The development of mouse zygotes after fusion with synchronous and asynchronous cytoplasm

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Cytoplasts with diameters of 40–45, 50–55 and 70–75 μm, derived from mouse oocytes at the germinal vesicle, metaphase II and zygote stages were incorporated into zygotes by electrofusion. Manipulated (n = 867) and culture-control (n = 1114) embryos were cultured in vitro and transferred to pseudo-pregnant recipients at the blastocyst stage. When synchronous cytoplasts measuring 40–45 and 50–55 μm in diameter were incorporated into 138 and 86 zygotes respectively, only one embryo in each group (not significant) became arrested at the 1-cell stage. A total of 124 (89.9 compared with 91.6% for controls) and 69 embryos (80%, P < 0.001 compared with 91.6% for controls) reached the blastocyst stage respectively. In the first group, 66 out of 106 blastocysts implanted (62.2 compared with 54.9% for controls; not significant), however, only 24 (22.6 compared with 40.2% for controls, P < 0.001) were viable in comparison with controls. There were four groups of zygotes that received metaphase II cytoplasts. In the first group, 200 zygotes were fused with 40–45 μm cytoplasts. The second group of 145 zygotes were fused with cytoplasts of the same size derived from aged oocytes. In the third and fourth groups, 38 and 36 zygotes were fused with 50–55 and 70–75 μm cytoplasts respectively. In the first two groups, none of the embryos arrested at the 1-cell stage, but in the other groups the rates were 15 out of 38 (39.5%) and 36 out of 36 (100%) respectively. These zygotes remained arrested at the pronuclear stage and contained large inflated pronuclei. The blastocyst formation rates were 183 out of 200 (91.5 compared with 91.6% for controls, not significant), 109 out of 145 (75.2% lower than controls, P < 0.05) and 14 out of 38 (39.5% lower than controls, P < 0.0001) respectively. In the first two groups 109 and 25 blastocysts were transferred, of which 76 (69.7%) and 15 (60.0%) implanted. This was higher than control embryos (54.9%, P < 0.01) for the first group and similar to controls for the second group. In the first group, 60 embryos (55%) were viable on day 10 of transfer in comparison with controls (40.2%, P < 0.05) while in the second group, 11 embryos (44.0%, not significant) were viable on day 10 of transfer. Zygotes that received germinal vesicle stage cytoplasts developed poorly and the implantation rate was significantly reduced. The present study confirms the importance of the ooplasmic domain in meiotic maturation and preimplantation development. Our results suggest that implantation may be enhanced by transfer of a small amount of metaphase II cytoplasm to the mouse embryo during the 1-cell stage; however, fusion of intact zygotes with cytoplasts >45 μm appeared largely detrimental. The mechanisms responsible for these changes are yet unknown.

Key words: cytoplast/electrofusion/implantation/meiosis/pre-implantation

Introduction

The status of nuclear and cytoplasmic components during meiosis, fertilization and early development has been investigated in the past decades with emphasis on function and timing of processes (Biggers, 1971; Biggers and Stern, 1973; Piko and Clegg, 1983; Johnson et al., 1984; Bachvarova, 1985). Quantitative and qualitative changes in the pattern of polypeptide synthesis occur during maturation in vivo and in vitro (Van Blerkom and McGaughey, 1978a,b). Each stage is characterized by a different pattern of specific polypeptides. Some polypeptides do not appear in vitro and synthesis of certain polypeptides is prolonged in vitro compared with oocytes matured in vivo. Translational proteins characteristic for the rabbit oocyte disappear gradually up to the 8-cell stage, and other embryonically activated proteins appear from that stage onwards. There are two mitochondrial reorganizations associated with two important stages of nuclear maturation (Van Blerkom and Runner, 1984). During metaphase I there is a translocation of mitochondria to the perinuclear region. Later there is another more dispersed translocation during polar body abstriction. It was found that these features are necessary and probably associated with processes requiring high levels of ATP.

