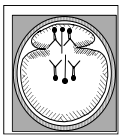
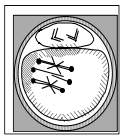
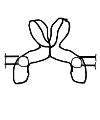


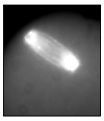


"Understanding the oocyte: Is there a way to recognize the best?"

U. Eichenlaub-Ritter

University of Bielefeld, 33501 Bielefeld, Germany



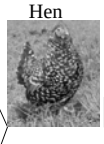


What comes first??

Nutrition/Survival

Life Style,Age

Hen *Meiosis* Fetus Baby Mother



Oocyte Quality

Embryogenesis begins during oogenesis (Paul Wassarman, 1988)
Oogenesis begins during embryogenesis (David Albertini)
Past and present is important- the ‚history' of the oocyte determines developmental potenial and health (Ursula Eichenlaub-Ritter)

„ History" of the Oocyte

Prinatal
- Erasure of Imprints during Primordial Germ Cell Formation

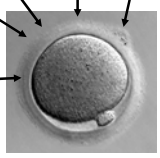
2. Pairing and Recombination and Genetic Exchange ⇒ Necessary for Chiasma Formation and Sister Chromatid Cohesion

Postnatal
- Follicle Formation and Follicular Dynamics- Chronological Ageing and Depletion of the Pool

Peri-ovulatory
- Follicular Development and Oocyte Growth- Extensive Cross-talk in Regulation of Gene Expression/Aquisition of ‚Maturity'

5. Oocyte Maturation- Chromosome Segregation and Preparation for Fertilization/ Support of Embryonal Development

1. Genetic background
2. Exposures/ Life style/ Nutrition
3. Hormonal Homeostasis
4. Handling/ Culture Conditions



„ History“ of the Oocyte

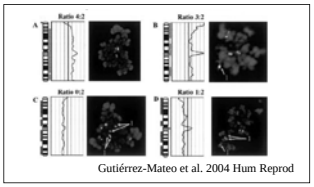
1. Erasure of Imprints during Primordial Germ Cell Formation **?**
2. Pairing and Recombination and Genetic Exchange ⇒ Necessary for Chiasma Formation and Sister Chromatid Cohesion

Chromosomal constitution (polar body analysis)

No direct test available!

Altered expression
Chromosomal imbalance

„ History“ of the Oocyte



Gutiérrez-Mateo et al. 2004 Hum Reprod

Chromosomal constitution (polar body analysis)

Fluorescent in situ Hybridisation (FISH)/arrays

Comparative Genomic Hybridisation (CGH)

Most dramatic changes seen with advanced age:

5% oocyte aneuploidy at mean 22 years
22% oocyte aneuploidy at mean of 32 years
65% aneuploid oocytes at mean 41 years

2.7 times more hypoploid than hyperploid

(Dagan Wells; Fragouli et al., in press)

AGE and Aneuploidy

„ History“ of the Oocyte

1. Erasure of Imprints during Primordial Germ Cell Formation **?**
2. Pairing and Recombination and Genetic Exchange ⇒ Necessary for Chiasma Formation and Sister Chromatid Cohesion

New approach:

Identify genes in aneuploid oocytes and cumulus cells of aneuploid oocytes:

a. Origin of high rate of nondisjunction in patient
b. Non-invasive markers of oocyte aneuploidy

<u>Pros and Cons in PB Testing:</u>
Pros:
Can aid in identifying euploid oocytes
Potential to reduce miscarriage and improve single embryo transfer
Cons:
Invasive
Requires time- cryopreservation
Not helpful with few oocytes

Altered Gene Expression Patterns reported by Wells and Coworkers

Altered gene expression in aneuploid oocytes:

Spindle dynamics — Cytoskeleton
Chromatin structure,
Cell cycle regulation
Cell surface — Secreted protein

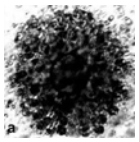
→ **Polarisation Microscopy**
→ **Polarisation Microscopy**
Morphology
GVBD/PB/PN Formation/Cleavage
→ **Polarisation Microscopy/FF**

