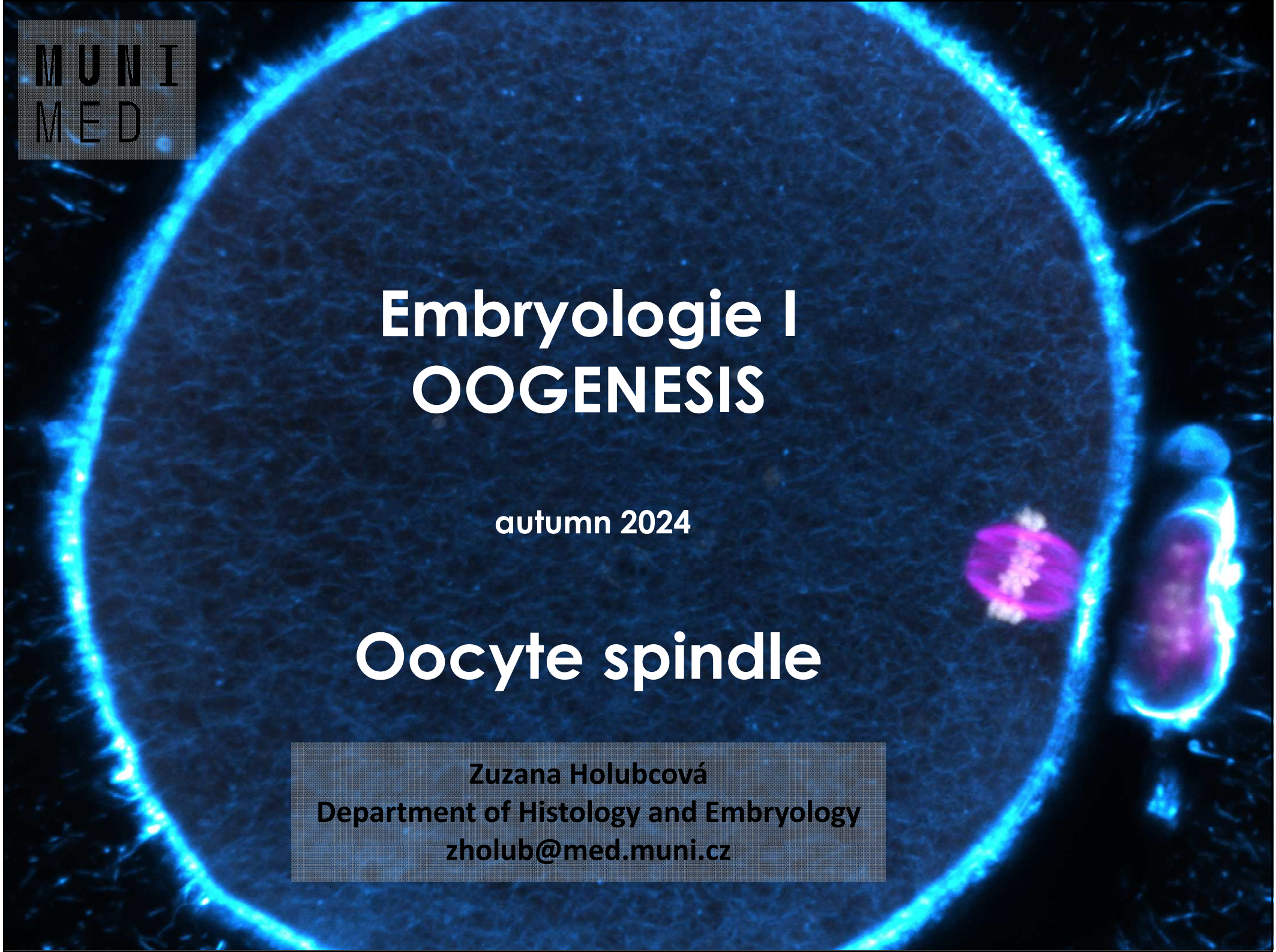


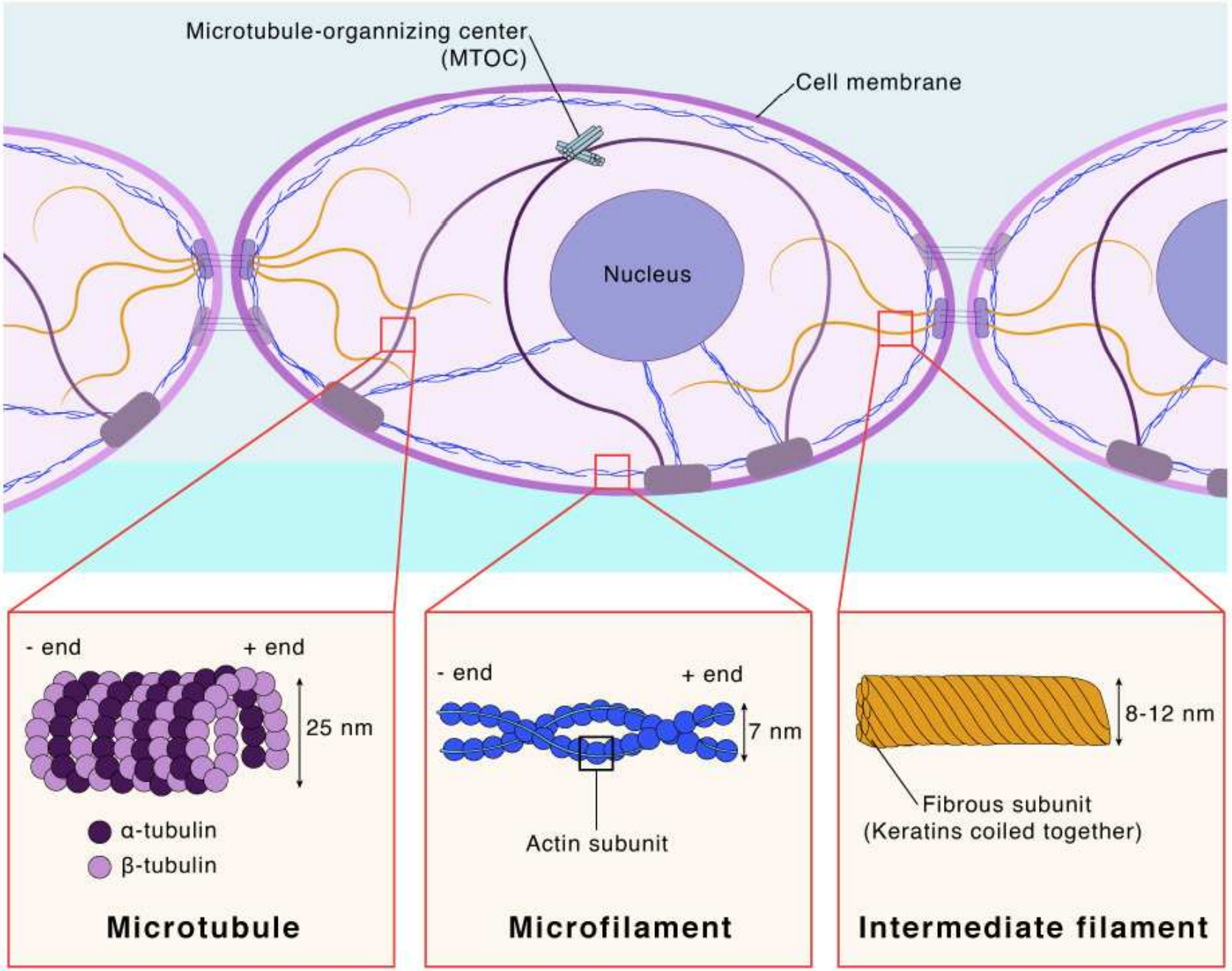
Embryologie I
OOGENESIS

autumn 2024

Oocyte spindle

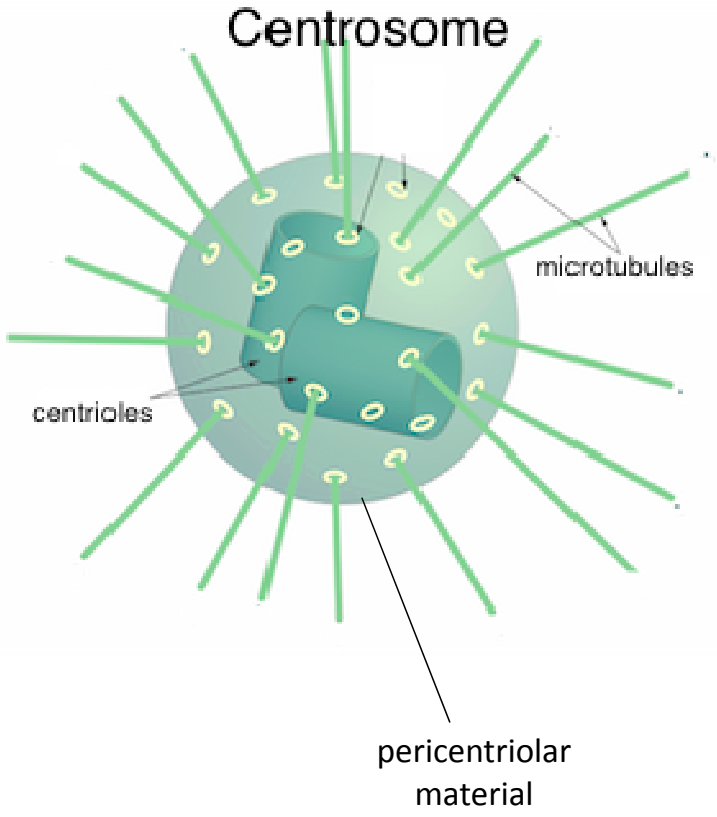
Zuzana Holubcová
Department of Histology and Embryology
