Review

Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF

Dr David Keefe

Dr David Keefe serves as Medical Director of the combined IVF programmes at New England Medical Center in Boston, Massachusetts, and Women and Infants Hospital in Providence, Rhode Island. Dr Keefe also serves on the faculty as an Associate Professor at Tufts and Brown University Medical Schools. He graduated from Georgetown University School of Medicine and completed his residency in Obstetrics and Gynecology and fellowship in Reproductive Endocrinology/Infertility at Yale University School of Medicine. He received research training through an NIH-funded grant in the Reproductive Biology Training Programme at Northwestern University and as the Kennedy–Dannreuther Research Fellow at Yale. Previously, he also trained in psychiatry at Harvard and University of Chicago. His research at Brown focuses on reproductive ageing in women and on the molecular and cellular basis of oocyte dysfunction. Before joining the Faculty at Brown, Dr Keefe was at Yale, where he directed the Oocyte Donation Programme and directed a laboratory funded by a Clinical Investigator Award from the NIH.

David Keefe[1], Lin Liu, Wei Wang, Celso Silva
Division of Reproductive Medicine and Infertility, Women and Infants Hospital, Providence, Rhode Island; Division of Reproductive Endocrinology, Tufts New England Medical Centre, Boston, MA; Laboratory for Reproductive Medicine, Marine Biological Laboratory, Woods Hole, MA, USA
[1]Correspondence: Department of Obstetrics and Gynecology, Women and Infants Hospital, 101 Dudley Street, Providence, RI 02905, USA. Tel: +1 401 2741122 ext. 1564; Fax: +1 401 4537599; e-mail: dkeefe@wihri.org

Abstract

Meiotic spindles tether the chromosomes of oocytes and have been found to be structurally abnormal in older women. Conventional methods to image the meiotic spindle, such as immunostaining or transmission electron microscopy, require prior fixation, so they cannot be used clinically, and their utility in developmental studies is limited. Spindles can also be imaged non-invasively based on their birefringence, an inherent optical property of highly ordered molecules, such as microtubules, as they are illuminated with polarized light. Polarized light microscopy has been gainfully applied to embryology for decades, but recently a digital, orientation-independent polarized light microscope, the polscope, has demonstrated the exquisite sensitivity needed to image the low levels of birefringence exhibited by mammalian spindles. Its use of nearly circularly polarized light also produces orientation-independent measures of spindle birefringence, thus providing a method to quantify spindle architecture in living oocytes. The safety and utility of polscope imaging has been demonstrated in mammalian oocytes, including those from women undergoing ICSI. Spindle imaging with the polscope provides structural information closely related to the more invasive immunostaining method, and also enables study of the dynamic architecture of spindles. Profound effects of cooling on meiotic spindles have also been shown, and polscope imaging has been used to optimize thermodynamic stability of oocytes during ICSI. It has been shown that embryos derived from oocytes with normal, intact meiotic spindles exhibit superior development after fertilization and in-vitro culture. The mechanisms underlying age-related disruption of meiotic spindles in women remain unclear, but may relate to factors residing within the chromosomes themselves, since mice engineered to shorten their telomeres exhibit structurally abnormal spindles in their oocytes, and their embryos undergo cell cycle arrest and apoptosis, a phenotype remarkably similar to that observed in oocytes and embryos from older women. A time-lapse video of a mouse oocyte imaged by polscope may be purchased for viewing on the internet at www.rbmonline.com/Article/824 (free to web subscribers).

Keywords: *IVF, meiosis, oocytes, reproductive ageing, spindles*

Introduction

At the time of ovulation, the mammalian oocyte is arrested at metaphase II of the meiotic cell cycle, when chromosomes are tethered by microtubule fibres of the meiotic spindle. During meiosis and fertilization meiotic spindles are responsible for proper segregation of the nuclear material, and abnormalities in this fragile structure can lead to infertility, miscarriage and genetic diseases, such as Down syndrome.

Up to 80% of aneuploidies found in embryos have their origin in the oocyte (Hassold and Hunt, 2001) and some evidence suggests that the physical state of the spindle reflects its function. Moreover, increased maternal age, the single most important predictor of female fertility, is associated with disruption of the spindle architecture (Battaglia *et al*., 1996).