Altered gene expression in cumulus of aneuploid oocytes:

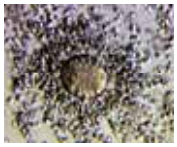
Hypoxia — Nutritional depriviation
Stress pathways
Cumulus proliferation — apoptosis — differentiation

→ **Vascularisation**
Markers in FF
Morphology
Non-invasive Markers

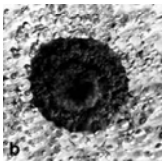
Cumulus Morpholgy



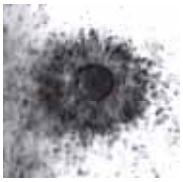
Corona radiata with mature oocyte

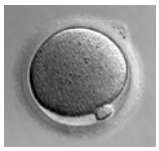


Normal expanded Cumulus



Compact corona immature oocyte





Normal Oocyte

Cycles with >50% mature grade 3 oocyte cumulus complexes yielded higher pregancy rates (Ng et al., 1999)

Courtesy: Lucinda Veeck Gosden, N.Y.

Non-invasive markers of oocyte quality: Follicle/ Cumulus/Granulosa Cells/Follicular Fluid

Classification	Parameters	References
Follicular Vascularisation /Blood Flow	*Transvaginal power-Doppler analysis of vascularisation and blood flow*	*Van Blerkom et al. 1997, - 2000; Borini et al., 2001; Palomba et al., 2006 (no);Paffoni et al., 2006; Raga et al., 2007 (no) Mercer et al.*
Morphology/Expansion of Cumulus	*C… A…*	
Apoptosis	*Apoptosis in cumulus or granulosa cell…*	*Piquette et al., 1994; Lee et al., 2001; Yang & Rajamahendran, 2002; Abu-Hassan et al., 2006 …*
Components in FF	*IGFs & IGFBPs;*	*Artini et al., 1994; Kawano et al., 1997, Oosterhuis et al., 1998; Fried et al., 2003; …*
	G… T… B… steroids,	*…r et …ncin …2008*
	leptin; nitric oxide, lipid peroxidases/ ROS	*Mantzoros et al., 2000, Wunder et al., 2005, DePlacibo et al., 2006 Bedaiwry et al., 2004, Lee et al., 2004; Pasqualotto et al., 2004*
Gene expression profiling	*Expression of Has2, Cox-2, Grem1, pentraxin 3 a… RBBP7, BUB… Cdc42; catepsin; BCL2l1/10;PCK1*	*Mckenzie et al., 2004; Zhang et al., 2005; Salustri et al., 2005; Gasca et al., 2007;Paffoni …; Cilo et al. 2007;Hamel et al.2008; Benegowda et al., 2008;Assou et al., 2009*

Branching of signalling pathways downstream from LH surge; this may cause uncoupling of oocyte maturation and from cumulus expansion

Is it feasable to assess apoptosis in individual follicles prior to/or shortly after fertilization?

Analysis of concentrations of protein or message in individual follicles and quantitation may be difficult and require too much time to be useful in routine clinical procedures to identify the„best" high quality oocyte; Some factors likeAMH rather related to depletion of pool

Omics are most promising

Adapted and extended from Wang & Sun, 2007, Reprod, Fertil. & Develop. 19, 1-12

Identifying Good Quality Oocytes at Oocyte Level

Requires isolation/ ICSI and in cases like chromosomal analysis also cryopreservation

2. *Oocyte*
   a. *Stage of development/nuclear maturation (GV/GVBD/PB)*
   b. *Chromosomal constitution (polar body analysis)*
   c. *Cytoplasmic maturation/ developmental potential (morphology/dysmorphisms)*
   d. *Oocyte secreted factors*

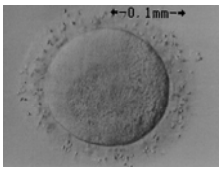
Assessment of the best after cryopreservation

Markers may differ from those of fresh oocytes!