zholub@med.muni.cz

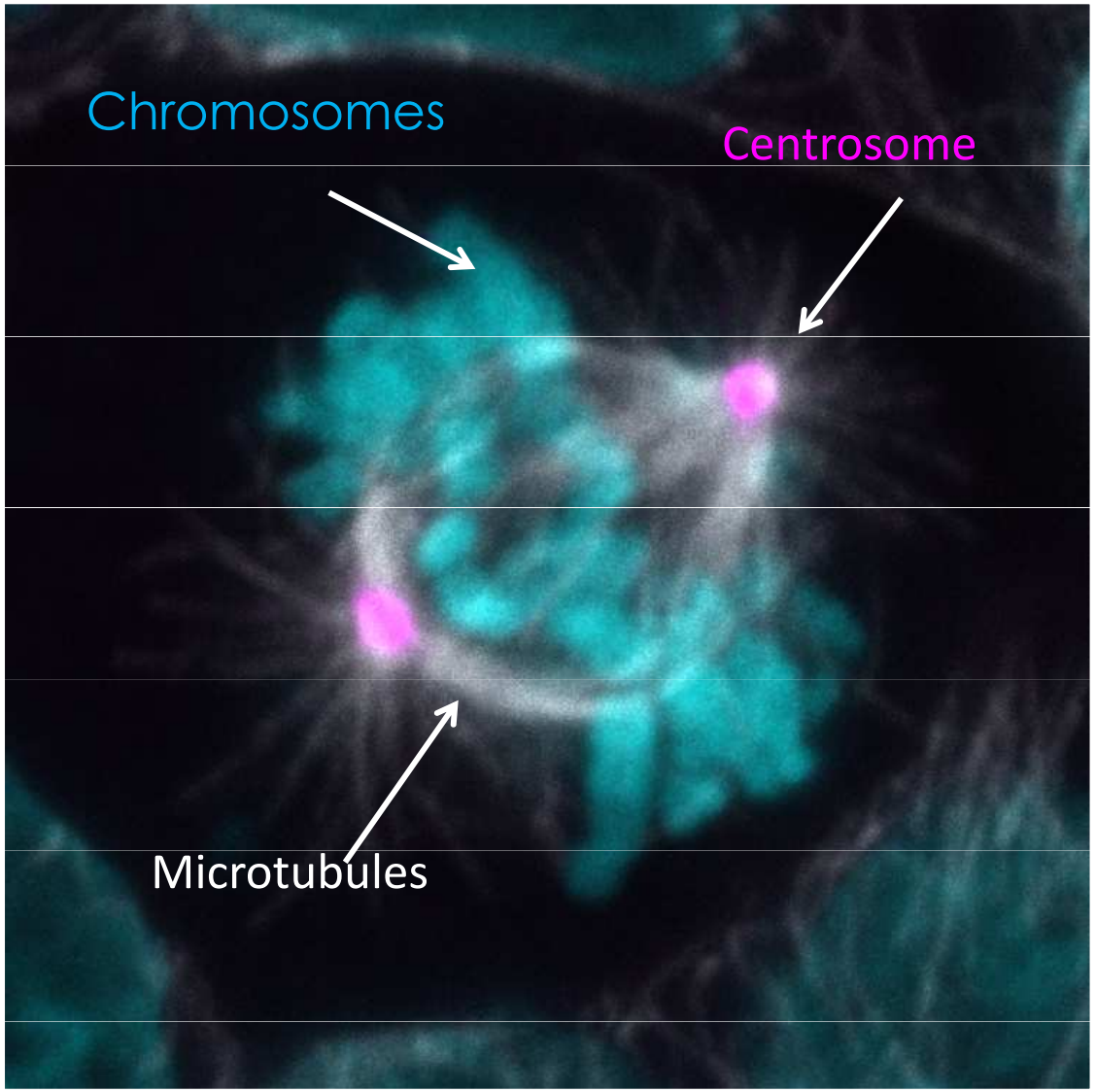
Cytoskeleton



Centrosome

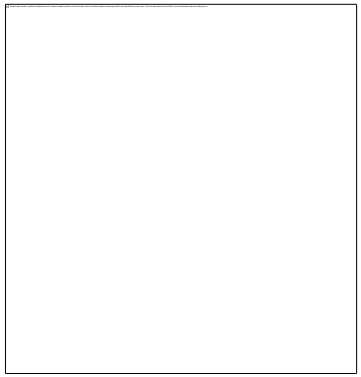
Centrosome = major Microtubule Organising Center (MTOC) in animal cells