Conventional methods of imaging the spindle (e.g. fluorescence labelling techniques) are invasive and not compatible with clinical use. On the other hand, a new orientation-independent polarized light microscope, the polscope, reveals the spindle's architecture non-invasively

(Oldenbourg, 1999; Liu *et al*., 2000). Instead of using exogenous dyes or exposure to damaging levels of light, the polscope generates contrast to image spindles based on a fundamental optical property of the spindle's molecules, called birefringence.

Reproductive wastage is a universal characteristic of biology, with all forms of life devoting enormous energies toward production of germ cells far in excess of the number that eventually develop into a new adult capable of repeating the life cycle. Ovaries of mammals, including women and domestic animal species, contain hundreds of thousands of germ cells at birth, the majority of which never ovulate, but rather undergo atresia at various stages of follicle development, before puberty or in adult life. Understanding the basic mechanisms that control the natural selection of the relatively few oocytes that are ovulated could provide key insights into how best to tap this enormous genetic resource. Applied to women, non-invasive spindle imaging could produce new insights into causes of oocyte dysfunction, lead to improved diagnosis of infertility, and reduce the risk of high multiple gestations generated by empiric infertility therapies.

Why focus on the condition of the oocyte as a way to improve IVF success rates?

While most viability scoring systems currently used in IVF laboratories focus on embryo morphology, oocytes are the progenitors of embryos and over 80% of aneuploidies that appear in preimplantation embryos originate in the oocyte (Hassold and Hunt, 2001). Finally, spindle structure appears to be a key determinant of oocyte potential.

Evaluation of oocyte quality has been difficult in humans. Attempts to estimate oocyte development potential demonstrate a number of morphologic features associated with poor developmental potential, such as darkness, granularity, vacuoles, fragmentation and irregularity (Bolton *et al*., 1989) but in fact standard imaging techniques used in the IVF laboratory do not provide a sensitive method of diagnosing oocyte dysfunction. Moreover, the pathobiological basis of these morphological markers is unclear.

IVF offers a unique opportunity to study the role of the meiotic spindle in human oocyte developmental potential, because oocytes are ovulated at the MII stage of development, when the chromosomes are poised on the metaphase plate, tethered by microtubules that are inherently unstable, and more birefringent than other structures in the oocyte. In patients who undergo immature oocyte retrieval and IVM (Cha and Chian, 1998), the MI spindle also is available for analysis. Unfortunately, the imaging methods currently used in the IVF laboratory, i.e. Hoffman, Nomarski or bright field microscopy, cannot image the meiotic spindle clearly (Oldenbourg, 1999) (**Figure 1**).

A previous study (Battaglia *et al*., 1996) compared spindles of oocytes from two groups of women, aged 20–25, and aged 40–45 years, using immunofluorescence, and found that meiotic spindles from older women exhibited significantly more abnormalities in chromosome placement and structure. In the older group, 79% of oocytes from the older group exhibited abnormal spindle structure, including abnormal tubulin placement and displacement of one or more chromosomes from the metaphase plate. In the younger group, only 17% exhibited such abnormalities. Spindles in the younger group appeared well ordered, and held chromosomes aligned on the metaphase plate. These data suggests that the architecture of meiotic spindles is altered in older women, possibly explaining their higher prevalence of aneuploidy.

While intriguing, these results originated from experiments that destroyed the oocytes by fixing and staining them and illuminating them with intense, high frequency light. Moreover, because it employed invasive imaging, it could not link spindle architecture to developmental outcome of individual oocytes.

Imaging spindles non-invasively with the polscope

Oocytes, like most living cells, are almost entirely translucent when viewed with a standard optical microscope (**Figure 1**). The meiotic spindle is not too small to be imaged by conventional light microscopy, but rather lacks contrast, making it necessary to employ methods to enhance contrast of the spindle against the translucent cytoplasm. Nomarski (also called differential interference contrast or DIC), Hoffman, and phase contrast microscopy use optical interference effects to create contrast. While non-invasive, they cannot image spindles (Oldenbourg, 1999). Other imaging methods mark spindles with exogenous, absorptive coloured stains or