3. *Cyopreserved Oocyte*
   a. *Intactness with respect to all cellular components*

Dysmorphisms

Giant Oocyte



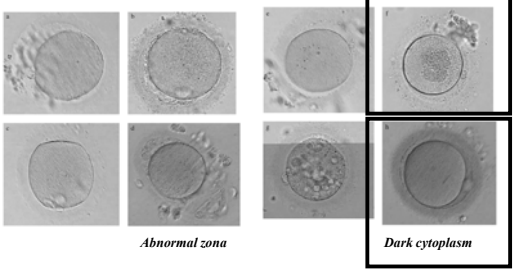
Mostly diploid oocytes that form digynic triploid embryos after fertilization
Incidence comparatively low (0.26% overall and 7.8% among cohorts from patients with normal oocytes plus giant oocytes (Rosenbusch et al., Hum. Reprod.)

Courtesy:Ying Shen and Kinderwunschzentrum, Dortmund

Half of embryos from oocytes with central granularity were aneuploid (Kahraman et al., 2000)

Large perivitelline space/ perivitelline debris

Central granularity

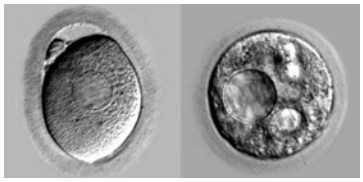


Abnormal zona

Dark cytoplasm

Presence of a dark cytoplasm decreased by 83% the likelihood of obtaining good quality embryos (Ten et al., 2007)

Courtesy: Ten et al., 2007, RBM Online



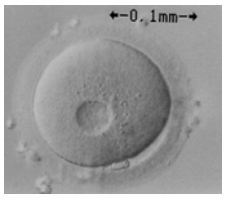
Large Vacuoles

Aberrant Zona

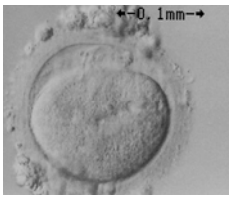
Presence of large vacuoles reduced fertilization rate (Ebner et al., 2006)
And multiple vacuoles were associated with increased degeneration after ICSI (De Sutter et al., 1996)

Courtesy: Lucinda Veeck Gosden, N.Y.

Oocyte with Vacuoles



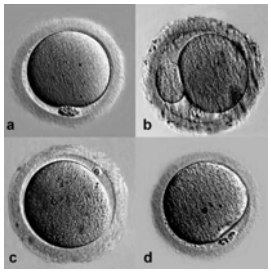
Large Vacuole



Small Vacuoles

Fragmented first PB
Large perivitelline Space

Courtesy:Ying Shen, Dieterle, Dortmund IVF



A. *Normal MII*
B. *Large PB*
C. *Small PB*
D. *Fragmented PB*

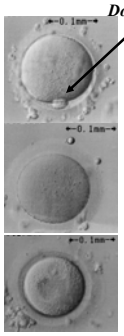
Large Pb may relate to sub-optimal culture conditions or genetic background in animal models
The presence of an enlarged PB was also related to poorer rates of fertilization, cleavage, and top quality embryos but not fragmentation (Navarro et al., 2008)

Fragmented polar body was associated with reduced blastocyst formation rate (Ebner et al., 2006; Balaban & Urman, 2006)- correlation to timing of 1PB formation??

Courtesy: Lucinda Veeck Gosden, N.Y.

Oocyte Maturational Stage- Nuclear Maturation

Do such oocytes contain a meiosis II spindle?

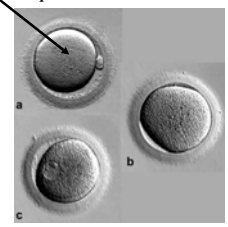


Polar Body

Germinal Vesicle Breakdown/ Metaphase I

Germinal Vesicle

Courtesy:Ying Shen, Drs. Dieterle, Neuer & Greb, Dortmund

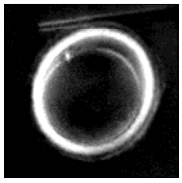


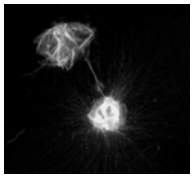
A: Normal MII
B: GVBD
C: GV

Courtesy: Lucinda Veeck Gosden, N.Y.

