Aberrant spindle structures responsible for recurrent human metaphase I oocyte arrest with attempts to induce meiosis artificially

B. Heindryckx1,*, S. Lierman1, C.M. Combelles2, C.A. Cuvelier3, J. Gerris1, and P. De Sutter1

1Department for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium 2Biology Department, Middlebury College, Middlebury, VT, USA 3Department of Pathology, Ghent University Hospital, Ghent, Belgium

*Correspondence address. Tel: +32-9-332-4748; Fax: +32-9-332-4972; E-mail: bjorn.heindryckx@ugent.be

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Background: In some couples, not all retrieved oocytes mature, even after prolonged in vitro culture. The underlying mechanisms are not known, although ionophore treatment may alleviate metaphase I (MI) arrest in some mouse strains. We attempted to induce first polar body (PB) extrusion and fertilization using assisted oocyte activation (AOA) after ICSI in maturation-resistant human MI oocytes.

Methods: Four ICSI patients are described in this retrospective study. A pilot study tested the calcium ionophore ionomycin (10 μM) on donated MI oocytes from patients with a normal number of metaphase II (MII) oocytes. Subsequently, ionomycin was used to induce first PB extrusion in two patients showing maturation-resistant MI oocytes. AOA, by calcium injection and ionomycin exposure, was applied when mature oocytes were available. Oocytes were analysed by polarized microscopy and immunostaining.

Results: Ionomycin induced the first PB extrusion in MI oocytes from patients with a normal number of retrieved MII oocytes, while extended in vitro culture failed to achieve the MII stage. Similarly, ionomycin induced first PB extrusion in one of two patients with recurrent maturation-resistant MI oocytes. Use of ICSI combined with AOA on MII oocytes matured in vitro or in vivo resulted in failed or abnormal fertilization with no further embryo cleavage potential. Highly abnormal spindle and chromosome configurations were observed in MI maturation-resistant oocytes, in contrast to control MI oocytes.

Conclusions: Ionophore induced first PB extrusion in MI oocytes from patients without maturation arrest but to a lower extent in maturation-resistant MI oocytes. Immunofluorescence staining and confocal analysis revealed, for the first time, highly abnormal spindle/chromosomal structures that may be responsible for this maturation arrest.

Key words: ionophore / oocyte maturation arrest / spindle analysis

Introduction

In assisted reproduction cycles, 15–20% of the collected oocytes are immature, either at the germinal vesicle (GV) or the metaphase I (MI) stage (Cha and Chian, 1998; Reichman et al., 2010); most of these are meiotically competent and may resume meiosis when cultured in appropriate conditions (Combelles et al., 2002; Nogueira et al., 2006; Heindryckx et al., 2007). Still, in some couples, it may recur that all collected oocytes are immature, either at the GV or at the MI stage, and remain resistant to further maturation even after prolonged in vitro culture (Beall et al., 2010). It was suggested that 0.1–1% of women may exhibit defective oocyte maturation, either qualitative or absolute (Beall et al., 2010). The underlying mechanisms and possible treatments of these rare human oocyte arrests remain to be addressed.

The complex process of meiotic oocyte maturation is under control of many important cell-cycle regulators and various molecular signal transduction pathways that converge on the activation of the maturation-promoting factor (MPF), the key regulator that catalyses MI to metaphase II (MII) transition (Eichenlaub-Ritter and Peschke, 2002; Schmitt and Nebreda, 2002). Using animal models, induced alterations via knockouts or inhibitors of these key regulatory proteins of meiotic progression have shown different types of meiotic arrest (Beall et al., 2010). Still it remains unclear how these artificial meiotic arrest phenotypes can be correlated to the manifestation of human clinical oocyte arrest. Abnormal spindle formation has been
associated with meiotic arrest at the MI stage in previous case reports (Windt et al., 2001; Combelles et al., 2003) but on a limited number of aged oocytes. Immunofluorescence combined with confocal microscopy allows for detailed assessment of the spindle but necessitates oocyte fixation. In contrast, non-invasive assessment of the oocyte spindle through polarized microscopy has allowed the visualization of the meiotic spindle in human oocytes on the basis of its birefringence in a non-destructive way (Keefe et al., 2003; Montag and van der Ven, 2008).

