Zygote **12** (August), pp. 241–249. © 2004 Cambridge University Press
DOI:10.1017/S0967199404002850 Printed in the United Kingdom

Spindle dynamics in living mouse oocytes during meiotic maturation, ageing, cooling and overheating: a study by polarized light microscopy

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Date submitted: 27.05.04. Date accepted: 9.07.04

Summary

A liquid crystal polarized light microscope (LC PolScope) was used to examine spindle dynamics in living mouse oocytes. Immature oocytes were cultured for 0–48 h and spindles were imaged with the PolScope at various time points of culture. Oocytes at metaphase I (M-I) and metaphase II (M-II) were also exposed to shifts of temperature from 25 to 41 °C to examine the effects of fluctuations of temperature on spindle dynamics. After examination with the PolScope, some oocytes were fixed and examined by immunocytochemical staining and confocal microscopy. After culturing for 6 h, 76% and 2% of the oocytes reached M-I and M-II stages and all oocytes had birefringent spindles. When the oocytes were cultured for 14–16 h, 88% and 6% of oocytes were at M-II and M-I stages respectively and all oocytes had birefringent spindles. However, when the oocytes were cultured for 22–48 h, the proportions of oocytes with birefringent spindles decreased as culture time was increased. Exposure of oocytes to 25 °C induced spindle disassembly within 10–20 min in both M-I and M-II oocytes. Most (93–100%) oocytes reassembled spindles after warming at 37 °C. Furthermore, exposure of oocytes at M-I stage but not at M-II stage, to 30 °C also induced significant microtubule disassembly. However, exposure of oocytes to 38–41 °C did not obviously change the quantity of microtubules in the spindles, which was measured by retardance. This study indicates that the PolScope can be used to examine spindle dynamics in living oocytes, and it has the advantage over the routine fluorescence microscope in that images can be obtained in the same individual oocyte and the quantity of microtubules can be measured by retardance in living oocytes. These results also indicate that the M-II spindle in mouse oocytes is sensitive to oocyte ageing and cooling, but not heating, and M-I spindle is more sensitive to temperature decline than M-II spindle.

Keywords: Microtubules, Mouse, Oocytes, PolScope, Spindle

Introduction

Maturation of immature oocytes into fertilizable matured oocytes involves a series of events including movements of chromosomes and organelles. Microtubules play an important role in these movements, such as the metaphase–anaphase transition, pronuclear migration and mixing of chromosomes (Maro *et al*., 1986). Inhibition of microtubule dynamics results in abnormal meiosis. When mammalian oocytes undergo meiotic maturation, two meiotic spindles are formed: one is at metaphase I (M-I) stage and the other at metaphase II (M-II) stage. Meiotic spindle is crucial for the configuration of chromosomes, hence disruption of spindles causes abnormal chromosome distribution, which may cause aneuploidy after fertilization. It has been reported that the dramatic drop in clinical pregnancy rates in patients with advanced maternal ages is closely related to the occurrence of aneuploidies (Munne *et al*., 1995; Benadiva *et al*., 1996). Indeed, it has been reported that abnormal spindle may

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contribute to the high prevalence of aneuploidy in older women (Battaglia *et al*., 1996). In addition, spindle architectures are also affected by other factors, such as oocyte age (Eichenlaub-Ritter *et al*., 1988; Pickering *et al*., 1988) and fluctuations in temperature (Almeida & Bolton, 1995; Aman & Parks, 1994; Baka *et al*., 1995; Moor & Crosby, 1985; Pickering & Johnson, 1987; Pickering *et al*., 1990; Sathananthan *et al*., 1988; Wang *et al*., 2001*c*). Cooling oocytes causes temporary or permanent disappearance of spindles and limited recovery has been found after warming in most mammals (Moor & Crosby, 1985; Aman & Parks, 1994; Pickering *et al*., 1990; Wang *et al*., 2001*c*). These results suggest that optimal conditions for manipulation of oocytes *in vitro* are important for production of normal embryos, especially in human *in vitro* fertilization (IVF) clinics.

