Activation of in vitro matured mouse oocytes arrested at first or second meiotic metaphase

ZBIGNIEW POLANSKI*

Department of Genetics and Evolution, Jagiellonian University, Kraków, Poland

ABSTRACT Some mammalian oocytes fail to complete maturation in vitro and arrest development at the first metaphase stage. The response of such blocked oocytes to sperm penetration was investigated. Ovarian mouse oocytes from two inbred strains, CBA/Kw and KE, were cultured in vitro for 20 h. Both oocytes arrested at the first metaphase (MI oocytes) and second metaphase (MII oocytes) were then inseminated. The majority of MII and MI oocytes reinitiated meiosis in response to sperm penetration, although those from the CBA strain did with higher frequency. Moreover, a high proportion of unpenetrated oocytes from CBA, but not the KE strain, resumed meiosis (33% for MII and 48% for MI oocytes, respectively). Parthenogenetic activation of MI-arrested oocytes was demonstrated in (CBAxKE)F1 mice; ovarian oocytes matured in vitro and then treated by electric shock were activated with a similar total frequency of 52.4% for MI and 47.8% for MII oocytes. The rate of activation increased equivalently for both MI and MII oocytes as the length of maturation prolonged. This demonstrates that mouse oocytes arrested at MI during their maturation in vitro continue cytoplasmic maturation and become capable of undergoing activation in a way similar to those maturing to MII. Additionally, in MII oocytes cultured for an equal time in vitro the rate of activation increased with the time lapse after first polar body (PB1) extrusion. This indicates that after PB1 extrusion, the oocyte requires some resting time before it may be activated, perhaps to restore the proper balance between elements of the cell cycle controlling the mechanism involved in first meiotic division.

KEY WORDS: mouse oocyte, meiotic maturation, metaphase arrest, activation, in vitro

Introduction

In mammals, when oocytes are released from antral follicles into culture medium and allowed to undergo spontaneous maturation in vitro, some fail to complete maturation. In such oocytes, rather than extruding the first polar body (PB1) and completing maturation to the second meiotic metaphase (MII), the progression of meiosis is arrested at the first (MI) metaphase (Bae and Foote, 1980; Tsuji et al., 1985; Motlik and Fulka, 1986; Polanski, 1986; Bagger et al., 1987; Didion et al., 1990; Meinecke and Meinecke-Tillmann, 1993). The proportion of MI-arrested oocytes may vary depending on different experimental conditions, for example, donor species (Motlik and Fulka, 1986; Didion et al., 1990), follicle or oocyte diameter (Sorensen and Wassarman, 1976; Tsuji et al., 1985; Motlik and Fulka, 1986), phase of the follicular cycle (Tsuji et al., 1985) and the composition of the medium used for oocyte isolation (Bagger et al., 1987) or culture (Bae and Foote, 1980). It has been recently reported that bovine oocytes arrested in MI during their maturation in vitro completed maturation when fertilized (Chian et al., 1992).

The aim of this study was to examine in what way mouse oocytes blocked at MI during maturation in vitro respond to sperm penetration. Two inbred strains of mice, CBA/Kw (henceforth called CBA) and KE, were used. These strains differ greatly in some oocyte characters as, for example, efficiency of fertilization in vivo (Krzanowska, 1970) and in vitro (Kaleta, 1977), or the number of cortical granules (Wabik-Sliz, 1979). Oocytes of these strains differ also in the speed of meiotic maturation measured both as the time of PB1 extrusion (Polanski, 1986) and as the time of acquisition of the capacity to transform sperm heads into pronuclei (Polanski, 1990). The use of such different types of mouse oocytes allowed us to study possible interstrain differences in oocyte behavior.

Results

During this study ovarian oocytes from CBA and KE mice were cultured in vitro to complete maturation. In both strains a...
The majority of penetrated oocytes, both MI and MII, reinitiated meiosis (Table 2). Such oocytes were found in anaphase or, more often, in telophase stage (first or second, depending on the type of inseminated oocyte; Fig. 1A, B). The proportion of penetrated oocytes resuming meiosis was higher in CBA strain, where 90.9% and 83.6% of inseminated oocytes left MI or MII arrest, respectively. These values in KE strain were 65% and 67.6%, respectively. Control non-inseminated oocytes remained (with one exception in KE strain) undisturbed in MI or MII stage (Fig. 1E, F). Moreover, in the CBA strain a relatively high proportion of both MI and MII inseminated oocytes reinitiated meiosis in the absence of sperm penetration (Fig. 1C, D), which was not observed in non-inseminated control oocytes from this strain (Table 2). Similar activating ability of sperm suspension without sperm penetration was previously described by Kaufman (1973).