Polscope in Assisted Reproduction:

Detection of Immature Oocytes with first Polar Body in Telophase I

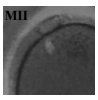




Human oocytes need about 2 hours to establish a metaphase II spindle after PB formation

75-90 min

40-60 min



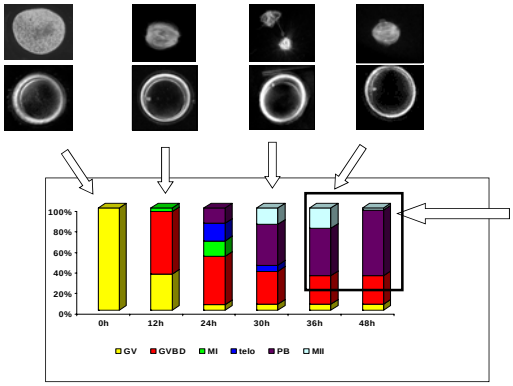
M.Montag, University of Bonn / OCTAX polarAIDE™

There is no information of ‚late' oocytes (telophase I).

GV or MI oocytes may be able to progress to meiosis II but are likely to be compromised in developmental potential even when able to emit a first polar body and become fertilized!

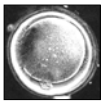
The detection of such oocytes may help to optimise treatment or counselling.

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

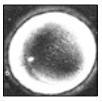


Shen et al., Mutat. Res. 2008

Fewer in vitro matured oocytes possess birefringent spindles compared to in vivo matured oocytes!



In vitro matured MII oocytes N =33	*In vivo* matured MII oocytes N = 203
10(30.3%)	177(86.8%)
23(69.7%)[a]	26 (13.2%)



[a] Significant difference to in vivo matured oocytes of the same group of patients (P < 0.001)

A high percentage of immature GV-stage oocytes that matured in vitro to Metaphase II are aneuploid!

70% vs. 54% aneuploidy in in vivo versus in vitro matured oocytes, P<.005)

62% vs. 40% complex aberrations; P<.001

55% vs. 34% chromatid containing oocytes; P<.001

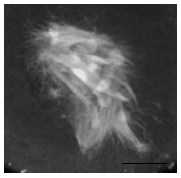
Magli et al., 2006 Fertil Steril. 86(3):629-35

Information on ‚delayed' oocytes is missing!

Accelerated or delayed maturation can be related to adverse exposures inducing checkpoint or changes in gene expression that predispose to errors in chromosome segregation!

Several genes in cell cycle regulation, spindle formation and chromosome separation are altered in expression in aged oocytes.

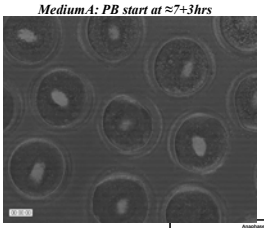
Depletion of such genes in animal model by RNAi causes altered maturation kinetics, spindle aberrations and aneuploidy!



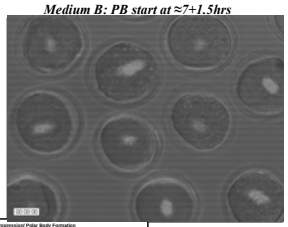
Eichenlaub-Ritter et al., in preparation

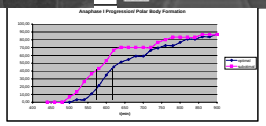
In in vitro maturation (and probably also in vivo) timing of progression to meiosis II is dependent on milieu- maturation conditions/ components in culture media