The studies on spindle dynamics in most mammalian oocytes have been conducted mainly using fixed samples examined by fluorescence microscopy. Although these studies have provided information for understanding of spindle morphology in mammals, they are of limited value in IVF clinics. Recently, a polarized light microscope combined with computerized imaging systems has made it possible to study the microtubule dynamics in living cells (Waterman-Storer, 1998). Polarized light microscopy is a technique that relies on a material's optical properties, the birefringence, to examine microstructure (Oldenbourg, 1996, 1999). It can be best applied to materials that are anisotropic and have two indices of refraction. Many biological materials that have a well-aligned molecular structure exhibit birefringence (Bragg & Pippard, 1953). Since spindles are composed of microtubules that exhibit birefringence, while most other cellular organelles and the cytoplasm do not exhibit apparent levels of birefringence (Oldenbourg, 1999), the spindle in oocytes can be detected using polarization microscopy. The conventional polarized microscope uses plain polarized light, whereby the operator must rotate the microscope stage and/or the liner analyser in order to visualize birefringent structures of interest. As the analyser is rotated, the structures with the property of birefringence can be detected when the molecules of that structure align with the angle of polarization of the microscope. This type of linear polarization microscopy is limited by orientation dependence (Oldenbourg, 1999). However, this technology has not been widely applied to mammalian embryology because of its orientation dependence and limited sensitivity in visualizing the spindle structure during specimen analysis.

The liquid crystal polarized light microscope (LC PolScope) augments the conventional polarized microscope by integrating liquid crystal variable retarders, electronic imaging and digital imaging processing tools to build a highly-sensitive, orientation-independent imaging system (Oldenbourg, 1996, 1999). In contrast to fluorescence microscopy, LC PolScope imaging does not require invasive preparative techniques such as fixation and staining and thus the fine structure of the spindle can be imaged in living mammalian oocytes (Silva *et al*., 1999; Liu *et al*., 2000; Wang *et al*., 2001*a*). In this study, experiments were designed to use the PolScope to study meiotic spindle dynamics in living mouse oocytes during meiotic maturation, ageing, cooling and heating. Furthermore, effects of temperature fluctuations on M-I spindles and M-II spindles were also compared by PolScope imaging as the PolScope can identify the presence of M-I spindles in living oocytes. In order to confirm these results, some oocytes were fixed and further examined by immunofluorescence staining and confocal microscopy after PolScope imaging.

Materials and methods

Collection and culture of oocytes

Three- to four-week-old CD1 female mice were killed by cervical dislocation. The ovaries were removed and transferred into Hepes-buffered CZB (HCZB) medium containing 10% fetal calf serum (FCS; Sigma Chemical, St Louis, MO). Oocytes were released from the follicles by puncturing with a 25-gauge needle and oocytes with cumulus cells were selected for *in vitro* maturation. *In vitro* maturation of oocytes was conducted at 37 °C, 5% CO_2 in air, in human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA) supplemented with 10% FCS, 1 IU/ml pregnant mare's serum gonadotrophin (PMSG) and 1 IU/ml human chorionic gonadotrophin (hCG).

In vivo oocytes were also collected from superovulated mice by injection of 10 IU of PMSG, which was followed 46–48 h later by injection of 10 IU of hCG. Mice were killed by cervical dislocation 16 h after hCG injection to collect oocytes. Oocytes enclosed with a cumulus mass were released from the oviductal ampullae into the modified HTF medium containing 10% fetal bovine serum. The cumulus cells were removed by gentle pipetting in modified HTF medium containing 80 IU/ml hyaluronidase (Sigma). Cumulus-free oocytes were washed in modified HTF medium three times, and then cultured *in vitro* in IVF-20 medium at 37 °C in 5% CO_2 and humidified air until examination.

Examination of spindles in living oocytes by the PolScope

Each oocyte was placed in a 5 μl drop of HCZB covered with warmed paraffin oil in a Bioptechs TC3 Culture Dish System (Bioptechs, Butler, PA). The system is comprised of a temperature controller, a stage adapter and a TC3 dish that has a specially coated clear glass (0.5 mm thick) bottom. The temperature was maintained at

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Table 1 Spindle dynamics in living mouse oocytes during maturation and ageing

