MOLECULAR REPRODUCTION AND DEVELOPMENT 58:180–185 (2001)

Viable Rabbits Derived From Reconstructed Oocytes by Germinal Vesicle Transfer After Intracytoplasmic Sperm Injection (ICSI)

GUANG-PENG LI, DA-YUAN CHEN,* LI LIAN, QING-YUAN SUN, MIN-KANG WANG, JI-LONG LIU, JIN-SONG LI, AND ZHI-MING HAN

State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China

ABSTRACT Abnormal oocyte spindle due to the improper function of ooplasm is associated with female infertility of advanced maternal age. A possible way to overcome this problem is to transfer an oocyte germinal vesicle (GV) which contains genetic materials of a patient with a history of poor embryo development to the cytoplast from a donor oocyte. Here we demonstrate that GV transfer is feasible using a rabbit model. When the GVs were transferred to auto- or heterocytoplasts of GV stage oocytes, around 80% of the reconstructed oocytes could mature in vitro and 7.1–9.4% of the oocytes developed to blastocyst stage after intracytoplasmic sperm injection (ICSI). Transfer of 93 fertilized eggs reconstructed via GV transfer into six recipients resulted in two live offspring. Results of this experiment indicate that GV transfer can potentially become a new approach in treatment of infertility because of advanced maternal age. *Mol. Reprod. Dev. 58:180–185, 2001.* © 2001 Wiley-Liss, Inc.

Key Words: germinal vesicle; nuclear transfer; intracytoplasmic sperm injection (ICSI); rabbit

INTRODUCTION

The relationship between nuclear and cytoplasmic components during oocyte meiosis, fertilization, and early development has been widely investigated and it has been proposed that cytoplasmic determinants play an important role in these processes (Muggleton-Harris et al., 1982; Pratt et al., 1988; Flood et al., 1990; Battaglia et al., 1996; Levron et al., 1996). In humans, it has been shown that abnormal oocyte spindle morphology is associated with female infertility; spindle abnormalities, such as abnormal chromosome alignment and a microtubule matrix that compromises the meiotic spindle function have been attributed to advanced maternal age (Battaglia et al., 1996). These data indicate that the regulatory mechanisms responsible for the assembly of meiotic spindle are significantly altered in older women, leading to the high prevalence of aneuploidy.

A possible way to overcome this problem is to transfer the cytoplasm from donor oocytes into the eggs of a patient with a history of poor embryo development (Cohen et al., 1998; Van Blerkom et al., 1998). Germinal vesicle (GV) transfer enables nearly complete cytoplasm exchange between oocytes. Researchers have been using a mouse oocyte GV transfer model to test the possibility of this technique in human assisted reproduction. When GVs were transferred into anuclear cytoplasts of GV stage oocytes, both auto- and hetero-GV transferred oocytes could mature normally in vitro to the metaphase II stage (Liu et al., 1999; Takeuchi et al., 1999). However, no further developmental progress was reported. This approach has also been tested in human oocytes (Zhang et al., 1999). Rabbit oocytes are similar to human oocytes in that they are more resistant to micromanipulation than mouse oocytes and their GVs can easily be seen under phase contrast microscope.

The aim of the present study was to establish a model to elucidate the cytoplasmic and nuclear interrelationships underlying the onset and progression of meiosis, and to determine the effect of GV transfer on the development of the reconstructed rabbit oocyte after ICSI.

MATERIALS AND METHODS

Animals and Oocyte Collection

Female Japan Big Eared White rabbits (White rabbit) and indigo blue Chinchilla rabbits (Blue rabbit) were injected intramuscularly with a total of 0.7–0.8 mg of follicle stimulating hormone (FSH) (Institute of Zoology, Academia Sinica) two times daily for 3 days and cumulus enclosed oocytes were collected from the ovaries 84 hr after the first injection. Cumulus cells were removed mechanically after exposure to M2 medium containing 500 IU/ml hyaluronidase (Sigma) for 5 min. The GV stage oocytes were cultured in tissue culture medium 199 (M199, Sigma) supplemented with

Grant sponsor: 973 Project; Grant number: G1999055902; Grant sponsor: Climbing Project; Grant number: 970211019-2; Grant sponsor: National Natural Science Foundation of China; Grant numbers: 39770098, 39970093.

Guang-Peng Li and Da-Yuan Chen contributed equally to this work.

