ESHRE SIG Embryology Course:
Session 2: Developmental kinetics and imaging

"Oocyte and zona imaging using polarisation microscopy "

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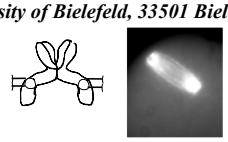
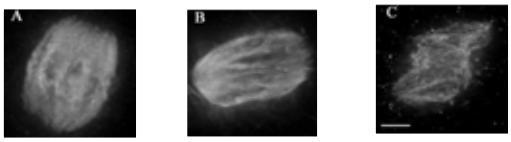


Selection of mature oocytes with high developmental competence to obtain good embryos and/or use oocyte scores to obtain additional information on embryo quality

1. ***Oocyte with normal genetic constitution (faithful separation of chromosomes on the meiotic spindle)***
2. ***Cytoplasmic mature oocyte (chromatin epigenetically remodelled during growth; full recruitment of maternal RNA and proteins, e.g. zona proteins; high number of functional mitochondria)***
3. ***Oocyte from healthy follicle (efficient paracrine signalling to provide optimal conditions for oocyte maturation but also all aspects of ovulation and remodelling of extracellular matrix which may support oocyte/sperm interactions and early development and implantation)***

Normal Genetic Constitution and Potential to Support Chromosome Segregation in the Preimplantation Embryo:

Aberrant spindles are a hallmark of low quality human metaphase II oocytes (e.g. from aged patients which are frequently aneuploid)

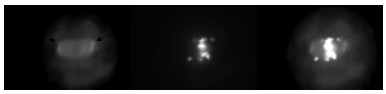


Non-invasive analysis of spindle morphology and formation

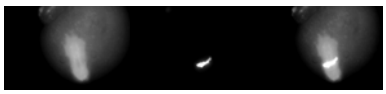
Eichenlaub-Ritter et al., RBMOnline, 2004 Jan;8(1):45-58.

Spindles are Sinks for many Components needed for Embryonic Development: Expression of EGFP-NuMA-Fusionproteins

EGFP-NuMA: Meiosis I



EGFP-NuMA: Meiosis II



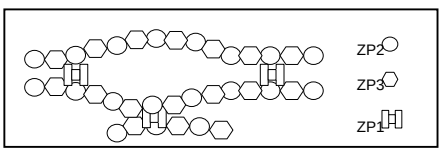
EGFP-NuMA an Spindeln und polaren MTOCs

Eichenlaub-Ritter & Peschke, Hum. Reprod. Update 2002

Cytoplasmic Maturity and Follicular Health:

The zona pellucida is secreted by the oocyte during oocyte growth and therefore should reflect oocyte and follicular health

A normal zona protects the embryo and therefore may contribute to normal development



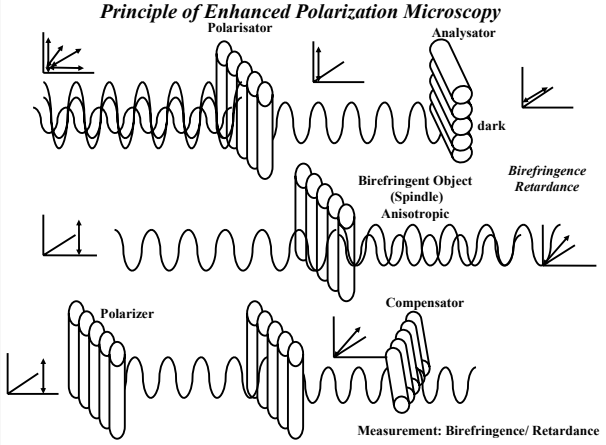
Non-invasive analysis of zona pellucida characteristics

1. *Oocyte Scoring at Meiosis II Arrest after Oocyte Retrival from the Follicle*

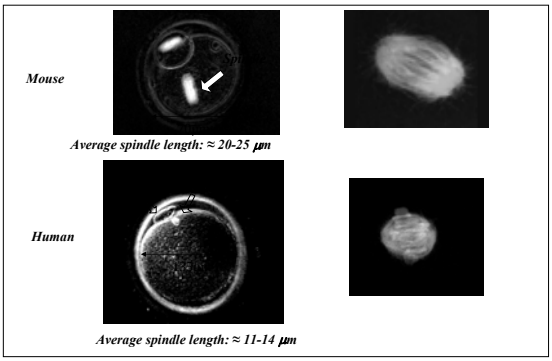
2. *Scoring by Analysis of Kinetics and Maturity at in vitro Maturation*