Time of culture (h)	No. of oocytes examined	No. of oocytes at GV(%)[a]	No. of oocytes at GVBD or M-I		No. of oocytes at M-II			No. of abnormal oocytes (%)[a]
			Total (%)[a]	With spindle (%)[b]	Total (%)[a]	With spindle (%)[b]	Without spindle (%)[b]	
0	15	15 (100)[c]	0 (0)[c]	–	0 (0)[c]	–	–	0 (0)[c]
6	52	1 (2)[d]	50 (96)[d]	38 (76)[c]	1 (2)[c]	1 (100)[c]	0 (0)[c]	0 (0)[c]
14–16	50	3 (6)[d]	3 (6)[d]	3 (100)[c]	44 (88)[d]	44 (100)[c]	0 (0)[c]	0 (0)[c]
22–24	82	6 (7)[d]	0 (0)[d]	–	67 (82)[d]	63 (94)[c]	4 (6)[c]	9 (11)[d]
46–48	91	4 (4)[d]	0 (0)[d]	–	55 (60)[e]	30 (55)[d]	25 (45)[d]	32 (35)[e]

[a] Percentage of number of oocytes examined.
[b] Percentage of number of oocytes at each category.
[cde] Values with different superscripts within each column are significantly different, $p < 0.05$.

37 °C during examination. Oocytes were examined under a Zeiss Axiovert 100 with a Neofluar ×40 strainfree objective and the LC PolScope (CRI, Wolbure, MA), combined with a computerized image analysis system (MetaMorph Universal Imaging System, West Chester, PA) or SpindleView system (CRI, MA) according to the methods reported previously (Wang *et al*., 2001*a*).

Examination of spindles in fixed oocytes by confocal microscopy

After imaging by the PolScope, some oocytes were fixed separately in 3.7% paraformaldehyde (Sigma) in phosphate-buffered saline (PBS) and treated overnight with 0.1% Triton X-100 in PBS. Oocytes were incubated in a blocking solution (PBS containing 2 mg/ml bovine serum albumin (BSA) and 150 mM glycine) for 30 min and then in PBS containing anti-β-tubulin antibody (1:300; Sigma) for 1 h. After being thoroughly washed in PBS containing 0.1% Tween-20, oocytes were incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (1:32; Sigma) in PBS-Tween for 1 h. Samples were then stained with 10 μg/ml propidium iodide in PBS for examination of chromosomes. Finally, the oocytes were mounted on slides and examined by laser confocal microscopy. Confocal microscopy was performed using a Carl Zeiss LSM 410 laser confocal imaging system equipped with an argon laser and mounted on a Zeiss microscope. The images were compared with those obtained with the PolScope.

Experimental studies

In experiment 1, spindle dynamics in oocytes during meiotic maturation and ageing were examined. At 0, 6, 14–16, 22–24 and 46–48 h after culture, oocytes were separated from cumulus cells, washed in HCZB medium and examined with the PolScope. For spindle confirmation, some oocytes were fixed and examined by confocal microscopy.

In experiment 2, effects of temperature on the spindle morphology were examined. *In vivo* matured oocytes or oocytes (M-II) cultured for 14 h were imaged with the PolScope and then cooled to room temperature (~25 °C) for various times. After cooling, oocytes were re-examined with the PolScope. Oocytes were then warmed at 37 °C for 10–15 min to examine spindle recovery.

In experiment 3, effects of fluctuations in the temperature on spindle dynamics in oocytes at M-I and M-II stages were compared. Only oocytes that had released the first polar body and showed birefringent spindles (M-II spindle) or oocytes that had not released the first polar body but showed birefringent spindles (M-I spindle) imaged at 37 °C were used in this study. Each oocyte was first imaged at 37 °C, then heated or cooled to the designated experimental temperature (25 °C, 30 °C, 38 °C, 39 °C, 40 °C or 41 °C) for 10 min. The temperature of microdrops was monitored and confirmed by a digital temperature thermometer. Thereafter, oocytes were returned to 37 °C for another 10 min for examination of spindle recovery. The maximum retardance of each spindle was measured and calculated by computer software (SpindleView system).

Statistical analysis

Comparisons were conducted by ANOVA. Percentage data were subjected to arcsine transformation before statistical analysis. A probability of $p < 0.05$ was considered to be statistically significant.

Results

Spindle dynamics in living mouse oocytes during maturation and ageing (experiment 1)

As shown in Table 1, all oocytes were at the germinal vesicle (GV) stage at the beginning of culture.