*Correspondence: Da-Yuan Chen, State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, #19 Zhongguancun Rd, Haidian, Beijing 100080, P. R. China.
E-mail: chendy@panda.ioz.ac.cn

Received 31 May 2000; Accepted 21 July 2000

10% fetal calf serum (FCS, Sigma) and 20 μg/ml 3-isobutyl-1-methylxanthine (IBMX, Sigma) for 1.5 hr. This inhibitor of cyclic nucleotide phosphodiesterase prevents spontaneous GVBD. Control GV oocytes were incubated with IBMX but were not micromanipulated prior to in vitro maturation.

Recipient rabbits of Japan Big Eared White breed were synchronized by superovulation via the same procedure described above and then injected intravenously with 100 IU hCG (Institute of Zoology, Academia Sinica) 72 hr after the first FSH injection. Their vaginae were stimulated using sterile glass probe at the time of hCG injection.

Preparation of GV-Karyoplasts

GV stage oocytes were cultured in 50 μl of M2 medium with 10% FCS, 7.5 μg/ml cytochalasin B (CB) and 20 μg/ml IBMX for 30 min at room temperature before GV aspiration. The method of GV removal was as described by Takeuchi et al. (1999) and Liu et al. (1999). Briefly, the zona pellucida was penetrated by pressing a glass microneedle tangentially into the perivitelline space against the holding pipette. The GV, surrounded by a small amount of cytoplasm (GV karyoplast), was repelled through a slit made in the zona as described by Meng et al. (1996) by increasing the pressure inside a holding pipette. The extruded GV-karyoplast remained connected with the oocyte as an indicator of the convenient retransfer of another, different GV karyoplast. The diameter of GV karyoplast was about 30–40 μm.

GV Transfer, Electrofusion, and Maturation

The White rabbit GV (WGV) and Blue rabbit GV (BGV) karyoplasts were transferred back to the same oocyte (W-auto-GV and B-auto-GV), or transferred to heterogeneous enucleated GV cytoplast (hetero-GV-GV), that is, transferred WGV to Blue rabbit GV-removed cytoplasm (WGV-BC) or BGV to White rabbit GV-removed cytoplast (BGV-WC) with a 25–30 μm inner diameter injecting pipette (Fig. 1A). The GV-cytoplast complexes were equilibrated in M199 for 30 min. Later, these complexes were 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) and treated with three electric pulses (180 V/mm DC for 80 μs) delivered by a Kefa Electro Cell manipulator (Academia Sinica). The fusion was examined 30 min later and the fused oocytes were cultured in M199 + 10 IU/ml PMSG (M199 + PMSG) for 18–20 hr. After culture, the oocytes emitting the first polar body (PB1) were regarded as mature, and subjected to intracytoplasmic sperm injection (ICSI) with Blue rabbit sperm.

Cytogenetic Analysis

In a preliminary study, the WGV-BC reconstructed oocytes extruded PB1 were prepared for chromosomal analysis as previously described (Tarkowski, 1966; Li et al., 1998). Briefly, oocytes were treated in a hypotonic solution (0.068 M potassium chloride with 1 mg/ml BSA) for 5 min, and swollen oocytes were transferred onto a clear slip and fixative I (methanol:acetic acid, 3:1, v/v) was added for 5 min. Then fixative II (methanol : acetic acid, 1 : 1, v/v) and fixative III (methanol : acetic acid, 1 : 3, v/v) were added for at least 5 min and dried in air. Fixed oocytes were stained with Giemsa to score chromosomes. Of the 27 treated matured oocytes, 22 had the normal 22 chromosomes (Fig. 1B).

ICSI and Culture of the Matured Oocytes

Rabbit spermatozoa were collected from the deferent ducts of a mature male blue Chinchilla rabbit. Spermatozoa were washed in M2 and then suspended for 1 hr in 1.5 ml of M2. Spermatozoa were centrifuged (300 *g*, 5 min) and resuspended in M2: 10% polyvinylpyrrolidone solution (1:1). The cover of a plastic dish (60 × 60 mm; Falcon Plastics, Oxnard, CA) was used as a microinjection chamber. Two rows of round droplets were placed along the center line of the dish, each row consisting of three 5 μl droplets. The first droplet was the sperm suspension in PVP medium. The other droplets were M2 medium containing 7.5 μg/ml CB for oocyte, each droplet with two oocytes. These droplets were covered with mineral oil (Sigma). The dish was placed on the stage of a Nikon inverted microscope with differential interference contrast and Nikon Narishige micromanipulators. The micromanipulation was as described by Li et al. (1999) (Fig. 1C). Briefly, the injection needle used was of 6–7 μm inner and 8–9 μm outer diameter. The polar body was at 6 or 12 O'Clock and the point of injection at 3 O'Clock. An oocyte was penetrated by the injecting micropipette, a small amount of cytoplasm was drawn into the micropipette, and then the cytoplasm together with the sperm was expelled into the oocyte. Immediately after ooplasmic injection, the injecting micropipette was withdrawn quickly, and the oocytes were released from the holding pipette to reduce the intracytoplasmic pressure exerted on the oocyte. After injection, all of the oocytes were transferred to M199 medium and cultured at 38°C, 5% CO_2 in air and examined every 24 hr.