The development of precise molecular tools and ability to sample and manipulate certain cellular structures now allows for in-depth investigation of the relationship between cytoplasm and nucleus. Ongoing research in our laboratory attempts to clarify specific functions of ooplasmic components during early development. In the mouse, the final stage of oocyte growth takes 2–3 weeks. During this period the oocyte diameter of ~12 μm grows to 80 μm at ovulation, resulting in a 300-fold increase in cell volume. The unfertilized mouse egg contains an extensive store of materials ready for use after fertilization.
Among other things, these stores include ~500 pg of RNA, 20–25 ng of protein and 150 pg of glycogen. The egg also contains ~10⁶ mitochondria, 10⁶ ribosomes, pools of tubulin (250 pg), actin (100 pg) and high activities of a variety of enzymes involved in macromolecule biosynthesis and energy metabolism. Compared with the 15–25 nmol found in a typical somatic cell, each egg has ~800 nmol of ATP. It appears that the initial concentration of all of these constituents does not differ significantly from that of somatic cytoplasm, since the oocyte volume is 60–70 times larger than the somatic cell. From the start of oocyte maturation, there is a progressive decrease in the total maternal RNA bulk and declining rate of protein synthesis (Bachvarova and DeLeon, 1980; Clegg and Piko, 1983a; Piko and Clegg, 1983; Bachvarova et al., 1985). During the transition from primary to secondary oocyte, maternal mRNA declines by 20% (430 pg per primary oocyte to 350 pg per secondary oocyte). Degradation of maternal mRNA continues over the next 1.5 days, and by the zygote stage almost half of the maternal mRNA is either degraded or de-adenylated. However, the most dramatic decline in maternal mRNA occurs at the 2-cell stage when the content of polyadenylated RNA (informational RNA) declines to 30% of that present at the zygote stage.

Until the 8-cell stage, the mass of the synthesized proteins is small compared with that contributed by the unfertilized egg. For instance, it is estimated that only 50 pg of tubulin is newly synthesized until this stage (6 pg/blastoocyte), which constitutes ~20% of the initial tubulin pool size (Wassarman, 1983; Schultz, 1986). Therefore it appears that after completion of oocyte growth, the subsequent stages of meiotic maturation, fertilization and, most importantly, the progression of the zygote through the first cleavage cycles, depend exclusively on stored signals without significant productive genomic transcription.

It is likely that development of pre-genomically activated embryos can be affected by cytoplasmic transfusion. The aim of this study was to determine the effect of ooplasmic transfer on pre- and post-implantation development of mouse zygotes. Variable quantities of cytoplasm at the germinal vesicle, metaphase II and zygote stages enclosed in a plasma membrane (cytoplasts) were electrofused to in-vivo fertilized mouse zygotes. Cytoplasmically hybrid embryos were cultured in vitro and assessed for cleavage rates, implantation and post-implantation development following embryo transfer to recipient animals.

**Materials and methods**

**Recovery of oocytes and zygotes**

Ovulation was stimulated in CB6F1 females aged 6–8 weeks by i.p. injection of 10 IU pregnant mare's serum gonadotrophin (PMSG; Sigma, St Louis, MO, USA) and 10 IU human chorionic gonadotrophin (HCG; Sigma) 48 h later. Zygotes were released by opening the ampullary wall of the excised Fallopian tubes of mated females 16–18 h post-HCG injection. Similarly, metaphase II oocytes were recovered 12–17 h post-HCG injection while germinal vesicle stage (GV) oocytes were recovered by puncturing ovarian follicles from naturally cycling mature females. The cumulus cells were removed from the zygotes and metaphase II oocytes by brief exposure to phosphate-buffered saline (D-PBS; Gibco, Grand Island, NY, USA) supplemented with 0.1% hyaluronidase (Sigma, type V from sheep testes) and 0.3% bovine serum albumin (BSA; Sigma, fraction V). Oocytes or zygotes that were assigned for cytoplasmic fusion with recipient zygotes had their zona pellucida removed by exposure to M2 medium (Quinn et al., 1982) supplemented with 0.3% protease (Sigma, type I from bovine pancreas) for 5–10 min at room temperature. In each experiment the recovered zygotes were divided into two groups, a culture-control group (zona-intact zygotes) and a study group (manipulated zygotes). All the zygotes were cultured in modified Whittingham medium (M16) (Whittingham, 1971) supplemented with 0.4% BSA under mineral oil (Squibb, Princeton, NJ, USA) and 5% CO₂ in air at 37°C.