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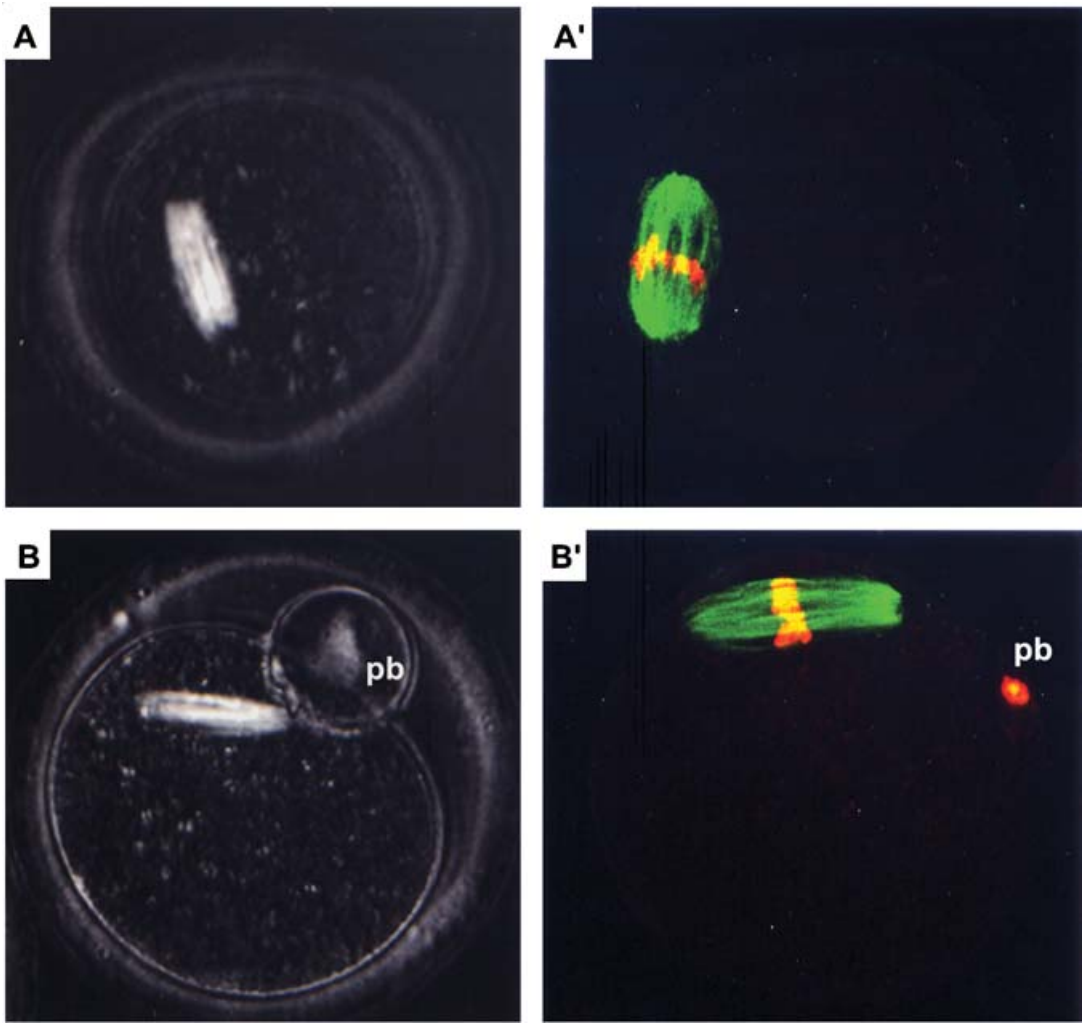

Figure 1 Meiotic spindles in mouse oocytes imaged by the PolScope and confocal microscope during meiotic maturation. (*A*) A birefringent spindle in a living oocyte at M-I stage imaged by the PolScope; (*A'*) an immunostained spindle in the same oocyte imaged by confocal microscopy after fixation. The oocyte was examined 6 h after culture. (*B*) A birefringent spindle in a living oocyte at M-II stage imaged by the PolScope; (*B'*) an immunostained spindle in the same oocyte imaged by confocal microscopy after fixation. The oocyte was examined 14 h after culture. Green images represent microtubules, stained by FITC conjugated with antibody to tubulin, and red images are chromosomes, stained by propidium iodide. pb, polar body.