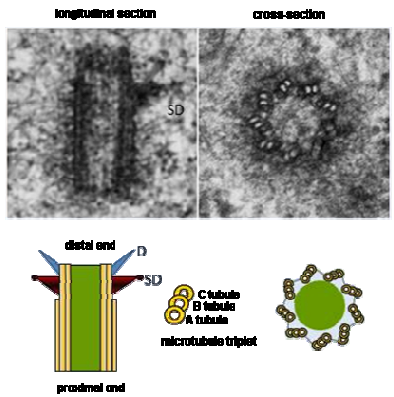


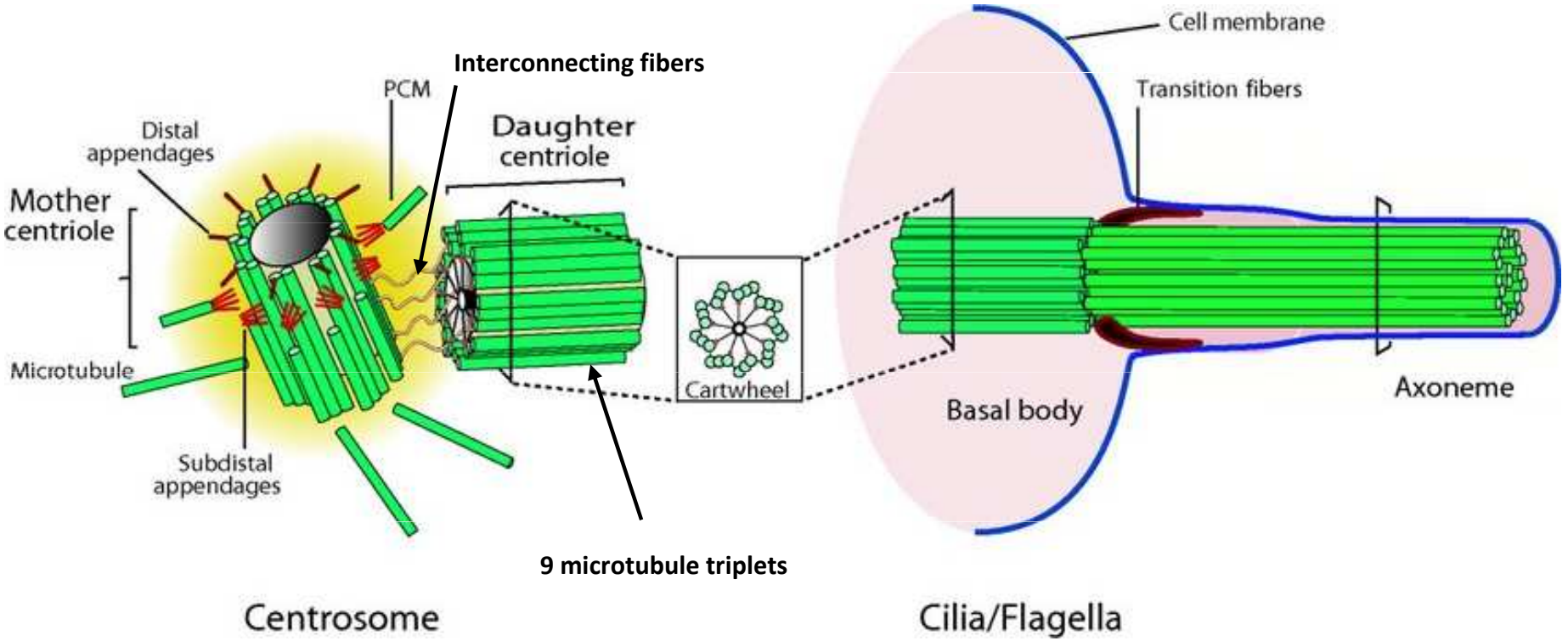


Centrosome

➢ Centrioles

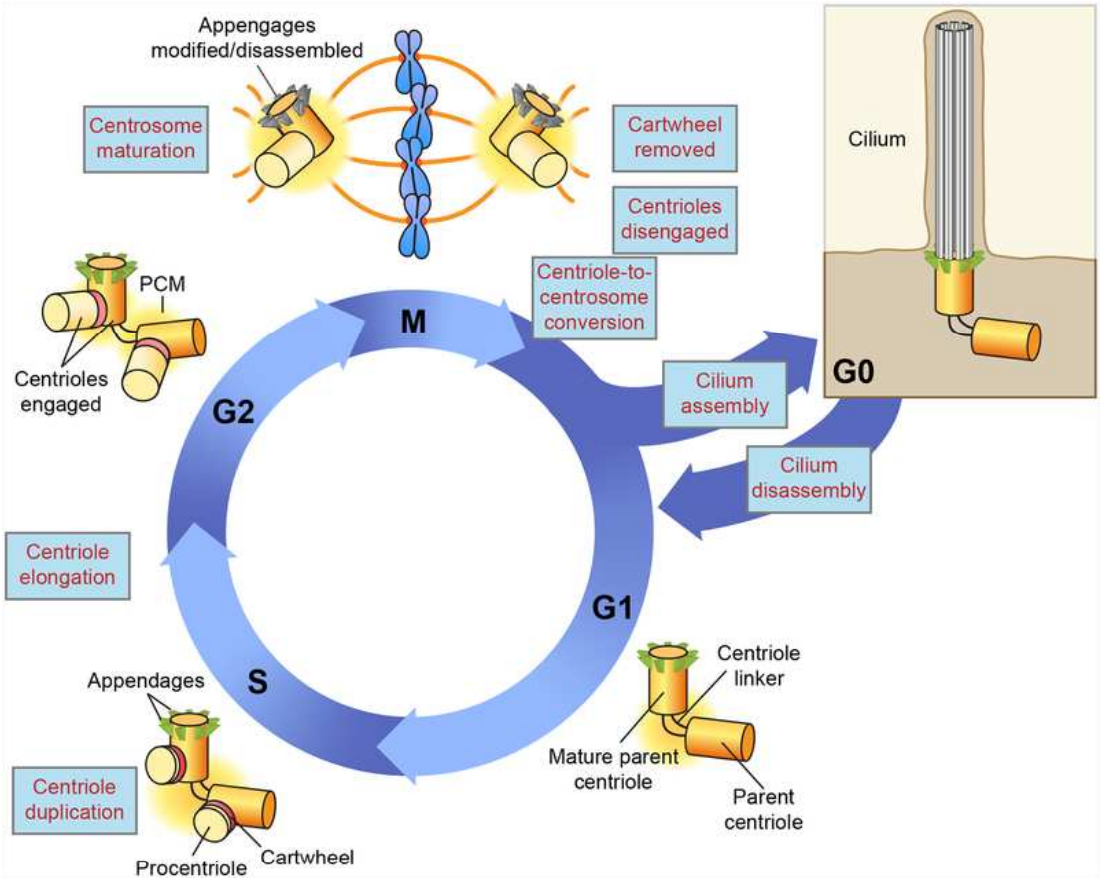


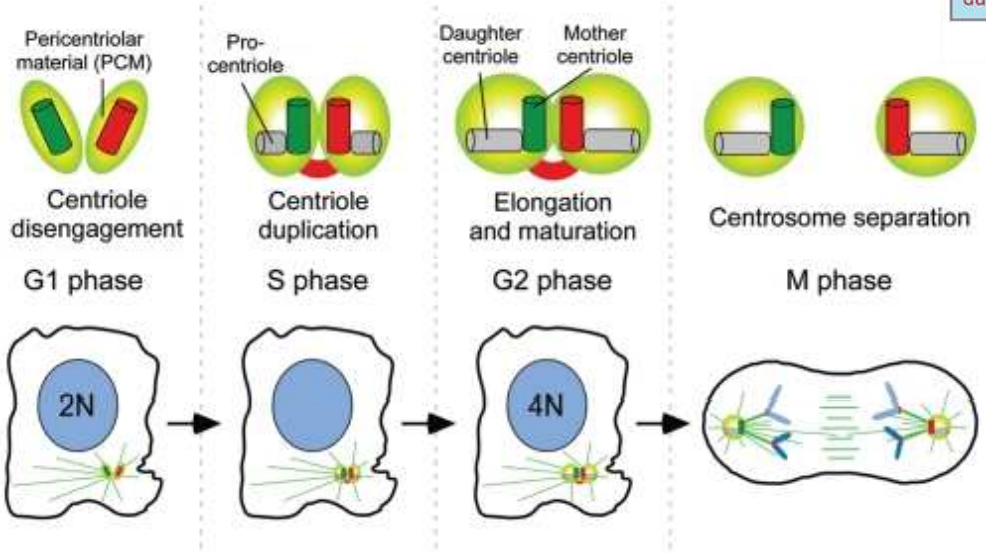




Centrosome

➢ Centrrosome duplication cycle



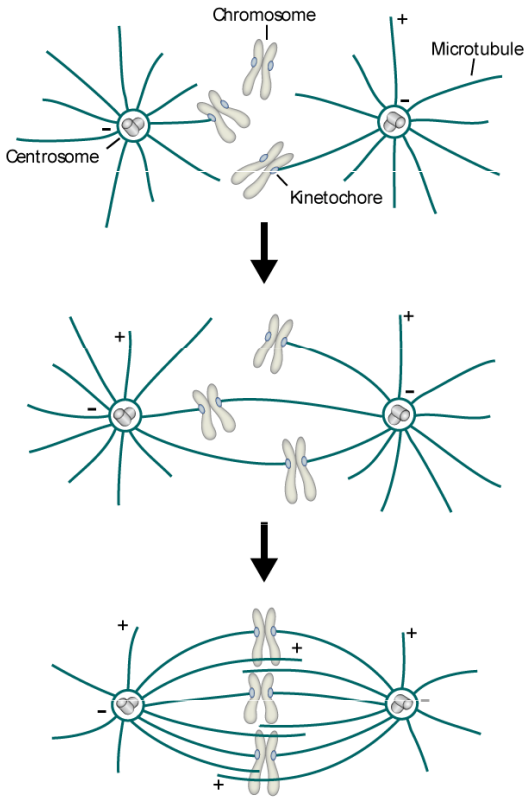


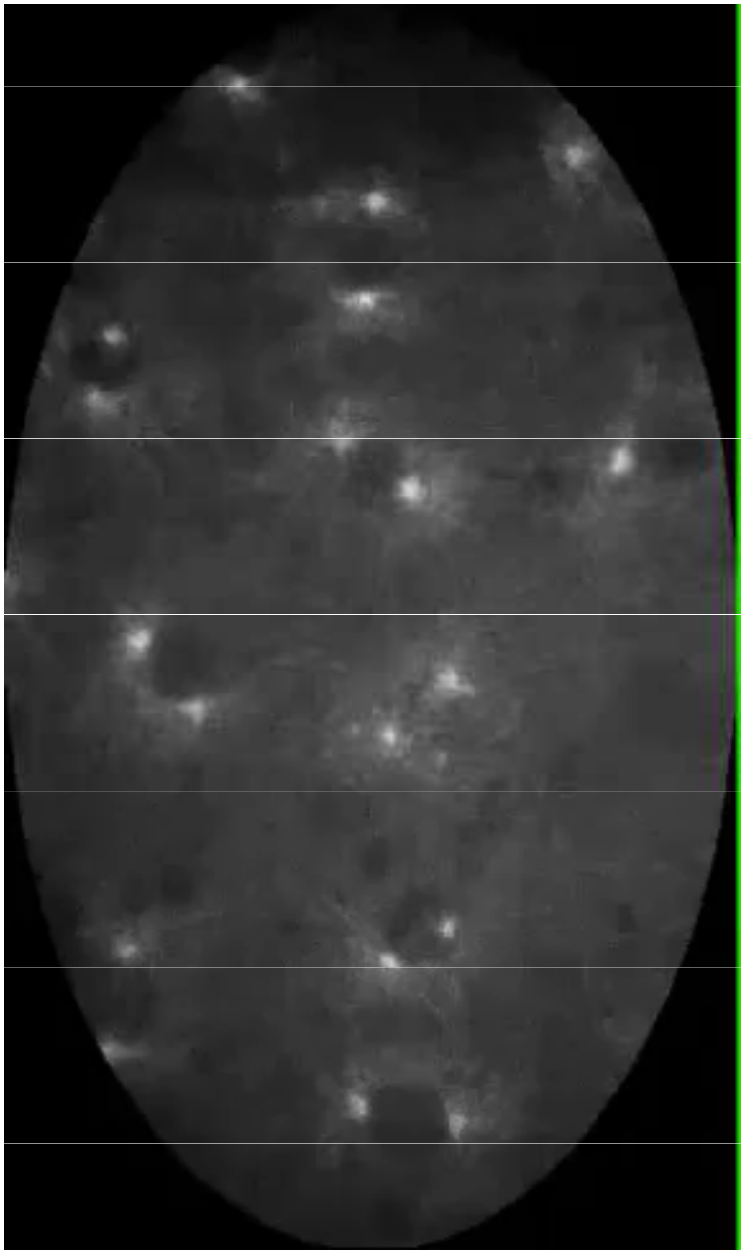