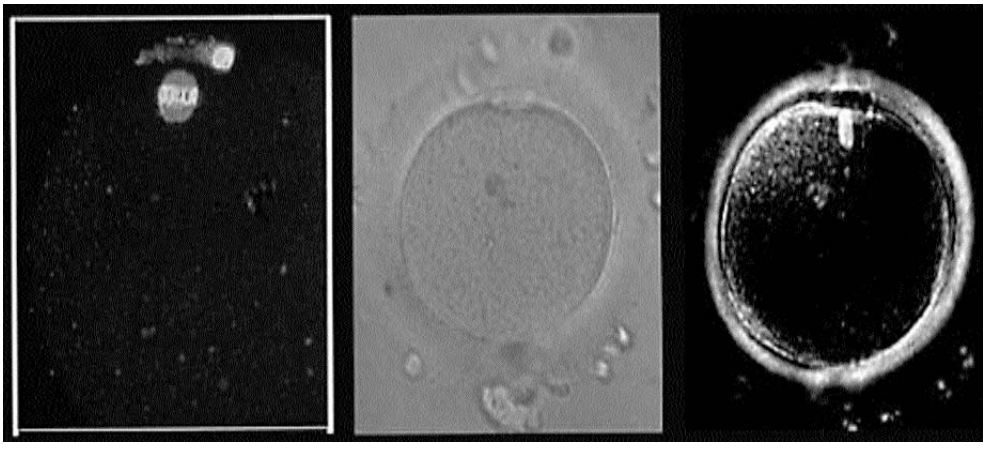

Figure 1. Spindle imaging in human oocytes by confocal (left), Nomarski differential interference contrast (DIC) (centre) and polscope microscopy (right).

fluorescent labels (**Figure 1**). While producing the high level of contrast needed to image the spindle within the oocyte, these latter methods kill the oocyte or disrupt its function, and therefore, provide limited value for clinical and/or developmental studies.

Birefringence is an optical property that derives from the molecular order found in such macromolecules as membranes, microtubules, microfilaments, and other cytoskeletal components (Sato *et al*., 1975; Oldenbourg, 1999). Polarized light microscopy has the unique potential to visualize and measure birefringent structures, such as spindles, dynamically and non-destructively in living cells. However, the low sensitivity of conventional polarized light microscopes make them marginally suitable for application to mammalian experimental and clinical embryology (Oldenbourg, 1999). Polarized light microscopy measures birefringence as retardance, which arises when the optical paths between two orthogonal, polarized light beams are differentially slowed as they pass through highly ordered molecules, such as microtubules, within the specimen. Birefringent objects, such as spindles, present differences in the paths encountered by polarized light beams as they traverse the specimen. Compared with non-biological materials, the birefringence of biological samples is weak, only a few nanometres, so the low level of birefringence in biological specimens requires the use of manually adjusted compensators and rotating stages, a complicated procedure (Sato *et al*., 1975; Oldenbourg, 1999), which is prohibitively slow for clinical applications. Moreover, quantification of retardance levels, necessary to compare spindles among oocytes, is complicated when using conventional, plane polarized light microscopes because the observed image originates from both the inherent birefringence of the specimen and the settings of the manually adjusted compensator and analyser.

The polscope uses digital image processing to enhance sensitivity, and nearly circularly polarized light, combined with electro-optical hardware, to achieve orientation independence, so even the low levels of retardance of mammalian spindles can be measured without the confounding orientation dependence (Oldenbourg, 1999). Colour-coded Doppler (CCD) technology, liquid-crystal compensator optics and computer algorithms are used to quantify birefringence magnitude (i.e. retardance) and orientation (called azimuth) at every image point in the field of view. The polscope's orientation independence enables quantification of retardance magnitude and azimuth of spindle fibres within microtubules, because differences in these parameters result from the tissue itself rather than settings of the compensators and stages. The polscope has been described previously (Oldenbourg 1999), however, since its application to mammalian embryology is still in its infancy it will be reviewed briefly here.

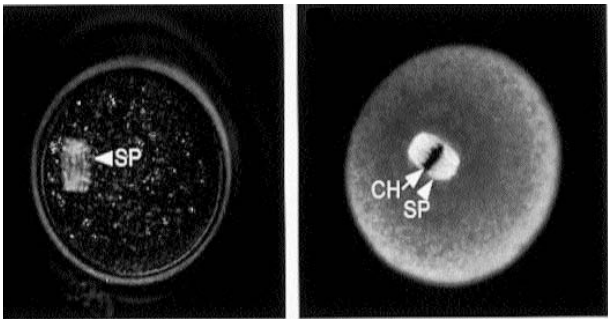

Figure 2. Comparison of spindle imaging by polscope (left) and fluorescence microscopy (right).

