes are visible in both the oocyte and the polar body. (B) Asymmetrically divided oocyte; interphase nuclei are present in both cells. (C) Condensed chromosomes scattered in both sister cells after symmetrical division. (D) Interphase nuclei present in both equal cells.

Table 4 Polar body extrusion and equal division in reconstructed divided oocytes cultured for 24 h

	Polar body		Equal division		
Type of cell	No. of oocytes	(%)	No. of oocytes	(%)	Total divided
O + 1/2	76	69.7	33	30.3	109
O + 1/4	42	65.6	22	34.4	64
O + 1/8	34	68	16	32	50
Control B	218	92.4	18	7.6	236
dbcAMP control	44	100	0	0	44
Untreated control	237	100	0	0	237

Table 5 Metaphase plates in reconstructed, divided (and fixed) oocytes cultured for 24 h

	No. of oocytes (%)				
	Metaphase		Interphase		
Type of cell	Polar body	Equal division	Polar body	Equal division	Total divided
O + 1/2	49 (64.5)	20 (26.3)	5 (6.6)	2 (2.6)	76
O + 1/4	26 (54.1)	19 (39.6)	2 (4.2)	1 (2.1)	48
O + 1/8	28 (63.6)	15 (34.1)	0	1 (2.3)	44
Control B	222 (94.1)	14 (5.9)	0	0	236
dbcAMP control	44 (100)	0	0	0	44
Untreated control	237 (100)	0	0	0	237

Discussion

Improvement in the method of GV ooplast collection

We have compared two methods that allow production of GV ooplasts, namely cutting and enucleation. Cutting of mouse oocytes is a relatively simple technique and was frequently used to obtain nucleate and anucleate halves of GV oocytes (Bałakier & Czołowska, 1977; Tarkowski & Bałakier, 1980; Bałakier & Masui, 1986; Fulka *et al.*, 1986, 1995, 2002). The latter were then reconstructed with nuclei of different cells.

Micromanipulation/enucleation technique has an advantage over cutting by reducing the volume of cytoplasm lost during removal of the nucleus. However, enucleation of mouse GV oocytes is difficult due to the large diameter of the GV and sensitivity of the plasma membrane. 'Indirect' enucleation of GV oocytes

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has been performed previously in the sheep (Sun & Moor, 1991) and rat (Meng *et al*., 1996) by cutting the zona pellucida and increasing the pressure inside the holding pipette to expel a GV karyoplast through the slit in the zona. More recently, improved methods of microsurgical enucleation of GV oocytes have been developed in the mouse (Liu *et al*., 1999, 2000; Takeuchi *et al*., 1999; Li *et al*., 2001*a,b*). However, these still involved prior cutting of the zona. A method described for removal of pronuclei from zygotes (Tsunoda *et al*., 1986) was also used earlier to obtain anucleate GV oocytes (Gao *et al*., 2002). This method too necessitates that the opening in the zona is made prior to enucleation.

We have used 16 mM glucose solution in mouse manipulation medium M2 for preincubation and enucleation. This resulted in enlargement of the perivitelline space in GV oocytes, which in turn allowed insertion of the micropipette without the need to produce a slit in the zona pellucida prior to the final stage of micromanipulation. Addition of 1 μg/ml CD (instead of cytochalasin B at 7.5–25 μg/ml, as in previously described methods) and 0.25 μg/ml of nocodazole, compounds that reversibly destabilize microtubule and actin networks in the cell, improved the mechanical properties of oocytes, allowing reduction of the diameter of the enucleation pipette to 15 μm. Overall, this approach allowed efficient and simple collection of anucleate GV oocytes that were deprived of only a minimal amount of cytoplasm and very likely retained all the factors that are normally required for proper development.

Maturation of reconstituted GV oocytes

The efficiency of maturation *in vitro* of reconstituted GV oocytes clearly depends on the type of medium used. Earlier reports showed that in one single experiment it differed from 55% (S1 medium), through 78% (M199), to 83% (HTF) (all media with 10% FBS; Liu *et al*., 2000). Surprisingly, in another trial, 92.3% maturation was reported after reconstituted GV oocytes were cultured in M199 (with 3 mg/ml BSA; Takeuchi *et al*., 1999). In these studies, use of KSOM medium, which was especially developed for culturing mouse preimplantation embryos, resulted in a maturation efficiency of oocytes reconstituted with GV nucleus of 42%, while in neither experimental nor control groups was the overall maturation efficiency higher that 60%. It is possible that KSOM is not optimal for supporting meiotic progress *in vitro*. However, other factors are likely to have added to such a low proportion of maturation.