Table 2 shows that in all groups oocytes from the CBA strain resumed meiosis with a higher frequency than oocytes from the KE strain. When the data for MI and MII oocytes were pooled (oocytes in both metaphases behave similarly) the strain difference was clearly significant both for penetrated as well as unpenetrated oocytes (Table 2).

The ability of MI-arrested oocytes to undergo parthenogenetic activation was demonstrated by applying a commonly used activating agent. Table 3 shows the efficiency of electric field mediated activation of oocytes from F1 mice (CBAXKE) matured in vitro for 14, 16 or 18 h. Two types of activation were observed: full activation (oocytes which developed pronuclei) and abortive activation characterized by the return of the oocyte to a metaphase state after polar body extrusion (Kubiak, 1989). A few of the control untreated MI oocytes extruded PB1 and progressed to the MII stage as a result of delayed maturation. This probably occurred also in the electric field-treated group of MI oocytes and should be taken into account. For this reason, the number of abortively activated MI oocytes in treated groups was corrected by subtracting the putative number of maturing oocytes (calculated from appropriate control groups) from the total number of oocytes found in metaphase and with extruded PB1. Thus, in Table 3 in the column referring to abortive activation of MI oocytes two values are given for each treated group, the first (no parentheses) being the corrected one. The last col-

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>oocyte status at insemination</th>
<th>No. of oocytes</th>
<th>No. (%) of penetrated oocytes</th>
<th>Mean no. of spermatozoa in penetrated oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBA</td>
<td>MI</td>
<td>32</td>
<td>11 (34.4)^a</td>
<td>2.40±0.56*</td>
</tr>
<tr>
<td></td>
<td>MII</td>
<td>79</td>
<td>61 (77.2)^a</td>
<td>1.56±0.10*</td>
</tr>
<tr>
<td>KE</td>
<td>MI</td>
<td>39</td>
<td>20 (51.3)^b</td>
<td>2.00±0.23</td>
</tr>
<tr>
<td></td>
<td>MII</td>
<td>80</td>
<td>71 (88.8)^b</td>
<td>1.90±0.16</td>
</tr>
</tbody>
</table>

Data pooled from 12 independent experiments; 3 involving only MI and 9 involving both MI and MII oocytes. Values with identical superscript differ significantly: ^a,b,P<0.0001 (\(\chi^2\) test of independence); "P<0.02 (ANOVA).
### Table 2

**Behavior of In Vitro Matured Oocytes from Inbred Mouse Strains CBA and KE after Insemination**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Oocyte Status at Insemination</th>
<th>Sperm Penetration Status</th>
<th>No. of Oocytes (MI,MII)</th>
<th>No. (%) of Oocytes that Resumed Meiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA</td>
<td>MI Penetrated</td>
<td>11</td>
<td>10 (90.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MI Unpenetrated</td>
<td>21</td>
<td>10 (47.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KE MI Penetrated</td>
<td>61</td>
<td>51 (83.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KE MI Unpenetrated</td>
<td>18</td>
<td>6 (33.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control MI</td>
<td>24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control KE</td>
<td>24</td>
<td>1 (4.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data pooled from 12 independent experiments: 3 involving only MI and 9 involving both MI and MIL oocytes. Values with identical superscripts differ significantly (χ² test of independence). Differences between control and inseminated oocytes within experimental groups: a,c,e,p<0.0001; b<0.002; c<0.01. Differences between strains: P<0.02; **P<0.01.

### Table 3

**Activation of Oocytes from F1, Mice After Different Periods of Maturation in Vitro**

<table>
<thead>
<tr>
<th>Oocyte Status</th>
<th>Duration of Culture (h)</th>
<th>Treatment</th>
<th>No. of Oocytes</th>
<th>No. of Activated Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>14</td>
<td>-</td>
<td>14</td>
<td>0**</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>+</td>
<td>12</td>
<td>0 (3)**</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>-</td>
<td>11</td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>+</td>
<td>17</td>
<td>0 (2)**</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>-</td>
<td>11</td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>+</td>
<td>20</td>
<td>2 (2)**</td>
</tr>
<tr>
<td>MII</td>
<td>14</td>
<td>+</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>+</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>+</td>
<td>46</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>-</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>MI Total 14-18</td>
<td>+</td>
<td>49</td>
<td>2 (7)**</td>
<td>20 (62.4)*</td>
</tr>
<tr>
<td>MII Total 14-18</td>
<td>+</td>
<td>113</td>
<td>29</td>
<td>25 (47.8)*</td>
</tr>
</tbody>
</table>