1. *Identify the oocytes from a cohort that have the „highest“ quality and developmental potential (without chromosomal aberrations)*
2. *Identify factors (e.g. stimulation protocol, handling/culture conditions) that may contribute to obtain ‚healthy‘ oocytes*
3. *Obtain information on whole cohort/treatment cycle to predict success and/or councel patients*

Principle of Enhanced Polarization Microscopy

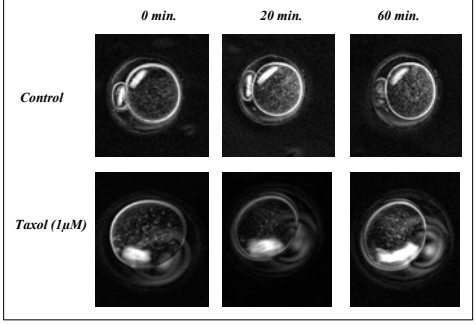


Novel tool to assess spindle expression, shape and high order structure: Polarisation microscopy with circularly polarised light and LC optics



Eichenlaub-Ritter et al. , 2002, RBMOnline 5, 117-124.

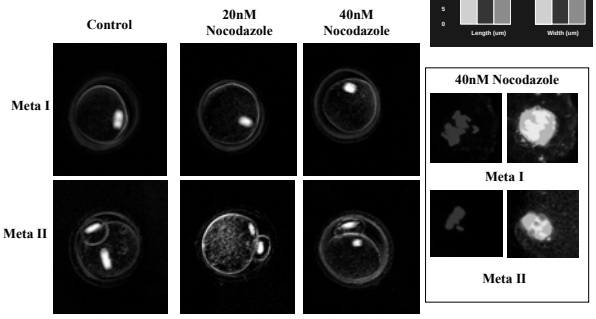
Dynamic Alterations in Metaphase II Spindles of Mouse Oocytes: Increase in Length during Taxol -Exposure

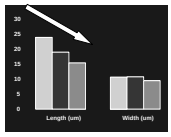


Eichenlaub-Ritter et al., (2002) RBM Online 5(2)117-124.

Direct Correlation to Microtubule Polymerization, Exposures and Risks for Errors in Chromosome Segregation:

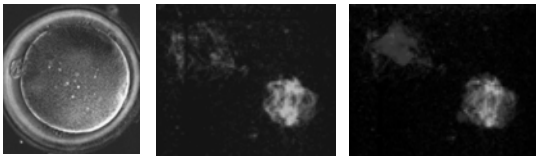
Nocodazole-induced Shortening of Spindles and Congression Failure in Mouse Oocytes



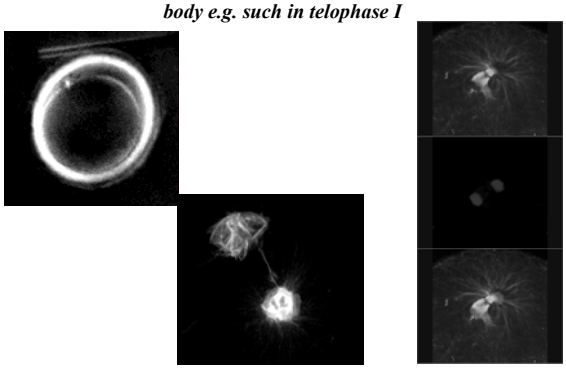


Shen et al., 2005, Reprod.Toxicol., 19(4):459-71.

Absence of a birefringent spindle is frequently associated with disturbed spindle organization and chromosome congression failure



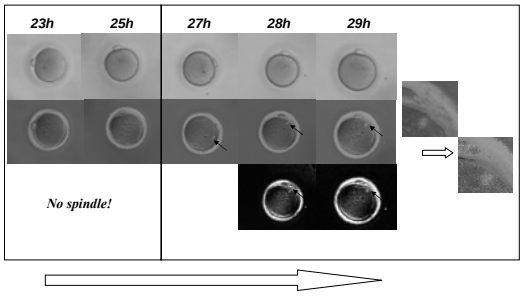
Polscope in Assisted Reproduction: Detection of immature or aberrant oocytes with first polar body e.g. such in telophase I