It has been claimed that when isolated GV karyoplasts are cultured *in vitro*, they undergo GVBD but with no extrusion of the first polar body. It was suggested that karyoplast size (in the range of > 30 μm to < 40 μm in diameter) influences the timing of GVBD initiation (the larger the karyoplast, the earlier GVBD begins) (Takeuchi *et al*., 1999). In our studies, 70% of 20 μm diameter GV karyoplasts divided. This discrepancy compared with the earlier reports may be due to the different medium used in our studies.

A lower ratio of meiotic maturation of reconstituted GV oocytes compared with a considerably higher maturation ratio in GV karyoplasts suggests a negative effect of CD and nocodazole on maturation. Furthermore, it indicates that this negative effect is conferred via the oocyte cytoplasm, since GV karyoplasts that have very little cytoplasm mature at almost as high a rate as untreated controls.

It has recently been shown that mouse GV ooplasts reconstituted with MI or MII karyoplasts extrude polar bodies in 68% and 35% of cases, respectively (Cheng *et al*., 2003). When G2 mouse zygotes are fused to maturing GV oocytes, they contribute chromosomes to metaphase I, and a fraction of them (38%) proceed further, joining the asymmetric division of the first polar body extrusion and then remaining arrested at metaphase II (Grabarek & Zernicka-Goetz, 2000). When zygotes are in M phase at the time of fusion with oocytes, a polar body is extruded in 91% of such hybrids, and this occurs 2 h earlier than in the control oocytes. Over a half of hybrids matured this way can be artificially activated. These results show that zygotic nuclei can participate in maturation of a GV ooplast.

Several attempts have been undertaken to use enucleated/cut immature (GV) oocytes as recipients of embryonic or somatic nuclei. A procedure consisting of culturing enucleated GV oocytes (ooplasts) for 13 h (presumably to complete maturation) did not culminate in their activation after introducing a nucleus from an embryonic stem (ES) cell (Gao *et al*., 2002). This is not surprising, however, since as shown by the authors, the donor nucleus did not progress beyond metaphase I (large, barrel-shaped spindle) before the reconstructed oocyte was subjected to activation by treatment with Sr^{2+} ions. This means that maturation was not completed before activation. Anucleate halves of GV oocytes that were immediately provided with ES or somatic (cumulus cell) cell nuclei did not form normal metaphase chromosomes (Fulka *et al*., 2002), which suggests that maturation of GV ooplasts is not supported by these types of cell nuclei.

Blastomere nuclei may be considered a half-way step between pronuclei (zygotic nuclei) and ES cell nuclei; they are early embryonic nuclei, whereas those of ES cells equate more with late embryonic nuclei. Our results show that nuclei from blastomeres of the 2-, 4-, and 8-cell stage can support meiotic maturation of GV ooplasts, resulting in first division. However, the timing of this process is altered. Oocytes reconstructed

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with nuclei of 2- or 4-cell-stage embryos divided considerably earlier than control treated or untreated oocytes. The least accelerated event was the extrusion of the first polar body in hybrids with 8-cell stage nuclei, although this still occurred 2 h before intact GV oocytes divide *in vitro*. Efficiency of division and the rate of asymmetric division are comparable among all three stages. The highest number of cells in metaphase (instead of interphase) was found in hybrids with 8-cell-stage nuclei. Therefore, nuclei from this stage appear to best conform to the cytoplasmic environment of GV ooplasts.

Further studies involving artificial activation followed by treatment with cytochalasin B or *in vitro* fertilization or intracytoplasmic sperm injection should reveal the mechanisms of developmental potency of GV ooplasts reconstructed with blastomere nuclei.

Note added in proof: In a recent paper by Chang *et al*. (2004; *Biol. Reprod.* **70**, 752–8) it has been shown that enucleated GV oocytes reconstituted with somatic nuclei can resume maturation with similar efficiency and temporal acceleration as those described in our work.

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