MediumA: PB start at ≈7+3hrs



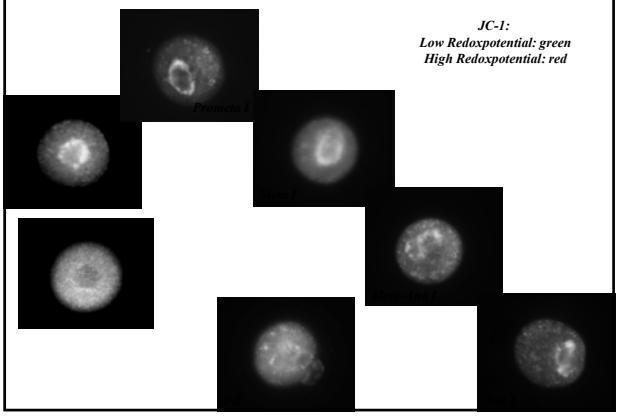
Medium B: PB start at ≈7+1.5hrs





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

Characteristic Distribution of Mitochondria in naked in vitro maturing Mouse Oocytes



JC-1:
Low Redoxpotential: green
High Redoxpotential: red

Expression of mitochondrial Genes (Rhesus Monkey)

Differences in the expression of mitochondrial genes in 2-cell embryos of rhesus monkey depending on hormonal stimulation and developmental potential

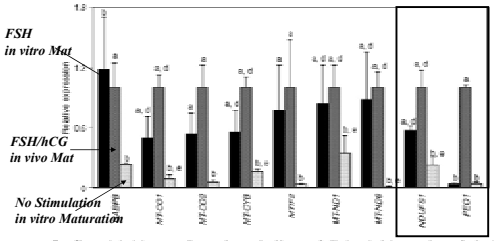
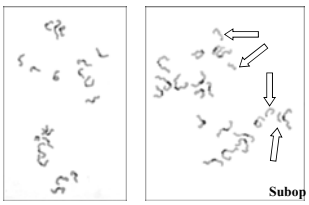


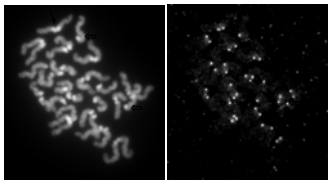
Fig. 1. Effect of hormonal stimulation on two-cell stage embryos produced by *in vitro* fertilization. Stimulation protocols: FSH, females given follicle-stimulating hormone (FSH) injection only, fully grown oocytes from large antral follicles matured *in vitro* (open columns); hCG, females injected with FSH and human chorionic gonadotrophin (hCG), fully grown oocytes from large antral follicles matured *in vivo* (solid columns); NS, females received no stimulation, fully grown oocytes from small antral follicles matured *in vitro* (stippled columns). Genes are listed alphabetically. Values for mean ± s.e.m. expression are normalized to the average hCG value (assigned a value of 1.0) for each mRNA. Different letters denote statistically significant differences within stage ($^{a-c}P < 0.05$, $^{A-C}P < 0.006$).

Mtango et al., 2008

Maturation in Sub-optimal Media induces Precocious Chromatid Separation!

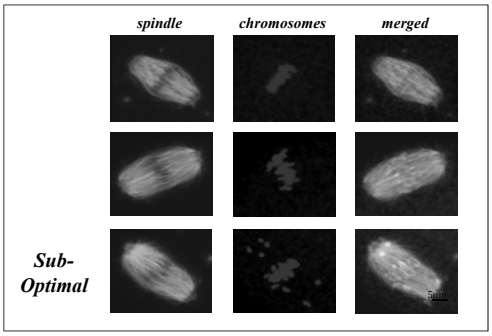


Redox potential in cytoplasm and/or mitochondria influences chromosome behaviour



Few chromatids (monads) in MII oocyte matured in optimal conditions

Subpotimal maturation conditions/ components in culture media may increase chromosome congression failure



Pragmatic Approach:

<u>IVF/ICSI</u>
Identify dysmorphisms, analyse spindle and zona

Are oocytes mature?

How many oocytes are ‚immature'

Change protocol
Decide on timing for ICSI/IVF
Identify ‚risk' patients

<u>IVM:</u>

Optimize culture conditions
Stimulation protocols/ timing of fertilisation etc.