Dynamics (timing and progression) of anaphase I and polar body formation/ metaphase II arrest can be assessed

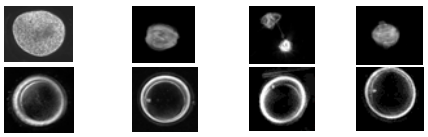
Movie

Immature human oocytes maturing in vitro without cumulus need even more than 2 hours to establish a robust metaphase II spindle after PB formation

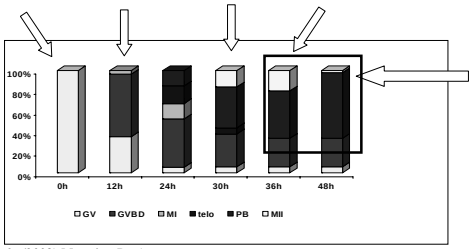


4 to 5 Hours!

A fairly low percentage of in vitro matured oocytes contain a spindle although the majority form a first polar body

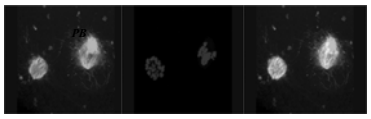


Increase aneuploidy in in vitro matured PB oocytes from ICSI cycles ! (Magli et al., 2006)



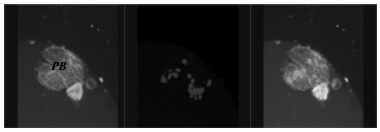
(Shen et al., (2008) Mutation Res.)

Oocytes with ‚mature' spindle more likely have well aligned chromosomes



Chromosomes aligned

Shrinking and loss of birefringence may result in displacement of chromosomes

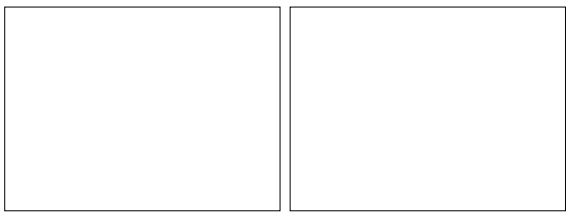


Unordered Chromosomes

Timing of progression to meiosis II is dependent on maturation conditions/ components in culture media and this may significantly affect developmental potential and quality of embryos!

MediumA: PB start at ≈7+3hrs

Medium B: PB start at ≈7+1.5hrs



Octax - Polarisation Microscopy:
Circularly polarised light/ elecronically controlled LC polarizing optics

Dynamics (timing and progression) of anaphase I and polar body formation/ metaphase II arrest can be assessed, for instance in relation to source, handling or culture conditions of oocytes and in particular for selection of fully mature oocytes and embryos with high developmental compentence

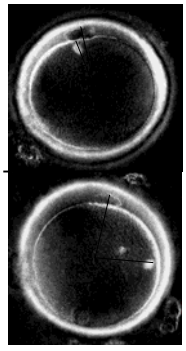
Influence of absence/presence of a birefringent spindle on fertilization and development

Fertilization of human oocytes with or without birefringent spindle

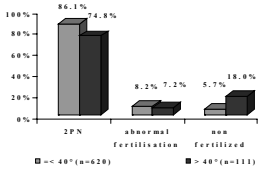
	n	% + spindle	%Fertilized + spindle	%Fertilized -spindle	Embryo quality
Wang et al. (2001) Fertil.Steril.	533	61.4	61.8	44.2[a]	
Wang et al. (2001) Hum.Reprod.	1544	82.0	69.4	62.9[a]	
Rienzi et al. (2003) Hum. Reprod.	532	91.0	74.8	33.3[b]	
Cooke et al. (2003) Hum.Reprod.	124	92.7	70.4	n.d.	
Moon et al.(2003) Hum.Reprod.	626	83.6	84.9	75.7[a]	(62.9/35.9)
Cohen et al. (2004) Hum.Reprod.	770	76.0	70.6	62.2[a]	
Konc et al. (2004)J.Ass.Reprod.Genet.	428	74.8	73.4	n.d.	
Shen et al. (2006) RBM Online	1369	83.9	88.5	66.4[b]	
Chamayou et al., RBM Online	967	42.9(?)	n.d.	n.d.	(43.5/48.5)
Rama Raju et al., (2007)RBM Onl.	205	88	82.5	31.1[a]	(48.5/14.3[a])