Centrosomes duplicate in coordination with DNA synthesis

Centrosome

In animal somatic cells, centrosomes

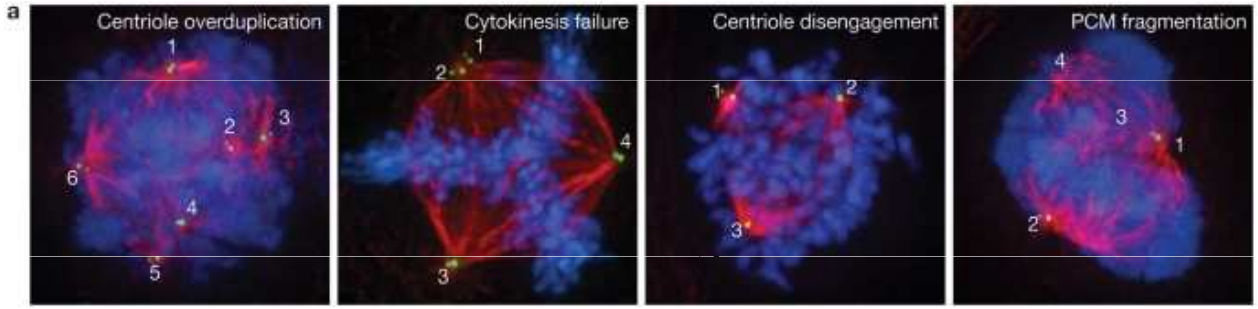
- **drive microtubule (MT) nucleation**
- **focus microtubule (-)ends at spindle poles and stabilize spindle poles**
- **assemble central bipolar spindle that evenly segregate sister chromatids during mitosis**