No birefringent materials (including spindle) were observed in the oocytes at GV stage. Six hours after culture, most oocytes (98%) underwent GV breakdown (GVBD), 76% of oocytes formed M-I spindles (Fig. 1A) and one oocyte released the first polar body and formed an M-II spindle. At 14–16 h of culture, 88% of the oocytes released the first polar body and all oocytes with polar body had M-II spindles (Fig. 1B). The images obtained with the PolScope were confirmed by confocal microscopy (Fig. 1A', B'). After 22–24 h of culture, 11% of oocytes degenerated and no birefringent spindle was observed, 6% of oocytes did not have birefringent spindles although no obvious abnormal morphology was observed, and 94% of oocytes at M-II stage still had a birefringent spindle. When oocytes were cultured for 46–48 h, however, the proportion of oocytes (55%) with birefringent spindles decreased significantly ($p < 0.05$) and 45% oocytes did not have a birefringent spindle although they had normal morphology and 35% of oocytes degenerated and did not have a birefringent spindle. When the oocytes without birefringent spindles were examined by confocal microscopy, it was found that the spindles in these oocytes had disassembled, or a few microtubules were distributed around the nucleus or microtubule asters were formed throughout the cytoplasm. Some oocytes were undergoing spontaneous activation, either to form a pronucleus or to divide. Due to the changes in both nuclei and microtubules during ageing, a birefringent spindle was not imaged in most aged oocytes.

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Table 2 Effects of temperature on spindle dynamics in living mouse oocytes at M-II stage

No. of oocytes examined	Cooling time (min)	No. of oocytes with spindle	
		After cooling	After warming[a]
33	10–20	9 (27)[b]	31 (94)
28	30–35	2 (7)[c]	28 (100)
42	40–50	1 (2)[c]	39 (93)

[a] Oocytes were warmed at 37 °C for 10–15 min.
[bc] $p < 0.05$.

Effects of temperature on spindle architecture in oocytes at M-II stage (experiment 2)

When oocytes with birefringent spindles identified with the PolScope were cooled (by keeping the dishes containing the oocytes on a table at a room temperature of ~25 °C) for 10–20 min, birefringent spindles were imaged in 27% of oocytes (Table 2). When cooling time was increased to more than 30 min, the proportions of oocytes with birefringent spindles dropped to 2–7%. However, when cooled oocytes were rewarmed at 37 °C for 10–15 min, birefringent spindles were imaged again with the PolScope in 93–100% of oocytes (Fig. 2). After recovery, the same spindle images (before cooling and after rewarming) were observed in the oocytes (Fig. 2A, C) and confocal microscope images indicated that chromosome alignment and spindle morphology were normal in these oocytes (Fig. 2D). However, some oocytes did not recover their spindles after cooling–warming (Fig. 2E, G) and confocal microscopy indicated that these oocytes did not have intact spindles (Fig. 2F, H).

M-I spindle is more sensitive to temperature decline than M-II spindle (experiment 3)

As shown in Table 3, spindle birefringent retardance was measured to quantify microtubules in the M-I spindles and M-II spindles after oocytes were exposed to various temperatures for 10 min. It was found that maximum retardance in M-II spindles reduced significantly ($p < 0.05$) when the oocytes were cooled to 25 °C. When the temperature was returned to 37 °C, the spindle recovered and the maximum retardance returned to original level (before cooling). No changes in maximum retardance were found when M-II oocytes were cooled to 30 °C or heated to 38–41 °C for 10 min. However, when M-I oocytes were cooled, it was found that the maximum retardance of the spindles was significantly ($p < 0.05$) decreased at both 25 °C and 30 °C. When the temperature was returned to 37 °C, the maximum retardance of spindles returned to its original level. However, incomplete recovery was observed in the oocytes cooled to 25 °C. As for the M-II oocytes, heating M-I oocytes to 38–41 °C did not change the maximum retardance of the spindles, indicating that the amount of microtubules did not change after heating.