<u>Altered gene expression in aneuploid oocytes:</u>

Spindle dynamics — Cytoskeleton → **Polarisation Microscopy**

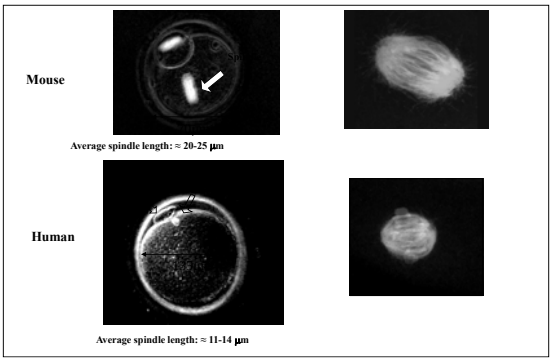
Chromatin structure → **Polarisation Microscopy**

Cell cycle regulation → **GVBD/PB/PN Formation/Cleavage**

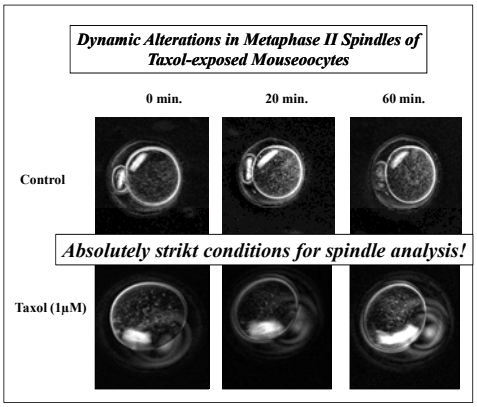
Cell surface — Secreted protein → **Polarisation Microscopy/FF**

Debate: Can presence, positioning, length and birefrigence of the meiotic spindle be predictive of oocyte quality?

Polarisation microscopy with circularly polarised light and LC optics

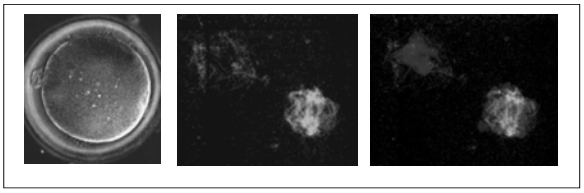


Eichenlaub-Ritter et al. , 2002, RBMOnline 5, 117-124.

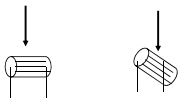


Eichenlaub-Ritter et al., (2002) RBM Online 5(2)117-124.

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



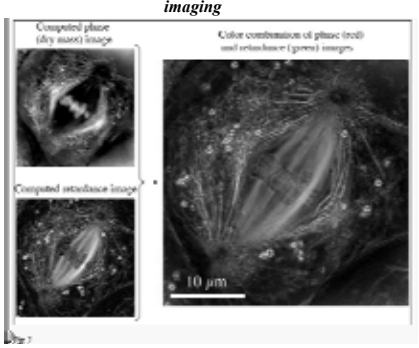
This does not implicate that presence of a spindle predicts chromosome alignment!



Retardance and length is dependent on orientation relative to plane of view

Orientation in plane of view is essential!

Study by Shribak et al., J. Biomed. Opt. 2008:
Spindles and chromosomes in crane fly spermatocyte viewed by combined orientation-independent polarisation microscopy plus computed phase (dry mass) imaging

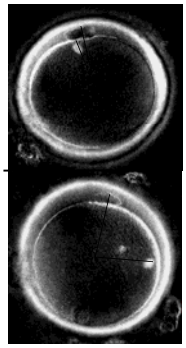


Fertilization rate of human oocytes without birefringent spindle is reduced

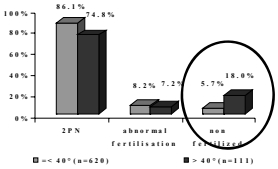
	n	*% + spindle*	*%Fertilized + spindle*	*%Fertilized -spindle*	*Embryo quality*
Wang et al. (2001) Fertil.Steril.	*533*	*61.4*	*61.8*	*44.2[a]*	
Wang et al. (2001) Hum.Reprod.	*1544*	*82.0*	*69.4*	*62.9[a]*	
Rienzi et al. (2003) Hum. Reprod.	*532*	*91.0*	*74.8*	*33.3[b]*	
Cooke et al. (2003) Hum.Reprod.	*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

