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Prolonged absence of meiotic spindles by birefringence imaging negatively affects normal fertilization and embryo development


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Abstract Meiotic spindle (MS) assembly in human oocytes is a dynamic process that can be visualized by computer-assisted microscopy. At extrusion of the first polar body a spindle bridge is detected until the completion of telophase I and its reformation requires approximately 1 h. This study analysed 396 oocytes from 112 cycles for fertilization and cleavage according to MS detection at two examinations, 39 and 41 h post-human chorionic gonadotrophin (HCG). All cycles had at least one injected oocyte lacking a visible MS at intracytoplasmic sperm injection (41 h post-HCG). To evaluate the results, oocytes were divided according to the presence (group A) or absence at both observations (group B) of the MS. Compared with group A, group B oocytes had lower normal fertilization rates, higher incidence of three pronuclei and two pronuclei in early dissolution and lower development to blastocyst. Some group A oocytes showed a late MS formation (not visualized at 39 h but at 41 h) and their performance was similar to that of the oocytes with a MS visible at both time points. Although some implantations occurred in group B, these findings suggest that prolonged MS non-detection could be a marker of reduced oocyte competence. 

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Introduction

Birefringence is an optical property associated with molecularly ordered structures such as membranes, microtubules and other cytoskeletal components that can be visualized by

computer-assisted polarization microscopy. As the detection of birefringence has been associated with cell integrity and viability, the non-invasive nature of birefringence imaging encouraged its application in assisted reproduction to evaluate the structure of oocytes (reviewed by Montag

and van der Ven, 2008) and spermatozoa (Gianaroli et al., 2008, 2010) with the aim of improving gamete selection during intracytoplasmic sperm injection (ICSI) by identifying those with a regular inner structure.

In the oocyte, the meiotic spindle (MS) and the zona pellucida are birefringent structures and data are emerging on their possible role in determining oocyte viability (Madaschi et al., 2009; Montag et al., 2008; Petersen et al., 2009; Shen et al., 2005). Special attention has been dedicated to investigate the MS, which are crucial structures composed of microtubules controlling the correct chromosome alignment in the metaphase plate and separation during meiosis I and II. Accordingly, disruption of the MS results in abnormal chromosome alignment and presumably in aneuploid embryos (Wang et al., 2001a).

In view of these considerations, several studies have been undertaken to verify the relevance of MS detection in the injected oocyte to support the hypothesis that the presence of birefringent spindles predicts oocyte quality. The reported results are quite contradictory, especially as far as clinical outcome is concerned (Chamayou et al., 2006; Madaschi et al., 2008). However, some additional factors need to be taken into consideration.

Firstly, spindle formation is a dynamic process during oocyte maturation as exemplified by video-cinematography (Montag et al., 2006). As soon as the first polar body is extruded, a spindle bridge is visible during the early telophase I and disappears at completion of this phase, its reformation requiring approximately 1 h. Therefore, it has been suggested that sequential examinations should be performed to ensure a correct classification of the oocyte, implying that timing of observation is a crucial factor for evaluating oocyte and embryo morphology and development (Cohen et al., 2004; Montag and van der Ven, 2008). This is especially true when different cycles are analysed and compared without taking into strict consideration the time of observation in relation to the hours post-human chorionic gonadotrophin (HCG). In this respect, patients' characteristics and types of ovarian stimulation protocol most likely have an effect in relation to the maturation stage of the ovulated oocytes.

The second important aspect concerning the appearance and location of the MS in oocytes entails its susceptibility to external conditions, such as temperature, pH and mechanical forces due to aggressive pipetting during oocyte denudation (Wang et al., 2001a). Strict control of these variables is important to avoid any damage to the MS structure (Montag et al., 2008).

The aim of the present study was to analyse, by sequential examination, the presence or absence of MS in human oocytes by using computer-assisted polarization microscopy to verify whether this parameter is related to fertilization and embryo development.

For this purpose, and to minimize differences due to patient variation, timing of assessment and conditions during oocyte handling and observation, the oocytes included in the study were generated from patients having at least one oocyte without a visible MS at the time of ICSI. In addition, all cycles underwent the same stimulation regime and were treated following the same protocol and time schedule. In this way, oocytes were analysed for fertilization and cleavage rates, number of top-quality embryos, number of

transferred embryos and implantation under the same conditions.

