

Oosight™ Imaging System:

Abdelmassih S, Abdelmassih R and Nagy P.Z. 2002. Meiotic spindle location relative to the first polar body is predictive for fertilization rate and for embryo quality: a prospective study of 25 ICSI-IVF cycles. Hum Reprod.V0566 04/04/02. Oral Presentation made at ESHRE 2002, Vienna, Austria.

Introduction: Meiotic spindles in living metaphase II (MII) human oocytes can be imaged non-invasively using PolScope based on their highly birefringent characteristic. It has been recently reported that the absence as well as the abnormal morphology of the spindle may be associated with a lower fertilization rate of oocytes and impaired embryonic development. In a preliminary investigation, we have observed that in many patients a relatively high percentage of oocytes display the meiotic spindle at various distances away from the first polar body. Therefore, the objective of this study was to investigate if the presence of a high proportion of MII oocytes with largely dislocated spindle in an ICSI-IVF cycle is associated with alterations in fertilization rate and embryo quality.

Boutin C, Hoyt C. The New Imaging Technique Applied to Human Reproduction. In: A Color Atlas for Human Assisted Reproduction, Laboratory and Clinical Insights, Philadelphia, PA: Lippincott Williams & Wilkins, 2003: 17-24.

In this chapter, the author's explore the use of a new imaging technique in the in vitro fertilization (IVF) laboratory, the SpindleView™ Imaging System, and address the theory behind its technology, system operation, and current and future applications. A review of applications is included in order to portray how the system is used to expand the utility of the standard microscope in the IVF lab.

Chen C.K., Wang C.W., Horng S.G., Huang H.Y., Wang H.S., Soong Y.K. Observation of meiotic spindles of MII oocytes before and after vitrification and thawing. Oral Presentation made at ASRM 2002, Seattle, WA, USA.

INTRODUCTION: During cryopreservation of MII oocytes, the integrity of meiotic spindles is crucial. Traditionally, the evaluation of the spindles has been through the immunofluorescent stain of tubulin. Polscope has emerged as a tool to view spindles in living oocytes. In this study we assessed the spindle status of oocytes before vitrification using Polscope and correlated their image again by the Polscope after vitrification and thawing. The thawed oocytes were finally assessed with immunofluorescent staining. **METHODS:** **DESIGN:** Prospective, controlled study, **SETTING:** Medical college animal laboratory, **SUBJECTS:** MII oocytes from outbred ICR mice superovulated by PMSG and hCG injections, **INTERVENTION:** The MII oocytes were first imaged by Polscope. They were subsequently undergoing vitrification thawing protocol. The oocytes were vitrified with 5.5 M Ethylene Glycol- and 1 M sucrose-containing Dulbecco phosphate buffered saline. They were loaded into thin walled pulled straw and were stored at liquid nitrogen till thawing. **MAIN OUTCOME MEASURE:** After thawing, the oocytes were evaluated by Polscope again. The spindle images were saved in computer for later analysis. The survived thawed oocytes were then fixed and incubated with anti-tubulin monoclonal antibody. Tubulin was stained by fluorescein isothiocyanate (FITC) conjugated anti-mouse IgG and chromatin was stained with Hoechst 33258. **RESULTS:** Spindle images by Polscope were classified as three groups. Group I oocytes had normal spindles. Group II oocytes had abnormal spindles. Group III oocytes had no image of spindles. Spindle morphology by immunofluorescent staining was classified as normal, reduced or disrupted. Totally, 250 MII oocytes were vitrified. After thawing, 146 oocytes were morphologically intact. Images by Polscope were classified as shown in table. With immunofluorescent stain, 35 were classified as having normal spindle morphology, 71 as having abnormal spindle morphology and 40 as having disrupted morphology. **CONCLUSIONS:** The spindles of oocytes before and after cryopreservation can be assessed using Polscope and the images are well correlated to the results by traditional immunofluorescent staining. MII oocytes with good spindle morphology revealed by Polscope could tolerate the procedure of vitrification and thawing better than those with poor spindle image. Polscope could be used as a tool to select oocytes with potential to withstand the cryopreservation procedure.

Cha JH, Hyun CS, Son WY, Yoon SH, Lim JH. How long is necessary to achieve complete cytoplasmic maturation after nuclear maturation in human oocytes? Fertility and Sterility. Sep 2003 (Vol. 80, Issue (Supplement 3), Page 7).

Chen CK, Wang CW, Tsai WJ, Hsieh LL, Wang HS, Soong YK. Evaluation of meiotic spindles in thawed oocytes after vitrification using polarized light microscopy. Fertil and Steril. Sep 2004 (Vol. 82, Issue 3, Pages 666-72).

Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Lin-Kou Medical Center, Tao-Yuan, Taiwan.

OBJECTIVE: To investigate the efficacy of the PolScope on imaging the spindle morphology in oocytes at the metaphase II stage before vitrification and after thawing. **DESIGN:** In vitro study. **SETTING:** University infertility clinic and academic research laboratory. **INTERVENTION(S):** Oocytes at the metaphase II stage that were obtained from superovulating mice were vitrified and then thawed. **MAIN OUTCOME MEASURE(S):** Morphological features of the spindle in oocytes were evaluated by both the PolScope and immunofluorescent staining. **RESULT(S):** Using the PolScope, the morphological features of the spindle of intact thawed oocytes were undetected by 3 hours of thawing in only 25% of cases. Most of the spindle images were recognized during the first hour of observation. Additionally, the statistical analysis of agreement of spindle morphology by both the PolScope and fluorescent staining showed a weighted Kappa value of 0.70, indicating good agreement. Oocytes with good spindle morphology verified by the PolScope before vitrification had a higher survival rate of intact oocytes

after thawing compared with those with poor or undetected spindle images. **CONCLUSION(S):** The morphological features of the spindle in oocytes evaluated by the PolScope before freezing and after thawing are significantly correlated with those assessed by immunofluorescent staining after fixation. With the assistance of the PolScope, thawed oocytes with good spindle morphology can be verified and selected for further manipulation without fixation and staining.

Chen N, SL Liow, WY Yip, LG Tan, GQ Tong, SC Ng. Early development of reconstructed embryos after somatic cell nuclear transfer in a non-human primate. Theriogenology. DOI: 10.1016/j.theriogenology.2006.04.012

To improve efficiency and assess variation in nuclear transfer techniques in non-human primates, we investigated the following factors: type of donor cell, interval between enucleation and cell injection, activation after electrical pulsing and cytokinesis inhibitors. An average of 16.4 oocytes were recovered from 91 retrievals; however, 15 (14%) additional retrieval attempts yielded no oocytes due to a failure of follicular stimulation. Oocyte maturation rates at 36, 38 and 40h post-hCG were 46.2, 52.6 and 61.2%, respectively. The MII spindle could be seen clearly using polarized microscopy in 89.1% (614/689) of oocytes. Nuclei were seen in 42% of the NT couplets, 53% of those cleaved to the 2-cell stage and 63% of the 2-cell embryos developed to the 8-cell stage by Day 3. There was no difference in the occurrence of nuclear formation between couplets created using fibroblasts or cumulus cells, although embryos were more reliably produced with fibroblasts. The interval (2, 3 and 4h) between enucleation and cell injection did not affect NT efficiency. Ethanol treatment after electrical pulses yielded more 2-cell NT embryos than did treatment with ionomycin, but the frequency of nuclear formation and development to the 8-cell stage was not different. Treatment of couplets with cycloheximide and cytochalasin B for 5h after activation had no impact on NT efficiency.

Cohen Y, M. Malcov, T. Schwartz, N. Mey-Raz, A. Carmon, T. Cohen, J.B. Lessing, A. Amit and F. Azem Spindle imaging: a new marker for optimal timing of ICSI? Human Reproduction. 19(3) 649

BACKGROUND: The LC Polscope facilitates visualization of the meiotic spindle in human oocyte. This study aimed to investigate meiotic spindle assembly in correlation to time elapsed after HCG administration, and to determine whether spindle imaging may serve to indicate the likelihood of fertilization and embryo cleavage. **METHODS:** Metaphase II (MII) oocytes from 103 couples who were being treated for male infertility were imaged with the Polscope prior to sperm injection. Spindle imaging was correlated to time elapsed from HCG administration, fertilization rate and embryo cleavage. The main outcome measures were spindle visualization, fertilization and embryo cleavage on day 3. **RESULTS:** A total of 770 MII oocytes were imaged. A spindle was imaged in a significantly higher number of oocytes from 38 h after HCG administration compared with those in the <38 h group (78.1–81.5% versus 61.6%; $P < 0.001$). The fertilization rate in oocytes with a visible spindle was statistically higher compared with oocytes in which spindle could not be detected (70.4% versus 62.2%; $P = 0.035$). We found no relationship between spindle imaging and embryo cleavage on day 3. **CONCLUSIONS:** Spindle imaging, in addition to first polar body appearance, is an accurate indicator for oocyte maturity. We suggest that spindle imaging be performed prior to sperm injection.

Cohen Y et al. Spindle imaging: a new marker for optimal timing of ICSI? Oral Presentation made at ESHRE 2003, Madrid, Spain.

Objective: IVF-ICSI success depends mainly on: Oocyte maturation and timing of sperm injection. Until now, however, oocyte quality and maturation were assessed by the absence of the GV and the presence of an extruded polar body. Timing of oocyte retrieval and sperm injection was based on a pre-determined, fixed time interval from hCG administration. Currently, the introduction of the Polscope, a non-invasive tool, enables demonstration of the meiotic spindle and cytogenetic estimation of the oocyte meiotic stage in living oocytes. This study was aimed at assessing whether spindle imaging can be used to determine the timing of sperm injection and the prediction of fertilization and embryo quality. **Design:** Prospective randomized study. The Polscope was used to image spindles in nude oocytes prior to sperm injection. Spindle imaging was correlated to: Time elapsed from hCG administration, fertilization rate and embryo quality (on day 3) **Materials and Methods:** Human oocytes, retrieved 36 hours after hCG administration, from women undergoing ICSI. Oocytes were cultured in a P1 medium, supplemented with 16% synthesized serum substitute (SSS), after aspiration. Cumulus cells were removed by hyaluronidase, washed and transferred to mHTF, supplemented with 5% SSS in glass petri dishes, for spindle observation using LC Polscope optics and controller. **Results:** 770 oocytes that extruded their first polar body were retrieved from 103 women. Average age was 33 (+5.6) years. In order to correlate between the time elapsed from hCG administration to spindle imaging, the oocytes were divided into 3 groups: Group-1 was imaged 35-37 hours after hCG administration, group-2 was imaged 38-39 hours after hCG administration and group-3 was imaged 40-42 hours after hCG administration. Spindle imaging in groups 1, 2 and 3 was 61.58%, 81.48% and 78.14% respectively. Spindle imaging in groups 2 and 3 was found to be significantly higher ($p < 0.001$), compared to group-1. In 585 oocytes (75.97%), spindle was imaged (group A) compared to 185 oocytes (24.03%) in which spindle was not imaged (group B). The fertilization rate in group A was 70.43% compared to 62.16% in group B ($p = 0.035$). Embryo developmental stage on day 3 was not statistically different. **Conclusion:** We conclude that meiotic spindle, imaged by the Polscope is a better indicator than the presence of the first polar body, for the determination of oocyte maturity. Furthermore, these results also indicate that the spindle constitutes an additional parameter for predicting future fertilization. Based on our data, we conclude that higher rates of oocyte maturity are achieved from 38 hours after hCG administration and onwards. Consequently, we suggest that the ICSI procedure should be postponed to 38-42 hours after hCG administration.

Cooke S, Tyler JP, Driscoll GL. Meiotic spindle location and identification and its effect on embryonic cleavage plane and early development. Hum Reprod. 2003 Nov; 18(11):2397-405

BACKGROUND: To examine the relationship between the meiotic spindle, the first cleavage plane and any resulting influence on embryonic development parameters. **METHODS:** Sibling oocytes (n = 246) were allocated to either a control [polar body (PB)-aligned] or a treatment (spindle-aligned) microinjection group by use of a random numbers table. Spindles were identified by PolScope((R)) and the early embryo development parameters, and angle of first cleavage plane in relation to a defined animal-vegetal pole were analysed. **RESULTS:** Most oocytes (92.7%) had a visible spindle at the time of microinjection; however, 62.6% of first PBs (1PBs) were not located above the spindle (average deviation 37.3 +/- 33.2 degrees; range 0-176.6), with 6.9% of 1PBs in the opposite hemisphere to the spindle. The second PBs (2PBs) can also have an unpredictable deviation from the position of the meiotic spindle (12.5 +/- 16.7 degrees; range 0-91.8). This increased when the 1PB was above the spindle, forming a physical barrier to extrusion (average 24.7 +/- 16.1 degrees; range 7.9-91.8). Embryos developing from the spindle-aligned microinjection group had significantly more blastomeres per embryo (P = 0.044), a higher morphology score per embryo (P = 0.008) and a significantly higher average embryo score parameter (P = 0.003), with more embryos developing without any detectable fragmentation (P < 0.05) than the PB-aligned control group. Non-fragmented embryos undergo meridional cleavage, with a small angle between the spindle location and first cleavage plane (16.4 +/- 14.0 degrees) compared with embryos with some degree of fragmentation (P = 0.002). This angle increased with the degree of fragmentation, with worst quality embryos having a spindle:cleavage angle of 45.1 +/- 17.7 degrees. **CONCLUSIONS:** The 1PB and, to a lesser degree, the 2PB can be unreliable predictors of the exact meiotic spindle location in human oocytes. Embryos from spindle-aligned oocytes have an increase in all measured development parameters over control siblings. When the animal pole is defined as the meiotic spindle location, non-fragmented embryos tend to develop from a meridional cleavage; with the most fragmented embryos developing from a more equatorial initial cleavage plane. This study proposes that the spindle accurately marks the animal pole in human oocytes, and provides evidence linking the meiotic spindle location to the first cleavage plane and resulting early embryo development parameters in human embryos.