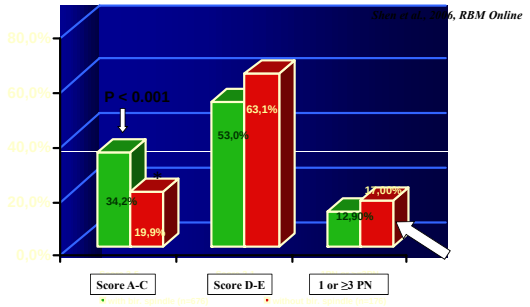


Significantly more oocytes with spindle <40° away from PB are fertilized, and more oocytes with ≥40° away from PB fail to become fertilized

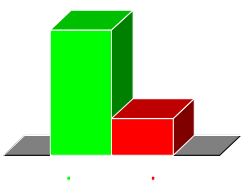


Significantly fewer oocytes without birefringent spindle form embryos with „good“ PN-score.

Shen et al., 2006, RBM Online

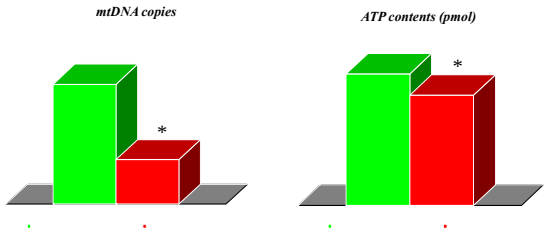


Significantly fewer oocytes without birefringent spindle develop to blastocysts



Rama Raju et al., 2007, RBM Online

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

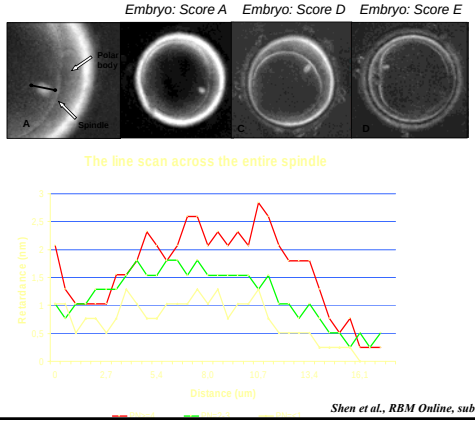


** Significant difference to group with spindle: P < 0.05*

mtDNA copy number and ATP content particularly low in maturation incompetent oocytes

Zeng et al., 2007, Hum Reprod

Quantitative Assessment of Average Retardance and Spindle Length



Shen et al., RBM Online, submitted

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 :

1. ***to embryo quality (Shen et al., 2006, RBM Online)***
2. ***conception cycle (Shen et al., 2006, RBM Online)***
3. ***to development to the blastocyst (Rama Raju et al., 2007, RBM Online)***
4. ***to mean maternal age (Rama Raju et al., 2007, RBM Online)***

Spindles are extremely metastable organelles!!!

High retardance and ‚normal' shape is not necessarily associated with aligned chromosomes and euploid state

Difficult to define general ‚cut-off' values for good/bad oocytes

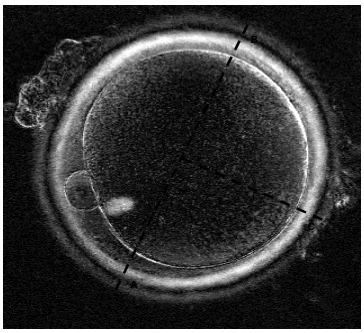
However, low retardance or absence of spindle may signal
Problems with handling
Problems with stimulation
Genetic predisposing factor
Helpful to identify ‚best' oocyte