Materials and methods

Patients

Between August 2008 and July 2010, 112 ICSI cycles from 104 infertile patients (37.1 ± 4.0 years) were included in the study. Ovarian stimulation was performed by administering exogenous gonadotrophins after a long desensitization protocol with long-acting gonadotrophin-releasing hormone analogues (Ferraretti et al., 1996, 2004). Oocytes were retrieved transvaginally via ultrasound guidance at 36 h after HCG administration and cultured in human tubal fluid (HTF; Quinn's Advantage Fertilization HTF medium; SAGE CooperSurgical, Pasadena, USA) supplemented with 5% human serum albumin (HSA; SAGE) at 37°C in a 5% CO₂ humidified gas atmosphere. The cycles included in the study had: (i) the MS not visible (see later in this article) in at least one oocyte that was destined to be inseminated; and (ii) no spare embryos cryopreserved.

For the analysis of data, oocytes were divided according to the presence (group A) or absence (group B) of the MS at imaging performed at the time of ICSI. As oocytes were scored twice for the presence of the MS, at 39 and 41 h post-HCG (Cohen et al., 2004), group A was further subdivided into A1 and A2 based on the time of MS detection. Therefore, the following oocyte categories were defined: group A1, MS visible at 39 and 41 h post-HCG; group A2, MS not visible at 39, but visible at 41 h post-HCG; and group B, MS not visible either at 39 or at 41 h post-HCG.

Oocyte visualization and insemination

All the following steps were performed under strict temperature and pH control by using heated working stages and buffered media.

Three hours after retrieval (39 h post-HCG) cumulus cells were removed by short exposure (a few seconds) to hyaluronidase (40 IU/ml, SAGE) at 37°C followed by gentle removal of corona cells, using calibrated pipettes of about 135 µm, and oocytes were assessed for morphology and nuclear maturation. These steps were performed in the same dish in which oocytes had been cultured, after replacing one drop of medium with equilibrated hyaluronidase. Oocytes, having extruded the first polar body, were selected to undergo meiotic spindle observation and were transferred to glass-bottomed culture dishes (Willco Wells, Amsterdam, The Netherlands), which had been prepared with a 5 µl drop of pre-warmed buffered Quinn's Advantage Fertilization HTF medium overlaid with pre-equilibrated mineral oil (SAGE). Normally, each dish contained no more than four oocytes. The meiotic spindle imaging was performed by an enhanced computer-assisted polarization microscopy system (Oosight; CRI; Woburn, MA, USA) installed in a Nikon Eclipse TE-2000 inverted microscope equipped with Hoffman interference optics and Narishige micromanipulators. The Oosight was combined with computerized image-analysis software for quantitative assessment of birefringence (CRI). Oocytes were imaged at $\times 400$

magnification in a temperature-controlled setting where heated working stages were set at a point ensuring a temperature of 37°C in the medium droplets contained in the dish. To have the MS and the polar body in focus in the oocyte equatorial plane, each oocyte was appropriately positioned using a holding pipette. Oocytes were then incubated until the time of ICSI which was performed 2 h later (41 h after HCG). At that time oocytes were imaged again to confirm the presence of the spindle that was placed at the 6 or 12 o'clock position with respect to the injection needle by rotating the oocyte as appropriate. For those oocytes in which the MS was not evident at the time of ICSI, the oocyte was rotated having the polar body as a point of reference and located at the 6 or 12 o'clock position. After injection, oocytes were thoroughly washed and cultured separately in cleavage medium (SAGE) supplemented with 10% HSA.