Cooke S, Tyler J, Driscoll G. Meiotic spindle location and identification and its effect on early embryonic development and polarity. Oral Presentation made at ESHRE 2003, Madrid, Spain.

Introduction: It is possible that lower morphology scores seen following ICSI 1, 2 (compared to IVF), may be due to the physical disruption of the oocyte experienced during microinjection, and disruption of ultrastructure components within the cytoplasm such as the meiotic spindle. This randomised prospective trial (utilizing improved culture media 3 and computer analysis to provide quantitative data for morphology grades) will examine the relationship between the meiotic spindle position and early embryonic development parameters. It will also assess any resulting influence on the control of early embryonic cleavage in relation to the animal-vegetal pole of the oocyte, and the morphology of the developing early embryos. **Materials and methods:** Sibling oocytes (n=246) were allocated to either a control (polar body aligned) or a treatment (spindle-aligned) microinjection group by use of a random numbers table. Spindles were identified by PolScope and early embryo development parameters, and the angle of first cleavage plane in relation to the animal-vegetal pole was analysed. **Results:** Of the 124 oocytes allocated to the spindle-aligned group 115 / 124 (92.7 %) had a visible spindle at the time of microinjection, however, 72 / 115 (62.6 %) were not located immediately underneath the 1st polar body, with 8 / 115 (6.9%) in opposite hemisphere. The day 2 embryos developing from spindle-aligned microinjection group have significantly more blastomeres per embryo (3.85 vs. 3.34; P=0.044); a higher morphology score per embryo (3.27 vs. 3.99; P = 0.008) a significantly higher average Embryo Score Parameter 4 (12.66 vs. 10.00; P=0.003); and more embryos develop without any detectable fragmentation (X2 = 4.8; P<0.05) than the polar body aligned control group. Non-fragmented embryos have a reduced angle between the animal-vegetal plane and first cleavage plane than those that formed embryos with some degree of fragmentation (16.39° ? 13.98° vs. 40.30° ? 14.99°; Z = -3.099, P = 0.002), and this angle increased with degree of fragmentation. **Conclusion :** The 1st polar body is a poor predictor of the meiotic spindle location. Embryos from spindle-aligned oocytes had an increase in all measured development parameters over control siblings. If the spindle location and sperm insertion point are controlled, embryos develop with a smaller angle between animal pole and cleavage plane, creating more embryos without any detectable fragmentation. This provides evidence linking oocyte spindle location, oocyte and embryo polarity and the control of early cleavage in human embryos, and the effect on embryo morphology.

Cooke, S, Tyler J, Driscoll G. Oocyte spindle identification and orientation prior to ICSI improves early human embryo development. IVF Australia, 12 Caroline Street, Westmead, NSW, 2145. Oral presentation at the Australian and New Zealand O&G Annual Scientific Meeting, September 2002.

Aim To determine if aligning an oocyte for microinjection based on the maternal spindle location instead of orientation of the first polar body at 12 o'clock improves early embryo development. **Methods** A prospective randomised study allocating sibling oocytes within patients to either a polar body aligned control group (n = 98 oocytes) or spindle aligned (n = 103 oocytes) group for microinjection. The spindle was identified by non-invasive polarised light microscopy (LC-PolScope Pro, CRI) and early development was subsequently assessed 41-42 hours post injection by comparing the number of blastomeres per embryo, embryo score and an embryo score parameter1. Statistical analysis was by Wilcoxon Signed-Rank Test of two related samples. **Results** In this study, 92.2% (95/103) of oocytes in the spindle aligned group had a visible spindle. However,

in 63.2% of these (60/95), the spindle was not located under the first polar body and in 7.4% (7/95) the spindle was visualised in the opposite hemisphere to the polar body. Following microinjection and culture, the spindle-aligned oocytes displayed significantly more blastomeres per embryo (3.97 vs. 3.38; $P=0.028$) and a significantly higher average embryo score parameter (13.43 vs. 11.04; $P=0.008$) than their control siblings. Conclusions The location of the first polar body is a poor determiner of the location of the oocyte spindle. Furthermore, in this preliminary study, non-invasive identification and alignment of the maternal spindle prior to microinjection appears to improve the early embryo development, perhaps by allowing a more ordered organization of the first animal-vegetal cleavage plane and by eliminating disruption of the maternal spindle during the microinjection process. 1 Steer et. al., Hum Reprod 1992; 7 (1); p 117-119.

De Santis L, Cino I, Rabellotti E, Calzi F, Persico P, Borini A, Coticchio G. Polar body morphology and spindle imaging as predictors of oocyte quality. *Reprod Biomed Online*. 2005 Jul;11(1):36-42.

It has been suggested that first polar body (PBI) morphology reflects oocyte competence. Oocytes with an intact normal-sized PBI have been described as generating better day 2 embryos, higher blastocyst yield, and increased pregnancy and implantation rates. In other studies, PBI morphology was found to be unrelated to fertilization rate, embryo quality, and blastocyst formation. In a prospective analysis, the predictive value of the PBI was investigated by comparing the development of oocytes retrieved from intracytoplasmic sperm injection patients and displaying different PBI morphology, classified according to the following characteristics: normal size and smooth surface (I), fragmented (II), rough surface (III), or large size (IV). Fertilization rates were 59, 57, 64 and 60% respectively. No significant differences were found between the various groups. The proportions of high quality (grade A) day 2 embryos were also comparable among groups I-III (14, 12 and 17% respectively), while the low number of grade A embryos in group IV (two embryos) did not allow comparison with the other classes. These data do not suggest that PBI selection can contribute to identification of embryos with high developmental ability. In order to establish alternative criteria for oocyte selection, a metaphase II (MII) spindle analysis was also conducted via Polscope. In oocytes of patients of different age, spindle retardance (which reflects the high order and density of microtubules) was compared with parameters of embryo development. In aged patients, a trend was observed between low retardance and poor embryo quality, although in general the association between retardance and oocyte developmental performance did not reach statistical significance.

Frankfurter, D et al. Follicular dynamics and ovarian stimulation influence meiotic spindle integrity during human IVF. *Fertil Steril* 2001 Sep; 76(3), Suppl. 1:S91, Abst O-242.

Objective: IVF cycle management relies on follicular dynamics, yet their effects on egg and embryo development remain unclear. As spindle integrity affects oocyte ploidy and oocyte ploidy affects egg and embryo competence, we use digital polarized light microscopy (polscope) to non-invasively view meiotic spindles. The polscope images the inherent optical birefringence of microtubules. Previously, we showed that spindle integrity predicts fertilization and blastulation (*Fertil Steril* 2001). Now, we report the effects of cycle specific factors on spindles from human metaphase II (MII) oocytes imaged prior to ICSI. Design: Retrospective cohort study at a university-based IVF center. Conclusions: Spindles tether the metaphase chromosomes and ensure their segregation at fertilization, and thus affect egg and embryo ploidy. Non-invasive imaging of meiotic spindles during IVF showed that stimulation and follicular dynamic factors associated with favorable IVF outcome i.e. PE2, number of lead follicles and late luteal GnRHa down regulation, affect spindle integrity. These results suggest that the polscope may help assess the impact of ovarian stimulation on developing oocytes and therefore help fine tuning IVF cycle management.

Gassner, P et al. Improved selection of developmentally competent oocytes in ICSI cycles using polarized light optics. Oral presentation at ESHRE 2003, Madrid, Spain.

Introduction: Circular polarized light optics (PolScope[®]) has been repeatedly applied to living human oocytes without detrimental side-effects to visualize the meiotic spindle. Recent publications suggested close relationships between the integrity and location of the spindle and treatment outcome in ICSI cycles. The aim of this prospective study was to investigate whether the selection of oocytes for further culture and embryo transfer can benefit from additional information provided by PolScope imaging. Materials and methods: 45 ICSI cycles were randomly assigned to either group A (control, n=21) or group B (PolScope, n=24). Inclusion criteria were as follows: female age <35 years, serum estradiol ≥ 2000 pg/ml and ≥ 10 follicles measuring ≥ 15 mm on the day of ovulation induction. Oocytes were analyzed for MII spindles immediately after cumulus removal and microinjected under conventional optics 1-2 hours later. At the pronuclear stage, up to three oocytes were selected for ongoing culture. Selection was based on oocyte and pronuclear morphology in group A. Spindle characteristics were an additional, decisive criterion in group B, where only oocytes were selected which had been presenting spindles situated close to the first polar bodies the day before. Statistical analyses were performed using t-test, Mann-Whitney test and Fisher's exact test. Results: Meiotic spindles were visible in 86,8% of the 302 oocytes analyzed in group B. Angles between spindles and polar bodies were $\leq 30^\circ$ in 77%, $30-60^\circ$ in 20% and $>60^\circ$ in 3% of the oocytes. No significant differences between groups could be found regarding oocyte numbers (mean values: group A: 16,1; group B: 17,6), proportions of MII stages (67,6 versus 75,1%), fertilization rates (71,6 versus 66,6%), numbers of transferred embryos (2,5 versus 2,6) and cumulative embryo scores (32,2 versus 27,7). There were clear, albeit not significant differences in clinical pregnancy rate (31,6 versus 50,0%) and abortion rate (22,2 versus 7,7%). Conclusion: The PolScope seems to be a valuable tool to improve the clinical outcome of ICSI cycles by active oocyte

selection. Dislocation or absence of the MII spindle might reflect disturbances in oocyte polarity or chromosome segregation and therefore could be related to decreased developmental competence.

Gavin, W et al. Comparison of enucleation methods for the production of transgenic dairy goats by somatic cell nuclear transfer. Oral presentation at The International Embryo Transfer Society meeting, 2003.

Introduction The production of recombinant therapeutics in the milk of transgenic dairy goats offers an alternative method compared to traditional manufacturing methods. While pronuclear microinjection has been the primary technique used to produce transgenic goats, somatic cell nuclear transfer may offer advantages for increasing the efficiency of generating transgenic goats. Typically, enucleation of mammalian oocytes is performed under epifluorescence by aspirating the cytoplasm surrounding the metaphase plate following staining the DNA with Hoescht 33342. However, deleterious effects of the DNA dye and ultraviolet (UV) illumination on the subsequent development of nuclear transfer embryos has been reported. A less invasive technique is available for spindle imaging under circular polarized light coupled with enucleation by the aspiration method, and may eliminate damage caused by the combined dye and UV light. The goal of these experiments was to compare enucleation of caprine oocytes utilizing UV and Hoescht 33342 or polarized light for the production of transgenic dairy goats by somatic cell nuclear transfer. Results: There were no significant differences in oocyte survival following either enucleation or reconstruction (1310 versus 1243 enucleated oocytes survived, or 1223 versus 1176 reconstructed couplets survived), fusion (1029 versus 960 couplets fused) or cleavage (384 versus 334 couplets cleaved) when comparing enucleation under UV or polarized light, respectively, $p > 0.05$. However, there was a tendency for twice as many ongoing pregnancies (Day 55 to 113 of gestation) to be established in recipient does that received nuclear transfer embryos generated using the polarized light compared to UV method of enucleation. Conclusion: The increased frequency of ongoing pregnancies produced from embryos generated under polarized light may result from the absence of the combined dye and UV illumination. In addition, the use of a heated stage required for spindle imaging under polarized light may have contributed to these results. Alternatively, any effects on the karyoplast donors of dye leaking from the oocytes, or UV light scatter during enucleation may have contributed to the reduced frequency of ongoing pregnancies produced from embryos generated under UV light. These results suggest that caprine oocytes enucleated under polarized light generate nuclear transfer embryos capable of establishing early fetal development, and may offer a method for increasing the efficiency of generating transgenic founder goats by somatic cell nuclear transfer.

Inoué, Shinya and Rudolf Oldenbourg. 1998. Microtubule dynamics in mitotic spindle displayed by polarized light microscopy. Molecular Biology of the Cell. 9:1603-1607.

This is a collection of mitotic images, viewed using the CRI PolScope™ in polarized light microscopy, such as the cell plate formation of the African blood lily, the Easter lily, primary spermatocytes of *Pardalophora apiculata* and newt lung cell mitosis. Video is available online at www.molbiolcell.org.

Kanyo K, Konc J, Solti L, Cseh S. Assisted reproductive research: laser assisted hatching and spindle detection (spindle view technique). Acta Vet Hung. 2004;52(1):113-23

Animal experiments are very important for the development of new assisted reproductive techniques (ART) for use in human and animal reproductive medicine. Most technical aspects of reproductive manipulation of humans and animals are very similar, and many components of successful human ART used nowadays have been derived from animal studies. In this study we examined (1) the use of 'non-contact' laser for assisted hatching, (2) whether spindles in living mouse oocytes could safely be imaged/examined by polarisation microscope (polscope) and (3) the influence of environment (e.g. temperature, in vitro culture, etc.) on spindle detection/visualisation. The data of the study presented here show that (1) laser assisted hatching (AH) is a fast, very accurate and safe procedure without any harmful effect on embryo development and it can support very effectively the implantation of embryos, (2) the use of polscope facilitates the evaluation of oocyte quality and the selection of oocytes with spindle, (3) by monitoring the spindle position during intracytoplasmic sperm injection (ICSI), we can reduce spindle damage and increase the chance of fertilisation. Further studies are underway to test the hypothesised connection between spindle birefringence and developmental capacity of oocytes/embryos.

Keefe, David. 1997. Non-invasive assessment of mammalian oocytes and embryo developmental potential. MBL/BUMP Seminar Series. Woods Hole, MA.