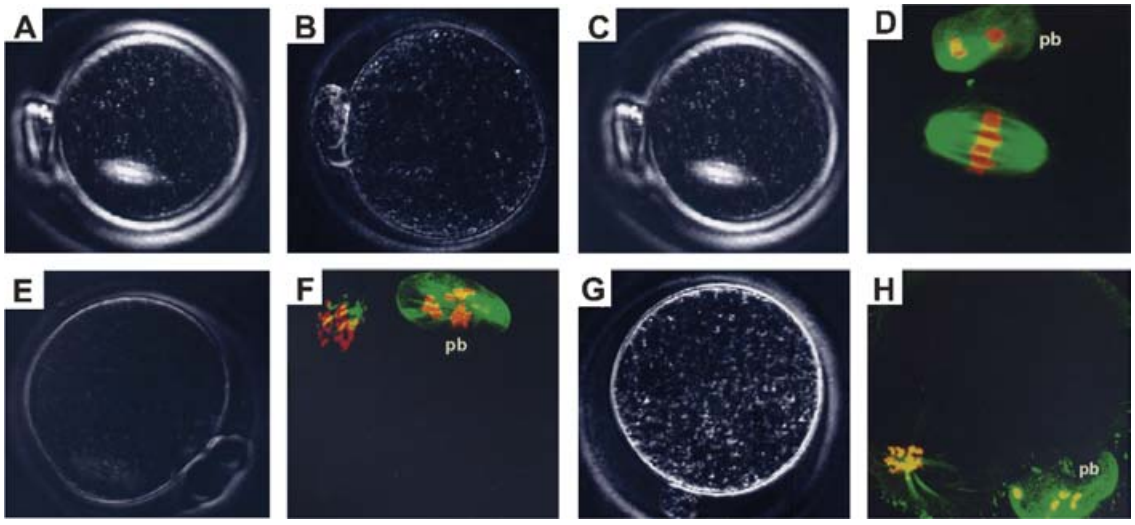

Figure 2 Morphology of meiotic spindles in mouse oocytes at M-II stage during cooling and warming examined by the PolScope and confocal microscope. (*A*)–(*C*) A birefringent spindle in an oocyte before cooling (*A*), after being cooled to 25 °C for 10 min (*B*), and after warming to 37 °C for 10 min (*C*). (*D*) An immunostained spindle (green) and chromosomes (red) in the same oocyte examined by confocal microscopy after fixation. (*E*), (*G*) PolScope images of oocytes cooled to 25 °C for 50 min and then warmed to 37 °C for 10 min. No birefringent spindles were observed in the oocytes. Confocal microscope images for immunostained spindle (green) and chromosomes (red) in the same oocytes indicated that few (*F*) or some microtubules (*H*) were polymerized in the oocytes. pb, polar body.

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Table 3 Maximum retardance of spindles in the oocytes at M-I and M-II stages treated at various temperatures

Treatments		M-II oocytes		M-I oocytes	
		No. of oocytes	Maximum retardance	No. of oocytes	Maximum retardance
37°C	0 min	5	2.0 ± 0.25	5	2.1 ± 0.06
	10 min		2.1 ± 0.04		2.0 ± 0.13
	20 min		2.0 ± 0.13		1.9 ± 0.05
25°C	37°C*	5	2.1 ± 0.04^{a}	5	2.1 ± 0.04^{a}
	25°C		1.3 ± 0.08^{b,A}		0.7 ± 0.09^{b,B}
	37°C**		2.1 ± 0.05^{a}		2.1 ± 0.04^{a}
30°C	37°C*	5	2.1 ± 0.02	6	1.9 ± 0.22^{a}
	30°C		2.0 ± 0.12^{A}		1.1 ± 0.09^{b,B}
	37°C**		2.1 ± 0.04		1.8 ± 0.09^{a}
38°C	37°C*	5	2.1 ± 0.02	6	2.0 ± 0.24
	38°C		2.1 ± 0.02		2.0 ± 0.13
	37°C**		2.1 ± 0.04		2.1 ± 0.04
39°C	37°C*	5	2.1 ± 0.05	6	2.1 ± 0.06
	39°C		2.1 ± 0.04		2.1 ± 0.01
	37°C**		2.1 ± 0.04		2.1 ± 0.04
40°C	37°C*	5	2.1 ± 0.15	5	2.1 ± 0.00
	39°C		2.1 ± 0.02		2.1 ± 0.04
	37°C**		2.1 ± 0.02		2.1 ± 0.04
41°C	37°C*	5	2.1 ± 0.02	5	2.1 ± 0.02
	41°C		2.1 ± 0.05		2.1 ± 0.03
	37°C**		2.1 ± 0.09		2.1 ± 0.00

Data are presented as Mean ± SD.
*Temperature for imaging oocytes before heating or cooling.
**Temperature for imaging oocytes after heating or cooling.
^{ab}Values are significantly different in the same column between treatments ($p < 0.05$).
^{AB}Values are significantly different in the same row between M-I and M-II spindles ($p < 0.05$).