**Micromanipulation**

Micromanipulation procedures were also carried out in drops of M2 medium supplemented with 0.4% BSA under mineral oil, but at room temperature using a Nikon Diaphot inverted microscope (Nikon Co., Tokyo, Japan) fitted with Hofman's contrast modulation optics (Modulation Optics Inc., Greensville, NY, USA).

Microtools were fabricated from Sutter glass tubes (1.0 mm outer diameter and 0.75 mm inner diameter), pulled on a Sachs Flaming micropipette puller (model PC-84; Sutter Instrument Co., Navato, CA, USA). The cytoplast pipettes were opened to a diameter of 50 μm and fire-polished on a microforge (model MF-9; Narishige, Tokyo, Japan). Zona dissection pipettes, which are closed at the tip, were prepared by pulling fine solid glass tubing from pre-pulled pipettes on the microforge.

**Preparation of anuclear cytoplasts from zygotes, metaphase II and germinal vesicle stage oocytes**

After zona removal, donor oocytes or zygotes were exposed to M2 medium containing cytochalasin B (CCB; Sigma) for 30 min at 37°C. Partitioning of metaphase II oocytes required lower CCB concentration than zygotes and GV stage oocytes (7 mg/ml compared with 25 mg/ml respectively). Cytoplasts of various sizes were separated from donor eggs by withdrawing a portion of cytoplasm enclosed in the plasma membrane. Cytoplasts were removed from areas contra-lateral to the polar body, in order to avoid nuclear material. This method was tested with DNA stains in a number of pilot experiments. Cytoplasts of three sizes were prepared, ~40–45, 50–55 and 70–75 μm in diameter. The large GV and metaphase II cytoplasts were prepared by enucleation of the germinal vesicle or the metaphase plate enclosed with the plasma membrane and a small amount of cytoplasm. Immediately after their preparation, the cytoplasts were washed five times in M2 medium and then left in M16 medium for 15–30 min before they were transferred under the zonae of the recipient zygotes. Prior to this step, ~50–75% of the zona circumference of the recipient zygotes were mechanically dissected. The second polar body was removed and a cytoplast was inserted under the zona with the aid of a mouth-controlled suction micropipette. When large metaphase II or GV cytoplasts were transferred, the pronuclei of the recipient zygotes were removed as described above before the donor cytoplast and the pronuclei were placed together inside an empty zona.

**Electrofusion**

A BTX Electro Cell Manipulator 200 (Biotechnologies and Experimental Research Inc., San Diego, CA, USA) was used to deliver the electrical stimulus. The fusion medium consisted of 0.3 M mannitol (d-mannitol; Sigma), 0.1 mM CaCl₂, 0.05 mM MgCl₂ (Sigma) and was supplemented with 0.1% BSA just before use. The zygotes were transferred to the fusion solution for 5 min before they were placed
between the electrodes of the cell fusion chamber. They were then exposed to an alternating current of 8 V, 1000 kHz for 8 s, followed by a single 24 V pulse of direct current lasting 99 ms. The electrofused zygotes were washed five times in M2 medium and were cultured in M16 medium for 3 days thereafter.

**Embryo transfer to foster recipients**

Day-3 pseudopregnant CD-1 females were used for transfer of blastocysts to the uterine horns. Up to five manipulated blastocysts were transferred to one horn, and the same number of control blastocysts were transferred to the other horn of the same recipient. All the transfers were carried out by the same person. The implantation rate was determined 10 days later from animals which had been killed.