Fertilization and embryo control

Fertilization was checked 16 h after ICSI for the presence of pronuclei and polar bodies. Pronuclear morphology was recorded according to the evaluation of: (i) pronuclear shape and location (five patterns: A, juxtaposed and centrally located; B, juxtaposed and peripheral; C, centrally located, but separated; D, different size; and E, fragmented); (ii) position and size of nucleoli within pronuclei (five patterns: 1, large size and aligned; 2, large size but scattered; 3, large size, aligned in one pronucleus and scattered in the other; 4, small size, scattered; and 5, all other configurations having totally different patterns in the two pronuclei); and (iii) the position of polar bodies with respect to the longitudinal axis of pronuclei (three patterns: α , in the longitudinal axis $\pm 30^\circ$; β , perpendicular to the longitudinal axis $\pm 30^\circ$; and γ , in a position $>30^\circ$ off the longitudinal or the perpendicular axis). According to previous studies, the configurations A1 α , A2 α , A1 β and A2 β were considered as top quality and having the highest chances of a normal chromosome status, embryo development and implantation (Gianaroli et al., 2007).

Normally fertilized oocytes were transferred to fresh cleavage medium supplemented with 10% HSA and individually cultured. Embryo transfer was usually performed on day 2 or 3, but it was postponed to day 4 or 5 when three embryos developed normally with the aim of favouring their selection in culture (Benagiano and Gianaroli, 2004, 2010). In this case, day-3 embryos were transferred to blastocyst medium (SAGE) supplemented with 10% HSA.

Embryo assessment was performed at 40 h post-insemination and then regularly at 24-h time intervals. Embryos were evaluated for cell number, blastomere appearance and fragmentation and were consequently graded as 1–4 (Magli et al., 2007). Day-2 top-quality embryos were defined as those grade 1 with four regular blastomeres and no fragments at the observation performed at 40-h post-insemination; day-3 top-quality embryos were grade 1 and had eight regular cells and no fragmentation at the observation performed at 64-h post-insemination; day-4 top-quality embryos were compacted morulae at the observation performed at 88-h post-insemination; day-5 top-quality embryos were morphologically normal blastocysts

(grade 3–4, A or B for inner cell mass and trophectoderm according to Gardner and Schoolcraft, 1999) at the observation performed at 112-h post-insemination. Non-viable embryos were defined as those that arrested in culture for at least 48 h with clear signs of degeneration.

Clinical outcome

Clinical pregnancies were defined by the presence of a gestational sac with fetal heartbeat at ultrasound. The implantation rate expressed the ratio between gestational sacs with fetal heartbeat and the number of transferred embryos.

Statistical analysis

Data were analysed by Student's *t*-test and chi-squared analyses applying the Yates' correction, 2×2 contingency tables.

General (multiple) comparisons were performed by chi-squared omnibus testing, individual comparisons were assayed by post-hoc Bonferroni correction. This correction calculates the comparisonwise level of significance (α_c) of experimentwise level of significance (α) on the basis of the number of comparisons (k) according to the formula $\alpha_c = 1 - (1 - \alpha)^{1/k}$ (Chiorri, 2010).

Results

As shown in Table 1, 396 oocytes were inseminated (3.5 ± 1.6 inseminated oocytes per cycle) and 312 (78.8%) were fertilized, generating 297 embryos (95.2%). Embryo

Table 1 Overall study data.

Characteristic	Population
Cycles and patients (n)	112/104
Age (years)	37.1 ± 4.0
Collected oocytes (n)	472
Inseminated oocytes (n)	396
Fertilized oocytes	312 (78.8)
Embryos generated	297 (95.2)
Day 2 ^a top quality	119 (40.1)
Day 3 ^a top quality/cultured	107/260 (41.1)
Day 4 ^a top quality/cultured	74/152 (48.7)
Day 5 ^a top quality/cultured	52/113 (46.0)
Transfer cycles	103 (92.0)
Embryos transferred	220 (2.1 ± 0.7)
Clinical pregnancies (n)	33
Per transfer cycle (%)	32.0
Per oocyte retrieval (%)	29.5
Implantation rate	40/220 (18.2)
Take-home baby rate per patient	29/104 (27.9)

Values are mean \pm standard deviation, *n* (%) or *n*/total (%), unless otherwise stated.

^aThe number of top-quality embryos was calculated from a static observation over the number of embryos in culture.

transfer was performed in 103 cycles resulting in 33 clinical pregnancies (32.0% per transfer cycle) with an implantation rate of 18.2%. There were three miscarriages and one extra-uterine pregnancy accounting for a take-home baby rate per patient of 27.9%.