Discussion

The results obtained in the present study indicate that spindles in living mouse oocytes can be imaged with the PolScope. The images obtained with the PolScope were confirmed by immunocytochemical staining and confocal microscopy, suggesting that such a technique is vital to the study of spindle dynamics in mammalian oocytes. Furthermore, this study also demonstrates that M-I spindle is more sensitive to a temperature decline than M-II spindles in mouse oocytes. This study indicates that the PolScope has the advantage over other microscopes in that the spindles can be observed in the living state, not only in M-II oocytes but also in M-I oocytes, and microtubules in the spindles can be quantified by maximum retardance in the living state.

The presence of a birefringent spindle is clearly related to oocyte age. All oocytes at M-I and M-II stage during oocyte maturation had a birefringent spindle. However, spindles disappeared in some aged oocytes due to partial and complete microtubule disassembly, and this process was accompanied by oocyte ageing. With confocal microscopy, we found that spindles in these oocytes were undergoing or had undergone disassembly, and some oocytes started to undergo spontaneous activation and degeneration. The results obtained with the PolScope in living oocytes were in agreement with those obtained by confocal microscopy in fixed oocytes. These results may suggest that the presence of a birefringent spindle in living oocytes may be a useful predictor of the quality or age of oocytes, which is useful in evaluating oocytes from their morphology without damaging them, such as occurs in biochemical analysis. This is extremely important for human oocytes in infertility clinics. Some infertility clinics have started to use this technology (Wang *et al*., 2001*a*,*b*; Moon *et al*., 2003; Cooke *et al*., 2003; Cohen *et al*., 2004; Rienzi *et al*., 2004). It has been found that PolScope spindle imaging in human oocytes can be used to evaluate oocyte quality, such as for fertilization and early development (Wang *et al*., 2001*a*,*b*: Moon *et al*., 2003), and to determine the optimal time for insemination (Cohen *et al*., 2004). We previously found that human oocytes with a birefringent spindle had higher fertilization and early developmental ability (blastocyst formation) than oocytes without a birefringent spindle, indicating that the presence of a birefringent spindle is a nuclear and cytoplasmic maturation marker for predicting oocyte quality (Wang *et al*., 2001*a*,*b*).

Microtubules are sensitive to fluctuations of temperature in mammalian oocytes (Moor & Crosby, 1985; Pickering & Johnson, 1987; Sathananthan *et al*., 1988; Pickering *et al*., 1990; Aman & Parks, 1994; Almeida & Bolton, 1995; Baka *et al*., 1995; Wang *et al*., 2001*c*; Liu *et al*., 2003). One common phenomenon is disappearance of spindles when oocytes are cooled (20–25 °C), which is accompanied by chromosome separation. It would appear that there are significant species differences. For example, bovine (Aman & Parks, 1994) and human (Pickering *et al*., 1990; Wang *et al*. 2001*c*) oocytes did not recover their spindles completely when they were exposed to room temperature, and the chromosomes in some oocytes were dispersed in the cytoplasm during cooling–warming. However, mouse oocytes can recover their spindles after rewarming (Pickering & Johnson, 1988; present data). These results indicate that spindles in mouse oocytes are more stable than those in other animals and a short exposure to room temperature does not affect the oocyte's ability to recover its spindles. In the present study, we found that 7% of oocytes cooled to room temperature did not

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recover their spindles completely, and misaligned chromosomes were found only in those oocytes cooled for 40–50 min. It is clear that spindle architecture altered the configuration of chromosomes and that limited (incomplete) recovery of the spindles may result in abnormal chromosome distribution. Although we did not inseminate these oocytes to examine their fertilization potential and embryo development in the present study, it is possible that these oocytes may fertilize abnormally. It has been found that aneuploid formation in human oocytes after insemination *in vitro* is partially due to abnormal spindle formation during oocyte maturation, and advanced maternal age is a major reason for this (Battaglia *et al*., 1996). Human oocytes without a birefringent spindle had significantly lower fertilization and developmental competence than those with a birefringent spindle. However, we still do not know the reason why approximately 20% of human oocytes do not have a birefringent spindle (Wang *et al*., 2001*b*; Moon *et al*., 2003). Factors such as oocyte ageing and suboptimal manipulation (temperature fluctuations) have been suggested (Wang *et al*., 2001*a*,*b*; Wang & Keefe, 2002), which was the same as the results observed in the present study with mouse oocytes.