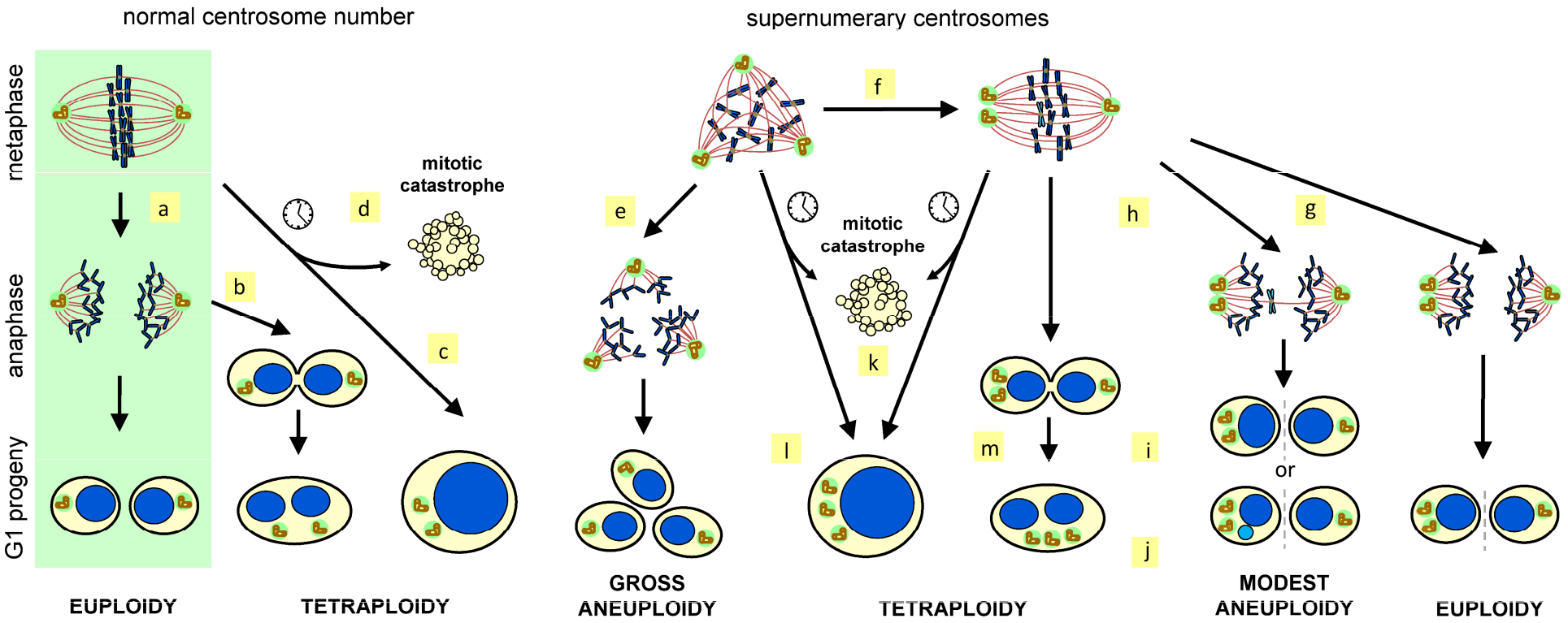




Centrosome overamplification

- occurs in cancer cells
- promotes genetic instability
- acentriolar centrosomes (only PCM) capable to nucleate and capture microtubules

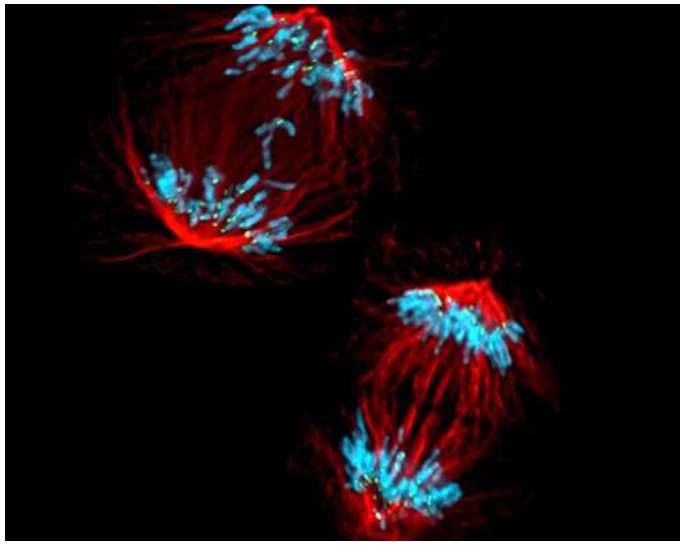


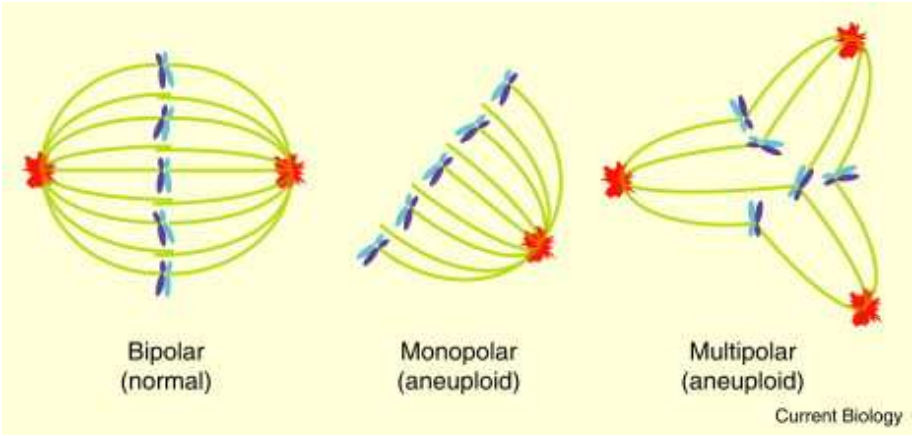


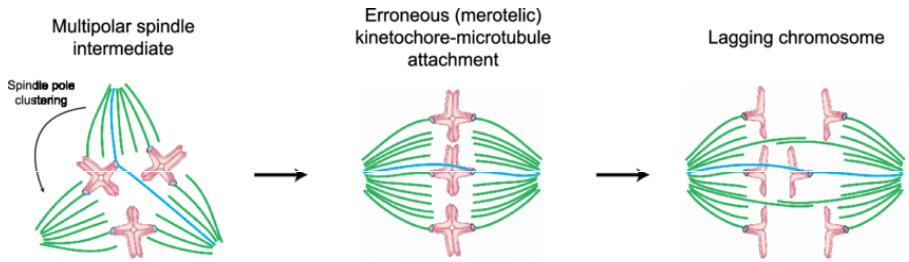
Centrosomes define spindle geometry

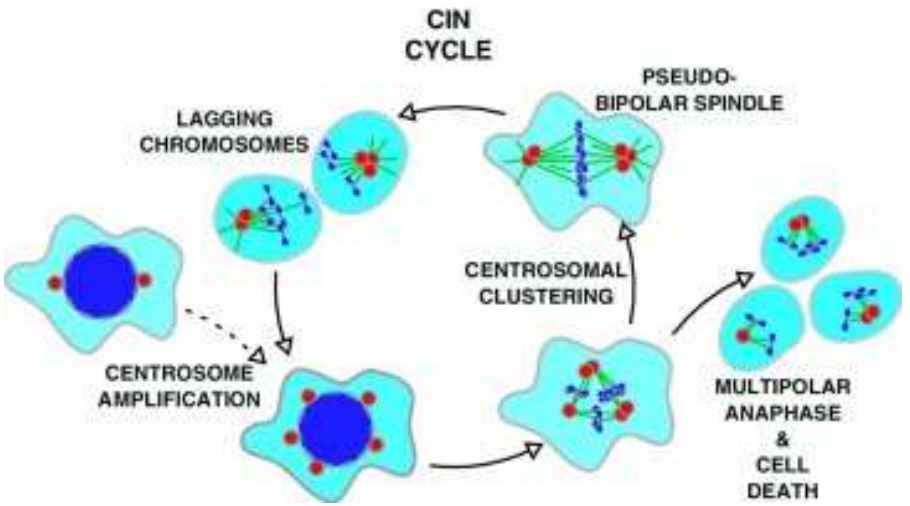
- overamplification of centrosomes generates multipolar spindle which produces gross aneuploidy

- clustering of centrosomes enables bipolarization but persisting merotelic attachments favour chromoosome lagging during anaphase and create risk of chromosome missegregation