Data pooled from three independent experiments (4 females/exp.). *not activated but delayed extrusion of the first polar body during the course of maturation. **Numbers in parentheses refer to the oocytes recognized as not activated but delayed in the maturation (see text in Results). *difference not statistically significant; P>0.7 (χ² test of independence).
meiosis by sperm penetration. Moreover, these data show that such oocytes are also able to undergo parthenogenetic activation and progress to the first mitotic metaphase (40 mitotic chromosomes, see Fig. 2D), similar to MI oocytes. In fact, first and second meiotic metaphases are very similar in their biochemical character both are maintained by a high level of activity of maturation promoting factor (MPF), a universal regulator of cell cycle control (Murray, 1992). The extrusion of both the first and the second polar body is accompanied by sudden disappearance of this activity (Choi et al., 1991; Kubiak et al., 1992) resulting from the rapid destruction of cyclin B, the regulatory subunit of MPF (Murray et al., 1989; Weber et al., 1991; Hampl and Eppig, 1995). However, triggering the pathway leading to cyclin degradation at second meiotic division depends on external factors (sperm penetration or parthenogenetic stimulus), which activate the egg, whilst at first meiotic division it is switched on by the maturing oocyte itself. It would seem therefore, that in the absence of an intrinsic factor triggering first anaphase, the agents known to activate the mature egg might substitute for it. However, in normal course of maturation (with the exception of some limited ability described in the CBA strain; Polanski, 1990), MI mouse oocytes are not capable of becoming activated (Iwamatsu and Chang, 1972; Clarke and Masui, 1986; McConnell et al., 1995). This capacity appears in MI oocytes as their postovulatory age increase (Kaufman, 1973; Kubiak, 1989). Indeed, the MI-arrested F1 oocytes in this study only acquired their ability to undergo activation later, and developed it gradually, in parallel to MI oocytes. This suggests that in spite of arrested nuclear maturation in MI oocytes, their cytoplasm matured reaching to a state at which they may respond to appropriate external activating stimuli. Moreover, the difference between CBA and KE strains in the susceptibility of MI oocytes to respond to spermatozoa reflects the difference between the MI oocytes from these strains. In addition, the total frequency of activation of oocytes from F1 mice does not differ significantly between MI and MI oocytes. This suggests a considerable similarity between the functional state of the cytoplasm from MI-arrested and MI oocytes.

Recently, whilst the results presented in this study were being prepared for publication, similar data were published by Eppig et al. (1994). These authors showed that MI-arrested oocytes upon insemination extruded PB1, formed pronuclei, cleaved and developed to the blastocyst stage. MI-arrested oocytes treated with calcium ionophore also underwent parthenogenetic activation and the pattern of protein synthesis in the MI-arrested oocytes was similar to that in MI oocytes. These authors concluded that cytoplasmic maturation, or at least some of its critical aspects, can occur in mouse oocytes whose nuclear maturation is arrested at MI (Eppig et al., 1994). The uncoupling of cytoplasmic and nuclear maturation in mouse oocytes was also demonstrated more recently by McConnell et al. (1995). This study, using mice of a different genetic origin, confirms the possibility of parthenogenetic activation of MI-arrested oocytes and gives additional evidence for the similarity between the cytoplasm of MI-arrested and MI oocytes. Thus, the ability to undergo activation in first meiotic metaphase is not exclusive to LT/Sv oocytes bearing a unique mutation (West et al., 1993) but, in certain conditions (after incomplete maturation in vitro), it may be a common feature of mouse oocytes regardless of their genetic composition.

As documented in Table 4, in MI oocytes cultured for an equivalent time, the activation ability increased with the time elapsed since PB1 extrusion. This shows that, independently of the cytoplasm maturity, the oocyte needs some resting time after PB1 extrusion before it can be activated. Kubiak et al. (1992) reported that formation of the second metaphase spindle starts 60-75 min after PB1 extrusion and the high level of MPF is re-established even later, about 2.5 h after PB1 extrusion. Thus, such a resting time seems to be necessary to restore a correct balance between factors of the cell cycle control machinery, following their involvement in the first meiotic division. This would also explain why among MI oocytes, in contrast to MI oocytes, many activated abortively.