Significant difference to oocytes with spindle, [a] P < 0.05; [b] P < 0.001

(5) Positioning of a birefringent spindle and rate of fertilization



Significantly more oocytes with spindle <40° away from PB are fertilized, and more oocytes with ≥40° away from PB fail to become fertilized



(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

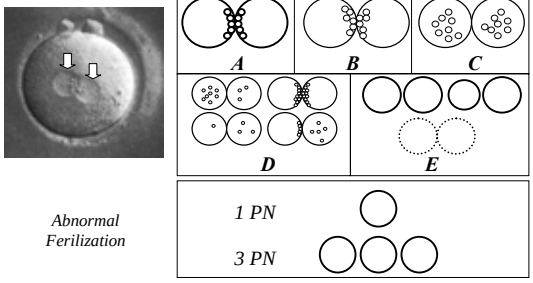
There may be less aneuploidy in embryos with good PN-score compared to bad PN-score:

Balaban et al., 2004, RBMOnline 8, 695-700.

Gianarolli et al., 2003, Fertil. Steril. 80, 341-349.

Kahraman et al., 2002, Hum Reprod. 17,3193-3200.

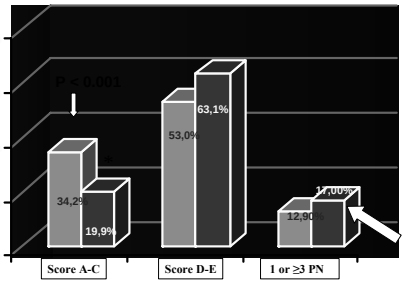
PN-Score (Scott et al., 1998) as indicator for quality and chromosomal constitution



Abnormal Ferilization

(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

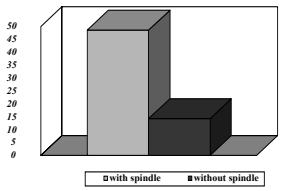
Significantly fewer oocytes without birefringent spindle form embryos with „good“ PN-score.



There is a tendency that oocytes without spindle more frequently develop into mono-or multinucleate embryos.

Shen et al., 2006, RBM Online

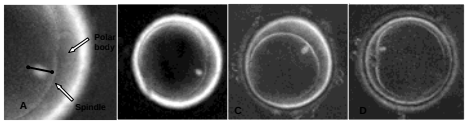
Significantly fewer oocytes without birefringent spindle develop to blastocysts

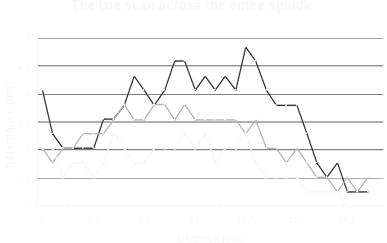


Rama Raju et al., 2007, RBM Online

Quantitative Assessment of Average Retardance and Spindle Length

Embryo: Score A Embryo: Score D Embryo: Score E





Shen et al., RBM Online, 2006

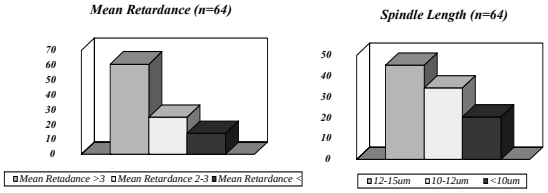
Mean retardance of light and spindle length correlate to PN-Score

	n	Retardance (nm)	Length (μm)
PN-Score A,B	180	1.72 ± 0.43	12.7 ± 1.8
PN-Score C	51	1.53 ± 0.40*	12.5 ± 1.6
PN-Score D	324	1.52 ± 0.44**	12.6 ± 1.7
PN-Score E and Abnormals	121	1.39 ± 0.46**	11.7 ± 1.7**

Significantly different to score A,B; * $p < 0.05$; **$p < 0.001$.