To produce a retardance image, the polscope generates four intensity images (which are in perfect register because there are no moving parts) at four liquid crystal compensator settings. This gives four numbers at each pixel of the 480 × 640 pixel image. These four values are used in a ratio metric calculation to determine the sample's retardance and azimuth at each pixel. An additional four images, without the sample in the optical field, are taken to serve as a background correction. Quantification of the specimen's retardance is carried out by grayscale thresholding. With this strategy, the polscope can measure retardances of as little as 0.05 nm, or 0.03 degrees of phase change (which corresponds to 10^{-4} wavelengths of light). In comparison, conventional polarized light techniques have a retardance sensitivity limit of 5–10 nm, barely enough to image the meiotic spindle of mammalian oocytes, which has a retardance of approximately 3 nm in mouse oocytes Moreover, since it illuminates specimens with the same intensity of light as used for Nomarski DIC, and since Nomarski DIC, also employs polarized light, the polscope should be non-toxic to oocytes. Indeed, no detrimental effects have been demonstrated on mouse or human oocytes or on mouse embryo developmental potential following exposure to the polscope (Wang *et al*., 2001a).

In collaboration with Dr Oldenbourg of the Marine Biological Laboratory at Woods Hole, Massachusetts (MBL), the polscope was adapted for use in mammalian embryology. First, the optical path of the polscope was optimized for imaging with an inverted microscope. Next, a number of unique features of oocyte morphology were observed, including a laminar structure of the zona pellucida, as well as the meiotic spindle (Keefe *et al*., 1997; Silva *et al*., 1997) of mouse and hamster oocytes with the polscope at the Women and Infants Laboratory for Reproductive Medicine at the Woods Hole Marine Biological Laboratory. After the safety of polscope imaging had been established in animals, polscopes were installed at IVF centres at Women and Infants Hospital in Providence, RI and at Tufts-New England Medical Centre in Boston to study human oocytes and embryos. Hoffman or Nomarski DIC microscopy reveals the position of the polar body, but not the meiotic spindle. Since clinical embryologists use the polar body to orient the injection site during ICSI, it was examined whether the location of the spindle could be predicted by the position of the polar body. Discordance was identified between the location of the spindle and the polar body in oocytes from hamster, mouse and humans (Silva *et al*., 1999; Liu *et al*., 2000) (**Figure 2**). To perform these experiments, oocytes were adjusted until the first polar body and spindle appeared in the same optical plane. Polscope images were saved and the angle formed between these two structures calculated by Metamorph (Universal Imaging, Chester, PA, USA). Using the polscope, it was found that the polar body does not predict accurately the location of the meiotic spindle in mouse, hamster or human oocytes. In only five of 18 hamster eggs did the polar body accurately predict the placement of the meiotic spindle. For approximately 30%

of the oocytes, if the polar body had been used to direct ICSI the needle would have approached the meiotic spindle. The risk of injury to spindle or chromosomes during needle insertion must be small, because the needle itself is so small relative to the oocyte, but injury would be more likely to occur when the oocyte's cytoplasm is aspirated just before the spermatozoon is injected. The lack of close relationship between the spindle and the polar body in MII oocytes raises the question of whether oocytes should be oriented on the holding pipette based on the location of the spindle rather than of the polar body. It also introduces the question of whether 'oocyte dysfunction' identified in some oocytes could result from abnormalities in the establishment of normal polarity during oogenesis.

The zona pellucida has a multilaminar structure

Since the zona pellucida is composed of three related, filamentous glycoproteins (ZP1, ZP2, ZP3) it was postulated that it would exhibit a high level of birefringence. The impairment of hatching identified in embryos after in-vitro culture also begged the question of whether the artificial conditions associated with IVF might change the biophysical properties of zona proteins and thus alter the retardance and/or azimuth of specific zona layers. When imaged under Hoffman or Nomarski DIC optics, the zona pellucida appears as a uniform, thick layer. However, when imaged with the polscope, the zona exhibits a multilaminar structure, with three layers differing in their degree of retardance and orientation (Keefe *et al*., 1997) (**Figure 3**). Moreover, when embryos were cultured *in vitro* or *in vivo*, the polscope revealed that in-vitro cultured embryos exhibited impaired thinning of the inner layer of the zona. Culturing embryos appears to disrupt the normal process of zona thinning by selectively affecting the inner layer of the zona, visible only with the polscope (Silva *et al*., 1997).