Embryo Transfer to Foster Recipients

In order to evaluate the ability of oocytes to undergo postimplantation development, oocytes after hetero-GV transfer, maturation, and ICSI were directly transferred into oviducts of the synchronized recipient rabbits.

Statistical Analysis

Data were analyzed using chi-square test. Significant difference was determined at $P < 0.05$.

RESULTS

Maturation of the Auto-GV-GV or Hetero-GV-GV Reconstructed Oocytes

The results are shown in Table 1. Fusion was observed in >80% of the auto-GV and hetero-GV complexes, with no significant difference among them.

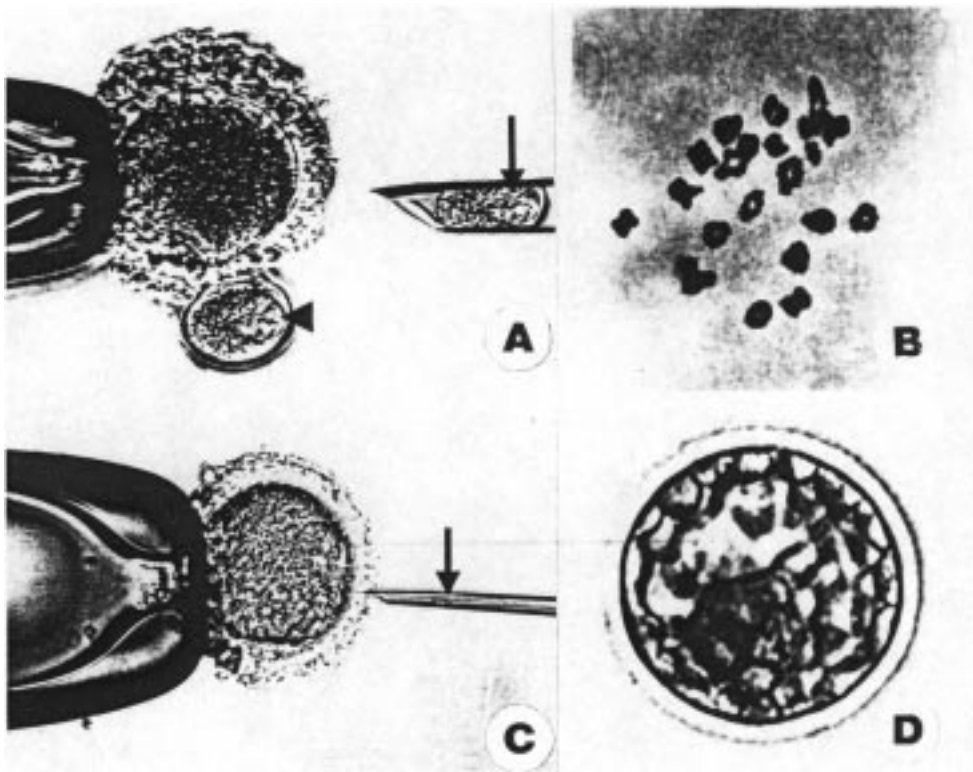

Fig. 1. A. Transfer of a GV-karyoplast (arrow) into a GV-removed oocyte through the same zona pellucida slit indicated by the GV-karyoplast (arrow head); **B**. Chromosomes of matured oocytes (PB1) reconstructed by GV transfer; **C**. ICSI of a reconstructed mature oocyte with a Chinchilla spermatozoon (arrow indicates the sperm); **D**. A blastocyst derived from ICSI of the GV-transferred oocytes.

Following incubation for 24 hr, the maturation rates revealed by PB1 emission were 80.4, 78.2, and 80.9% for W-auto-GV, WGV-BC, and BGV-WC, respectively, which are not significantly different from that of the control group (90.2%). The maturation rate of B-auto-GV reconstructed oocytes (67.6%) was significantly lower than that of control.