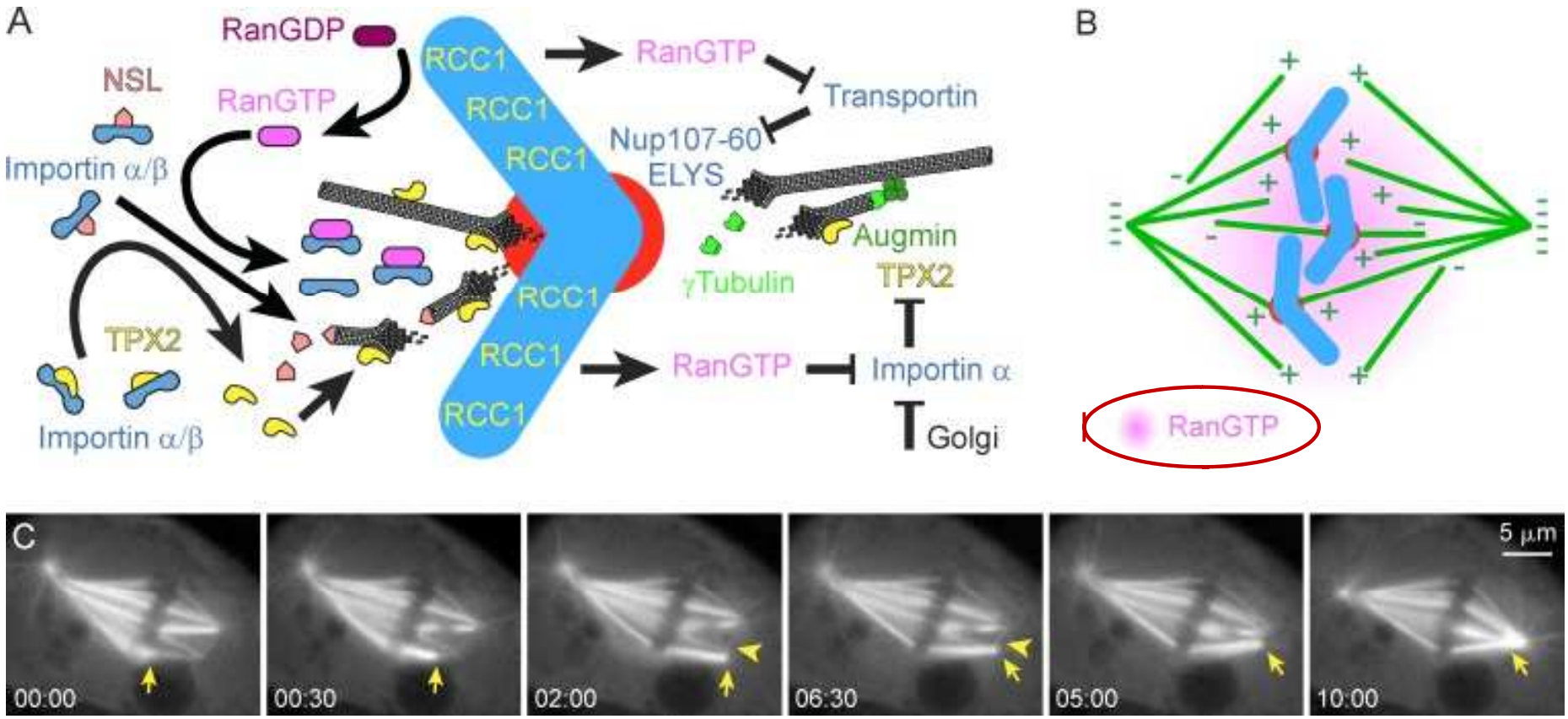


Microtubule nucleation pathways

Microtubule nucleation pathways

❖ **Chromatin-driven microtubule (MT) nucleation**

- **RanGTP** gradient promotes both de novo MT nucleation near kinetochores and amplification of MT growth toward chromosomes

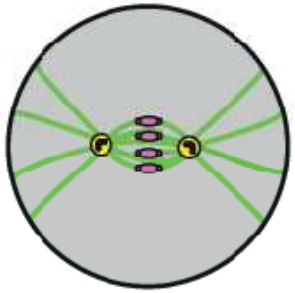


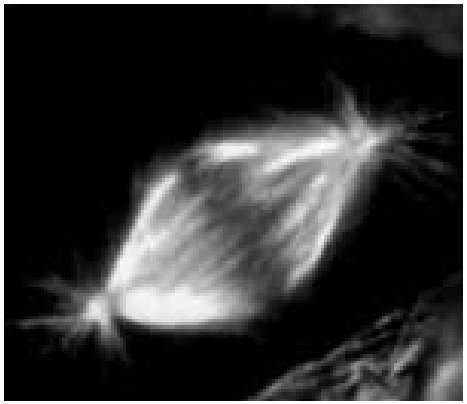
Heald and Khodjakov, J Cell Biol, 2015

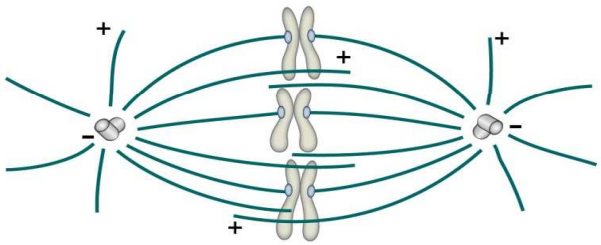
Female meiotic spindles lack centrosomes