Abnormal oocyte developmental potential, i.e. ability to fertilize, divide, hatch and give rise to pregnancy, is an important cause of infertility in some women. Although the incidence of oocyte infertility in the population is known, it plays an important part in the age-related loss of fertility, which is increasing in society because of the tendency for women to delay attempts at childbearing until their middle years. Furthermore, oocyte infertility is one of the most refractory causes of infertility using standard therapies. A non-destructive technique that could be used to assess developmental potential of oocytes and pre-implantation embryos would facilitate the diagnosis of oocyte infertility, and help women decide whether to pursue costly reproductive therapies which depend upon their own oocytes or to pursue alternative such as adoption or oocyte donation. Assessment of embryo morphology currently is the most widely used clinical method of assessing embryo developmental potential, but

conventional morphology grading is limited by its qualitative nature and by its dependence on sub-optimal optical configurations. His research employs digital image processing and analysis to study oocyte and embryo developmental potential in mammals. Recent developments in polarization optics (CRI PolScope™) combined with digital image processing have improved the microscopic resolution of living cells. Furthermore, with the new polarization microscope, microtubules, membranes and filaments within cells are highlighted, without fixation or staining, owing to their optical anisotropy.

Keefe, David, et al. 1997. Polarized light microscopy and digital image processing identify a multilaminar structure of the hamster zona pellucida. Human Reproduction. 12(6):1250-1251.

The zona pellucida (zona) is a glycoprotein coat that envelops the oocyte and embryo, binds sperm during fertilization and facilitates transfer of the embryo through the Fallopian tube. Before implantation can occur, the blastocyst must hatch from the zona. Several lines of evidence suggest that the zona is multilaminar. The authors hypothesized that the multilaminar structure of the zona filaments could be imaged non-destructively with the polarized light microscope. A recent modification of the polarized light microscope (the CRI PolScope™), which combines innovations in polarization optics with novel image processing software, allows measurement of birefringence at all points of the image. Hamster metaphase II oocytes were placed on glass coverslips which replaced the bottom of culture dishes. Imaged under differential interference contrast (DIC) and PolScope optics, then digitized and processed to measure birefringence magnitude and orientation. The PolScope revealed the zona to be divided into outer and inner layers separated by a zona of low retardance. This finding is consistent with filaments in the outer layer oriented tangentially and in the inner layer oriented radically. The multilaminar structure of the mammalian zona suggested by differential lectin binding and by scanning electron microscopy could be imaged non-destructively with the PolScope. Because the PolScope provides a non-destructive method to identify macro-molecular organization of the zona, it may prove useful in developmental studies of hatching and to direct resection of the zona.

Keefe, David, et al. 2000. Rigorous thermal control during ICSI stabilized the meiotic spindle and improves fertilization and pregnancy rates. ESHRE 2000. Bologna, Italy.

Meiotic spindles are formed from microtubules comprised of polymerized tubulin and are exquisitely temperature-sensitive. When an orientation-independent polarized light microscope (PolScope, CRI Boston, MA.) [CRI SpindleView] was used to image spindles non-invasively during human ICSI, a high proportion of oocytes exhibited abnormal or missing spindles and oocytes with abnormal spindles had decreased fertilization rates (Abstract#1, ASRM Annual Meeting, Oct. 1999). Because during handling for IVF and ICSI the oocyte's temperature can drop transiently, we employed a thermistor to measure temperature near the oocyte and compared thermal control with a conventional heating stage and a novel heating stage (Bioptech Delta T, Butler, PA). The rigorous thermal control produced by a novel heating system stabilized spindles and increased the fertilization and clinical pregnancy rates achieved after ICSI. Without the objective heater, the objective lens at ambient temperature cooled the egg as it was brought into focus, even if the dish itself was warmed. The objective heater overcame this heat sink effect of the objective lens and stabilized the oocyte's temperature. Although microtubules can repolymerize after transient cooling, presumably the function of the reconstituted spindles can be disrupted, since the resulting pregnancy rates were half those produced by rigorous thermal control during ICSI. Imaging the meiotic spindle with the PolScope provides an intracellular thermostat during ICSI.

Keefe D, Liu L, Wang W, Silva C. Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF. Reprod Biomed Online 2003 Jul-Aug;7(1):24-9. Review

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).

Kilani SS, Cooke S, Kan AK, Chapman MG. Do age and extended culture affect the architecture of the zona pellucida of human oocytes and embryos? *Zygote*. 2006 Feb;14(1):39-44.

Advanced female age and extended in vitro culture have both been implicated in zona pellucida (ZP) hardening and thickening. This study aimed to determine the influence of (i) the woman's age and (ii) prolonged in vitro culture of embryos on ZP thickness and density using non-invasive polarized light (LC-PolScope) microscopy. ZP thickness and density (measured as retardance) were determined in oocytes, embryos and blastocysts in women undergoing intracytoplasmic sperm injection (ICSI) in two age groups (older, > 38 years; younger, < or = 38 years). A total of 193 oocytes from 29 patients were studied. The younger group contained 100 oocytes and the older group 93 oocytes. The ZP was significantly thicker in metaphase II oocytes in the older group compared with the younger group (mean +/- SD: 24.1 +/- 2.5 microm vs 23.1 +/- 3.3 microm; $p = 0.01$) but ZP density was equal (2.8 +/- 0.7 nm). By day 2 of culture, embryos from the two groups had similar ZP thickness (22.2 +/- 2.2 microm vs 21.7 +/- 1.6 microm; $p = 0.28$) and density (2.9 +/- 0.7 nm vs 2.8 +/- 0.8 nm; $p = 0.57$). For the embryos cultured to blastocyst (older: $n = 20$; younger: $n = 18$) ZP thickness was similar in the two groups (19.2 +/- 2.7 microm vs 19.1 +/- 5.0 microm; $p = 0.8$) but thinner than on day 2. The older group had significantly denser ZP than the younger group (4.2 +/- 0.5 nm vs 3.3 +/- 1.0 nm, $p < 0.01$). Blastocysts from both groups had significantly denser ZP than their corresponding day 2 embryos (older: 4.2 +/- 0.5 nm vs 2.9 +/- 0.7 nm, $p < 0.001$; younger: 3.3 +/- 1.0 nm vs 2.8 +/- 0.8 nm, $p = 0.013$). It is concluded that there is little relationship between ZP thickness and its density as measured by polarized light microscopy. While ZP thickness decreases with extended embryo culturing, the density of the ZP increases. ZP density increases in both age groups with extended culture and, interestingly, more in embryos from older compared with younger women.

LaFountain JR Jr, Oldenbourg R, Cole RW, Rieder CL. 2001. Microtubule Flux Mediates Poleward Motion of Acentric Chromosome Fragments during Meiosis in Insect Spermatocytes. *Mol Biol Cell* 2001 Dec;12(12):4054-4065.

We applied a combination of laser microsurgery and quantitative polarization microscopy to study kinetochore-independent forces that act on chromosome arms during meiosis in crane fly spermatocytes. When chromosome arms located within one of the half-spindles during prometaphase or metaphase were cut with the laser, the acentric fragments (lacking kinetochores) that were generated moved poleward with velocities similar to those of anaphase chromosomes ($\sim 0.5 \mu\text{m}/\text{min}$). To determine the mechanism underlying this poleward motion of detached arms, we treated spermatocytes with the microtubule-stabilizing drug taxol. Spindles in taxol-treated cells were noticeably short, yet with polarized light, the distribution and densities of microtubules in domains where fragment movement occurred were not different from those in control cells. When acentric fragments were generated in taxol-treated spermatocytes, 22 of 24 fragments failed to exhibit poleward motion, and the two that did move had velocities attenuated by 80% (to $\sim 0.1 \mu\text{m}/\text{min}$). In these cells, taxol did not inhibit the disjunction of chromosomes nor prevent their poleward segregation during anaphase, but the velocity of anaphase was also decreased 80% ($\sim 0.1 \mu\text{m}/\text{min}$) relative to untreated controls. Together, these data reveal that microtubule flux exerts pole-directed forces on chromosome arms during meiosis in crane fly spermatocytes and strongly suggest that the mechanism underlying microtubule flux also is used in the anaphase motion of kinetochores in these cells.

Liu, Lin. et al. 2000. A reliable, noninvasive technique for spindle imaging and enucleation of mammalian oocytes. *Nature Biotechnology*. 18(2):223-225.

Factors affecting the efficiency of animal cloning remain to be elucidated. Enucleation of recipient oocytes is a critical step in cloning procedures and typically is performed by aspirating a portion of the cytoplasm underlying the first polar body. Enucleation is evaluated using epifluorescence after Hoechst staining for DNA, which may disrupt functions of the cytoplasm, especially mitochondria. Mitochondrial DNA in Dolly and other cloned sheep has been shown to derive exclusively from recipient oocytes. Not only might evaluation of the aspirated karyoplast portion inadequately reflect the state of the cytoplasm, it is also time consuming. Here the authors report a reliable, noninvasive technique for spindle imaging and enucleation of oocytes using a new microscope, the PolScope. The efficiency of enucleation was 100%, and only 5.5% of the oocytes' mitochondria entered the karyoplast upon PolScope-directed removal of the spindle. Moreover, PolScope imaging of spindles and micromanipulation did not compromise the developmental competence of reconstituted oocytes and cytoplasts.

Liu, Lin. et al. 2000. Increased birefringence in the meiotic spindle provides a new marker for the onset of activation in living oocytes. *Biology of Reproduction*. 63:251-258.

The newly developed Pol-Scope allows imaging of spindle retardance, which is an optical property of organized macromolecular structures that can be observed in living cells without fixation or staining. Experiments were undertaken to examine the changes in meiotic spindles during the initial stages of activation of living mouse oocytes using the Pol-Scope. Parthenogenetic activation of oocytes treated with calcium ionophore evoked a dynamic increase in meiotic spindle retardance, particularly of the midregion, before spindle rotation and second polar body extrusion. The pronounced increase in spindle retardance, which could, for the first time to our knowledge, be quantified in living oocytes, was maintained during polar body extrusion. Spindle retardance of newly in vivo fertilized oocytes was significantly higher than that of ovulated, metaphase II oocytes. Pol-Scope imaging of fertilized oocytes did not affect subsequent development. These results establish that increased spindle

retardance precedes polar body extrusion and pronuclear formation. The increased birefringence in the spindle provides an early indicator of oocyte activation. Thus, noninvasive, quantitative imaging of the onset of activation in living oocytes might improve the efficiency of assisted fertilization and other embryo technologies.

Liu L, Keefe DL. Ageing-associated aberration in meiosis of oocytes from senescence-accelerated mice. Hum Reprod 2002 Oct;17(10):2678-85

BACKGROUND: The senescence-accelerated mouse (SAM) has been shown to exhibit ageing-associated mitochondrial dysfunction and oxidative stress, and early decline in fertility. **METHODS:** We compared meiotic progression of germinal vesicle oocytes between young (2-3 months) and old (10-14 months) SAM mice using triple immunostaining and fluorescence microscopy as well as Pol-Scope imaging. **RESULTS:** At 8-9 h of in-vitro maturation (IVM), most young SAM oocytes (86%, 32/37) were at meiosis I (MI) stage, with chromosomes aligned in the mid-region of MI spindles, whereas disrupted MI spindles and/or chromosome misalignments (45%, 18/40) and a few oocytes (20%, 8/40) with abnormal MII spindles were found in old SAM oocytes. At 15-17 h of IVM, old SAM oocytes, despite errors at MI stage, extruded a first polar body at an incidence of 88% (n = 85), which did not differ from that (92%, n = 106) of young SAM oocytes. However, oocytes from old SAM (64%, 32/50) showed aberrant MII, with chromosome misalignment and dispersal, in contrast to normal MII in most young SAM oocytes (87%, 65/75), showing chromosome alignment at the metaphase plate of MII spindles. Moreover, Pol-Scope imaging non-invasively detected disrupted or absent visible spindles and possibly aberrant chromosome alignment. **CONCLUSIONS:** Spindle disruption and/or chromosome misalignments at both MI and MII are associated with maternal ageing in the SAM mouse. Our findings also suggest that meiotic division lacks a competent checkpoint for spindle integrity and chromosome alignment during reproductive ageing-associated oocyte senescence.

Lu F, Shi D, Wei J, Yang S, Wei Y. Development of embryos reconstructed by interspecies nuclear transfer of adult fibroblasts between buffalo (*Bubalus bubalis*) and cattle (*Bos indicus*). Theriogenology. 01 October 2005. (Vol 64, Issue 6, Pages 1309-1319).

The objective of this study was to explore the feasibility of employing adult fibroblasts as donor cells in interspecies nuclear transfer (NT) between buffaloes and cattle. Buffalo and bovine oocytes matured in vitro for 22 h were enucleated by micromanipulation using the Spindle View system. An ear fibroblast, pretreated with 0.1 microg/mL aphidicolin for 24 h, followed by culture for 2-9 days in Dulbecco's Modified Eagle's Media+0.5% fetal bovine serum, was introduced into the cytoplasm by microinjection. Reconstructed oocytes were activated by exposure to 5 microM ionomycin for 5 min and 2 mM 6-dimethylaminopurine for 3 h. When buffalo adult fibroblasts were used as donor cells, there were no differences (P < 0.75) in the cleavage rate (66.2% versus 64.0%) between bovine and buffalo recipient oocytes, but more embryos derived from bovine cytoplasts developed to blastocysts than from buffalo cytoplasts (13.3% versus 3.0%, P < 0.05). When bovine adult fibroblasts were used as donor nuclei, both cleavage rate (45.3%) and blastocyst yield (4.5%) of NT embryos derived from buffalo cytoplasts were lower than those of NT embryos derived from bovine cytoplasts (65.5 and 11.9%, P < 0.05). The proportion of parthenogenetic buffalo (29.1%) or bovine (35.6%) oocytes developing to blastocysts was higher than those of NT embryos (P < 0.01). Interspecies NT embryos were derived from the donor cells and 55.0-61.9% of them possessed a normal diploid karyotype. In conclusion, embryos reconstructed by interspecies NT of adult fibroblasts between buffaloes and cattle developed to blastocysts, but bovine cytoplasts may direct embryonic development more effectively than buffalo cytoplasts, regardless of donor cell species.