Shen et al., 2006, RBM Online

Mean retardance of light and length of oocyte spindle correlate to development to blastocyst



Rama Raju et al., 2007, RBM Online

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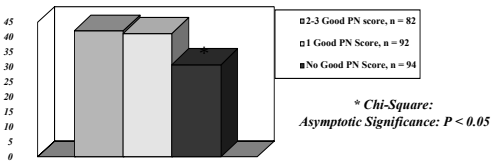
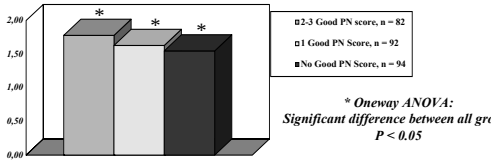
254 transfer oocytes from 103 cycles, mean age 32.5±4.4 years

Percent of Embryos in Conception Cycles



** Chi-Square:*
Asymptotic Significance: P < 0.05

Mean Retardance of Oocyte Spindle in Conception Cycles



** Oneway ANOVA:*
Significant difference between all groups
P < 0.05

Shen et al., 2006, RBM Online

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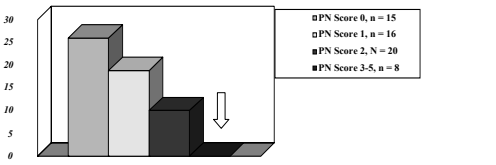
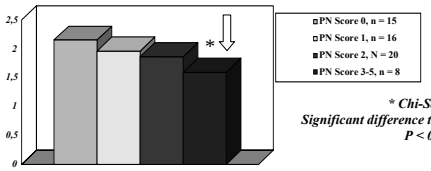
59 transfer oocytes from 28 cycles, mean age 33.7±4.3 years

Percent of Embryos in Conception Cycles



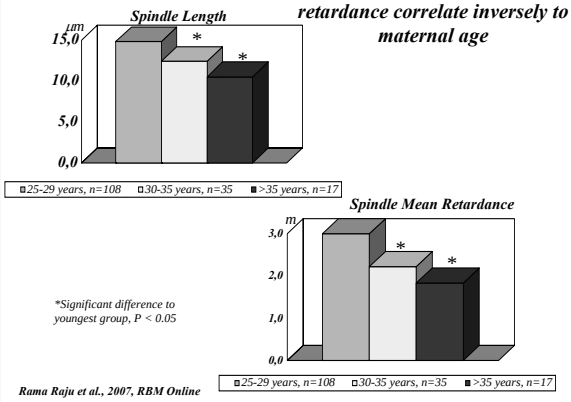
Mean Retardance of Oocyte Spindles



** Chi-Square:*
Significant difference to group one and two:
P < 0.05

Shen et al., 2006, RBM Online

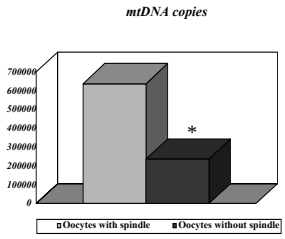
Spindle length and mean retardance correlate inversely to maternal age

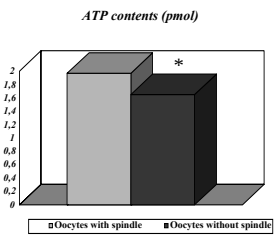


*Significant difference to youngest group, P < 0.05

Rama Raju et al., 2007, RBM Online

Presence of meiotic spindle and mitochondria and ATP content of in vitro matured human oocytes





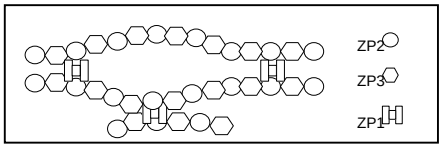
** Significant difference to group with spindle: P < 0.05*

Zeng et al., 2007, Hum Reprod

Magnitude of retardance and length of the human oocyte spindle may be used to identify non-invasively oocytes from a cohort forming embryos with good PN-score, which may be chromosomally normal and possess high developmental potential (e.g. high number and activity of mitochondria)

Mean magnitude of retardance and length of the human oocyte spindle may be used to identify indivisual patients/cycles with higher or lower chance to conceive

Imaging of Zona Pellucida



- ***All cell organelles with highly ordered paracrystalline lattice like the spindle (possessing parallel microtubule bundles) but also the zona pellucida exhibit intrinsic optical properties in polarized light:***

  a. Birefringence (shift in plane of vibration)

  b. Retardance (phase shift, retardantion of polarized light)

  c. Magnitude of retardance (birefringence) is proportional to numbers of fibres

<u>Quantitative Imaging of the Zona Pellucida of Human Oocytes (Pelletier et al. 2004, Fertil. Steril):</u>