Calcium (Ca\(^{2+}\)) rises also play a role in meiotic progression (Homa et al., 1993; Tosti, 2006; Ajduk et al., 2008). In the absence of intracellular calcium rises, spontaneous meiosis resumption does not occur in vitro (Carroll and Swann, 1992; Jones et al., 1995). Cell-cycle control checkpoints are in turn modulated by transient increases of intracellular calcium. Similar to human oocytes (Goud et al., 1999, 2002), spontaneous calcium oscillations in maturing mouse oocytes exhibit a specific inositol triphosphate IP3-dependent pattern (Deng et al., 1998).

Lee et al. (2004) have reported the absence of calcium-releasing channels in both GV-arrested and GV breakdown-arrested mouse oocytes, pointing to a defect in expression or translation of calcium-releasing channels. Importantly, different parthenogenetic stimuli were able to complete the first meiotic division in MI-arrested LT/Sv mouse oocytes, indicating that polar body (PB) extrusion can be triggered experimentally (Archacka et al., 2008; Hupalowska et al., 2008).

Another type of oocyte arrest involves patients whose oocytes show the MII phenotype but fail to fertilize after ICSI (Levran et al., 2002). When caused by an oocyte activation deficiency, assisted oocyte activation (AOA) has been shown to efficiently restore fertilization and pregnancy rates in the majority of cases of failed fertilization after ICSI (Heindryckx et al., 2005, 2008). In the cases of complete oocyte maturation arrest, some immature oocytes that did progress to the MI stage after extended in vitro culture mostly showed fertilization failure after routine ICSI (Combelles et al., 2003). Given the importance of calcium release during oocyte activation (Ducibella et al., 2002), it remains to be demonstrated whether AOA could also overcome fertilization failure in the patients with maturation-resistant oocytes when immature oocytes do progress to the MII stage.

Our study targeted detailed assessment of chromatin and microtubule organization in four patients whose oocytes showed maturation arrest by both non-invasive (polarized microscopy) and invasive (immunoﬂuorescence) analysis. Additionally, we investigated whether calcium ionophore treatment was able to artificially induce PB extrusion in these oocytes and if AOA could establish normal fertilization.

### Materials and Methods

The analysis of oocytes that failed to resume meiotic maturation and the application of AOA was approved by the Ethical Committee of the University Hospital after obtaining written informed consent from the patients. Patient characteristics and the number of previous treatments in another department are presented in Table I. All reagents were from Sigma (Sigma-Aldrich, Bornem, Belgium) unless stated otherwise.

#### Stimulation protocol and oocyte retrieval

Stimulation was performed with the short GnRH agonist protocol for 7 days (Decapeptyl®, Ipsen, Paris, France) and hMG (Menopur®, Ferring, Saint-Prex, Switzerland) or recombinant FSH (Puregon®, Organon, Oss, The Netherlands or Gonal-F®, Merck-Serono, Geneva, Switzerland) until hCG administration (Pregnyl®, Organon, Oss, The Netherlands) when at least half of the follicles were 18–20 mm in diameter. Cumulus–oocyte complexes were collected by ultrasound-guided transvaginal aspiration 36-h post-hCG, except for the third ICSI cycle in patient C, with oocyte puncture at 38-h post-hCG in an attempt to increase oocyte maturity. About 2–3 h after retrieval, oocytes were denuded using hyaluronidase with mechanical pipetting and assessed for nuclear maturation: (i) GV stage with an intact GV; (ii) MI stage showing the absence of a GV or first PB and (iii) MII stage with the presence of the first PB. Oocytes were maintained in Cook Fertilization Medium (Cook Ireland Ltd, Ireland) at 37°C in 6% CO\(_2\) and air atmosphere until further processed. As described in detail in the ‘Results’ section, some immature oocytes were cultured in in vitro maturation (IVM) medium, a supplemented tissue culture medium (TCM 199) that normally results in efficient maturation (Heindryckx et al., 2007).

#### Induced meiotic progression and AOA

As a pilot study, we collected 36 MI-donated oocytes from ICSI patients with a normal number of mature oocytes retrieved (>80% MII stage).

### Table I Characteristics of patients with maturation resistant MI oocytes.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Primary infertility duration (years)</th>
<th>Genetic analysis</th>
<th>Sperm characteristics</th>
<th>Previous treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28</td>
<td>5</td>
<td>♂ normal</td>
<td>Mild OAT</td>
<td>Eight cycles: MI-arrested oocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>♀: 8% mosaic Turner syndrome*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>23</td>
<td>2</td>
<td>♂ normal</td>
<td>Mild OAT</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>♀ normal</td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>26</td>
<td>2</td>
<td>♂ normal</td>
<td>T</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>♀ normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>3</td>
<td>♂ normal</td>
<td>T</td>
<td>Three cycles: high rate of immature oocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>♀ normal</td>
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</tr>
</tbody>
</table>

Sperm characteristics: OAT, oligo astheno terato zoospermia; T, teratozoospermia; MI, metaphase I.