The polscope does not disrupt pre-implantation mouse embryo development

Since the polscope uses light of approximately the same intensity as Nomarski DIC and Hoffman (unpublished observation) and since Nomarski DIC uses polarized light, yet exerts no significant detrimental effects on embryo development, it was postulated that the polscope would be safe for embryos. To test this hypothesis, the implantation rates of mouse embryos following imaging with the polscope were compared with control embryos not imaged with the polscope, after transfer into the uterus of pseudo-pregnant young recipients. Results confirmed that the light exposure levels encountered during polscope operation have no apparent affect on blastocyst rate, cell number or pregnancy rates after transfer (Liu *et al*., 2000).

Spindle observation in living human oocytes with polarized microscope

Spindles imaged by immunofluorescence of human oocytes appear to be smaller than spindles from mouse oocytes, so it was necessary to determine whether the polscope could image spindles in human oocytes. Human oocytes aspirated from stimulated ovaries of consenting patients undergoing oocyte retrieval for ICSI were examined. Five hundred and thirty-three oocytes from 51 cycles were examined by the polscope. Spindles were imaged in 61.4% of oocytes (Wang *et al*., 2001b). Interestingly, after ICSI, more oocytes with spindles fertilized and developed normally than oocytes lacking spindles (Wang *et al*., 2001b).

It was also necessary to confirm that the polscope was safe for human oocytes, so an observational study was conducted to compare development *in vitro* of embryos that either had or had not been imaged with the polscope at the oocyte stage of development (Wang *et al*., 2001a). The average age was 33.3 ± 3.9 years for patients whose oocytes were exposed to the polscope and 34.7 ± 3.7 years for the patients whose oocytes were not exposed to the polscope. Three hundred and thirty seven oocytes from 35 cycles were examined with the polscope, and spindles were imaged in 59.3% of oocytes. The fertilization rate of imaged oocytes was 62.3%, the same as that (64.0%) of oocytes (433 from 52 cycles) that had not been exposed to the polscope. The rate of viable embryos (57.1%) from oocytes that had been exposed to the polscope did not differ statistically from that of oocytes that had not been exposed to the polscope (59.6%). These results indicate that exposure of human oocyte to polscope for spindle images is not detrimental to oocytes.

Comparison of polscope and confocal microscope images of spindles from oocytes

The polscope measures spindle retardance while immunocytochemistry directly images immunolabelled-microtubules, so it was necessary to compare spindles imaged by both methods. Two sources of oocytes were used: unfertilized oocytes from mice (**Figure 2**) and spare human oocytes from women undergoing ICSI (**Figure 1**). While immunocytochemistry provided greater detail of spindle microtubules, excellent overlap could be seen when the same mouse oocytes were imaged with the polscope before fixation

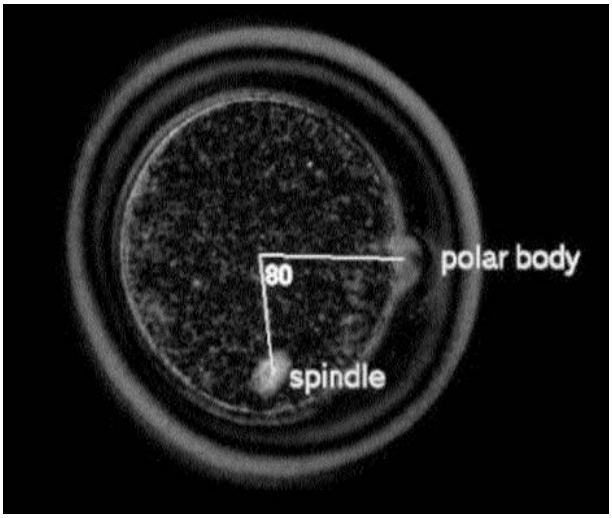

Figure 3. Examination of a hamster oocyte by polscope. The polar body does not always predict the location of the spindle. The trilaminar structure of the zona pellucida can be seen.