Development of the Reconstructed Oocytes After ICSI

As the published reports have only focused on examining the maturation of the reconstructed oocytes after germinal vesicle transfer in mouse (Liu et al., 1999; Takeuchi et al., 1999) and human (Zhang et al., 1999), we tested the developmental ability of the matured reconstituted oocytes after ICSI. After ICSI, high survival rates (68–74.5%) were observed among the four groups of reconstructed oocytes, which are comparable to that of the control group (85.1%). The cleavage rates of the reconstructed oocytes showed no significant differences with the control group. More than 7% of the embryos resulting from auto-GV or hetero-GV reconstructed oocytes developed to blastocyst stage (Fig. 1D), which was not significantly

TABLE 1. Maturation of Rabbit Reconstructed Oocytes After GV Transfer

Types of reconstructed oocytes	Replicates	Number of GV–cytoplast complexes	Number of fused (%)	Number of (%) emitted PB1
W-auto-GV	6	115	92 (80)[a]	74 (80.4)[a]
B-auto-GV	4	43	37 (86.0)[a]	25 (67.6)[b]
WGV-BC	6	114	101 (88.6)[a]	79 (78.2)[a]
BGV-WC	6	79	68 (86.1)[a]	55 (80.9)[a]
Control (W oocytes)	6	82	—	74 (90.2)[a]

Abbreviations: W, white rabbit of Japan Big Ear breed; B, indigo blue rabbit of Chinchilla breed; GV, germinal vesicle; WGV, germinal vesicle from white strain; BC, cytoplast from indigo blue strain; BGV, germinal vesicle from indigo blue strain; WC, cytoplast from white Strain.
[a] vs. [b] $P < 0.05$.

TABLE 2. In Vitro Development of Matured Reconstructed Oocytes After ICSI

Types of oocytes matured to MII	Total oocytes	Number of oocytes survived after ICSI (%)	Cleavage (%)	Blastocyst (%)
W-auto-GV	74	53 (71.2)[a]	31 (58.5)[a]	5 (9.4)[a]
B-auto-GV	25	17 (68)[a]	11 (64.7)[a]	1 (9.1)[a]
WGV-BC	79	56 (70.9)[a]	34 (60.7)[a]	4 (7.1)[a]
BGV-WC	55	41 (74.5)[a]	23 (56.1)[a]	3 (7.3)[a]
Control (W oocytes)	74	63 (85.1)[a]	42 (66.7)[a]	12 (19.0)[a]

Abbreviations are the same as in Table 1.

different from that of the control group (19.0%) (Table 2).

We transferred 93 fertilized oocytes into 6 recipient rabbits, one of which received 17 embryos and produced 2 young puppies (females) on January 16, 2000 (Fig. 2). One is black and white, 92.6 g in weight, 13 cm in length. The other one, 90.6 g in weight and 13.5 cm in length, is almost white but with several black dots on the back which were masked by white two weeks later. The birth weight and length were not significantly different compared with Japan Big Eared White rabbits (88–110 g in weight, 12–14.6 cm in length) and indigo blue Chinchilla rabbits (90–105 g in weight, 11–14 cm in length).

DISCUSSION

Progression from the incompetent to the competent state during oocytes growth does not occur abruptly but includes an intermediate stage during which meiosis progresses only to metaphase I stage (Fulka et al., 1998). When metaphase I – arrested mouse oocytes are fertilized or parthenogenetically activated their chromosomes to decondense, a pronucleus forms and triploid blastocysts develop from fertilized eggs (Eppig et al., 1994). This seems to show that cytoplasmic rather than nuclear differentiation influences meiotic progression in oocytes during the penultimate stages of growth. That the GV plays a role parallel to that of the cytoplasm in conferring full competence on these oocytes has been demonstrated by the introduction of incompetent GV nuclei into cytoplasts prepared from fully grown mouse immature oocytes (Kono et al., 1996). Although the reconstructed oocytes by GV transfer progressed to metaphase II in mouse (Kono et al., 1999; Liu et al., 1999; Takeuchi et al., 1999), they were subsequently totally unable to support either normal sperm decondensation or male pronuclear formation (Kono et al., 1996). When metaphase II plates from reconstructed oocytes were retransferred to cytoplasts prepared by the enucleation of normal