3 Layers; retardance initially increases at fertilization; zona then becomes thinner during early development

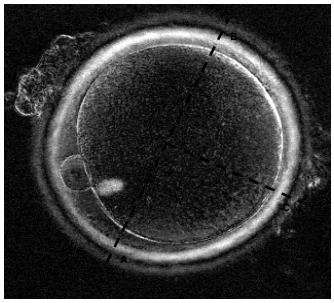
Quantitative Imaging of the ZP prior to fertilzation by ICSI (Shen et al., 2005, Human Reproduction, 20:1596-1606)

•Analysis of Mean Thickness of the Layers of the Zona Pellucida

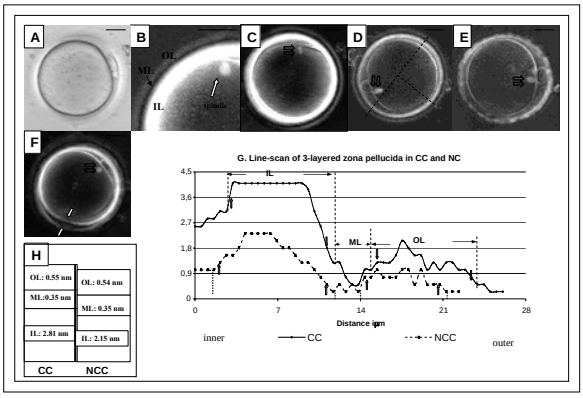
•Analysis of Mean Retardance of Layers of the Zona Pellucida

•Comparison of Data between Oocytes used in Transfer in Conception Cycles (CC) versus Non-Conception Cycles (NCC)

Quantitative analysis of the Zona Pellucida



(Shen et al., (2005) Hum. Reprod. 20:1596-1606)



(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

No significant difference between conception cycle (CC) and non-conception cycle (NCC) in:

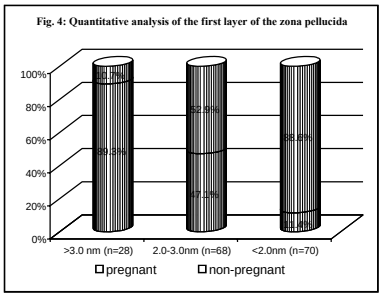
- ***Patient age***
- ***Maternal smoking***
- ***Number of attempts***
- ***Peak Oestradiol levels***
- ***Number of follicles***
- ***Number of oocytes***
- ***Fertilization rate***
- ***Oocytes with birefringent spindle***
- ***Number of embryos transferred***

Thickness (µm)	2.81±0.60#	2.15±0.41
	11.35±1.61#	9.35±1.71

Mean retardance of zona inner layer about 30% higher in CC compared to NCC!

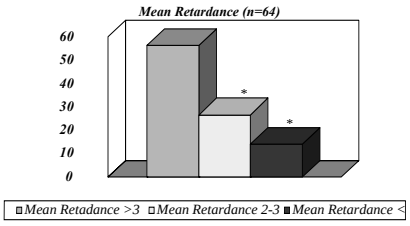
(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

Cut-off at average 3 nm of retardance predictive for CC in nearly 90% of cases.
Cut-off at average of 2 nm of retardance predictive of NCC in nearly 90% of cases.



(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

Mean retardance of the inner zona layer correlates to development to blastocyst



**Significant difference beteen groups, p < 0.05*

Rama Raju et al., 2007, RBM Online

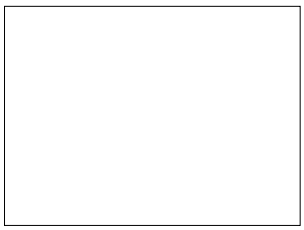
Study by Munne et al. 2007:
Embryo grading is not very predictive for presence of chromosomal aberrations

Chromosomal aberrations in good grade (1) embryos: 30%

Chromosomal aberrations in low grade (4) embryos: 44%

Qualitative and quantitative analysis of spindle and zona retardance has additional predictive value to assess chromosomal aberrations of the oocyte and may help to identify those ‚best' oocytes with presumably full nuclear and cytoplasmic maturity related to good developmental potential leading to implantation, conception cycles and birth of a healthy child.

Delayed progression into anaphase I, polar body formation and establishment of a metaphase II spindle can be an indicator of a mutation or adverse exposure during oocyte maturation!



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MTG: Octax Polarisation Microsc.