**Oocyte fixation**

The fusion products of zygotes with GV and metaphase II cytoplasts arrested at the 1-cell stage were fixed ~24 h after fusion. These embryos were mounted between two glass slides, fixed with aceto-orcein dye (Sigma) and examined under phase microscopy (×1000) (Tarkowski, 1971).

**Statistical analysis**

Cleavage and implantation rates were compared by Fisher’s exact test.

**Results**

**The culture control group**

A total of 1114 control embryos were used for the experiments. Only 12 (1.0%) control embryos arrested at the 1-cell stage, while 1020 (91.6%) became blastocysts (Tables I, II and III). A total of 246 embryos with up to five embryos in each group were transferred concomitantly with the manipulated ones to opposite uterine horns of the same animal. While 135 (54.9%) embryos implanted, 99 (40.2%) were viable on day 10 of embryo transfer. These results are compared with those of the experimental groups below.

**Transfer of synchronous (pronuclear stage) cytoplasm**

Zygote cytoplasts were fused with 224 intact zygotes (Table I). In the first group, cytoplasts 40–45 μm in diameter were fused with 138 zygotes. Of these embryos, 137 passed the 2-cell stage and 124 (89.9%) became blastocysts. This is not significantly different from the untreated controls. A total of 106 embryos were transferred, 66 (62.2%: not significant) implanted, but only 24 (22.6%) were viable on day 10 of embryo transfer, which is significantly lower than the controls (P < 0.001). In a second group, cytoplasts of 50–55 μm diameter were fused with 86 recipient zygotes. Of these embryos, 85 passed the 2-cell block, while 69 (80.0%) became blastocysts; which is lower in comparison with control embryos (P < 0.001).

**Transfer of asynchronous, germinal vesicle stage cytoplasm**

Zygotes (n = 224) were fused with GV stage cytoplasts (Table III). In the first group, cytoplasts of 40–45 μm diameter were fused with 123 zygotes. A total of 22 (17.8%) embryos (more than control group; P < 0.0001) arrested at the 1-cell stage with no visible pronuclear structures on the second day of culture; 101 embryos passed the 2-cell stage, but growth was reduced and only 78 (63.4%; P < 0.0001) became blastocysts. Of the 40 embryos transferred, only 11 (27.5%) implanted. This was significantly lower when in comparison with controls (P < 0.05) and only five embryos (12.5%; P < 0.01) were viable on day 10 of embryo transfer in comparison with controls.

In the second group, cytoplasts of 50–55 μm in diameter were fused with 38 zygotes. Of the manipulated embryos, 15 arrested at the 1-cell stage, showing large pronuclei on the second day of culture. The remaining 23 embryos passed the 2-cell stage, but only 14 (36.8%; P < 0.0001) became blastocysts in comparison with controls.

In the third group, both pronuclei were enucleated from 36 zygotes and fused with metaphase II oocytes from which the metaphase plate had been removed. All the embryos in this group arrested at the 1-cell stage showing large inflated double pronuclei on the second day of culture.

**Transfer of asynchronous, metaphase II cytoplasm**

Zygotes (n = 419) were fused with metaphase II cytoplasts (Table II). In the first group, cytoplasts with a diameter of 40–45 μm were fused with 200 zygotes. In all, 198 manipulated embryos passed the 2-cell stage and 183 (91.5%; not significant) became blastocysts. A total of 109 embryos were transferred, 76 (69.7%) implanted (compared positively with controls; P < 0.01), and 60 (55.0%) were viable on day 10 of embryo transfer which compared positively with controls.

In the second group, 145 zygotes received cytoplasts of the same size derived from metaphase II oocytes that were aged in vivo and had been recovered ~17 h after HCG injection. All the embryos in this group passed the 2-cell stage, but growth was reduced compared to controls, since 109 (75.0%; P < 0.05) developed to the blastocyst stage. Of the 25 embryos transferred, 15 (60.0%; not significant) implanted, and 11 (44.0%; not significant) were viable on day 10 of embryo transfer.