As represented in **Table 2**, the repeated imaging demonstrated that the MS was present at both observations in 222 oocytes (group A1); it was not detected at 39 h post-HCG, but it was present at 41 h post-HCG in 51 oocytes (group A2); while in the remaining 123 oocytes the MS was undetected at both observations (group B). The distribution of the oocytes having different timing of meiotic spindle detection (groups A1, A2 and B) was independent of the patient's age.

When comparing groups A1 and A2, no differences were detected in terms of fertilization (86.0% in group A1 versus 86.2% in group A2) or cleavage rates (96.3% versus 100%, respectively).

Based on these observations of fertilization and cleavage rates, oocytes from groups A1 and A2 were gathered in one group denominated group A (273 metaphase II, MII), having in common the detection of the MS at the time of ICSI, and then compared with oocytes from group B (123 MII). In group A oocytes, the position of the MS with respect to the first polar body was $\pm 45^\circ$ in 97.1% of cases. As represented in **Table 3**, the comparison of groups A and B demonstrated that the normal fertilization rate was significantly higher in group A than in group B (86.1% versus 62.6%, respectively; $P < 0.001$), while the proportion of top-quality pronuclear configurations was similar in the two groups (group A 63.4%, group B 61.0%). Group B showed an increased incidence of abnormal fertilization with three pronuclei and one polar body (8.9% versus 0.4% in group A; $P < 0.001$) and a higher proportion of oocytes with two polar bodies and two pronuclei that were almost dissolving (at least one of them) at the fertilization check performed at 16 h post ICSI (11.7% versus 0.4% in group A; $P < 0.001$).

A higher cleavage rate was detected in group A (97.0% versus 89.6%; $P < 0.025$), whilst the presence or absence of the MS did not affect the proportion of day-2 top-quality embryos (group A 41.2%; group B 36.2%) or day-3 top-quality embryos (group A 42.8%; group B 35.6%) (**Figure 1**). However, when the culture period was extended, top-quality embryos were significantly more frequent in group A than in group B both on day 4 (53.7% versus 27.6%; $P < 0.025$) and day 5 (52.7% versus 18.2%; $P < 0.01$).

Looking at the clinical outcome, the number of embryos transferred from group-A oocytes was 171, and 49 from group B, implying that inseminated oocytes with a visible MS at the time of ICSI had a significantly higher potential of generating transferable embryos (171/273, 62.6% in group A versus 49/123, 39.8% in group B; $P < 0.001$).

To evaluate the implantation potential of the studied oocytes, the 103 transferred cycles were divided according to the group of oocytes that generated the transferred embryos. In all, 55 cycles were transferred with embryos generated from group-A oocytes, 10 cycles had embryos from group-B oocytes and 38 received a mixed transfer with embryos from oocytes belonging to both groups A and B (mixed group). Female age and similar mean number of inseminated oocytes did not differ in the three groups, while the proportion of cycles having two or more inseminated oocytes lacking the MS at both observations was 5.5% (3/55) in cycles transferred with group-A oocytes, 50.0% (5/10) in cycles having embryos from group-B oocytes and 13.2% (5/38) in the mixed group. Twenty-one clinical pregnancies derived from group-A oocytes (38.2% pregnancy rate) after the transfer of three embryos in 12 cycles (21.8%), two embryos in 32 cycles (58.2%) and one embryo in 11 cycles (20.0%), accounting for 21.6% (24/111) implantation rate and 34.5% take-home baby rate. A similar performance was detected in the mixed group with 12 clinical pregnancies (31.6% pregnancy rate) with 20 cycles transferred with three embryos (52.6%) and 18 with two embryos

Table 2 Fertilization and embryo cleavage according to the presence or absence of the meiotic spindle in relation to the time of oocyte imaging.