Mitosis

Symmetric division



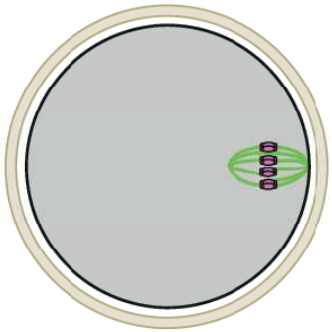


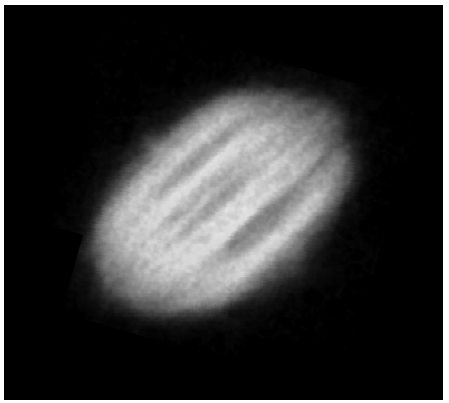


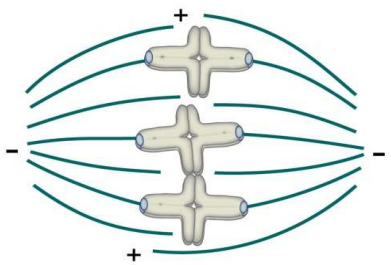
Centrosomal spindle

Female meiosis

Asymmetric division



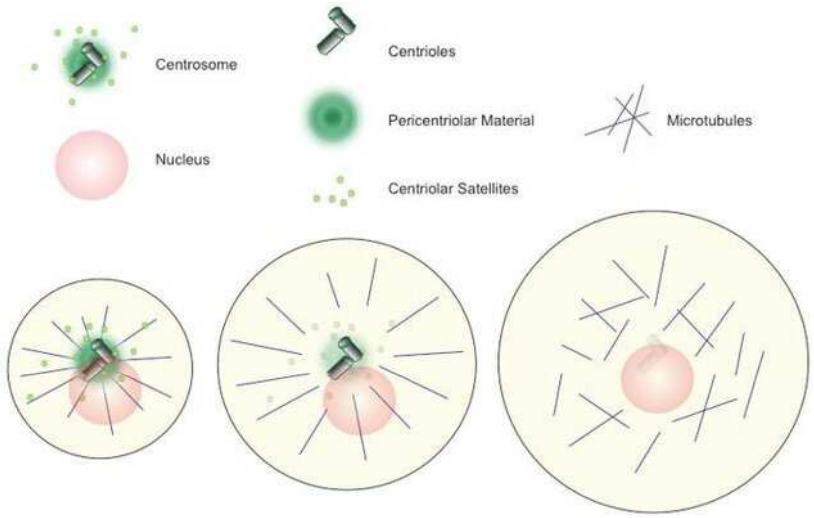




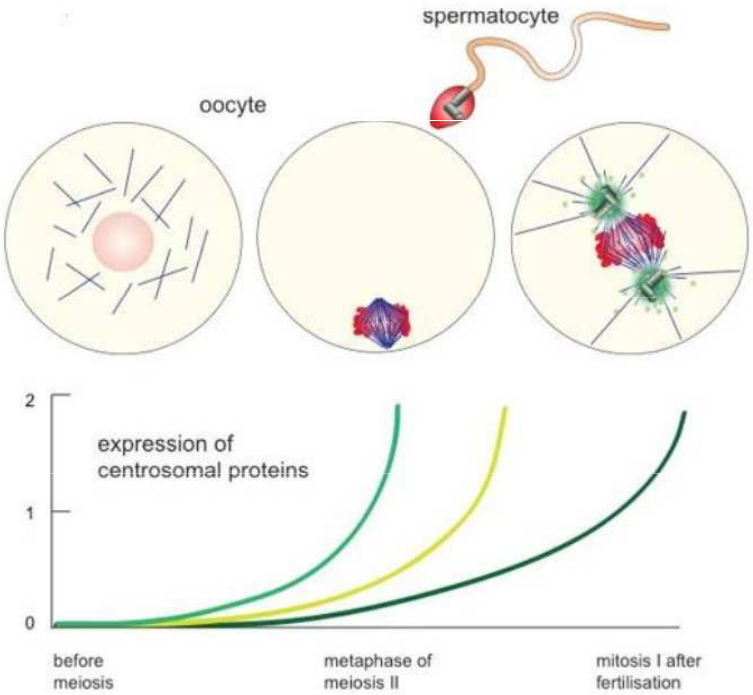
Acentrosomal spindle

Centrioles are eliminated during oogenesis

Metazoan oocytes eliminate centrosomes during oogenesis in order to
(1) ensure highly asymmetric cell division
(2) avoid a superior number after fertilisation



- PCM synthesized during oocyte maturation
- Centrioles paternally inherited

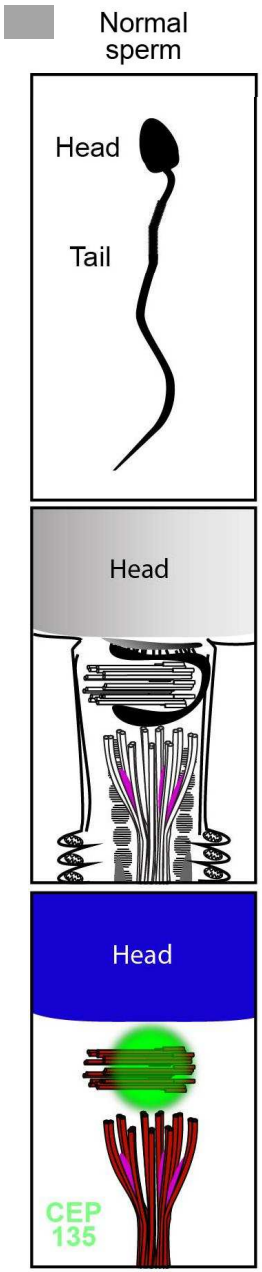


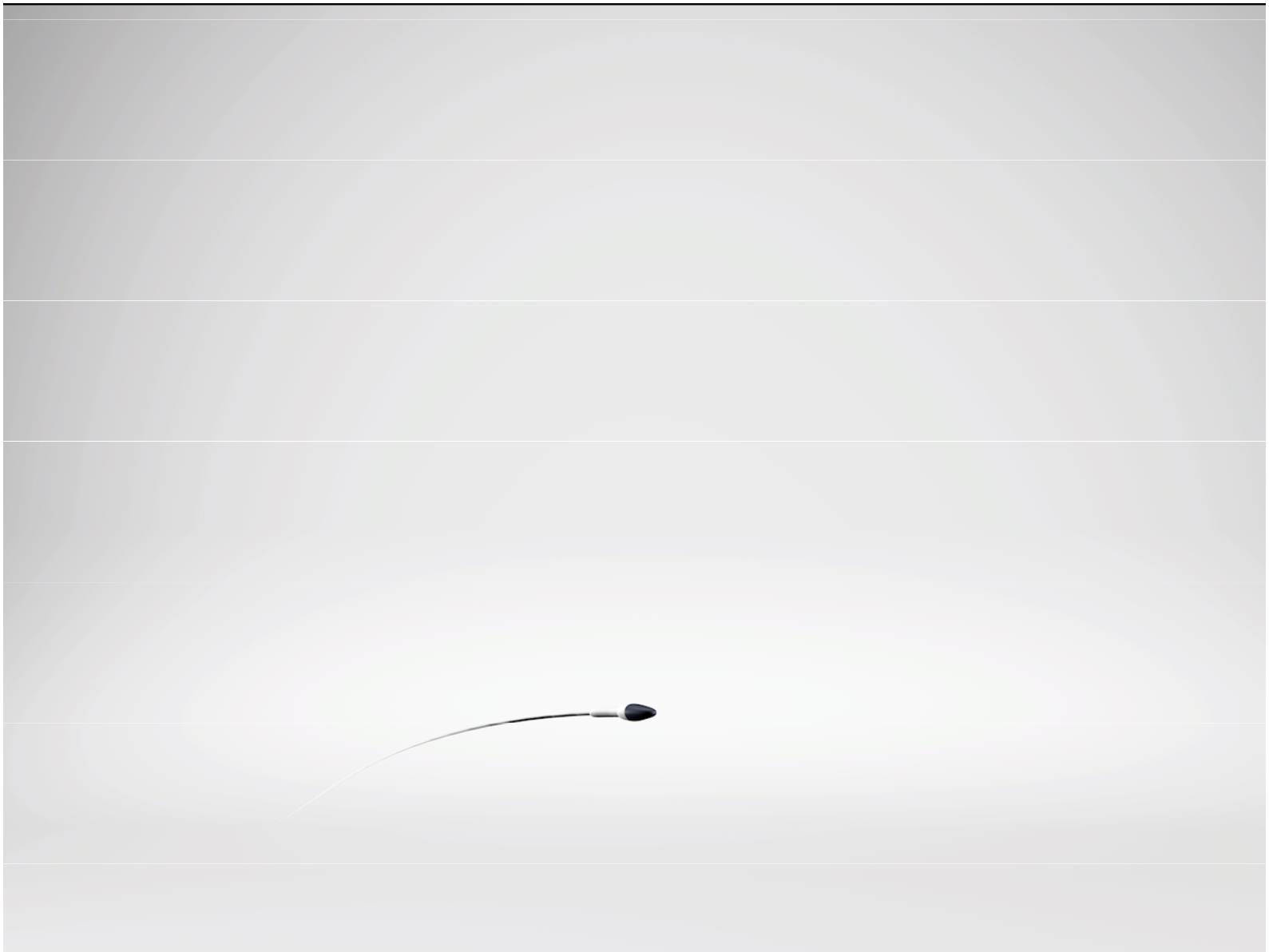
- sperm-derived centrioles recruit maternal PCM after fertilization to assemble first mitotic spindle

- sperm-derived centrioles are destroyed
- first mitosis with acentrosomal spindle
- de novo centriole assembly during embryo cleavage stage