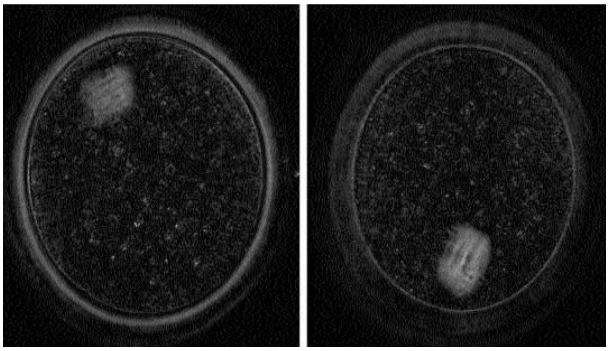

Figure 4. Time lapse video of a mouse oocyte, imaged by the polscope during activation with strontium. Non-invasive imaging with the polscope allows study of the architectural dynamics of spindle birefringence. Also note the multilaminar appearance of the zona pellucida. The layers suggest different levels of retardance (molecular organization) within the inner, middle and outer zona layers. In another mode (not shown), the polscope can measure azimuth (the direction of the long axis of macromolecules). Studies of zona pellucida's azimuth demonstrate that macromolecules within the intermediate layer are oriented nearly randomly. Those from the outer layer are oriented tangentially relative to the oocyte.

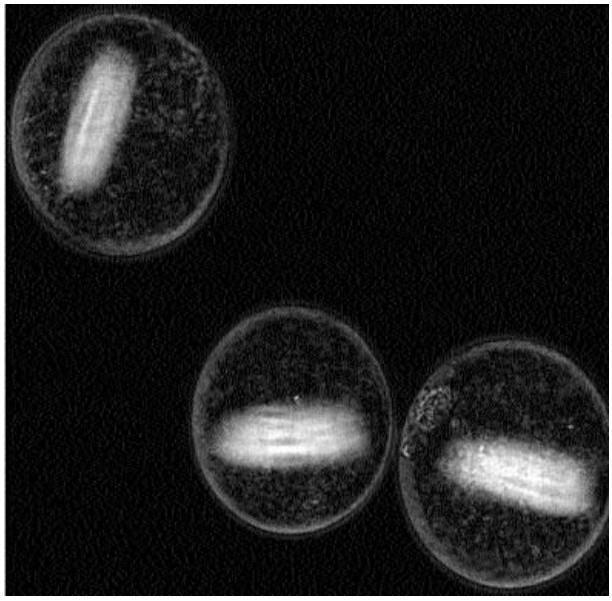

Figure 5. Karyoplasts formed by centrifugation with the centrifuge polscope exhibit nearly normal spindle birefringence, and when activated with strontium, exhibit normal karyokinesis and cytokinesis.

and immunostaining (Liu *et al*., 2000) (**Figure 2**). Imaging spare human oocytes, on day 1 after ICSI, spindles could be imaged in 73% oocytes with the polscope (Wang *et al*., 2001b). Interestingly, spindles from aged oocytes were shorter (8.08 ± 0.84 mm) than from fresh oocytes (11.2 ± 3.4 mm). Spindle structure obtained with the polscope was comparable to that imaged by confocal microscopy. Of the 27% oocytes on day 1, and all oocytes from days 2–4, in which no birefringent spindles could be imaged by the polscope, confocal immunostaining also failed to demonstrate meiotic spindles, suggesting that the sensitivities of the two methods to detect the presence of a spindle were comparable. Indeed, oocytes lacking spindle birefringence, exhibited only disassembled microtubules and dispersed chromosomes upon confocal immunostaining.

Culture of immature human oocytes yielded M-I and M-II stage oocytes in which spindles could readily be observed with the polscope. When oocytes with birefringent spindle were fixed and examined by confocal microscopy, 75% of oocytes at M-I and 71% of oocytes at M-II had typical metaphase spindles with two polar bodies. Most oocytes (75%) with birefringent spindles had normal chromosome configuration, which was confirmed by a series of confocal microscopic images. However, all oocytes without a birefringent spindle and 25% of oocytes with a birefringent spindle exhibited abnormal chromosome configurations. Thus, the polscope provides nearly the same information about spindle structure, but is not as sensitive as confocal immunostaining to detect chromosome misalignment.