Malcov, Mira. Polscope- a Tool for Determining Oocyte Maturation and Improving the Fertilization Rate in ICSI ? IVF Unit, Sourasky Medical Center, Tel-Aviv, Israel. Abstract submitted to SGI Conference, Washington D.C., April 2003.

Objective: Chromosomal aberrations, as a result of oocyte meiotic division errors, are associated with implantation failure and early pregnancy loss. Spindle formation in the mature human oocyte is crucial for correct chromosomal segregation. Polscope, a non-invasive tool, enables demonstration of the meiotic spindle in living oocytes. This study was aimed at assessing whether spindle imaging can be used for evaluation of oocyte maturation, accurate timing of sperm injection and prediction of fertilization and embryo quality. **Design:** Prospective randomized study. The Polscope was used to image spindles in nude oocytes prior to sperm injection. Spindle imaging was correlated to: fertilization rate, embryo quality (on days 1 and 3) and time elapsed from HCG administration. **Materials and Methods:** Human oocytes, retrieved from women undergoing ICSI, were cultured in a P1 medium, supplemented with 16% synthesized serum substitute (SSS), after aspiration. Cumulus cells were removed by hyaluronidase, washed and transferred to mHTF, supplemented with 5% SSS in glass petri dishes, for spindle observation using LC Polscope optics and controller. **Results:** 369 oocytes were retrieved from 45 women. Average age was 33.4 + 6.19 years. All 369 oocytes released their first polar body. In order to correlate between the time elapsed from HCG administration and spindle imaging, oocytes were divided into 2 groups: Group-1 was imaged 36 - 39.5 hours after HCG administration, Group-2 was imaged 39.5 - 42 hours after HCG administration. Spindle imaging was statistically higher in group-2, 141/174 (81.03%) compared to 128/195 (65.64%) in group- 1, P=0.001. Correlation between spindle imaging and fertilization rate after ICSI: Spindle was imaged in 269 oocytes (72.90%), compared to 100 oocytes (27.10%) in which spindle was not imaged. Fertilization rate in oocytes with spindle was 73.6% (198/269) compared to 59% (59/100) in oocytes without spindle. Fertilization rate in oocytes with spindle is significantly higher (P=0.0067). Embryo developmental stage on day 3 was not statistically different. **Conclusion:** These preliminary results indicate that meiotic spindle, imaged by the Polscope, can constitute an additional parameter for the prediction of

future fertilization. According to the correlation between time elapsed between HCG administration and spindle imaging, we conclude that spindle imaging may be a better indicator than the presence of the first polar body, for the determination of oocyte maturity, prior to sperm injection.

Mandelbaum J, Anastasiou O, Levy R, Guerin JF, de Larouziere V, Antoine JM. Effects of cryopreservation on the meiotic spindle of human oocytes. Eur J Obstet Gynecol Reprod Biol. 2004 Apr 5;113 Suppl 1:S17-23.

The microtubular meiotic spindle of most mammals, including humans, is very sensitive to cooling [Hum. Reprod. 16 (2001) 2374; Fertil. Steril. 54 (1990) 102; Fertil. Steril. 75 (2001) 769; Zygote 3 (1995) 357] and is rapidly depolymerised even after a slight reduction in temperature to 33 degrees C. Spindle disassembly is dependent on the extent of temperature decrease and its duration. After rewarming, the recovery is far from complete. Cryoprotectants themselves may alter the spindle structure, depending on the duration and temperature of exposure, the duration of recovery at 37 degrees C and the species [Hum. Reprod. Update 2 (1996) 193]. Damage to the meiotic spindle is considered to be the cause of aneuploid embryos, by inducing chromatid non-disjunction and chromosome scattering and by disturbing the sequence of events leading to the completion of meiosis and fertilisation. Nevertheless, a consensus arose from all the studies: appropriate exposure to cryoprotectants and appropriate rates of cooling and thawing allow the cryopreservation of mature oocytes without any significant changes in their second meiotic spindle organisation and without any increase in the rate of aneuploid embryos [Mol. Hum. Reprod. 2 (1996) 445; Hum. Reprod. 8 (1993) 1101; Hum. Reprod. 9 (1994) 684; Microsc. Res. Technol. 27 (1994) 165; Fertil. Steril. 75 (2001) 354]. These fundamental studies in humans, showing good preservation of cell structures after freeze-thaw procedures opened the way to new successful clinical trials with embryos derived from cryopreserved mature oocytes [Fertil. Steril. 68 (1997) 724]. Considering immature oocyte freezing at prophase I (germinal vesicle (GV) stage), a stage which was thought to be less sensitive to cryoinjury, pooled data from the literature showed no advantage in terms of survival rates, fertilisation rates of in vitro matured oocytes and developmental ability of the resulting embryos, especially in unstimulated cycles. Moreover, conflicting results are reported on the effects of freezing on the spindle-chromosome configuration of immature oocytes or in vitro matured oocytes, highlighting the need for large scale studies [Hum. Reprod. 10 (1995) 1816; Hum. Reprod. 13 (Suppl. 3) (1998) 161; Hum. Reprod. 17 (2002) 1885; Microsc. Res. Technol. 27 (1994) 165; Fertil. Steril. 68 (1997) 920]. One child has been born after the use of cryopreserved immature oocytes at GV stage, matured in vitro and fertilised by ICSI [Hum. Reprod. 13 (1998) 3156], demonstrating at least the feasibility of this technique. Improvements are required so as to make mature and immature oocyte cryopreservation an established and safe technique for ART.

Martinez, F et al. Metaphase II human meiotic spindle visualization during freezing thawing-procedure. Oral presentation made at ESHRE 2003, Madrid, Spain.

Introduction: Detrimental changes in the meiotic spindle structure of metaphase II (MII) human oocytes have been reported after cryopreservation procedure. These changes have been suggested to be responsible for the limited success rate reported after oocyte cryopreservation combined with ICSI. With the use of a computer-assisted polarization microscopy system (PolScope), able to visualise the meiotic spindle in living oocytes, the birefringent structure was evaluated during the freezing and thawing procedure. Materials & Methods: Only supernumerary fresh MII human oocytes obtained after ovarian hyperstimulation and ovum pick-up in ICSI patients, with a clear meiotic spindle visualized in the oocyte cytoplasm were included in this study. The oocytes were cryopreserved in 1,2-propanediol (PROH) 0.1M sucrose (Su) (Freeze-Thaw Kit I, Vitrolife) using a slow-freezing-rapid-thawing programme. The meiotic spindle presence has been evaluated for each step of the freezing-thawing procedure. Results: The meiotic spindle of the studied oocytes (N= 40) remained detectable during the different incubations in the freezing solutions at room temperature. Immediately after thawing, only half of them (20 oocytes) had still a detectable meiotic spindle. Interestingly, all the oocytes had lost their spindle when incubated in the last two thawing solutions at room temperature. Part of the frozen-thawed oocytes (40%) were nevertheless able to restore a detectable spindle after incubation in culture medium at 37°C. Conclusions: The polscope can be used to select oocytes that have restored the meiotic spindle after cryopreservation to be used for ICSI. This system is also useful for experimental biological studies aimed of the optimization of the freezing-thawing protocol.

Moon JH, Jee BC, Ku SY, Suh CS, Kim SH, Choi YM, Kim JG, Moon SY. Spindle positions and their distributions in in vivo and in vitro matured mouse oocytes. Hum Reprod. 2005 Aug;20(8):2207-10. Epub 2005 Apr 28.

BACKGROUND: This study was carried out to compare spindle locations and their developmental competencies both in vivo and in vitro in matured mouse oocytes. Spindle locations were identified using a polscope. Since meiotic spindles in living oocytes are highly birefringent, their structures can be viewed non-invasively by using a polscope. METHODS: In vivo matured metaphase II oocytes were collected from the oviducts of mice. Immature oocytes were collected from mouse ovaries, and then cultured in YS medium until the first polar body (PB) extrusion. In vitro and in vivo matured oocytes were classified into four categories according to their spindle positions relative to the first PB (0 degrees , 0-90 degrees , 90-180 degrees and without a spindle image), and rates of fertilization and blastocyst formation were assessed. In vivo matured oocytes with a 0 degrees spindle disposition relative to PB were cultured in vitro for 24 h, and then their spindle positions were re-assessed. RESULTS: Most in vivo matured oocytes (89.1%) had a 0 degrees spindle position. Only 6 and 3% of oocytes had spindle positions of 0-90 degrees and 90-180 degrees , respectively. No spindle image was observed in 2%. However, most in vitro matured oocytes (83.1%) had a 0-90

degrees spindle position and, in contrast, only 6.5% of these oocytes had a 0 degrees spindle position. The rate of fertilization and blastocyst rate were significantly higher for in vivo matured oocytes than in vitro matured oocytes (87.1 versus 64.9% and 76.1% versus 66.0%, respectively, $P < 0.05$ for each). We also observed that 71.7% of the in vivo matured oocytes with the 0 degrees spindle position showed a spindle position change to 0-90 degrees after 24 h of culture. These oocytes had a poor fertilization rate (43%) and a zero blastocyst rate. CONCLUSION: In vitro matured mouse oocytes showed quite different spindle positions compared with in vivo matured oocytes. Moreover, in vivo matured oocytes cultured for 24 h had a spindle position distribution that was similar to that of in vitro matured oocytes. The different spindle positions observed in in vivo and in vitro matured oocytes may reflect differences in their cytoplasmic maturation processes. These findings have implications regarding the lower developmental competency of in vitro matured oocytes.

Moon JH et al. Identification of metaphase II spindle in living human oocytes using Pol-Scope enables the prediction of embryonic developmental competence after ICSI. *Fertility and Sterility*. Sept 2001 (Vol 76, Issue 3 (Supplement 1), Page S3).

BACKGROUND: Meiotic spindles in living human oocytes can be visualized by the Polscope. This study investigated the relationship between the presence/location of the spindle in metaphase II (MII) oocytes and developmental competence of embryos in vitro. METHODS: The spindles in 626 MII oocytes were examined by the Polscope and divided into six groups (A±F) based on the presence or absence of the spindles and the angle between the spindle and the 1st polar body. After ICSI, the fertilization and embryo development were evaluated. RESULTS: Meiotic spindles were imaged in 523 oocytes (83.5%), while 103 (

Moon JH et al. Visualization of the metaphase II meiotic spindle in living human oocytes using the PolScope enables the prediction of embryonic developmental competence after ICSI. *Human Reproduction* 2003 Apr;18(4):817-820.

BACKGROUND: Meiotic spindles in living human oocytes can be visualized by the Polscope. This study investigated the relationship between the presence/location of the spindle in metaphase II (MII) oocytes and developmental competence of embryos in vitro. METHODS: The spindles in 626 MII oocytes were examined by the Polscope and divided into six groups (A±F) based on the presence or absence of the spindles and the angle between the spindle and the 1st polar body. After ICSI, the fertilization and embryo development were evaluated. RESULTS: Meiotic spindles were imaged in 523 oocytes (83.5%), while 103 (16.5%) did not have a visible spindle (group F). The majority of oocytes (68.8%) had the spindle directly beneath or adjacent to the 1st polar body (groups A and B: 48.2 and 20.6%). Oocytes in group C (11.2%) had the spindle located between 60 and 120° angle away from the 1st polar body, those in group D (2.4%) had the spindle located between 120 and 180° angle and those in group E (1.1%) had the spindle located at 180° angle to the 1st polar body. The fertilization and embryonic development were similar in the oocytes with spindles regardless of spindle position. However, the rate of high quality embryos was significantly higher in the oocytes (64.2%) with visible spindles than in the oocytes (35.9%) without spindle and multipronuclear proportion showed a slight tendency to increase in oocytes without spindles. (10.7 versus 5.9%, $P = 0.12$; NS). CONCLUSIONS: the presence of a bi-refringent meiotic spindle in human oocytes by using the Polscope can predict a higher embryonic developmental competence. However, the relative position of the spindle within the oocyte doesn't appear to influence the developmental potential of embryos.

Navarro PAAS, Liu L, Ferriani RA, Keefe DL. Non-invasive imaging of spindle dynamics during mammalian oocyte activation. *Fertility and Sterility*. April 2005 (Vol. 835, Issue 4 (Supplement), Pages 1197-1205).

Navarro PAAS, Liu L, Ferriani RA, Keefe DL. Arsenite induces aberrations in meiosis that can be prevented by coadministration of N-acetylcysteine in mice. *Fertility and Sterility*. April 2006 (Vol. 85, Issue (Supplement 1), Pages 1187-1194).

OBJECTIVE: To evaluate in vitro effects of arsenite and of arsenite plus N-acetylcysteine on mouse oocyte meiosis. DESIGN: Morphological study using mouse oocytes submitted to in vitro maturation (IVM). SETTING: Laboratory of reproductive biology. ANIMAL(S): Six-week-old CD-1 mice superovulated with pregnant mare serum gonadotropin. INTERVENTION(S): During IVM, mouse oocytes were exposed to arsenite alone or to arsenite plus N-acetylcysteine. MAIN OUTCOME MEASURE(S): Meiotic anomalies were assessed using immunofluorescence microscopy and PolScope (Cambridge Research and Instrumentation, Boston, MA) imaging. RESULT(S): In vitro arsenite administration produced dose-dependent and time-dependent meiotic anomalies, characterized by spindle disruption or chromosome misalignment. After 12-14 hours of IVM, exposure to 2 microg/mL of arsenite for 12-14 hours or to 8 microg/mL of arsenite for 2 hours arrested oocyte maturation at the germinal vesicle or germinal-vesicle breakdown stage. Exposure to 4 microg/mL of arsenite for 2 hours arrested oocyte maturation at metaphase I stage in 95% of exposed oocytes (80% exhibiting abnormalities) after 12-14 hours in IVM. After 12-14 hours in IVM, of the oocytes exposed to 2 microg/mL of arsenite for 2 hours, only 15% reached the meiosis II stage (5% exhibiting abnormalities). After 15-17 hours in IVM, however, of the oocytes exposed to 2 microg/mL of arsenite for 2 hours, 65.2% reached the meiosis II stage (43.5% exhibiting abnormalities). Co-administration of N-acetylcysteine prevented the arsenite-induced meiotic abnormalities and the delayed IVM. CONCLUSION(S): In vitro arsenite exposure caused meiotic abnormalities that were prevented by co-administration of N-acetylcysteine, suggesting that arsenite-induced meiotic aberrations are mediated by reactive oxygen species.