*Turner syndrome (45, X0/46, XX) but not confirmed in another centre.
This selected subset of MI oocytes failed to mature spontaneously after 30 h of IVM in Cook Cleavage medium (Fig. 1). This time interval of 30 h was chosen to verify the effect of oocyte ageing on spindle staining outcome, since MI maturation-resistant oocytes from our case series could mostly be fixed after extended in vitro culture of 24 h. Furthermore, we have observed that donated MI oocytes that did not mature after 30 h of in vitro culture will not progress further to the MII stage even after extended in vitro culture (see Results), so they can serve as a model for MI maturation-resistant oocytes for our case series. Thirteen of these oocytes were exposed to 10 mM ionomycin for 10 min to examine meiotic progression 16-h post-ionomycin treatment, while 10 MI oocytes were fixed after 30 h for immunostaining analysis. For comparison, freshly collected MI oocytes were fixed for immunostaining \((n = 10)\) immediately after assessment of nuclear maturity and polarized microscopy.

In patients A and C, MI oocytes were treated with the calcium ionophore ionomycin (10 μM, 10 min) to induce PB extrusion. Oocytes were assessed for nuclear maturation 6- (day of retrieval) to 24-h post-activation, based on the first PB extrusion. AOA was applied to oocytes that were at the MII stage on the day of retrieval, or after induced or spontaneous meiotic maturation. For this, a mechanically immobilized sperm was injected in combination with 0.1 M CaCl₂ (Heindryckx et al., 2005, 2008). Thirty minutes after ICSI, oocytes were exposed twice to ionomycin (10 μM) for 10 min, with a 30-min time interval. Oocytes were checked for normal fertilization 16–20 h after ICSI.

### Oocyte analysis

Polarized microscopy imaging (Oosight Imaging System, CRi Inc., Woburn, MA, USA) was applied to oocytes from the pilot study and patients B–D. Several rotation attempts were carried out with a partial zona dissection needle, and pictures were saved at \( \times 400 \) final magnification. Some oocytes were simultaneously fixed and extracted in a microtubule-stabilizing buffer, as described elsewhere (Mattson and Albertini, 1990). Oocytes were incubated in the presence of a 1:1 mixture of mouse monoclonal anti-α, β-tubulin (Molecular Probes, Eugene, OR, USA), followed by Alexa Fluor-conjugated goat-anti-mouse immunoglobulin while chromatin was stained with Ethidium Homodimer-2 (Molecular Probes). Labelled oocytes were mounted on microscope slides and observed using a laser scanning confocal microscope (Biorad Radiance 2000, Tokyo, Japan) with a 60 × oil immersion objective. A three-dimensional image of the microtubular structure and chromosomes was rendered from Z-axis stacks (0.5–0.75 μm steps) by using ImageJ software.

### Results

#### Pilot study

The pilot study showed that of the 13 MI oocytes that were exposed to ionomycin 30 h after IVM, 4 showed extrusion of the first PB without pronucleus (PN) formation while the rest showed PN formation.

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**Figure 1** Experimental overview of the pilot study: MI oocytes were donated by ICSI patients with a normal number of mature MII oocytes retrieved. Abbreviations: IVM, in vitro maturation; CC, Cook Cleavage medium; PN, pronucleus/pronuclei; PB, polar body; BP, bipolar spindle formation; AL, normal chromosome alignment on equatorial plate; NAL, non-alignment: not all chromosomes properly aligned on equatorial plate.
formation or cleavage at 16-h post-activation (Fig. 1). The rest of the MI oocytes not exposed to ionomycin remained arrested after a total of 54 h of IVM. Confocal analysis of MI oocytes, freshly collected or cultured in vitro for 30 h, showed normal bipolar spindle formation with aligned (Fig. 2A) or non-aligned chromosomes; polarized microscopy detected normal MI spindles located at the oocyte periphery (Fig. 2B).

Case series
Detailed information of the ICSI cycle attempts, interventions and outcomes are presented in Table II.