Spindle microtubules can be quantified by retardance measurements

Because of its orientation independence, the polscope provides quantitative information about spindle structure. Conversely, quantification of spindle structure following fixation and immunostaining is problematic because of the confounding effects of fixation and fluorescence quenching. The quantitative features of the polscope were used to image mouse oocytes during activation and demonstrated increased assembly of microtubules in the spindle's mid-piece, suggesting that the spindle's microtubules may be pushing as well as pulling chromosomes during anaphase (Liu *et al*., 2000).

The feasibility of studying spindle kinetics was also demonstrated. Since polscope imaging is non-invasive, time-lapse polscope imaging of activated mouse oocytes was performed (**Figure 4**). It was also possible to perform time lapse polscope imaging of meiotic spindles in karyoplasts generated either by enucleation (Liu *et al*., 2000) or centrifugation (**Figure 5**).

During oocyte aging in-vitro meiotic spindle structure may deteriorate. Retardance was measured in human spindles, and large differences were found in retardance among oocytes as a function of in-vitro aging. Aged oocytes had smaller spindles with less birefringence than freshly aspirated oocytes (Wang *et al*., 2001a). The quantity of spindle birefringence may prove useful to monitor oocyte quality or cytoplasmic maturation. These results indicate that spindle retardance can be qualified in living human oocytes and that retardance may be an important parameter for evaluation of oocyte quality and to predict cytoplasmic maturation.

Thermodynamic regulation of spindle assembly

Mammalian spindles are remarkably temperature sensitive. Previous studies had demonstrated profound effects of even transient cooling on spindle assembly, so the exquisite

temperature sensitivity of human meiotic spindles was confirmed, and reassembly upon rewarming was demonstrated to be only partial following significant cooling (Wang *et al*., 2001a). Moreover, conventional heating plates employed in most IVF laboratories only poorly maintain thermal stability of oocytes while they are being imaged and injected. A system which maintained rigorous thermal control during ICSI, by thermostaining both the Petri dish and the microscope's objective, nearly doubled the development of embryos to morula and blastocyst stages following the ICSI procedure (Wang *et al*., 2002).

Prediction of aneuploidy by polscope imaging of the meiotic spindle

Abnormal spindle structure has been indirectly implicated in the generation of aneuploidy by the association of advanced maternal age and structurally abnormal spindles (Battaglia *et al*., 1996), and by the obviously important role of the meiotic spindle in partitioning chromosomes during meiotic maturation and fertilization. An association between chromosome misalignment and spindle abnormalities also has been observed in immunostained spindles (Battaglia *et al*., 1996). It was demonstrated that non-invasive imaging of the MI spindle in human oocytes provides an excellent predictor of chromosome misalignment (Wang and Keefe, 2002). It was also demonstrated that abnormal spindle birefringence in oocytes from the senescence-accelerated mouse (SAM), a naturally occurring strain of mouse which exhibits precocious reproductive senescence compared with wild-type strains. Although the retardance of chromosomes is much less than that of microtubules, it was also possible to image chromosomes non-invasively in SAM oocytes and by doing so, higher rates of chromosome misassembly were demonstrated in oocytes from SAM compared with oocytes from wild-type strains (Liu and Keefe, 2002).

How aging disrupts spindles of oocytes from older females remains unclear. It has been postulated that the effects of reproductive aging on oocyte function are mediated by telomere shortening in oocytes ovulated late in the life of the female. Late-ovulating oocytes exited late from the production line during fetal oogenesis (Henderson and Edwards, 1968; Polani and Crolla, 1991), and therefore must have traversed more cell cycles, so they would be expected to have shorter telomeres than oocytes ovulated early in the life of the female, which exited early from oogenesis and therefore traversed fewer cell cycles. Moreover, during the prolonged interval between entry into prophase and completion of meiotic maturation in the female, telomeres anchor the oocyte chromosomes in the nuclear membrane, where they are susceptible to chronic exposure to reactive oxygen species, the inevitable by-products of cellular metabolism, which are thought to accelerate aging in virtually every type of cell. Reactive oxygen species can further shorten telomeres and induce chromosome fusions (Liu *et al*., 2002a) in mouse zygotes. Moreover, mice genetically engineered to have short telomeres exhibit abnormalities in the structure of their meiotic spindles, but only when the telomere length reaches a critical length (Liu *et al*., 2002b). Oocytes with short telomeres also give rise to embryos that exhibit cell cycle arrest and apoptosis (Liu *et al*., 2002c), a phenotype remarkably similar to that of embryos from older women.