Fig. 2. The foster mother and the two offspring. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

metaphase II oocytes then both decondensation and pronuclear formation occurred in a normal manner, and produced live mouse offspring (Kono et al., 1996). These experiments demonstrated that differentiation events in both the cytoplasmic and nuclear compartments are essential for the acquisition of meiotic competence in mouse oocytes (Fulka et al., 1998). It is, however, somewhat different in rabbit. In the present study, the GV of a rabbit oocyte was found to be able to undergo GVBD and form a structurally normal first meiotic metaphase following its removal and electrofusion into an enucleated cytoplast at the same developmental stage. The maturation rates of auto- or hetero-GV transfer oocytes were 67.6–80.9%. After ICSI, more than 7% of the embryos resulting from auto-GV or hetero-GV reconstructed oocytes were capable of developing to the blastocyst stage, with no significant difference compared to the control group (19.0%). When 93 fertilized oocytes were transferred into six recipient rabbits, two young offspring were produced, which represent the first animals produced directly from GV-transferred reconstructed oocytes.

Chromosome function during oocyte maturation appears to be linked directly to a spindle assembly process which becomes increasingly dysfunctional in older individuals (Battaglia et al., 1996). It has been shown in the CBA mouse that the pole–pole distance in the meiotic spindle becomes shorter than normal during maternal aging (Eichenlaub-Ritter et al., 1988a,b). Spindles in mouse oocytes also appear to suffer from considerable chromosome misalignment as maternal age increases (Eichenlaub-Ritter and Boll, 1989). The possible reason of spindle disorder in oocytes from older individuals may be due to altered regulatory factors that produce abnormal spindle components and/or alter the timing of the phases of meiosis resulting in microtubule irregularities and unusual chromosome placement (Battagalia et al., 1996). In mouse (Mattson and Albertini, 1990; Messinger and Albertini, 1991) and rat oocytes (Albertini, 1992), numerous microtubule organizing centers, the centrosomes, were found adjacent to the germinal vesicle. They appear to be recruited for spindle assembly as the transition from prophase to metaphase begins.

In humans, it has been shown that abnormal oocyte spindle morphology is associated with female infertility, and advanced maternal age has been attributed to spindle abnormalities which are probably caused by improper function of regulating mechanisms for spindle assembly in the ooplasm (Battaglia et al., 1996; Kono et al., 1996; Levron et al., 1996; Angell, 1997). Cytoplasmic factors may be the major reason for these reproduction failures. Cytoplasmic factors initiate and organize the construction of the meiotic spindle during final maturation and after sperm penetration. This problem can be overcome in two possible ways: ooplasm transfer and germinal vesicle transfer. In order to restore normal growth and viability in developmentally comprised embryos judged to be ooplasmic deficiency, Cohen et al. (1997, 1998) transfered ooplasm from donor eggs at MII into synchronous patient eggs by electrofusion of an anuclear donor fragment into each patient egg, or direct injection of a small amount of ooplasm from a donor egg into each patient egg. Normal fertilization was significantly higher after injection of ooplasm (63%) in comparison with fusion (23%). Pronuclear anomalies appeared to be enhanced after fusion with ooplasts. Embryo morphology was not improved with electrofusion and patients did not become pregnant. An improvement in embryo morphology was noted in two patients after injection of ooplasm and both become pregnant, one baby was born. These results showed that ooplasmic transfer at the MII stage may be promising in patients with compromised embryos. Using micromanipulation techniques in monkeys, Flood et al. (1990) removed ooplasm from MII oocytes and injected it into prophase I oocytes. After nuclear maturation, oocytes were transferred to the fallopian tube for fertilization. After ooplasmic transfusion, prophase I oocytes showed in a delivery rate of 13%. In contrast, when mouse GV nuclei were transferred and electrofused into enucleated MII oocytes or zygotes only a few reconstructed oocytes underwent GVBD, none reached the MII stage (Liu et al., 1999; Takeuchi et al., 1999).

The GV transfer method presented here is another possible way to overcome ooplasmic deficiency. Previous and the present studies have shown that the reconstructed oocytes by GV transfer could mature to MII stage and the matured oocytes had normal chromosomal constitution (mouse: Liu et al., 1999; Takeuchi et al., 1999; human: Zhang et al., 1999; rabbit: present paper). Transplantation of germinal vesicle nuclei of women at risk of transmitting mitochondrial disease into normal ooplasts of enucleated recipient cells should provide a unique opportunity, since it may discontinue any transmission of these genetic diseases in affected families (Cohen et al., 1998). Germinal vesicle transfer enables nearly complete cytoplasm exchange between oocytes, and avoids the risk of transmitting potentially harmful factors into the host ooplasm.