Patient A
During the second ICSI attempt, ionomycin exposure of MI oocytes resulted in three MII-stage oocytes 6-h post-activation. Immediately thereafter, fertilization was attempted using ICSI in combination with AOA, which resulted in two unfertilized oocytes and one zygote showing five PN without further cleavage potential. Oocytes that did not show maturation 24 h after retrieval were fixed for immuno-analysis from both the ionomycin-treated group (n = 12) and without ionomycin (n = 5). In the former group, five categories of spindles could be distinguished: (A) spindle positioned in the centre of the oocyte, chromosomes aligned and no bipolar formation (n = 1); (B) cortical location, dispersed chromosomes, weak microtubules and absence of defined poles (n = 3) (Fig. 3); (C) similar to category B but almost absent microtubules (n = 3); (D) similar to category C but central positioning of chromosomes (n = 3); and (E) PN formation (n = 2). The group of MI maturation-resistant oocytes that were not exposed to ionomycin all showed category B spindle and chromosomal structures (Fig. 3). Owing to the inability to obtain normal fertilization and the aberrant spindle structures, the couple was advised to undergo a donor oocyte cycle. This third ICSI cycle resulted in normal fertilization and embryo development, and a successful twin pregnancy was established.

Patient B
In the second ICSI attempt, three MI oocytes were used immediately after cumulus stripping for polarized microscopy followed by fixation and confocal analysis to avoid oocyte ageing in vitro. An abnormal spindle located more towards the centre of the oocyte with very low birefringence was observed by polarized microscopy in two oocytes (Fig. 4A), while no spindle was detected in the third oocyte. In the two oocytes with a detectable spindle after polarization microscopy, fixation and confocal analysis revealed a dispersed and very aberrant spindle structure without pole formation, although abundant polymerized microtubules were present and arranged in an astral configuration (Fig. 4B). In the one oocyte with no detectable spindle, the chromosomes were lying in the centre of a microtubule-free region from which microtubules were arranged radially (Fig. 4C).

Patient C
During the second ICSI attempt, 13 MI oocytes were collected all with a visible spindle located at the periphery after polarized microscopy (similar to Fig. 2B). Ionomycin treatment of seven oocytes did not result in PB extrusion. In the third ICSI attempt, ICSI with AOA on two oocytes with a presumptive first PB but without detectable spindles after polarized microscopy resulted in failed fertilization. No confocal analysis was carried out.

Patient D
During the first ICSI attempt, ICSI in combination with AOA on five IVM MII stage oocytes resulted in four oocytes showing one PN containing no second PB or a fragmented PB and one oocyte with two PN formation (minus second PB) without further cleavage potential. Two MI oocytes that did not mature after 8 h were fixed immediately on the day of retrieval. One was lost during the staining procedure while the other oocyte showed an abnormal spindle configuration with few associated microtubules and condensed chromosomes clustered together (Fig. 5A). Confocal analysis of MI oocytes that were cultured in vitro for 24 h revealed one oocyte with one PN and a PB, while two MI oocytes showed abnormal microtubule formation comparable with Fig. 5A. At the second ICSI attempt, one MI oocyte with no visible spindle was immediately fixed after denudation, and staining showed the same abnormal pattern as in Fig. 5A. ICSI with AOA resulted in one zygote showing one PN formation without
extrusion of the second PB but no further cleavage was observed. Confocal analysis of two MII oocytes that did not mature at Day 1, and one GV that became MI, revealed an abnormal spindle and dispersed chromosome configurations (Fig. 5B) and a typical GV nucleus in the GV-arrested oocyte.

Discussion

Thus far, no therapeutic approaches have been described to overcome rare human oocyte maturation arrest. Higher doses of hCG, extending the interval between hCG and retrieval and extended in vitro culture in an adapted culture IVM environment all have been shown to fail to overcome this maturation arrest (Harrison et al., 2000; Levran et al., 2002). Levran et al. (2002) reported on eight patients showing maturation arrest either at the GV, MI or MII stage: in patients from whom all collected oocytes were at either the GV or the MI stage, no spontaneous meiotic maturation was observed. On the other hand, when MII oocytes were collected beside a high number of immature oocytes in three patients, poly nucleated zygotes and failure of second PB extrusion were observed.
In our case series of patients, prolonged in vitro culture or adapted IVM conditions mostly failed to stimulate meiotic progression except for patient D with some MI oocytes that progressed to the MII stage. Calcium ionophore treatment was able to alleviate MI arrest in patient A, but a fertilization attempt by AOA gave rise to a polynucleated zygote. Patient B showed exclusively MI oocytes that were not able to progress to the MII stage. In two other patients, ICSI with AOA in presumptive MII oocytes (patient C) and mature or IVM MII oocytes (patient D) resulted in failed or abnormal fertilization without further cleavage potential. It is relevant to note that previous attempts to mature MI maturation-resistant oocytes in vitro were performed on denuded oocytes, as was the case in our study. Given the importance of normal oocyte–somatic cell interactions during the final hours of oocyte maturation (Albertini et al., 2001; Eppig, 2001; Gilchrist et al., 2004), it may be of interest to attempt meiotic maturation in MI-arrested oocytes before denudation or in an IVM cycle.