	Group A		Group B	General comparison P-value
	A1	A2		
Inseminated oocytes (n)	222	51	123	—
Normally fertilized oocytes	191 (86.0) ^a	44 (86.2) ^b	77 (62.6) ^{a,b}	<0.01
1PN + 1PB oocytes	2 (0.9)	1 (2.0)	0	NS
3PN + 1PB oocytes	0 ^c	1 (2.0) ^d	11 (8.9) ^{c,d}	<0.01
0PN + 1PB oocytes	29 (13.1) ^e	5 (9.8) ^f	35 (28.5) ^{e,f}	<0.01
Top-quality 2PN oocytes	123 (64.4)	26 (59.1)	47 (61.0)	NS
Dissolving 2PN	1 (0.5) ^g	0 ^h	9 (11.7) ^{g,h}	<0.01
Embryos	184 (96.3) ⁱ	44 (100) ^j	69 (89.6) ^{i,j}	<0.01

Values are n (%) unless otherwise stated.

Group A1 = meiotic spindle (MS) visible at 39 and 41 h post-human chorionic gonadotrophin (HCG); group A2 = MS not visible at 39 h but visible at 41 h post-HCG; group B = MS not visible either at 39 or at 41 h post-HCG.

General (multiple) comparisons were performed by chi-squared omnibus testing; individual comparisons were assayed by post-hoc Bonferroni correction (see Materials and methods).

Values with the same superscripts are significantly different: ^{a-c,e-j} $P < 0.001$; ^d $P < 0.05$.

PB = polar body; PN = pronuclei; NS = not statistically/significant.

Table 3 Fertilization and embryo cleavage according to the presence or absence of the meiotic spindle at the time of intracytoplasmic sperm injection.

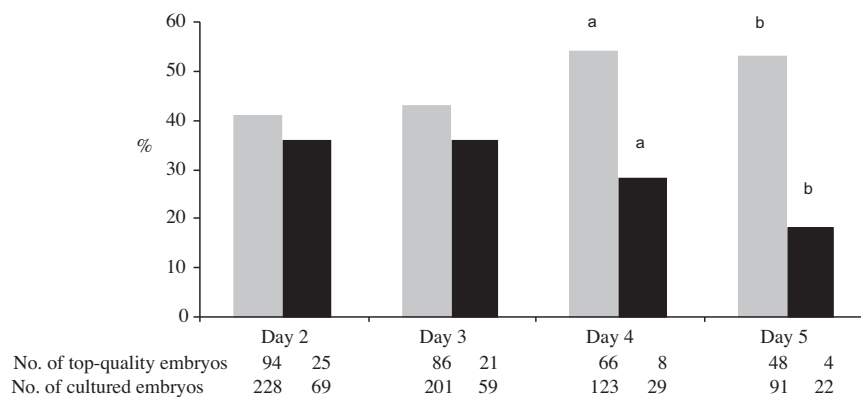
	Group A	Group B	P-value
Inseminated oocytes (n)	273	123	—
Normally fertilized oocytes	235 (86.1) ^a	77 (62.6) ^a	<0.001
1PN + 1PB oocytes	3 (1.1)	0	—
3PN + 1PB oocytes	1 (0.4) ^b	11 (8.9) ^b	<0.001
OPN + 1PB oocytes	34 (12.4) ^c	35 (28.5) ^c	<0.001
Top-quality 2PN oocytes	149 (63.4)	47 (61.0)	—
Dissolving 2PN	1 (0.4) ^d	9 (11.7) ^d	<0.001
Embryos	228 (97.0) ^e	69 (89.6) ^e	<0.025

Values are n (%) unless otherwise stated.

Group A (group A1 + group A2 as defined in Table 2) = meiotic spindle (MS) visible at 41 h post-human chorionic gonadotrophin (HCG); group B = MS not visible either at 39 or at 41 h post-HCG.

Values with the same superscripts are significantly different:

^{a-c} $P < 0.001$; ^d $P < 0.05$. PB = polar body; PN = pronuclei.

**Figure 1** Proportion of top-quality embryos on days 2–5 in oocytes in which the meiotic spindle was visible (group A, grey bars) or not visible (group B, black bars) at the time of intracytoplasmic sperm injection. Values with the same superscripts are significantly different: ^a $P < 0.025$; ^b $P < 0.01$.