In the third group, cytoplasts of 50–55 μm in diameter were fused with 86 zygotes. Of the manipulated embryos, 14 (36.8%; P < 0.0001) became blastocysts in comparison with controls.

The development of mouse zygotes after cytoplasmic transfer

| Table I. The development of embryos receiving synchronous cytoplasm transferred at the 1-cell stage. Figures in parentheses are percentages |
| Group size | Manipulated embryos | Control embryos |
| 40–45 μm | 50–55 μm |
| Number arrested at 1-cell stage | 138 | 66 (62.2%) |
| Number blastocysts | 124 (89.9%) | 69 (80%) |
| Number embryos transferred | 106 | 77 |
| Number embryos implanted | 66 (62.2%) | 50 (64.9%) |
| Number viable embryos | 24 (22.6%) | 32 (41.5%) |

P < 0.001.
Table II. The developmental outcome of embryos receiving metaphase II stage (asynchronous) cytoplasmic transfer at the 1-cell stage. Figures in parentheses are percentages

<table>
<thead>
<tr>
<th>Manipulated embryos/cytoplasm size</th>
<th>40-45 μm</th>
<th>50-55 μm</th>
<th>70-75 μm</th>
<th>Culture controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h after HCG</td>
<td>200</td>
<td>145</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>17 h after HCG</td>
<td>200</td>
<td>145</td>
<td>38</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group size</th>
<th>No. arrested at 1-cell stage</th>
<th>No. blastocysts</th>
<th>No. embryos transferred</th>
<th>No. embryos implanted</th>
<th>No. viable embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-17 h after HCG</td>
<td>0 (0.0)</td>
<td>183 (91.5)</td>
<td>109 (75.2)</td>
<td>76 (69.7)</td>
<td>60 (55.0)</td>
</tr>
<tr>
<td>17 h after HCG</td>
<td>0 (0.0)</td>
<td>136 (76.8)</td>
<td>5 (3.2)</td>
<td>15 (39.5)</td>
<td>11 (44.0)</td>
</tr>
</tbody>
</table>

HCG = human chorionic gonadotrophin.
*P < 0.0001.
bP < 0.05.
^P < 0.01.

Table III. The developmental outcome of embryos receiving germinal vesicle (GV) stage (asynchronous) cytoplasmic transfer at the 1-cell stage. Figures in parentheses are percentages

<table>
<thead>
<tr>
<th>Manipulated embryos</th>
<th>Cytoplasm size</th>
<th>Culture controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40-45 μm</td>
<td>50-55 μm</td>
</tr>
<tr>
<td>Group size</td>
<td>No. arrested at 1-cell stage</td>
<td>No. blastocysts</td>
</tr>
<tr>
<td>12-17 h after HCG</td>
<td>123</td>
<td>79</td>
</tr>
<tr>
<td>17 h after HCG</td>
<td>27 (17.8) a</td>
<td>38 (41.1) a</td>
</tr>
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</table>

*P < 0.0001.
^P < 0.05.
bP < 0.01.

zygotes and fused with enucleated GV stage oocytes. On the second day of culture all the manipulated embryos arrested at the 1-cell stage. Of the 22 embryos, 10 extruded a polar body of ~20-30 μm in diameter. After fixing and staining the embryos, metaphase chromosomes were observed in the cytoplasm. In the zygotes that extruded a polar body, metaphase chromosomes were present within both the larger and the smaller cells, while in the remainder the chromosomes were arranged in a typical spindle structure.