Intrinsic oocyte factors involving abnormal cell-cycle control, spindle and/or cytoskeletal function are likely to be responsible for MI oocyte arrest, although the underlying mechanisms remain unknown. Some case reports have performed cytogenetic and spindle analysis on a very low number of mostly aged human oocytes (24 or 48 h post-retrieval). Besides a persistent absence of spindle formation demonstrated by transmission electron microscopy on two oocytes (Windt et al., 2001), only limited conclusions could be drawn in other reports (Neal et al., 2002; Schmiady and Neitzel, 2002). A more detailed analysis of spindle and chromosome structures in two patients with maturation-resistant oocytes was reported by Combelles et al. (2003). In the first patient, half of the oocytes that did not mature after 29 h showed spindles approximating a bipolar arrangement but unorganized spindle poles and mostly displaced chromosomes; in the other half, microtubules were organized in a non-bipolar mass within which chromosomes were located. Still, some MI oocytes progressed to the MII stage after prolonged in vitro culture but after ICSI, no fertilization was observed (Combelles et al., 2003). Similarly, in patient D, few MI oocytes progressed to the MII stage, but gave rise to failed or abnormal fertilization even after application of AOA. Patient D showed microtubules assembled in a non-bipolar arrangement with condensed chromosomes. Combelles et al. (2003) also...
reported on a second patient with predominantly MI-resistant maturation oocytes, which possessed normal bipolar spindle formation and correctly aligned chromosomes. In our case series of patients, no normal bipolar spindle formation with correctly aligned chromosomes could be observed. Alternatively, in one case report, haploid MI chromosomes were observed after Day 1 fixation of collected oocytes with no detectable first PB, which might be due to the result of an abnormally rapid maturation and coincident degeneration of the first PB (Eichenlaub-Ritter et al., 1995). Until now, no study has performed spindle analysis on freshly collected MI oocytes; this might be a critical limitation of past studies since oocyte ageing is associated with spindle malformations, in particular the loss of bipolarity and chromosome displacement (Eichenlaub-Ritter et al., 2004). Given the comparable abnormal spindle configurations in patient D in both fresh (6 h after denudation) and aged (24 h after denudation) oocytes, the influence of ageing does not seem to account for the abnormal spindle configurations observed in our case series. In support of this, the highly abnormal spindle structures observed in patient B also originated from MI oocytes 6 h after collection.