Neri QV, Takeuchi T, Rosenwaks Z, Palermo GD. Imaging of the metaphase spindle in vitrified human and mouse oocytes matured in vitro. *Fertility and Sterility*. Sept 2003 (Vol 80, Issue (Supplement 3), Pages 143-144).

Piquette GN. The in vitro maturation (IVM) of human oocytes for in vitro fertilization (IVF): is it time yet to switch to IVM-IVF? *Fertility and Sterility*. April 2006 (Vol 85, Issue 4, Pages 833-835)

The recent study by Li et al. observed that human oocytes from patients with polycystic ovary syndrome (PCOS) matured in vitro exhibited a higher proportion of abnormal spindle structures and disturbed chromosomal configurations compared with in vivo-matured oocytes from a control group of PCOS patients. This article discusses the obstacles that must be overcome and factors that must be monitored when attempting to optimize conditions for the in vitro maturation of human oocytes, with particular attention to the strengths and weaknesses of the study by Li et al.

Pelletier C, DL Keefe and JR Trimarchi. Noninvasive polarized light microscopy quantitatively distinguishes the multilaminar structure of the zona pellucida of living human eggs and embryos. *Fertility and Sterility* Volume 81, Issue (Supplement 1), Pages 850-856 (March 2004)

Abstract. Objective To characterize the architecture of the zona pellucida in living human eggs and embryos, noninvasively with the PolScope, a digital polarizing light microscope. **Design** The PolScope was used to examine zonae pellucida of living human eggs and embryos. **Setting** Academic IVF clinic. **Patient(s)** Patients undergoing fresh, nondonor infertility treatment who underwent egg aspiration, fertilization by intracytoplasmic sperm injection or traditional IVF, and cleavagelstage embryo transfer (day 3). **Intervention(s)** The PolScope imaged the zona of eggs before intracytoplasmic sperm injection and in cleavage-stage embryos before transfer. **Main outcome measure(s)** Thickness and retardance of three zona layers were measured from eight quadrants. Mean and variance in thickness and retardance were calculated for individual eggs and embryos, between eggs and embryos of a cohort, and across the sample patient population. **Result(s)** Cleavagelstage (day 3) embryos have thinner zonae ($15.2 \pm 2.9 \mu\text{m}$) than both immature ($20.4 \pm 2.4 \mu\text{m}$) and mature ($19.5 \pm 2.2 \mu\text{m}$) eggs. The zona of embryos is thinner, primarily owing to thinning of the outer layer. The thicker the zona layer, the greater its retardance. Considerable variation exists in the thickness and retardance of zona layers around individual eggs and embryos and between members of a cohort. The zona of eggs and embryos from different patients differ in thickness, retardance, and variation. **Conclusion(s)** Thickness and organization of zonae pellucida of human eggs and embryos varies considerably and can be quantitatively imaged with the PolScope.

Plante BJ, Liu L, Trimarchi JR, Jurema M, Keefe DL. Deuterium Oxide stabilizes meiotic spindles in living human oocytes during cooling. *Fertility and Sterility*. September 2005 (Vol. 84, Issue (Supplement 1), Pages S63-S64).

Rienzi L et al. Relationship between meiotic spindle location with regard to the polar body position and oocyte developmental potential after ICSI. *Human Reproduction* 2003 Jun;18(6):1289-1293.

BACKGROUND: The recent development of a computer-assisted polarization microscopy system (Polscope) with which the meiotic spindle can be visualized in living oocytes on the basis of its birefringence permits analysis of the meiotic spindles of oocytes subjected to ICSI. Previous studies have shown that the meiotic spindle is not always aligned with the λ Erst polar body (PB) in metaphase II human oocytes prepared for ICSI. In the present study, the relationship between the degree of meiotic spindle deviation from the λ Erst PB location and ICSI outcome was analysed. **METHODS:** Oocytes were divided into four groups according to the angle of meiotic spindle deviation from the PB position. The angle of deviation was $0 \pm 5^\circ$, $6 \pm 45^\circ$, $46 \pm 90^\circ$ and $>90^\circ$ for groups I to IV respectively. **RESULTS:** The rates of normal [2 pronuclei (PN)] and abnormal (1PN or >2PN) fertilization did not differ between groups I, II and III. However, the rate of normal fertilization was lower among oocytes in which the meiotic spindle deviation angle was $>90^\circ$; this led to an increased proportion of tripronucleated zygotes that failed to extrude the second PB. When embryos developed from normally fertilized oocytes were evaluated on day 3 after ICSI, no relationship was found between the angle of meiotic spindle deviation and embryo quality. The meiotic spindle was not detected in only 9% of oocytes, and these showed a higher incidence of fertilization and cleavage abnormalities than did oocytes in which the spindle was detected. When oocytes at metaphase I after cumulus oophorus and corona radiata removal were matured in vitro, the meiotic spindle was detected in 53.8% of those that reached metaphase II. In these in-vitro-matured oocytes the meiotic spindle was always aligned with the λ Erst PB, suggesting that misalignment seen in those oocytes matured in vivo resulted from PB displacement during manipulations for cumulus and corona removal. **CONCLUSION:** High degrees of misalignment between the meiotic spindle and the λ Erst PB predict an increased risk of fertilization abnormalities. However, when normal fertilization had occurred, the cleavage potential of embryos developing from such oocytes was not impaired. These λ Endings facilitate the selection of oocytes for ICSI in situations when the creation of supernumerary embryos is to be avoided.

Rienzi L et al. Metaphase II meiotic spindle location of human oocytes affects ICSI results. Oral presentation made at ESHRE 2003, Madrid, Spain.

Introduction: A computer-assisted polarization microscopy system (Polscope) as been recently developed to visualise the meiotic spindle in living

oocytes on the basis of its birefringence. It has been already shown that the meiotic spindle is not always aligned with the first polar body in metaphase II human oocytes prepared for ICSI. In present study the relationship between the degree of meiotic spindle deviation from the first polar body location and ICSI outcomes is analysed. Materials & Methods: Five hundred thirty two metaphase II oocytes prepared for ICSI were divided into 4 groups according to the angle of meiotic spindle deviation from the polar body position. The angle of deviation was 0-5° (254 oocytes), 6-45° (104 oocytes), 46-90° (102 oocytes), and >90° (24 oocytes) for groups I-IV, respectively. Results: The fertilization rate did not differ between groups I, II, and III (74.0%, 75.0% and 82.3% respectively) while it was statistically lower (50.0%) in the group of oocytes with the meiotic spindle deviation angle of >90° (P<0.05). Furthermore, an increase in the percentage of tripronucleated oocytes that failed to extrude the second polar body was recorded in group IV (16.7% versus 3.1%, 3.8% and 2.0%; P<0.05). When embryos that developed from normally fertilized oocytes were evaluated on day 3 after ICSI, no relationship between the angle of meiotic spindle deviation and embryo quality was found. The oocytes in which meiotic spindle could not be detected (48 oocytes; 9.0%) displayed a higher incidence of fertilization and cleavage abnormalities as compared with oocytes in which the meiotic spindle was detected. Conclusions: High degrees of misalignment between the meiotic spindle and the first polar body predict an increased risk of fertilization abnormalities. However, when the normal fertilization has occurred, the cleavage potential of embryos developing from such oocytes is not impaired. These findings facilitate the selection of oocytes for ICSI in situations in which the creation of supernumerary embryos is to be avoided.

Rienzi L et al. Polscope analysis of meiotic spindle changes in living metaphase II human oocytes during the freezing and thawing procedures. Hum. Repro. Advance Access originally published online on January 29, 2004 Human Reproduction, Vol. 19, No. 3, 655-659, March 2004

BACKGROUND: The clinical efficacy of the current methods used for cryopreservation of metaphase II human oocytes is low. Meiotic spindle disorders are thought to be largely responsible for this situation. **METHODS:** Supernumerary fresh metaphase II human oocytes were cryopreserved in 1,2-propanediol with 0.1 M sucrose using a slow freezing/rapid thawing programme. Meiotic spindles were analysed in these living metaphase II oocytes at sequential steps of the freezing and thawing procedures with the use of a computer-assisted polarization microscopy system (PolScope). **RESULTS:** The meiotic spindle was detected in all 56 oocytes (from 16 patients) before freezing and remained visible in all these oocytes throughout the preparation for freezing up to the time that they were loaded into cryopreservation straws. Immediately after thawing, the spindle was visible in 35.7% of oocytes, but it disappeared in all of the thawed oocytes during the subsequent washing steps. However, the spindle reappeared in all surviving thawed oocytes after washing (57.4%), by 3 h of incubation at 37°C in culture medium. **CONCLUSIONS:** The current techniques of oocyte freezing and thawing inevitably cause meiotic spindle destruction. All spindles observed in thawed oocytes result from post-thaw reconstruction.

Shen Y, Staf T, Mehnert C, De Santis L, Cino I, Tinneberg HR, Eichenlaub-Ritter U. Light retardance by human oocyte spindle is positively related to pronuclear score after ICSI. Reprod Biomed Online. 2006 Jun;12(6):737-51

Disturbed spindle assembly increases risks of chromosome mal-segregation. Non-invasive polarization microscopy (PolScope) was employed in two centres to assess spindle integrity for the first time quantitatively in human oocytes from consenting patients undergoing intracytoplasmic sperm injection (ICSI) with respect to pronuclear (PN) score after fertilization. In one centre oocytes were selected before ICSI, in another selection was after ICSI according to PN score. In both centres, mean retardance of light by birefringent spindles in oocytes forming a pre-embryo with good PN score after ICSI was significantly higher compared with spindles in oocytes developing into a lower PN score pre-embryo with limited developmental potential (P < 0.001). Transfers involving oocytes with high retardance and at least one good PN score embryo resulted more frequently in a conception than transfers from oocytes with spindles of lower mean retardance and lower PN score embryos. There was a trend for an inverse relationship between age and magnitude of retardance in a small oocyte cohort. The study suggests that quantitative evaluation of mean retardance of light by the oocyte spindle predicts oocyte health, is related to PN score of the embryo and may be especially useful to assess oocyte quality in countries with legal restrictions to select after fertilization.

Shen Y, Betzendahl I, Sun F, Tinneberg HR, Eichenlaub-Ritter U. Non-invasive method to assess genotoxicity of nocodazole interfering with spindle formation in mammalian oocytes. Reprod Toxicol. 2005 Mar-Apr;19(4):459-71.

Trisomies due to nondisjunction in oogenesis are still a major cause of genetic diseases in humans. In this study, we analysed spindle morphology of in vitro matured nocodazole-exposed mouse oocytes by novel non-invasive Polscope-microscopy, and compared images to those obtained by anti-tubulin immunofluorescence of fixed oocytes. Polscope revealed a reduction in the numbers of oocytes expressing a birefringent spindle, and alterations in spindle morphology at concentrations of nocodazole below those inducing detectable aberrations in immunofluorescence. Hyperploidy increased significantly at a concentration of 40 nM nocodazole in mouse metaphase II oocytes, similar to thresholds inducing nondisjunction in cultured human lymphocytes. In conclusion, Polscope represents a novel highly sensitive, non-invasive method to identify chemicals inducing severe spindle aberrations that predispose mammalian oocytes to nondisjunction. Polscope may provide information on the functionality of the spindle in experimental studies but is also compatible with clinical trials in human assisted reproduction due to its non-invasive nature.

Shen Y, Stalf T, Mehnert C, Eichenlaub-Ritter U, Tinneberg HR. High magnitude of light retardation by the zona pellucida is associated with conception cycles. Hum Reprod. 2005 Jun;20(6):1596-606. Epub 2005 Feb 25.

BACKGROUND: Failures in expression of zona proteins correlate to subfertility in animals. Low expression of the zona proteins by the growing human oocyte may indicate reduced developmental potential. Therefore, we non-invasively analysed the thickness and the structure of the zona pellucida (ZP) of human oocytes with respect to embryo fate after ICSI. METHODS: Retardance magnitude and thickness of the inner, middle and outer layers of the ZP were quantitatively analysed by a Polscope in 166 oocytes selected for transfer after ICSI (63 patients; 32.8 +/- 4.4 years) on the basis of pronuclear score at day 1. Blastomere number was determined at day 2. Data were compared between conception cycles (CC; 65 oocytes/23 patients) and non-conception cycles (NCC; 101 oocytes/40 patients) and with respect to maternal age. RESULTS: The thickness was slightly elevated ($P < 0.001$), and the mean magnitude of light retardance was nearly 30% higher ($P < 0.001$) in the inner layer of the zona pellucida of oocytes contributing to CC compared to NCC. Embryos in the CC group tended to develop faster. CONCLUSIONS: The magnitude of light retardance by the zona pellucida inner layer appears to present a unique non-invasive marker for oocyte developmental potential.

Shi Der-Shen, GungXi University, China. A rabbit cloned from an embryonic cell was born in GungXi University, China. Time: 28th May, 2002; 11:00:06.0 Source: New China Net.