Discussion
The interval between oocyte maturation and fertilization is of critical importance for successful implantation after clinical in-vitro fertilization. Embryos derived from reinsemination of oocytes that fail to become fertilized initially due to severe male factor infertility, or matured in vitro from the GV stage, demonstrate significantly reduced implantation potential (Flood et al., 1990; Cohen et al., 1992). The cytoplasmic control over nuclear behaviour during the cell cycle has been demonstrated by nuclear transplantation and cytoplasmic transfer in the frog (Masui and Markert, 1971), the starfish (Kishimoto, 1986), in mammals such as the mouse (Balakier and Czolowska, 1977; Muggleton-Harris et al., 1982), bovine and sheep (Willadsen, 1986, 1989). The ability of human ooplasm to support pronuclear decondensation is also dependent on the stage of maturation (Tesarik and Kopečny, 1989). It has been proposed recently that the cytoplasmic determinant may also play an important role for implantation. By cytoplasmic transusion of mature ooplasm in primates, prophase (GV) oocytes became developmentally competent once they completed maturation and were fertilized in vivo (Flood et al., 1990). Two remarkable experiments demonstrating the importance of cytoplasmic factors and organelles during meiosis and cleavage come from the work of Muggleton-Harris and Brown (1988) and Pratt and Muggleton-Harris (1988). In the first study, it was found that mitochondria associate with the mitotic spindle in normally dividing blastomeres; a distribution which was absent when the blastomeres arrested. Resumption of mitosis and normal mitochondrial distribution occurred after injection of G2 cytoplasm from normal blastomeres. The second study involved two cell mouse embryos from random bred strains which are known to arrest at the G2 phase of the 2-cell stage. Injection of non-blocking cytoplasm obviated this phenomenon (Muggleton-Harris et al., 1982). Using the mouse model also, the present study was designed to evaluate the possibility of altering zygote development by cytoplasmic transfer. Cleavage and implantation rates were determined after cytoplasts of variable sizes and maturational stages were incorporated into zygotes by electrofusion.

The results of synchronous cytoplasmic transfer from zygote to zygote show that cleavage and blastulation rates are not
different from those of the culture control group. Although the implantation rates of the control group are similar, the embryo viability after synchronous cytoplasmic fusion was reduced. This difference may be related in part to the procedure but probably also to the added mass of cytoplasm. An artificial increase in zygote volume may affect cytokinesis and chromosomal balance since we observed a significantly lower blastocyst formation and higher reabsorption, when the cytoplasm size was increased from 40–45 to 50–55 μm. Karyotyping of embryos resulting from such procedures may be needed to further clarify this question. The present results confirm those of others, who found that removal of large quantities of mouse cytoplasm and injection into a recipient egg may cause a decrease in viability. Smaller transferred amounts appeared to be more compatible with development (Petzoldt and Muggleton-Harris, 1987).

The consequences of metaphase II stage cytoplasm fusion were related to the amount of transferred cytoplasm. Embryos that received cytoplasts 40–45 μm in diameter, demonstrated normal in-vitro development and significantly higher implantation and embryo viability when compared with the sibling culture control group. There appeared to be an inverted correlation between the amount of transferred cytoplasm and the development of recipient embryos, because as the cytoplast diameter was increased from 50–55 to 70–75 μm, a significant proportion of the embryos arrested at various stages of development. It appears that cell cycle arrest may occur after incorporation of metaphase II cytoplasm in the zygote, but this is entirely dependent on the proportion of fused material (>45 μm). Zygotes that were arrested at the 1-cell stage did not proceed to syngamy and mitosis but rather demonstrated large inflated pronuclei, possibly suggesting DNA synthesis without karyogenesis. The factor responsible for metaphase arrest during meiosis II may be able to induce non-specific cell-cycle arrest in other cells once present in sufficient quantities.

The cytoplasmic factor(s) that may support early development and implantation are unknown; they likely constitute a combination of material, such as membrane lipids, cytoskeletal elements, specific organelles and proteins. It has been proposed by others (Flood et al., 1990) that these factor(s) are heat sensitive. In a small group of embryos which received metaphase II cytoplasm which was aged in vivo, the implantation rate was higher than in the control group, but embryo viability was not significantly different. This may suggest that most of these beneficial factors are present shortly after completion of meiotic maturation and are rapidly exhausted during the early stages of embryogenesis. It is probable, therefore, that these factors are related to the family of cell-cycle regulators.