In patient A, peripherally or centrally positioned spindles were observed without normal bipolar formation and weak or almost absent microtubules and mostly displaced chromosomes. The correct formation and positioning of the spindle requires MPF activity, through the activity of cyclin B and activation of Mos/mitogen-activated protein kinase (Madgwick et al., 2004; Brunet and Maro, 2005). Defects in these regulatory proteins may account for the aberrant spindle configurations in our case series. In support of this theory, some aberrant spindles were positioned in the centre in both patients A and B. Studies in mice have further shown that in the absence of a normal meiotic spindle, homologue chromosome separation does not occur and oocytes remain arrested at the MI stage (Soewarto et al., 1995). The spindle checkpoint will be activated when sister chromatids are not aligned at the metaphase plate or not properly attached by their kinetochores (Musacchio and Hardwick, 2002). However, human oocytes are prone to missegregate their homologue chromosomes in meiosis I, especially in aged patients (Angell, 1994), and this high error rate may argue against the functionality of a spindle assembly checkpoint (SAC) during meiosis I. Still, mammalian oocytes have been shown to possess a functional and active spindle checkpoint during the first meiotic division under regulation of Bub 1, Bub3 and Mad2 kinases (Wassmann et al., 2003; Tsurumi et al., 2004; Li et al., 2009). Interestingly, overexpression of Mad2 in meiosis I leads to a cell-cycle arrest in MI with some degree of abnormal spindle configurations (Wassmann et al., 2003), but not the same as the spindle aberrations observed in our case series. Knock out of the polyubiquitin B gene (Ubb) resulted in MI arrest, with the majority of the oocytes possessing malformed spindles or even absent microtubules (Ryu et al., 2008): the reported spindle phenotypes resembled the
Highly abnormal spindle configurations were detected in these inducing first PB extrusion in MI oocytes that would not progress by the periphery of the oocyte. Although calcium ionophore was capable of inducing meiotic progression in one of the two patients (Hupalowska et al., 2008). These oocytes are also characterized by a lack of normal degradation of p34cdc2 and cyclin B, which is required for the MI to anaphase I transition (Hampl and Eppig, 1995). By application of calcium ionophore, MI-arrested LT/Sv oocytes could progress to the MI stage, indicating that first PB extrusion can be induced experimentally (Archacka et al., 2008); however, aneuploid MI plates were observed (Hupalowska et al., 2008), pointing to other subtle mechanisms underlyling MI arrest. It was suggested that the most likely explanation of this sustained SAC activity involves discrete anomalies in spindle architecture, although possible intrinsic failures in the molecular machinery cannot be ruled out. In our case series, calcium ionophore was only capable of inducing meiotic progression in one of the two patients in a few oocytes, pointing to another possible defect in meiotic progression. Given the highly abnormal MI spindle formations and the lack of potential to overcome MI arrest using AOA by ionophore, more research is necessary to reveal the cause of these meiotic arrests. Before any clinical attempts are undertaken, such as the use of ionophore to resolve these maturation arrests, more information should be gained about the possible role of calcium signalling and the downstream processes during IVM of human oocytes, and in particular related to meiotic arrest. Therefore, it would be useful to study first the calcium-releasing potential, the calcium signalling or the changes in calcium contents in maturation-resistant mouse models. In a second step, these methods may be applied to human maturation-resistant oocytes to elucidate the role of calcium during meiotic progression. Furthermore, the proteins involved in correct spindle formation need to be further explored as clinical attempts to fertilize oocytes containing highly abnormal spindles are not acceptable.

There is still controversy about the biological relevance of an absent spindle in human oocytes and its predictive capacity of human oocyte quality and implantation potential (Woodward et al., 2008; Petersen et al., 2009; unpublished observations). Still, in two of three patients of our case series analysed by polarized microscopy, abnormally positioned and/or very low birefringent spindle structures were observed that correlated with highly abnormal spindle configurations after confocal imaging.

In conclusion, using polarized microscopy for the first time to assess maturation-resistant MI oocytes, we have shown a possible correlation between abnormal birefringent spindles and coincident highly abnormally organized spindles after immunostaining. In contrast, control MI oocytes immediately fixed after denudation and derived from IVF patients with a normal number of collected oocytes (pilot study group) showed a normal, highly birefringent spindle located at the periphery of the oocyte. Although calcium ionophore was capable of inducing first PB extrusion in MI oocytes that would not progress further after a prolonged time of in vitro culture (pilot study) and it induced PB extrusion in some maturation-resistant MI oocytes, the application of AOA resulted in failed or abnormal fertilization. Highly abnormal spindle configurations were detected in these MI-resistant oocytes, which have not been reported previously. Owing to these highly abnormal MI spindles, the chances of obtaining normal meiotic progression, or even normal fertilization and subsequent embryo development, are very low. Future studies should concentrate on identifying the molecular defects present in these oocytes, which are involved in the MI to MII stage transition. An improved molecular understanding of meiotic progression will also help in the design of approaches to overcome maturation resistance; such approaches should be investigated first in available mouse MI maturation-resistant models and, once validated and shown to be successful, then extrapolated to human oocytes showing maturation arrest. In this context, supplementation of exogenous H$_2$O$_2$ has been reported to inhibit first PB emission in rat and mouse oocytes cultured in vitro (Chauve et al., 2005; Tamura et al., 2008). Therefore, optimization of a superior IVM medium that maintains an adequate pro-/antioxidant balance may prove beneficial in overcoming meiotic arrest (Combelles et al., 2009). However, the availability of these human oocytes is limited as maturation arrest has only been shown in a handful of case reports (Beall et al., 2010). Based on our present findings and the lack of a clear understanding of the causes and consequences of these rare meiotic arrests, no attempts at AOA should be carried out until the involvement of calcium is first proven and validated.

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