The Animal Reproduction Institute of GungXi University announced today that their research personnel had successfully reproduced a rabbit cloned from an embryonic cell. The rabbit cloned from the embryonic cell was born in the lab on 13th May. As witnessed by the reporters, this less than two-week-old rabbit was rather active, and appeared healthy and aggressive. Professor Shi Der-Shen, director in charge of this project, said that they obtained an early-stage cell from a rabbit, proceeded to inject it into a mature oocyte that had its nuclear material removed, where it then merged to become a healthy embryo. This constructed embryo was cultured *ex vivo* then later transplanted into a surrogate rabbit. After 34 days of average pregnancy, the cloned rabbit was born. When the cloned rabbit was born, it weighed 82 grams. It is now several folds heavier. As understood, PRC has already mastered the technology of cloning goat, cow, and rabbit from embryonic cells since the early 90s, and is now working on enhancing the efficiency of this technology in order to lay foundation for more complex cloning using tissue cells. Up to the moment, no rabbit has been born from tissue cells, while the first cow cloned from tissue cells was born earlier this year. As different from goat and cow, the transparent membrane of the oocytes of rabbit is rather tough. During the process of cloning, it is quite difficult to remove the nuclear material by suction after piercing through it. If the nuclear material is not removed completely, cloning will fail. Prof. Shi said, "During the experiment, we used a new and advanced observation technique, The SpindleView™ Imaging System, to look for the meiotic spindle, which has greatly enhanced the efficiency of removing the nuclear material. This is providing us with assurance that we are getting high quality cloning, which we see as the achievement today." As acknowledged, the application of cloning rabbit is very helpful to scientific research because rabbit, in terms of its physiology, is much closer to man than cow is, and is therefore a more ideal animal species for such experiments. Since the heredity of the cloned rabbit is completely the same as the original cell, the marginal difference due to individual mutation can be reduced to none. In addition, the employment of cloning technology on rabbits can be used to produce transgenic rabbits that contain specific genes associated with a given terminal disease. This allows for subsequent cause-and-effect studies to be done in order to positively identify potential cures, thus proving quite powerful advancements in medical research.

Silva, C., V. Silva, K. Kommeneni and D. Keefe. 1997. Effect of in vitro culture of mammalian embryos on the architecture of the zona pellucida. Developmental, Cellular and Molecular Biology. 193:235-236.

The mammalian zona is composed of glycoprotein filaments organized in three layers that differ in their orientation and birefringence. The authors hypothesized that in vitro culture may alter zona architecture. It was determined that in vitro cultured embryos did exhibit thicker zonas as viewed using the CRI PolScope™.

Silva CA, Swain J, Acevedo N, Smith GD. Influence of vitrification on metaphase II spindle dynamics, spindle morphology, and chromatin alignment. Fertility and Sterility. September 2004 (Vol. 82, Issue (Supplement 2), Page S111).

Silva, C., K. Kommineni, R. Oldenbourg and D. Keefe. 1999. The first polar body does not predict accurately the location of the metaphase II meiotic spindle in mammalian oocytes. Fertility and Sterility. 71(4):719-721.

This study was done to identify the meiotic spindle in living, unfixed hamster oocytes and determines spindle location relative to the polar body. Spindles were imaged, using the CRI PolScope™, in 30 oocytes and only in 5 of them could the polar body predict the spindle localization. In the remaining oocytes, the spindles presented a random distribution within the cytoplasm. These data show that the polar body location is not an accurate predictor for meiotic spindle location in mammalian oocytes. These data are replicated in human oocytes. It should be possible to locate spindles before ICSI to test directly the hypothesis that localization of the metaphase II spindle will improve ICSI success rates.

Sun F, Yin H, Eichenlaub-Ritter U. 2001. Differential chromosome behaviour in mammalian oocytes exposed to the tranquilizer

diazepam in vitro. *Mutagenesis* 2001 Sep;16(5):407-17.

There are several reports demonstrating that aneuploids may preferentially affect segregation of particular chromosomes in somatic cells. Much less is known on specific susceptibility of individual chromosomes to non-disjunction in mammalian meiosis in response to chemical exposures. To explore possible chromosome-specific behaviour and susceptibility to errors in chromosome segregation in mammalian oogenesis we employed spindle immunofluorescence in combination with FISH with chromosome-specific probes to analyse congression of chromosomes X, 8 and 16 in diazepam (DZ)-treated, meiotically delayed meiosis I oocytes of the mouse. Concomitantly, we assessed the susceptibility of homologues to precociously segregate prior to anaphase I during DZ-induced meiotic arrest. About 50% of all oocytes exposed to 25 microg/ml DZ became meiotically delayed. Chromosomes failed to congress at the spindle equator in one-third of these meiosis I oocytes. The X chromosome was significantly more often located away from the spindle equator as compared with the expected random behaviour. Concomitantly, DZ exposure induced untimely segregation of homologous chromosomes of the gonosome and the autosomes in meiosis I. This occurred with similar frequencies. The observations confirm that DZ perturbs cell cycle progression, interferes with chromosome alignment, causes predivision and thus may predispose mammalian oocytes to errors in chromosome segregation. For the first time, chromosome-specific behaviour is reported in female meiosis in response to exposure to an aneuploidic chemical.

Tran, PT, et al. 1997. Effect of gossypol on *Spisula* sperm observed with real-time confocal microscopy, polarized light microscopy, and video microscopy. *Biol. Bull.* 193(2):227-228.

Trimarchi JR, Karin RA, Keefe DL. Average spindle retardance observed using the PolScope predicts cell number in day 3 embryos. *Fertility and Sterility*. September 2004 (Vol. 82, Issue (Supplement 2), Page S268).

Wang WH, et al. Selection of morphologically normal human oocytes by the living spindle PolScope images. *Fertil Steril* 2000 Sep; 74(3), Suppl. 1:S146, Abst P-162.

Objective: To examine spindle morphology as imaged by the Polscope and confocal microscopy and chromosome configuration in human oocytes matured in vitro without cumulus cells. To determine whether the Polscope can be applied to human IVF to help select normal oocytes for insemination. **Design:** The LC Polscope was used to examine spindle images in living human oocytes matured in vitro without cumulus cells. Polscope images of spindle were compared with the chromosome and spindle images obtained by confocal microscopy. **Materials and Methods:** Approval was obtained from the Women and Infants Hospital Institutional Review Committee to study unfertilized human oocytes and to study images of oocytes obtained during human IVF with the Polscope. Oocytes were obtained from stimulated ovaries of consenting patients undergoing oocyte retrieval for ICSI. After retrieval, oocytes were cultured in P1 medium containing 6% synthesized serum substitute for 5–6 h. Cumulus cells were removed by pipetting in modified HTF containing 80 IU/ml hyaluronidase. Oocytes with the first polar body were used for ICSI, but immature oocytes were cultured further to examine maturation and spindle morphology. For imaging spindles, each oocyte was placed in a 5 ml drop of modified HTF covered with warm paraffin oil in a Bioptechs DTC3 Culture Dish System. Dishes were maintained at 37°C during examination with Bioptechs heating stage. Oocytes were examined under a Zeiss Axiovert 100 with a Neofluar 40 3 strainfree objective and LC Polscope optics and controller, combined with a computerized image analysis system. After imaging, oocytes were fixed separately and used for microtubule and chromosome staining and examined by confocal microscopy. **Results:** One hundred and thirteen oocytes were retrieved from 6 women and 67 (59.3%) oocytes were at metaphase II stage and were inseminated by ICSI. Thirty five immature oocytes (31.0%) with normal morphology were exposed to further culture. After culture for 22–24 h (from retrieval), 27 (77.1%) oocytes reached metaphase II stage, with 51.9% of the oocytes exhibiting birefringent spindles by the Polscope. Examination of oocytes by confocal microscopy indicated that 71% of oocytes with birefringent spindles had normal spindle architecture and chromosome configuration. 29% of oocytes with birefringent spindles and all oocytes without birefringent spindles had abnormal spindle architectures and abnormal chromosome configuration. **Conclusions:** These results indicate that most immature oocytes found at ICSI can mature in vitro, even without cumulus cells. More than half of the matured oocytes showed abnormal chromosome configuration, possibly resulting from the absence of cumulus cells and/or in vitro maturation condition. The birefringent spindle images obtained by the Polscope can be used to select the morphologically normal oocytes which may be useful for rescue insemination.

Wang, W.H. et al. 2000. Spindle observation and its relationship with fertilization after ICSI in living human oocytes. *Fertil Steril* 2001 Feb;75(2):348-53.

OBJECTIVE: To image spindles in living human oocytes and to examine the relation between spindles and fertilization after ICSI. **DESIGN:** The LC polscope was used to examine spindles in an observational study of living oocytes. **SETTING:** Academic IVF clinic. **PATIENT(S):** Women being treated for infertility. **INTERVENTION(S):** Oocytes retrieved from patients for infertility treatment were examined before ICSI. Aged, unfertilized oocytes after IVF or ICSI were examined with polscope and confocal microscopes to compare the two methods. **MAIN OUTCOME MEASURE(S):** Spindle structure in living oocytes and fertilization after ICSI. **RESULT(S):** Spindles could be imaged in 61.4% of oocytes. More oocytes with spindles than oocytes without spindles fertilized normally after ICSI (61.8% vs. 44.2%). Spindles in most aged oocytes were partially

or completely disassembled, and only a few microtubules around the chromosomes or dispersed microtubules in the cytoplasm were observed. Confocal images of immunostained spindles were almost identical to polscope images of spindle birefringence. CONCLUSION(S): Spindles in living human oocytes can be imaged by using the polscope. A birefringent spindle in human oocytes may clinically predict the quality and age of oocytes. This method also can be used to monitor spindle position during ICSI.

Wang WH, Meng L, Hackett RJ, Keefe DL. Developmental ability of human oocytes with or without birefringent spindles imaged by Polscope before insemination. Hum Reprod 2001 Jul;16(7):1464-8.

Birefringent spindles imaged with the Polscope can predict fertilization rates after intracytoplasmic sperm injection (ICSI). The present study examined the development of human oocytes with or without birefringent spindles, imaged with the Polscope before ICSI. METHODS: Oocytes were obtained from stimulated ovaries of consenting patients undergoing oocyte retrieval for ICSI. Spindles were imaged with the Polscope combined with a computerized image analysis system. After imaging and ICSI, oocytes with or without spindles were cultured separately for examination of fertilization and embryo development. A total of 1544 oocytes from 136 cycles were examined with the Polscope and inseminated by ICSI. RESULTS: Spindles were imaged in 82% of oocytes. After ICSI, more oocytes ($P < 0.05$) with spindles (69.4%) fertilized normally, forming 2 pronuclei, than oocytes without spindles (62.9%). At day 3, more oocytes ($P < 0.01$) with spindles (66.3%) developed to 4-11 cell stages than oocytes without spindles (55.4%). Significantly more ($P < 0.001$) oocytes with spindles developed to morula and blastocyst by day 5 (51.1 versus 30.3%) and day 6 (53.2 versus 29.3%) compared with oocytes without spindles. CONCLUSIONS: The results indicate that the presence of a birefringent spindle in human oocytes can predict not only higher fertilization rate, but also higher embryo developmental competence.

Wang WH, Meng L, Hackett RJ, Odenbourg R, Keefe DL. Limited recovery of meiotic spindles in living human oocytes after cooling-rewarming observed using polarized light microscopy. Hum Reprod 2001 Nov;16(11):2374-8.

Spindles are formed from microtubules and are exquisitely sensitive to changes in temperature. An orientation-independent polarized light microscope, the Polscope, can be used to image spindles in living oocytes allowing analysis of spindle kinetics in the living state. This study examined the effects of cooling on spindle disassembly in living human oocytes and spindle recovery after rewarming. METHODS: Oocytes were imaged continuously with the Polscope during cooling and rewarming. The quantity of microtubules in the spindles was measured by its birefringence using the Polscope. RESULTS: Spindles had completely disassembled by 5 min after cooling and recovered by 20 min after rewarming to 37 degrees C if rewarming started soon after the oocyte's temperature dropped to room temperature. However, when oocytes were cooled and kept at 33, 28 or 25 degrees C for 10 min and then warmed, it was found that warming allowed 5/5, 2/5 and 0/5 oocytes of the spindles to recover respectively. CONCLUSIONS: These results indicate that human meiotic spindles are exquisitely sensitive to alterations in temperature. The maintenance of temperature at 37 degrees C during in-vitro manipulation is important for spindle integrity and, therefore, is likely to be important for normal fertilization and subsequent embryo development.

Wang WH, Meng L, Hackett RJ, Odenbourg R, Keefe DL. Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. Fertility and Sterility 2002 Jun;77(6)

Objective: To examine the effects of different thermodynamic control systems on the temperature stability of human eggs during in vitro manipulation, with the integrity of meiotic spindles imaged using the LC-PolScope (CRI, Inc. Woburn, MA). Design: We performed intracytoplasmic sperm injection (ICSI) and/or imaging of eggs with the temperature regulated by three different systems: thermostated coverslip (system 1), thermostated coverslip combined with objective heater (system 2), and conventional stage warmer (system 3). Setting: Academic in vitro fertilization clinic. Patient(s): Oocytes were aspirated from stimulated ovaries of patients undergoing oocyte retrieval for ICSI. Intervention(s): Measurement of temperature regulation in media surrounding eggs during in vitro manipulation and imaging. Main Outcome Measure(s): Rate of oocytes with spindles, fertilization rates, and clinical pregnancy rates after ICSI. Result(s): We imaged spindles in more oocytes with system 2 (81.2%) than with system 1 (61.4%). Spindles could not be imaged for system 3 because of technical limitations. Fertilization rates were significantly higher when oocytes were imaged and used for ICSI with system 2 (78.8%) than with system 1 (56.7%) or system 3 (64.0%). Most importantly, a significantly higher clinical pregnancy rate was observed when oocytes were manipulated with system 2 (51.7%) than with system 1 (25.0%) or system 3 (23.1%). No differences were found in average ages, number of previous cycles, number of eggs, or day 3 FSH or E2 levels among groups. Conclusions: Imaging meiotic spindles with the LC-PolScope provides an intracellular thermostat during ICSI. Rigorous thermal control during ICSI stabilized spindles and increased fertilization and clinical pregnancy rates achieved after ICSI. The presence of birefringent spindles in living human eggs served as a monitor for in vitro conditions.