The response of microtubules in meiotic spindles to cooling was different between oocyte stages. Sheep oocytes at M-I to A-I stages cooled below 29 °C had the least degree of disruption, while oocytes undergoing GVBD were particularly sensitive to cooling (Moor & Crosby, 1985). It has been suggested that the sensitivity of microtubules to cooling may be related to some other components in the cytoplasm, such as changes in protein synthesis, protein phosphorylation and membrane transport (Crosby *et al*., 1984). Cooling bovine oocytes at the GV stage for 10 min at 31 °C or 24 °C did not significantly alter the formation of microtubules and meiotic spindles (Wu *et al*., 1999). However, when bovine oocytes at M-II stage were cooled at 25 °C for 5 min, the microtubules in the meiotic spindle started to depolymerize (Aman & Parks, 1994). On the other hand, M-II mouse oocytes that had been subjected to cooling at approximately 25 °C for 60 min and then warmed to 37 °C for 60 min showed a normal spindle organization (Pickering & Johnson, 1987; present data). Also, mouse oocytes subjected to cooling at 4 °C for 60 min followed by 60 min at 37 °C recovered normal spindles in 89% of cases (Pickering & Johnson, 1987). However, limited spindle recovery was observed in human oocytes after cooling–rewarming, which was examined more precisely in individual oocytes with polarized light microscopy (Wang *et al*., 2001*c*). When porcine oocytes at GV stage were cooled to room temperature, and then warmed and exposed to culture, both nuclear and cytoplasmic maturation were significantly reduced (Liu *et al*., 2004). In the present study, we found that M-I spindle is more sensitive to temperature decline than M-II spindle in mouse oocytes, although oocytes at both stages can recover spindles after warming. Overall, it would appear that an oocyte's ability to recover a spindle depends on its stage, and that species-specific differences are present in mammals.

With the PolScope, we can identify not only M-II spindles but also M-I spindles in oocytes. In the present study, oocytes at M-I or M-II stage were cooled to room temperature for just 10 min in order to measure subtle differences in maximum retardance. We found that cooling M-II oocytes to 30 °C did not affect microtubule quantity in the spindles as maximum retardance was not reduced significantly. However, when M-I oocytes were cooled to 30 °C, the maximum retardance in the spindles was reduced significantly, to about half of that at 37 °C. These results indicate that the M-I spindle is more sensitive to a temperature decline than the M-II spindle. The differences in the results at 25 °C between M-I and M-II oocytes also supported such a conclusion. When oocytes at M-II stage were cooled to 25 °C, the maximum retardance was reduced to about 60% of that at 37 °C. However, when oocytes at M-I stage were cooled to 25 °C, the maximum retardance was reduced to about 30% of that at 37 °C. Although we still do not know the reason why spindles at different oocyte stages have different abilities to tolerate temperature changes, as discussed above other cytoplasmic components may participate in the regulation of spindle sensitivity to environment change.

Many experiments have been conducted to examine the effects of low temperature on meiotic spindle dynamics (Moor & Crosby, 1985; Pickering *et al*., 1990; Aman & Parks, 1994; Wang *et al*., 2001*c*; Liu *et al*., 2003; 2004). However, little attention has been paid to the effects of high temperature. Previous studies using of bovine oocytes indicated that heating oocytes at M-II stage to 40.5–43 °C did not affect oocyte morphology (Kawarsky & King, 2001) and subsequent fertilization or early embryo development (Ju *et al*., 1999). In the present study, we heated mouse oocytes at both M-I and M-II stage up to 41 °C and found that spindle morphology and the maximum retardance of the spindles were not changed. However, recently we found that the meiotic spindles in human oocytes at M-II stage were also sensitive to a rise in temperature, and abnormal spindles were observed when the temperature was increased to 39 °C (Sun *et al*., 2004). It was suggested that spindles in human oocytes are more sensitive to higher temperature than lower temperature (Sun *et al*., 2004). From these studies, it would appear that species-specific differences also exist among animals. It is probable that the thermolability of oocytes is related to the presence heat shock proteins in the oocytes (Ju *et al*., 1999; Kawarsky & King, 2001).

In summary, our present study indicates that spindles in living mouse oocytes at both M-I and M-II stage can be imaged by the PolScope and that this imaging system is useful for the study of spindle dynamics in living oocytes. Our results also indicate that the presence of a birefringent spindle in oocytes is related to oocyte age and temperature changes. Ageing and cooling can induce spindle disassembly in both M-I and M-II oocytes, but M-I spindle is more sensitive to a temperature decline than M-II spindle, suggesting that other cytoplasmic components may be related to the sensitivity of microtubules to temperature.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 30225010).

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