In mammals vast numbers of small primary oocytes are present in the ovary from before birth, however only a small portion of them are used by the female throughout her reproductive life. Thus, primary oocytes are a potentially large source of material for experimental or zootechnical purposes. There is an increasing number of reports of the successful maturation in vitro of primary or preantral oocytes after their growth in culture (Eppig and Schroeder, 1989; Hirao et al., 1990; Carroll et al., 1991). Under these conditions, however, the number of oocytes attaining an MI status may be reduced. For example, of 40% of oocytes undergoing GVBD, half blocked subsequently in MI (Carroll et al., 1991). However, this study shows that such oocytes may be activated either fully, entering first meiotic interphase, or abortively, stopping at the second meiotic metaphase. It would be tempting to speculate the development of a system for abortive activation of such oocytes, which might improve efficiency of maturation in cases where it is reduced due to MI arrest.

### Materials and Methods

**In vitro oocyte maturation**

Adult females from the inbred mouse strains KE and CBA or from their F1 crosses (in both directions) were killed by cervical dislocation regardless of the stage of the estrus cycle. The ovaries were dissected...
and placed in M2 culture medium (Fulton and Whittingham, 1978). Oocytes were released into the medium by puncturing antral ovarian follicles. The cumulus cells were removed mechanically and oocytes at the germinal vesicle (GV) stage were collected. Zona pellucida was removed from oocytes to be inseminated by brief exposure to a 0.05% solution of a-chymotrypsin (Sigma Chemical Co.). This was necessary because oocytes matured in medium without serum become impermeable to spermatozoa due to hardening of the zona pellucida (Downs et al., 1986). Finally, after rinsing 3 times in M2, the oocytes were cultured in this medium at 37°C for 20 h (for insemination) or 14, 16 and 18 h (for parthenogenetic activation). At the end of the assigned culture period, oocytes were analyzed for the progression of meiotic maturation. Oocytes still at the GV stage were discarded. Oocytes in MI and MI, as indicated by the absence or presence of PB1, were inseminated or parthenogenetically activated.

**Fertilization in vitro**

Spermatozoa were collected from the cauda epididymis and vas deferens of adult CBA males into a 0.4 ml drop of fertilization medium (Toyoda et al., 1971) and incubated for 70-90 min at 37°C in an atmosphere of 5% CO2 in air to allow for capacitation. Oocytes were rinsed and placed in drops of fertilization medium to which aliquots of preincubated sperm suspension were added. The final concentration of spermatozoa in different experiments ranged between 1x10⁴ and 2.5x10⁵. After incubation with spermatozoa for 1 h, the oocytes were washed and incubated for a further 3 h in fertilization or M16 medium (Whittingham, 1971) before fixation. Control (non-inseminated) oocytes were processed in the same way as experimental ones except that no spermatozoa were added to fertilization drops.

**Parthenogenetic activation**

Oocytes were activated by electric shock in an electrofusion chamber. The chamber was filled with common electrofusion solution of 0.25 M glucose in water, supplemented with a small amount of M2 (100 μl M2/3 ml glucose solution). Oocytes were placed between two parallel platinum wire electrodes (1 mm apart) and treated with an electric current (DC, 100V, duration 25 μs, two pulses 100 ms apart). They were then washed 3 times and cultured in M2 for 5-6 h before fixation.

**Oocyte fixation**

Oocytes were processed for air dried preparations and stained with toluidine blue according to Krzanowska and Lorenc (1983), which permits penetration of ovvi to be easily demonstrated. Only sperm heads which entered the vitellus became stained, whether the chromatin starts to decondense or remains in a condensed state (Krzanowska and Lorenc, 1983). Chromosome preparations were made according to the method of Tarkowski (1966).

**Statistical analysis**

The data were analyzed using the $\chi^2$ test of independence adjusted for low numbers (Snedecor, 1955), or one-way analysis of variance (ANOVA).

**Acknowledgments**

I thank Dorota Buda-Lewandowska for photographic work, Drs. Jacek Kubiak and Bernard Marot for helpful comments and Drs. Halina Krzanowska and Nicola Winston for critical reading of the manuscript and helpful advice. This work was supported by the Jagiellonian University grant DS/IZ'19/93.

**References**


Accepted for publication: September 1995