Wang WH, Keefe DL. Spindle observation in living mammalian oocytes with the polarization microscope and its practical use. Cloning Stem Cells 2002;4(3):269-76.

The meiotic spindle is crucial for normal chromosome alignment and separation of maternal chromosomes during meiosis. Conventional methods to image spindles rely on fixation and transmission electron microscope or immunofluorescence staining and fluorescence microscope,

so they provide limited value to studies of spindle dynamics and human clinical in vitro fertilization. A new orientation-independent polarized light microscope, the LC Polscope, was used to examine the bi-refracting spindles in living mammalian oocytes. It was found that spindles could be imaged with the Polscope in living oocytes in all mammals so far examined, including hamster, mouse, cattle, human, and rat. The first polar body did not accurately predict the spindle location in most metaphase II oocytes. Intracytoplasmic sperm injection (ICSI) could be performed by monitoring spindle position. Studies in humans indicated that, after ICSI, higher fertilization and embryonic developmental rates could be achieved in oocytes with than without bi-refracting spindles. Because spindles in most mammalian oocytes are extremely sensitive to slight changes in temperature, maintenance of temperature at 37 degrees C is crucial for normal spindle function. As chromosomes are usually associated with microtubule fibers in the spindles, the position of chromosomes could be indirectly located by imaging spindles. Removing spindles under the Polscope can achieve an efficiency rate of 100% in mouse oocytes. The Polscope can also be used to examine the spindle dynamics, detect spindle morphology, predict chromosome misalignment, and perform spindle transfer.

Wang WH, Keefe DL. Prediction of chromosome misalignment among in vitro matured human oocytes by spindle imaging with the PolScope. Fertil Steril 2002 Nov;78(5):1077-81

OBJECTIVE: To examine whether spindle morphologic features imaged with the LC-PolScope (Cambridge Research and Instrumentation, Woburn, MA) in living human oocytes matured in vitro can be used to predict chromosome configuration and select oocytes with normal chromosomes. **DESIGN:** Morphological study. **SETTING:** Academic IVF clinic. **PATIENT(S):** Women undergoing oocyte retrieval for ICSI treatment. **INTERVENTION(S):** Oocytes were examined after in vitro maturation. **MAIN OUTCOME MEASURE(S):** The study examined meiotic spindle morphologic features and chromosome alignments. **RESULT(S):** After culture for 22 to 24 hours, 77.1% of oocytes reached metaphase II stage, with 51.9% of oocytes showing birefringent spindles. Confocal microscopy revealed that 71% of oocytes with the birefringent spindles had normal chromosome alignment, and 29% of oocytes with birefringent spindles and all oocytes without birefringent spindles had abnormal microtubule organization and abnormal chromosome alignment. **CONCLUSION(S):** The spindle images obtained with the PolScope in living human oocytes are coordinate with those in fixed oocytes as imaged by confocal microscopy. Spindle images with the PolScope can be applied to human in vitro fertilization to help predict chromosomally normal oocytes for insemination.

Wang WH, Day B, Wu GM. How does polyspermy happen in mammalian oocytes? Microscopy Research and Technique 2003 Jul;61(4):335-41.

Polyspermy is one of the most commonly observed abnormal types of fertilization in mammalian oocytes. In vitro fertilization (IVF) provides approaches to study the mechanisms by which oocytes block polyspermic fertilization. Accumulated data indicate that oocyte, sperm and insemination conditions are all related to the occurrence of polyspermic fertilization. A high proportion of immature and aged oocytes showed polyspermy as compared with mature oocytes. Preincubation of oocytes and/or sperm with oviductal epithelial cells or collected oviductal fluid before IVF reduces polyspermic penetration. Recently, it was found that an abnormal zona pellucida is one of main causes of polyspermy in human eggs. A high proportion of polyspermy has resulted from the use of a high concentration of capacitated spermatozoa at the site of fertilization, irrespective of in the in vivo or in vitro environment. Oviductal secretions or oviductal epithelial cells themselves can regulate the number of spermatozoa reaching or binding to the zona pellucida thus reducing multiple sperm penetration. Suboptimal in vitro conditions, such as supplementations in IVF media, pH, and temperature during IVF, also induce polyspermic fertilization in some mammals. Species-specific differences are present regarding the relationship between insemination conditions and polyspermy. Copyright 2003 Wiley-Liss, Inc.

Wang WH, Gill J, Boutin C. Spindle Imaging in Living Mammalian Oocytes Using the Liquid-Crystal Polarized Light Microscope and Its Practical Use. 2003. Not yet in print.

This chapter gives a general overview of the performance and applications of the SpindleView System. Particular attention is given to software protocol and sample handling, followed by an overview of SpindleView-related applications currently performed in the field of reproductive biology.

Yoon HJ, Moon JH, Son WY, Lee SW, Yoon SH, Lim JH. The survival and fertilization of human MII-stage oocytes cryopreserved by vitrification. Fertility and Sterility. Sept 2002 (Vol. 78, Issue (Supplement 1), Page S14).

Zhan Q, Zhuang G, Li R, Cai Z, Kuwayama M, Zhang J. Reconstruction of mouse oocytes with somatic cell nuclei produces abnormal meiotic spindles. Fertility and Sterility. September 2005. (Vol. 84, Issue (Supplement 1), Page S381).

Zhu L, Kong LH, Li H, Chen SL, Xing FQ. The first polar body of human oocytes does not help predict the precise location of the spindle. Assisted Reproductive Technology Center, Nanfang Hospital, First Military Medical University, Guangzhou 510515, China. zhu_liang75@sina.com

OBJECTIVE: To observe the precise position of the metaphase II spindle for facilitating clinical practice of assisted reproduction. **METHODS:**

LC-PolScope imaging system was used for observing the spindles in human metaphase II oocytes matured in vitro or in vivo, and the angle between the center of the oocyte, the spindle and the first polar body was measured and compared between in vitro and in vivo matured oocytes. RESULTS: In vitro and in vivo matured oocytes were significantly different in the angle between the spindle and the first polar body ($P=0.006$). CONCLUSION: The deviation of the spindle from the polar body in in vitro matured oocyte was significantly smaller than that in in vivo matured oocyte, and the first polar body does not help predict the precise location of the metaphase II spindle. The imaging system is safe and effective for observing the spindles.

Zimmerman AL, Keefe DL, Pelletier C, Trimarchi JR. Correlation between the innermost layer of the zona pellucida (SP) and age using computerized image analysis of polarized light microscope (Polscope). Fertility and Sterility. September 2004 (Vol 82, Issue (Supplement 2), Page S57).

Abrio Imaging Systems – Cell Biology and General References

1996. Biology in pictures: polarized views. *Current Biology*. 6(10):1205.

A series of detailed images illustrating the dynamic architecture of living cells obtained using a new, improved polarization microscope, the CRI PolScope™, which uses a combination of electro-optical devices, polarization algorithms and digital image processing.

Flamarique, I. N. 1995. Transmission of polarized light through sunfish double cones reveals minute optical anisotropies. *Biol. Bull.* 189(2):220-222.

Allison M. D. Wiedemeier, Jan E. Judy-March, Charles H. Hocart, Geoffrey O. Wasteneys, Richard E. Williamson, and Tobias I. Baskin Mutant alleles of *Arabidopsis* RADIALLY SWOLLEN 4 and 7 reduce growth anisotropy without altering the transverse orientation of cortical microtubules or cellulose microfibrils *Development* 129: 4821-4830.

The anisotropic growth of plant cells depends on cell walls having anisotropic mechanical properties, which are hypothesized to arise from aligned cellulose microfibrils. To test this hypothesis and to identify genes involved in controlling plant shape, we isolated mutants in *Arabidopsis thaliana* in which the degree of anisotropic expansion of the root is reduced. We report here the characterization of mutants at two new loci, RADIALLY SWOLLEN 4 (RSW4) and RSW7. The radial swelling phenotype is temperature sensitive, being moderate (rsw7) or negligible (rsw4) at the permissive temperature, 19°C, and pronounced at the restrictive temperature, 30°C. After transfer to 30°C, the primary root's elongation rate decreases and diameter increases, with all tissues swelling radially. Swelling is accompanied by ectopic cell production but swelling is not reduced when the extra cell production is eliminated chemically. A double mutant was generated, whose roots swell constitutively and more than either parent. Based on analytical determination of acid-insoluble glucose, the amount of cellulose was normal in rsw4 and slightly elevated in rsw7. The orientation of cortical microtubules was examined with immunofluorescence in whole mounts and in semi-thin plastic sections, and the orientation of microfibrils was examined with field-emission scanning electron microscopy and quantitative polarized-light microscopy. In the swollen regions of both mutants, cortical microtubules and cellulose microfibrils are neither depleted nor disoriented. Thus, oriented microtubules and microfibrils themselves are insufficient to limit radial expansion; to build a wall with high mechanical anisotropy, additional factors are required, supplied in part by RSW4 and RSW7.

Baskin T, Herman T.H.M. Meekes, Benjamin M. Liang, and Robert E. Sharp Division of Biological Sciences (T.I.B., H.T.H.M.M.) and Department of Agronomy, Plant Science Unit (B.M.L., R.E.S.), University of Missouri, Columbia, Missouri, 65211 Regulation of Growth Anisotropy in Well-Watered and Water-Stressed Maize Roots. II. Role of Cortical Microtubules and Cellulose Microfibrils *Plant Physiol.* Feb(1999) 119: 681-692

We tested the hypothesis that the degree of anisotropic expansion of plant tissues is controlled by the degree of alignment of cortical microtubules or cellulose microfibrils. Previously, for the primary root of maize (*Zea mays* L.), we quantified spatial profiles of expansion rate in length, radius, and circumference and the degree of growth anisotropy separately for the stele and cortex, as roots became thinner with time from germination or in response to low water potential (B.M. Liang, R.E. Sharp, T.I. Baskin [1997] *Plant Physiol* 115:101-111). Here, for the same material, we quantified microtubule alignment with indirect immunofluorescence microscopy and microfibril alignment throughout the cell wall with polarized-light microscopy and from the innermost cell wall layer with electron microscopy. Throughout much of the growth zone, mean orientations of microtubules and microfibrils were transverse, consistent with their parallel alignment specifying the direction of maximal expansion rate (i.e. elongation). However, where microtubule alignment became helical, microfibrils often made helices of opposite handedness, showing that parallelism between these elements was not required for helical orientations. Finally, contrary to the hypothesis, the degree of growth anisotropy was not correlated with the degree of alignment of either microtubules or microfibrils. The mechanisms plants use to specify radial and tangential expansion rates remain uncharacterized

Curis, A. Cell forces in tissue. *Medical Engineering and Physics*. November 2005 (Vol. 27, Issue 9, Pages 773-779).

This article reviews the measurement, the effects and the biological importance of forces that cells exert on each other. It does not review the effects of forces originating from movement of tissues, muscular activity, movement and gravity.

Hoyt, Clifford. 1996. Birefringence imaging reveals cell components. Biophotonics International. Sept/Oct: 21-22.

The CRI PolScope™ is used to view living cells, specifically the meiotic spindle fibers due to the birefringent nature of the microtubules from which they are composed. The PolScope provides fast acquisition, high sensitivity, high dynamic range, clarity and resolution, including orientation-independent contrast.

Hoyt, Clifford and Rudolf Oldenbourg. 1999. Structural analysis with quantitative birefringence imaging. American Laboratory. 31(14):34-42.

Many materials in biomedical and industrial applications are transparent or translucent. They appear homogeneous and seem to have no structure when back-illuminated by white light. However if placed between crossed polarizers, structures become evident; some regions in the sample enable light to pass through the two crossed polarizers. Birefringence provides a unique opportunity to measure and analyze the structural order of samples, without having to treat them with exogenous dyes, fluorescence labels, or stains, or to resort to destructive and expensive electron microscopy. The development of the LC-PolScope (CRI and Marine Biological Laboratory, Woods Hole) greatly facilitates birefringence measurement and enhances the utility of the conventional polarized light microscope. This article reviews quantitative birefringence imaging, cell biology applications, biomedical applications, material science applications and the operation of the system.

Inoué, Shinya. Windows to dynamic fine structures, then and now. FASEB J 1999 Dec;13 Suppl 2:S185-90.

How can we learn about dynamic fine structures that are far too small to be resolved with the light microscope without destroying the active living cell? Examples spanning the last half century show how polarized light microscopy can and should continue to provide an attractive window for such studies. Long before microtubules were found with electron microscopy, or their assembly properties were biochemically characterized in isolated cell-free systems, the dynamic fine structure of the mitotic spindle and assembly properties of its microtubules were revealed in living cells by polarized light microscopy. More recently, the polarizing microscope was improved, by invention of the new Pol-Scope, so that quantitative measurements of birefringence retardation and axes could be made rapidly for all image pixels independent of their birefringence axis orientation. In addition, the centrifuge polarizing microscope, just developed, allows us to follow the dynamic ordering of fine structures in living cells as they become stratified or restructured by centrifugal acceleration of up to ten thousand times gravity. The significance of these technological advances is discussed-Inoué, S. Windows to dynamic fine structures, then and now.

Katoh, K. et al. 1997. Actin bundles in neuronal growth cone observed with the Pol-Scope. Biol. Bull. 193(2):219-220.