All the embryos that received GV stage cytoplasm demonstrated an increasing incidence of metaphase arrest which correlated to the relative amount of GV cytoplasm incorporated. All the fusion products of the large GV cytoplasts and the removed pronuclei remained at metaphase and almost half of them extruded a polar body-like cell. Since oocytes at the GV stage are able to spontaneously complete meiotic maturation in vitro (Mukherjee, 1972), it appears that the stored cytoplasmic signals responsible for the typical meiotic cytokinesis are able to transform both pronuclei back to the metaphase stage and separate the major cell and its corresponding polar body as the meiotic maturation is completed. Although we did not perform chromosomal counts in these fusion products, the significance of this observation is that cell-cycle manipulations such as induction of metaphase transformation and haploidization of diploid nuclei are possible using this technique, as was described previously (Willadsen, 1992).

Although most of the embryos receiving 40–45 μm GV cytoplasts developed to the blastocyst stage, only few implanted and even less were viable. This lowered implantation rate among the embryos that escaped metaphase arrest and reached the blastocyst stage may be related to cytostatic factors present in the cytoplasm of the GV-stage oocyte (Meyerhof and Masui, 1979).

In conclusion, the present observations suggest that replenishing the cytoplasm of mouse zygotes with fresh metaphase II cytoplasm may result in improved development for a significant proportion. The mechanism by which metaphase II cytoplasmic fusion increases the preimplantation developmental competence is unknown, but may be related to cell-cycle regulation, mRNA replenishment or ATP increase. We also observed cell-cycle related phenomena that were induced by the introduction of asynchronous cytoplasm to early embryos. These results reinforce previous observations on the important role of the cytoplasmic determinant in oocyte maturation and embryonic development. The relevance of the present observations to clinically assisted human reproduction remains to be determined.

References


Meyenr, P.G. and Masui, Y. (1979) Properties of a cytostatic factor from

influence mitochondrial reorganization and resumption of cleavage during

control of preimplantation development in-vitro in the mouse. *Nature*, 299,
460–461.

Mukherjee, A. (1972) Normal progeny from fertilization in-vitro of mouse
oocytes matured in culture and spermatozoa capacitated in-vitro. *Nature*,
237, 397–398.

that promote mitosis in the cultured two-cell mouse embryo. *Development*,
104, 115–120.

nucleocytoplasmic ratio on protein synthesis and expression of a stage-
specific antigen in early cleaving mouse embryos. *Development*, 99,
481–491.

Piko, L. and Clegg, K.B. (1983) Quantitative changes in total RNA, total
poly(A) and ribosomes in early mouse embryos. *Dev. Biol.*, 89,
362–78.

oocytes to assay the fertilizing capacity of human spermatozoa. *J. Reprod.
Fertil.*, 66, 161–168.

Schultz, R.M. (1986) Molecular aspects of mammalian oocyte growth and
maturation. In Rossant, J. and Pedersen, R.A. (eds), *Experimental
Approaches to Mammalian Embryonic Development*. Cambridge University

(ed.), *Methods in Mammalian Embryology*. W.H.Freeman, San Francisco,


the rabbit ovum. I. During oocyte maturation in vivo and *vitro*. *Dev. Biol*.,
63, 139–150.

the rabbit ovum. II. During the preimplantation development of in vivo and

resumption of arrested meiosis in the mouse oocyte. *Anat. J. Anat.*, 171,
335–355.

Wassarman, P. (1983) Oogenesis: synthetic events in the developing
mammalian egg. In Hartman, J. (ed.), *Mechanism and Control of Animal

(Suppl.), 7–21.

63–65.

956–963.

Willadsen, S.M. (1992) Observations on the behavior of foreign nuclei
introduced into in-vitro matured oocytes. *Symposium on Cloning Mammals
by Nuclear Transfer*. Colorado, USA.

*Received on November 29, 1995; accepted on March 29, 1996*