(47.4%), an implantation rate of 16.7% (16/96) and a take-home baby rate of 26.3%. Finally, no pregnancies resulted in the 10 cycles transferred with embryos derived from group-B oocytes, but in this group, where only three cycles had two embryos transferred and seven had a single embryo transfer, the mean number of transferred embryos (1.3 ± 0.5) was significantly lower when compared with the other two groups (2.0 ± 0.6 in group A, $P = 0.001$; 2.5 ± 0.5 in mixed group, $P < 0.001$).

Due to the occurrence of mixed transfers, only for 28 sacs with a fetal heartbeat was an evaluation of the relationship with the corresponding oocytes possible; 24 embryos derived from group A and four from the mixed group. As in the mixed group, these sacs were generated in two twin pregnancies after the transfer of two embryos, one coming from group-A oocytes and the other from group-B oocytes, the final result was that 26 sacs derived from group-A oocytes (24 + 2 from the mixed group) and two sacs from group-B oocytes (the two oocytes from mixed group). In addition, looking at group A in more detail, three of the sacs with a known origin derived from oocytes belong-

ing to group A2 (in which the MS was observed for the first time at 41 h post-HCG).

Discussion

Spindle birefringence is a physical property of the microtubule-based structure that might reflect oocyte quality representing a marker for its cytoplasmic maturation, inner structure and possible stress during manipulation (Montag and van der Ven, 2008; Wang et al., 2001a). Several studies have demonstrated that oocytes with a MS detected by birefringence imaging are more prone to give higher rates of fertilization, cleavage and development to top-quality embryos (Moon et al., 2003; Wang et al., 2001b), but a correlation with the clinical outcome seems not to be so evident (reviewed by Petersen et al., 2009). This could be for several reasons, including the variability of patients and their response to hormonal stimulation, as well as to differences in the time of oocyte observation, handling and insemination. With the aim of minimizing the majority of

these variables, in this study the developmental outcome in relation to the detection or non-detection of the MS was evaluated in oocytes belonging to the same cohorts in ICSI cycles and generated by patients according to the same stimulation and laboratory protocols. In other words, instead of evaluating embryo growth and implantation in 'good' patients (meaning those having only oocytes with a visible MS) in comparison to 'poor' patients (those with abnormal MS birefringence characteristics), sibling oocytes having different MS birefringence properties were studied here.

The incidence of group-B oocytes was 31.1% (123/396 inseminated oocytes) and, as expected, they showed a significantly lower rate of normal fertilization when compared with group-A oocytes (Table 3). In agreement with other results (Rienzi et al., 2003), the absence of a visible MS at the time of ICSI was more frequently associated with three pronuclear fertilizations and with a condition of starting dissolution of pronuclei at the observation performed at 16-h post-insemination. These findings are indicative of some event desynchronization in these oocytes compared with what happened in the majority of their sibling oocytes and seem to be in analogy to what occurs in in-vitro matured oocytes, of which approximately 50% do not have an evident MS and are prone to abnormal fertilization (Wang and Keefe, 2002). It has been reported that oocytes lacking a MS during imaging could have just entered the late telophase I after which the spindle disappears for approximately 40–60 min and then reforms typically underneath the first polar body (Montag et al., 2006). The dynamic nature of spindle assembly implies the existence of a lag time between the entire structure organization and birefringence detection, during which the partial assembly of microtubule-associated proteins is ongoing and possibly needs to reach a cut-off level before yielding a birefringence effect (Gomes et al., 2008). Therefore, in the normal transition from metaphase I to metaphase II, if oocytes lacking a MS at imaging are left in culture for a couple of hours and imaged again (as for group-A2 oocytes), they should display a normal MS demonstrating that they were relatively immature. Conversely, the prolonged lack of MS visualization (as for group-B oocytes) is most probably indicative of an abnormal condition. In the first case, the oocytes might suffer from the defects deriving from a delayed maturation such as higher predisposition to chromosomal abnormalities, altered methylation patterns and higher incidence of DNA fragmentation (Bosco et al., 2005; Magli et al., 2006; Muzii et al., 2009), but their fertilization capacity and embryo development do not appear to be significantly affected (Table 2). In the second case, the condition of abnormality is more severe and this is reflected in a reduced capacity of being normally fertilized (Table 3). These findings are in agreement with observations by others (Montag et al., 2008), including the lack of association with maternal age (Wang et al., 2001b).