After transferring 93 fertilized oocytes into six recipients only two rabbits were produced. The rate of birth was very low. The possible explanation is that this is due to some mechanical trauma resulting from the micromanipulation. Although care was taken to avoid damage to germinal vesicle or the first polar body during GV removal and transfer or ICSI, respectively, some damages were unavoidable. Results of in vitro development (Table 2) showed that only 7.1 and 7.3% of the WGV-BC and BGV-WC reconstructed oocytes developed to blastocyst stage, respectively, after ICSI.

In conclusion, GV transfer oocytes can be fertilized normally and develop to term. This research has provided a model that may be useful in analyzing the relationship between nucleus and cytoplasm in oocytes, and also may have potential application to human assisted reproduction. However, evaluation of ooplasmic anomalies and optimization of techniques

will require further investigation prior to clinical application.

ACKNOWLEDGMENTS

We thank Dr. Flank J. Longo, College of Medicine, University of Iowa, for helpful comments and critical reading of the manuscripts.

REFERENCES

Albertini DF. 1992. Regulation of meiotic maturation in the mammalian oocyte—interplay between exogenous cues and the microtubule cytoskeleton. Bioessays. 14:97–103.

Angell RR, Xian J, Keith J, Ledger W, Baird DT. 1994. First meiotic division abnormalities in human oocytes: mechanism of trisomy formation. Cytogenet Cell Genet 65:194–202.

Battaglia DE, Goodwin P, Klein NA, Soules MR. 1996. Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. Hum Reprod 11:2217–2222.

Cohen J, Scott R, Schimmel T, Levron J, Willadsen S. 1997. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. Lancet 350:186–187.

Cohen J, Scott R, Alikani M, Schimmel T, Munne S, Levron J, Wu L, Brenner C, Warner C, Willadsen S. 1998. Ooplasmic transfer in mature human oocytes. Mol Hum Reprod 4:269–280.

Eichenlaub-Ritter U, Boll I. 1989. Nocodazole sensitivity, age-related aneuploidy, and alterations in the cell cycle during maturation of mouse oocytes. Cytogenet Cell Genet 52:170–176.

Eichenlaub-Ritter U, Chandley AC, Gosden RG. 1988a. The CBA mouse as a model for age-related aneuploidy in man: studies of oocyte maturation, spindle formation and chromosome alignment during meiosis. Chromosoma 96:220–226.

Eichenlaub-Ritter U, Stahl A, Luciani JM. 1988b. The microtubular cytoskeleton and chromosomes of unfertilized human oocytes aged in vitro. Hum Genet 80:259–264.

Flood JD, Chillic CF, Van Uem JF, Iritani A, Hodgen GD. 1990. Ooplasmic transfusion: prophase germinal vesicle oocytes made developmentally competent by microinjection of metaphase II egg cytoplasm. Fertil Steril 53:1049–1054.

Fulka J Jr, First NL, Moor RM. 1998. Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol Hum Reprod 4:41–49.

Kono T, Obata Y, Yoshimzu T, Nakahara T, Carroll J. 1996. Epigenetic modifications during oocyte growth correlates with extended parthenogenetic development in the mouse. Nat Genet 13:91–94.

Levron J, Willadsen S, Bertoli M, Cohen J. 1996. The development of mouse zygotes after fusion with synchronous and asynchronous cytoplasm. Hum Reprod 11:1287–1292.

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 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.

Mattson BA, Albertini DF. 1990. Oogenesis: chromatin and microtubule dynamics during meiotic prophase. Mol Reprod Dev 25:371–383.

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.

Messinger SM, Albertini DF. 1991. Centrosome and microtubule dynamics during meiotic progression in the mouse oocyte. J Cell Sci 100:289–298.

Muggleton-Harris A, Whittingham DG, Wilson L. 1982. Cytoplasmic control of preimplantation development in vitro in the mouse. Nature 299:460–462.

Pratt HPM, Muggleton-Harris AL. 1988. Cycling cytoplasmic factors that promote mitosis in the cultured 2-cell mouse embryo. Development 104:115–120.

Takeuchi T, Ergun B, Huang TH, Rosenwaks Z, Palermo GD. 1999. A reliable technique of nuclear transplantation for immature mammalian oocytes. Hum Reprod 14:1312–1317.

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

Van Blerkom J, Sinclair J, Davis P. 1998. Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. Hum Reprod 13:2857–2868.

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.