Katoh, K. et al. 1999. Arrangement of radial actin bundles in the growth cone of Aplysia bag cell neurons shows the immediate past history of filopodial behavior. Proc. Natl. Acad. Sci. USA. 96:7928-7931.

Filopodia that protrude forward from the lamellipodium, located at the edge of a neuronal growth cone, are needed to guide the extension of a nerve cell. To directly observe actin bundles in living unstained growth cones non-invasively, the authors measured and displayed the optical anisotropy (birefringence) of the cellular fine structures using the CRI PolScope™. The PolScope achieves high sensitivity and high resolution by enhancing the traditional polarizing microscope with electro-optical devices, electronic imaging and digital image analysis.

Katoh, K. et al. 1999. Birefringence imaging directly reveals architectural dynamics of filamentous actin in living growth cones. Molecular Biology of the Cell. 10: 197-210.

The authors have investigated the dynamic behavior of cytoskeletal fine structure in the lamellipodium of nerve growth cones using a new type of polarized light microscope (the PolScope™). PolScope images display with exquisite resolution and definition birefringent fine structures, such as filaments and membranes, without having to treat the cell with exogenous dyes or fluorescent labels. Furthermore, the measured birefringence of protein fibers in the thin lamellipodial region can be interpreted in terms of the number of filaments in the bundles. They confirmed that birefringent fibers are actin-based using conventional fluorescence-labeling methods. By recording movies of time-lapsed PolScope images, they analyzed the creation and dynamic composition of radial fibers, filopodia, and intrapodia in advancing growth cones. The strictly quantitative information available in time-lapsed PolScope images confirms previously deduced behavior and provides new insight into the architectural dynamics of filamentous actin.

Madibally SV, Solomon V, Mitchell RN, Van de Water L, Yarmush ML, Toner M. Influence of insulin therapy on burn wound healing in rats. Journal of Surgical Research. February 2003 (Vol. 109, Issue 2, Pages 92-100)

BACKGROUND: Insulin is proposed as a therapy for suppressing muscle wasting after burn trauma although the long-term effects of this therapy on wound healing are not yet known. The present study was designed to investigate the effect of systemically administered insulin therapy on burn wound healing. **MATERIALS AND METHODS:** Young rats weighing 80-150 g were subjected to 15-20% total body surface area burn injury on their shaved dorsum. The insulin dosage was increased over the first 3 days in each rat from 0.25 U (Day 1), 0.5 U (Day 2), and 1.0 U (Day 3)

per 100 g body wt. The rats were euthanized at the fourth or fifteenth day postinjury. Skin sections were analyzed by histochemistry and quantitative polarization microscopy. RESULTS: Histology showed a decreased number of inflammatory cells and increased vasodilation in the insulin-treated animals at Day 4 relative to untreated rats; at Day 15 there was increased reepithelialization. Quantitative analysis using polarization microscopy and picrosirius red staining showed an increased collagen deposition in wounds by Day 4 in insulin-treated rats relative to untreated burn controls. CONCLUSION: These results indicate that insulin induces accelerated wound healing associated with diminished inflammation and increased collagen deposition.

Miura M, Osako M, Elsner AE, Kajizuka H, Yamada K, Usui M. Birefringence of intraocular lenses. Journal of Cataract and Refractive Surgery. July 2004 (Vol. 30, Issue 7, Pages 1549-1555).

Oldenbourg, Rudolf. Polarization Microscopy with the LC-PolScope. In: Live Cell Imaging: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 2003. Not yet in print.

This chapter contains an extensive review of the LC-PolScope System, including descriptions of the optical setup, design specifics, acquisition and measurement parameters, and performance characteristics. The chapter also includes an overview of the desired microscope setup, including selection of optics most appropriate for polarization imaging. A description of sample preparation and background imaging offers useful insight into optimizing image quality.

Oldenbourg, Rudolf and G. Mei. 1995. New polarized light microscope with precision universal compensator. J. Microscopy. 180:140-147.

A new type of polarized light microscope for fast and orientation-independent measurement of birefringent line structure has been developed. The design of the new pol-scope incorporates a precision universal compensator made from two liquid crystal variable retarders. A video camera and digital image processing system provides fast measurements of specimen anisotropy (retardance magnitude and azimuth) at all points of the image forming the field of view. The images document line structural and molecular organization within a thin optical section of the specimen. The sensitivity of the current instrument is 0.1 nm of specimen retardance measured with data gathered in 0.43 s at all 640 x 480 image points. Examples of birefringence measurements in biological (microtubule arrays) and industrial (magneto-optical disc substrate) specimens are presented.

Oldenbourg, Rudolf et al. 1998. Birefringence of single and bundled microtubules. Biophys. J. 74(1):645-654.

We have measured the birefringence of microtubules (MTs) and of MT-based macromolecular assemblies in vitro and in living cells by using the new Pol-Scope. A single microtubule in aqueous suspension and imaged with a numerical aperture of 1.4 had a peak retardance of 0.07 nm. The peak retardance of a small bundle increased linearly with the number of MTs in the bundle. Axonemes (prepared from sea urchin sperm) had a peak retardance 20 times higher than that of single MTs, in accordance with the nine doublets and two singlets arrangement of parallel MTs in the axoneme. Measured filament retardance decreased when the filament was defocused or the numerical aperture of the imaging system was decreased. However, the retardance "area", which we defined as the image focus and of numerical aperture. These results are in good agreement with a theory that we developed for measuring retardances with imaging optics. Our theoretical concept is based on Wiener's theory of mixed dielectrics, which is well established for non-imaging applications. We extend its use to imaging systems by considering the coherence region defined by the optical set-up. Light scattered from within that region interferes coherently in the image point. The presence of a filament in the coherence region leads to a polarization dependent scattering cross section and to a finite retardance measured in the image point. Similar to resolution measurements, the linear dimension of the coherence region for retardance measurements is on the order $\lambda/(2 NA)$, where λ is the wavelength of light and NA is the numerical aperture of the illumination and imaging lenses.

Oldenbourg, Rudolf. 1996. A new view on polarization microscopy. Nature. 381(27 June):811-812.

Improvements to the traditional polarization microscope have enhanced the direct analysis of the molecular architecture in living cells with new electro-optical devices, polarization algorithms and digital image processing. The Pol-Scope™ was recently developed at the Marine Biological Laboratory in Woods Hole, MA for the analysis of sub-microscopic molecular architecture directly in living cells by analyzing the change in polarization of transmitted light. The system adapts to an upright microscope and a schematic is included. Because of its fast speed, high sensitivity, versatility and ease of use for measuring optical anisotropies, the new instrument significantly advances the analytical power of the polarizing microscope in all its traditional application areas.

Oldenbourg, Rudolf. 1999. Polarized light microscopy of spindles. In Methods in Cell Biology. 61(Chapter 10):175-208.

This chapter contains general information about the meiotic spindle; the basic set-up of the polarized light microscope, including retardance, image contrast, signal to noise ratio, the importance of high extinction, liquid crystal variable retarders and the CRI PolScope™; the analysis of spindle birefringence, including molecular origin of microtubule birefringence; birefringence of single, bundled, arrays of parallel microtubules,

birefringence of individual spindle components; and, optimum cell types for polarized light microscopy of spindles. This reference contains an excellent collection of CRI PolScope images and 76 references.

Oldenbourg R, Salmon ED, Tran PT. Birefringence of single and bundled microtubules. Biophys J 1998 Jan;74(1):645-54.

We have measured the birefringence of microtubules (MTs) and of MT-based macromolecular assemblies in vitro and in living cells by using the new Pol-Scope. A single microtubule in aqueous suspension and imaged with a numerical aperture of 1.4 had a peak retardance of 0.07 nm. The peak retardance of a small bundle increased linearly with the number of MTs in the bundle. Axonemes (prepared from sea urchin sperm) had a peak retardance 20 times higher than that of single MTs, in accordance with the nine doublets and two singlets arrangement of parallel MTs in the axoneme. Measured filament retardance decreased when the filament was defocused or the numerical aperture of the imaging system was decreased. However, the retardance "area," which we defined as the image retardance integrated along a line perpendicular to the filament axis, proved to be independent of focus and of numerical aperture. These results are in good agreement with a theory that we developed for measuring retardances with imaging optics. Our theoretical concept is based on Wiener's theory of mixed dielectrics, which is well established for nonimaging applications. We extend its use to imaging systems by considering the coherence region defined by the optical set-up. Light scattered from within that region interferes coherently in the image point. The presence of a filament in the coherence region leads to a polarization dependent scattering cross section and to a finite retardance measured in the image point. Similar to resolution measurements, the linear dimension of the coherence region for retardance measurements is on the order $\lambda/(2 NA)$, where λ is the wavelength of light and NA is the numerical aperture of the illumination and imaging lenses.

Sundararajan V. Madibally Ph.D., Vered Solomon Ph.D., Richard N. Mitchell M.D., Ph.D. , Livingston Van De Water Ph.D., Martin L. Yarmush M.D., Ph.D. and Mehmet Toner Ph.D. Influence of insulin therapy on burn wound healing in rats. J Surg Res 2003 Feb;109(2):92-100, Wound healing/Plastic surgery.

Background. Insulin is proposed as a therapy for suppressing muscle wasting after burn trauma although the long-term effects of this therapy on wound healing are not yet known. The present study was designed to investigate the effect of systemically administered insulin therapy on burn wound healing. Materials and methods. Young rats weighing 80-150 g were subjected to 15-20% total body surface area burn injury on their shaved dorsum. The insulin dosage was increased over the first 3 days in each rat from 0.25 U (Day 1), 0.5 U (Day 2), and 1.0 U (Day 3) per 100 g body wt. The rats were euthanized at the fourth or fifteenth day postinjury. Skin sections were analyzed by histochemistry and quantitative polarization microscopy. Results. Histology showed a decreased number of inflammatory cells and increased vasodilation in the insulin-treated animals at Day 4 relative to untreated rats; at Day 15 there was increased reepithelialization. Quantitative analysis using polarization microscopy and picrosirius red staining showed an increased collagen deposition in wounds by Day 4 in insulin-treated rats relative to untreated burn controls. Conclusion. These results indicate that insulin induces accelerated wound healing associated with diminished inflammation and increased collagen deposition.

Tang, Jay X., Oldenbourg, Rudolf , Allen, Philip G. , and Janmey, Paul A. Tactoidal granules in concentrated actin gels: a solid-like state of protein filaments.

We report here the first observation and microscopic characterization of tactoidal granules of actin in concentrated gels of actin filaments (F-actin). Phase contrast microscopy shows these stable tactoids of densely packed F-actin to be of various sizes on the order of 10 μ m. The background gel of F-actin is optically birefringent, indicating orientational order of the filaments consistent with theoretical predictions. In contrast, no birefringence is detected through the tactoids, suggesting very distinct yet undetermined packing of the filaments inside. The tactoids demonstrate elastic response upon micromanipulation. The microscopic segregation of F-actin in two states of different protein concentrations is consistent with a first order phase transition between a nematic gel of lower concentration on the order of ten mg/ml and a highly packed, possibly columnar state of protein filaments. In addition to the excluded volume effects which are known to drive thermodynamic phase transitions as a function of particle concentration, additional molecular forces must also play important roles since the formation was found to be irreversible upon dilution.

Tran, P. et al. 1994. Muscle fine structure and microtubule birefringence measured with a new pol-scope. Biol. Bull. 187(2):244-245.

Tran, P. 1995. Quantifying single and bundled microtubules with the polarized light microscope. Biol. Bull. 189(2):206.

Yano T, Miura T, Whittaker P, Miki T, Sakamoto J, Nakamura Y, Ichikawa Y, Ikeda Y, Kobayashi H, Ohori K, Shimamoto K. Macrophage colony-stimulating factor treatment after myocardial infarction attenuates left ventricular dysfunction by accelerating infarct repair. Journal of the American College of Cardiology. 07 Feb 2006 (Vol. 47, Issue 3, Pages 626-634).

OBJECTIVES: We aimed to determine the effects of macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF) treatment on both the repair process and ventricular function after myocardial infarction (MI). **BACKGROUND:** The M-CSF and G-CSF



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Selected Annotated References Using CRI PolScope Technology

have multiple potential effects on cells involved in wound repair. **METHODS:** Myocardial infarction was induced by 45- or 90-min coronary occlusion and reperfusion in rats with or without subsequent injection of M-CSF (10(6) IU/kg/day) or G-CSF (50 microg/kg/day) for five days. We examined histology and messenger ribonucleic acid (mRNA), and assessed left ventricular function in situ using a conductance catheter. **RESULTS:** Five days after MI, M-CSF increased the number of ED-1-positive cells, mRNA levels of transforming growth factor-beta-1, collagen I and III, and collagen fibers within the infarct. Fourteen days after MI, induced by 45-min ischemia, left ventricular end-systolic elastance (Ees) was reduced (1,191 +/- 87 mm Hg/ml vs. 1,812 +/- 150 mm Hg/ml) and both isovolumic relaxation time constant (tau) (11.9 +/- 0.9 ms vs. 8.5 +/- 0.4 ms) and left ventricular end-diastolic volume (LVEDV) (0.225 +/- 0.014 ml vs. 0.172 +/- 0.011 ml) increased versus sham-operated rats. These alterations after MI were attenuated by M-CSF (Ees = 1,650 +/- 146, tau = 9.7 +/- 0.7, LVEDV = 0.199 +/- 0.012) but not by G-CSF. This beneficial effect of M-CSF on Ees was also detected in hearts with MI induced by 90-min ischemia. Furthermore, M-CSF increased collagen content within infarcts and reduced the proportion of thin collagen fibers 14 days after MI. The Ees significantly correlated with infarct collagen content. Nevertheless, neither M-CSF nor G-CSF modified infarct size. **CONCLUSIONS:** The M-CSF treatment attenuates deterioration of left ventricular function after MI by accelerating infarct repair.