All cases of oocytes with three pronuclei (one in group A and 11 in group B, Table 3) had only one polar body, while the oocytes in which pronuclei had already started to dissolve (one in group A and nine in group B, Table 3) had two polar bodies. There is no doubt that sometimes counting the number of polar bodies after fertilization is not easy because of the frequent fragmentation and degeneration of

the first polar body. Nevertheless, a large experience in polar-body biopsy for chromosomal and gene analysis and the systematic imaging of oocytes before and during ICSI, as well as at the fertilization control, made this assessment quite robust.

Failure of second polar-body extrusion after fertilization causes digynic triploidy, a situation that occasionally arises after ICSI. The mechanisms responsible for this condition are not fully understood and deterioration of the MS during injection has been postulated as a possible cause (Macas et al., 1996). In the current study, possible mechanical damage to the MS by the injection needle was theoretically avoided by positioning the MS at the 6 or 12 o'clock position in group-A oocytes, but this strategy could not be followed in those cases (group-B oocytes) in which the MS was not visible. Other mechanisms can be postulated and interesting considerations derive from experimental studies which have confirmed that the formation of the MS requires a time-dependent interaction of several proteins with specific targets (Eichenlaub-Ritter et al., 2004). It was demonstrated in mice that if microfilament depolymerization is blocked by drugs like cytochalasin B for a period of 3 h, second polar-body extrusion fails to occur (Zhu et al., 2003). These data suggest the need for temporal and spatial requirements that are essential for the completion of the two meiotic divisions to occur correctly. In other words, should any of the factors entering the process of meiosis not be present at the right time, irregular development would follow.

In a similar concept of timing, early dissolution of pronuclei could indicate a series of desynchronized events leading to a slow or accelerated cleavage rate with consequent high risk of generating embryos having reduced competence possibly related to aneuploidy (De Santis et al., 2005; Magli et al., 2007). It is well known that the first mitoses of the human embryo are organized by the sperm centrosome, but it is also clear that the structural and functional elements forming the microtubular network are provided by the oocyte (Sathanathan et al., 1996). Therefore, the absence of the MS in a situation in which it should be clearly visible and organized could be the sign of a profound disturbance in the mechanisms controlling the two meiotic divisions that normally depend on the coordination between the cell-cycle progression and the MS assembly and function. According to the current data, this condition has a significant effect both on the formation of pronuclei, with the concomitant extrusion of the second polar body, and on embryo cleavage. Actually, it is when the embryonic genome is switched on that the situation of abnormality becomes especially evident with a reduced growth to blastocysts (group-B oocytes in Figure 1). Nevertheless, some of these oocytes can develop further and give rise to implantation and this finding justifies their clinical use.

In conclusion, the current results indicate that the detection of the MS at the time of ICSI has a role in determining normal fertilization of the oocyte, while the prolonged absence of the MS at birefringence imaging seems to be associated with a higher incidence of abnormal fertilization because of failed extrusion of the second polar body or early dissolution of the two pronuclei. These data together with a lower development to blastocyst suggest that the non-detection of the MS could reflect a reduced competence

of the oocyte that is possibly related to an asynchronous course of nuclear and cytoplasmic maturation.

On the other hand, the physiologically dynamic nature of MS formation confirms that there is a time interval closer to HCG administration during which some oocytes are still in the transition from metaphase I to metaphase II. Although no conclusive evidence has been published so far regarding the best time to perform sperm injection, postponing ICSI to 41 h post-HCG gives time for most MII oocytes to complete their maturation phase (Cohen et al., 2004).

According to the current results, the repeated imaging of MII oocytes would permit the establishing of whether the absence of the MS is an indicator of physiological progression through meiosis, rather than an abnormal condition. Even though further development will deselect most embryos derived from abnormal oocytes, performing ICSI at the most appropriate time could provide a comprehensive assessment of oocyte maturation.

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