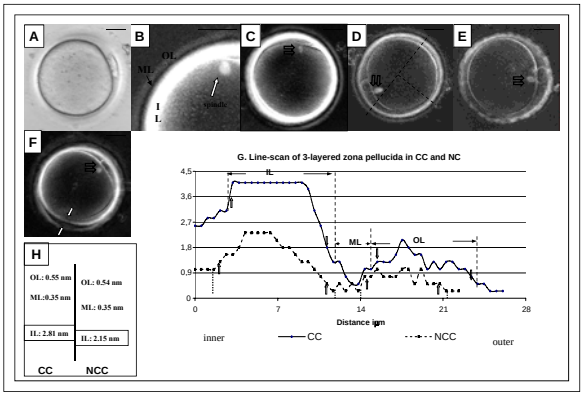
Cryopreservation: Reduced birefringence indicative of reduced fibre density after cyopreservation (Cotticio)

Altered gene expression in aneuploid oocytes:

Spindle dynamics Cytoskeleton	→ **Polarisation Microscopy**
Chromatin structure, Cell cycle regulation	→ **Polarisation Microscopy GVBD/PB/PN Formation/Cleavage**
Cell surface Secreted protein	→ **Polarisation Microscopy/FF**

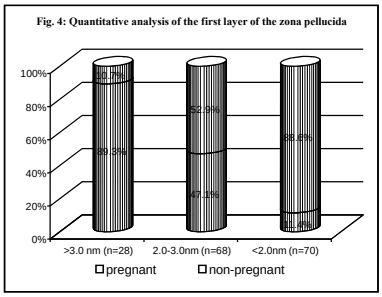
Quantitative analysis of the Zona Pellucida





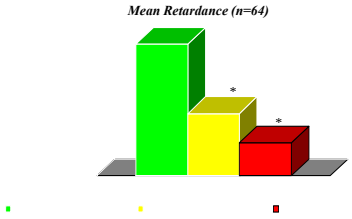
(Shen et al., (2005) Hum. Reprod. 20:1596-1606)

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)

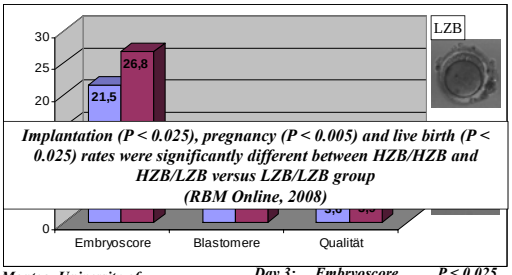
Mean retardance of the inner zona layer correlates to development to blastocyst



**Significant difference beteen groups, p < 0.05*

Rama Raju et al., 2007, RBM Online

Relative high retardance of the inner zona layer correlates to high embryo quality on day 3



Implantation (P < 0.025), pregnancy (P < 0.005) and live birth (P < 0.025) rates were significantly different between HZB/HZB and HZB/LZB versus LZB/LZB group (RBM Online, 2008)

M.Montag, University of Bonn
OCTAX polarAIDE™

Day 3:	*Embryoscore*	*P < 0.025*
	Blastomeres	*P = 0.089*
	Quality	*P < 0.001*

Assessment of Oocyte Quality

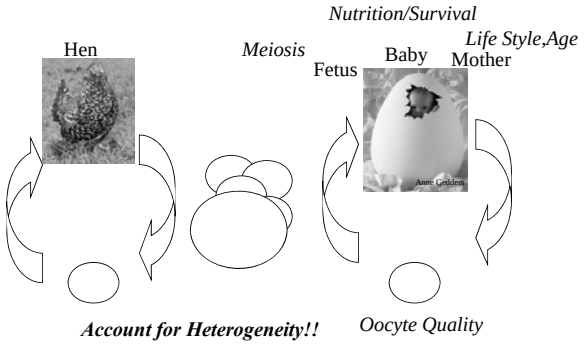
Morphological markers:
interference optics
polarisation microscopy
imaging of kinetics

Molecular markers:
FF
Cumulus
Metabolites/oxygen consumption

Genetic markers:
Screening for polymorphisms/ susceptibilty genes

Personalised Treatment in Routine IVF!
New approaches in routine treatment!

What comes first??



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Thank you for your attention!