References

Battaglia DE, Goodwin P, Klein NA, Soules MR 1996 Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. *Human Reproduction* **11**, 2217–2222.

Bolton VN, Hawes SM, Taylor CT, Parsons JH 1989 Development of spare human preimplantation embryos in vitro: an analysis of the correlations among gross morphology, cleavage rates, and development to the blastocyst. *Journal of In Vitro Fertility and Embryo Transfer* **6**, 30–35.

Cha KY, Chian RC 1998 Maturation in vitro of immature human oocytes for clinical use. *Human Reproduction Update* **4**, 103–120.

Hassold T, Hunt P 2001 To err (meiotically) is human: the genesis of human aneuploidy. *Nature Reviews Genetics* **2**, 280–291.

Henderson SA, Edwards RG 1968 Chiasma frequency and maternal age in mammals. *Nature* **217**, 22–28.

Keefe DK, Tran P, Pelligrini C 1997 Polarized light microscopy and digital image processing identify a multilaminar structure of the hamster zona pellucida. *Human Reproduction* **12**, 1250–1252.

Liu L, Keefe DL 2002 Aging-associated aberration in meiosis of oocytes from senescence-accelerated mouse (SAM). *Human Reproduction* **17**, 2678–2685.

Liu L, Oldenbourg R, Trimarchi JR, Keefe DL 2000 A reliable, noninvasive technique for spindle imaging and enucleation of mammalian embryos. *Nature Biotechnology* **18**, 223–225.

Liu L, Blasco M, Keefe DL 2002a Requirement of functional telomeres for metaphase chromosome alignments and integrity of meiotic spindles. *EMBO Reports* **3**, 230–234.

Liu L, Blasco M, Trimarchi JR 2002b An essential role for functional telomeres in mouse germ cells during fertilization and early development. *Developmental Biology* **249**, 74–84.

Liu L, Trimarchi JR, Smith PJS 2002c Mitochondrial dysfunction leads to telomere attrition and genomic instability. *Aging Cell* **1**, 40–46.

Oldenbourg R 1999 Polarized light microscopy of spindles. *Methods in Cellular Biology* **61**, 175–208.

Polani PE, Crolla JA 1991 A test of the production line hypothesis of mammalian oogenesis. *Human Genetics* **88**, 64–70.

Sato H, Ellis GW, Inoue S 1975 Microtubular origin of mitotic spindle for birefringence. *Journal of Cell Biology* **67**, 501–517.

Silva CP, Silva V, Kommineni K, Keefe D 1997 Effect of in vitro culture of mammalian embryos on the architecture of the zona pellucida. *Biological Bulletin* **193**, 235–236.

Silva CP, Kommineni K, Oldenbourg R, Keefe DL 1999 The first polar body does not predict accurately the location of the metaphase II meiotic spindle in mammalian oocytes. *Fertility and Sterility* **71**, 719–721.

Wang WH, Keefe DL 2002 Prediction of chromosome misalignment among in vitro matured human oocytes by spindle imaging with the polscope. *Fertility and Sterility* **78**, 1077–1081.

Wang WH, Meng L, Hackett RJ, Keefe DL 2001a Developmental ability of human oocytes with or without birefringent spindles imaged by polscope before insemination. *Human Reproduction* **16**, 1464–1468.

Wang WH, Meng L, Hackett RJ *et al*. 2001b The spindle observation and its relationship with fertilization after intracytoplasmic sperm injection in living human oocytes. *Fertility and Sterility* **75**, 348–353.

Wang WH, Meng L, Hackett RJ *et al*. 2002 Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. *Fertility and Sterility* **77**, 1274–1277.

Paper based on a contribution presented at the Serono Symposium 'Towards Optimizing ART: a Tribute to Howard and Georgeanna Jones' in Williamsburg, Virginia, USA, April 2002.

Received 3 December 2002; refereed 20 December 2002; accepted 24 March 2003.