Gruss 2018

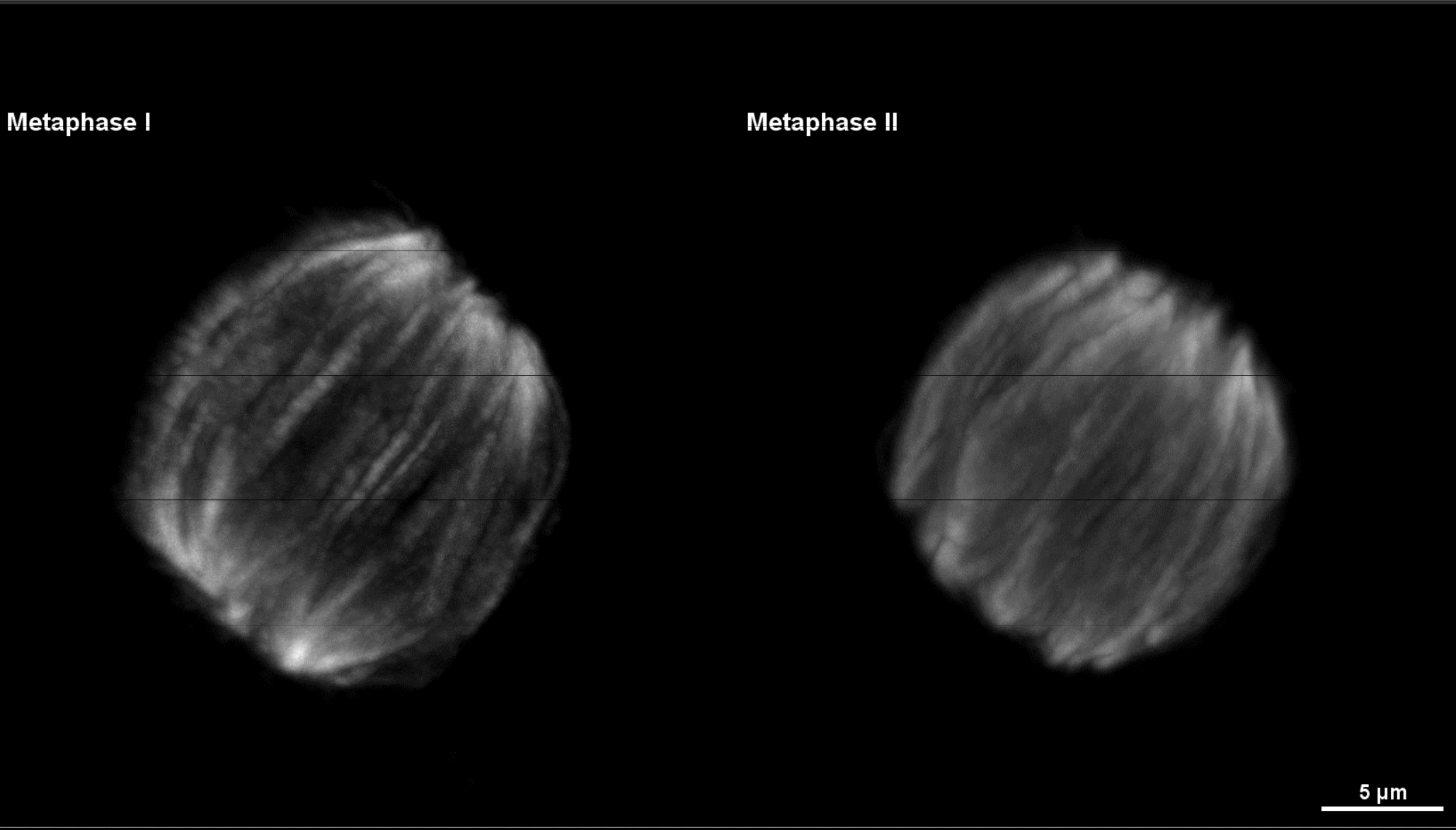
Centrioles are delivered by sperm during fertilization





Andrea Procházková (bakalářská práce 2020) , Katedra vizuální informatiky, Fakulta informatiky MU

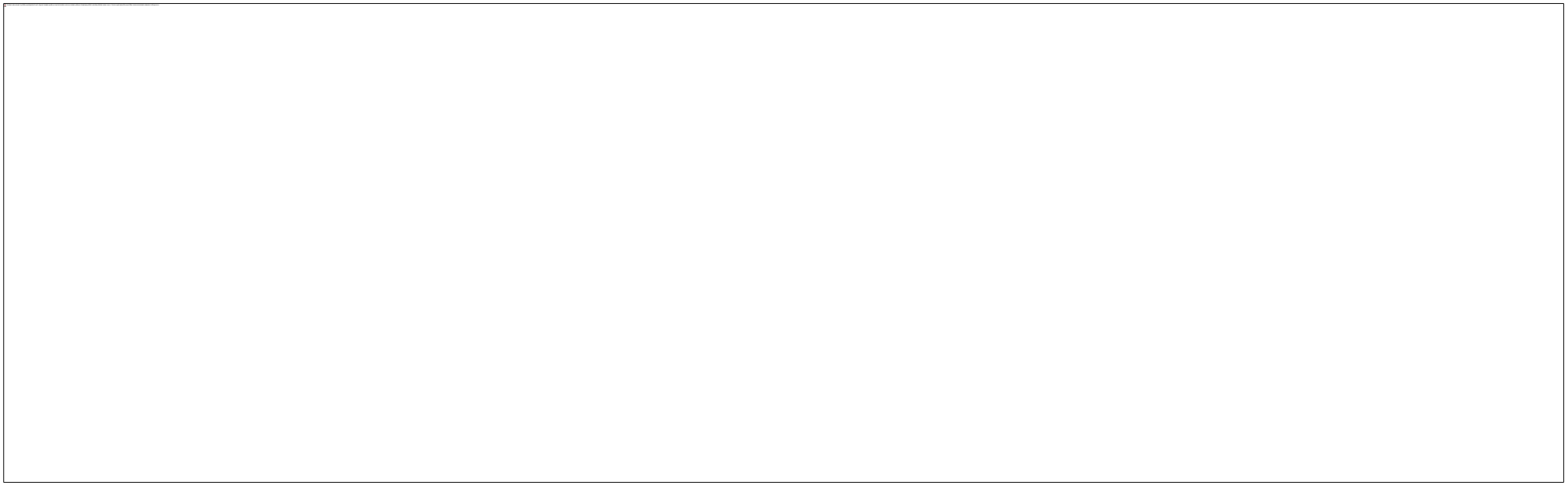
Human oocyte spindle lacks centrosome



How are meiotic spindle poles assembled
in the absence of centrosomes?

Holubcova et al, 2015

Acentrosomal spindle drives chromosomal segregation during female meiosis



GERMINAL VESICLE (GV) | METAPHASE I (MI) | ANAPHASE I | METAPHASE II (MII) | ANAPHASE II

Functional spindle is required for chromosome segregation fidelity

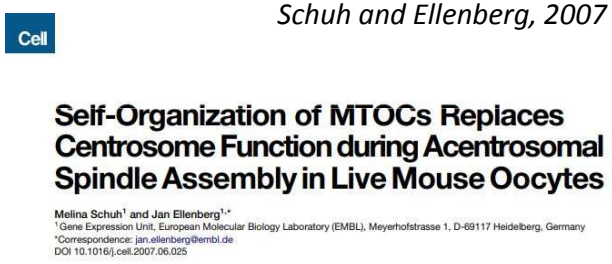
errors in 1st meiotic division → egg aneuploidy

errors in 2nd meiotic division → embryo aneuploidy

Acentrosomal spindle assembly in mouse oocytes

- high-resolution confocal live cell imaging of mouse oocytes maturing in vitro showed that mouse oocyte spindle is assembled by multiple small acentriolar MTOCs that functionally replace canonical centrosomes

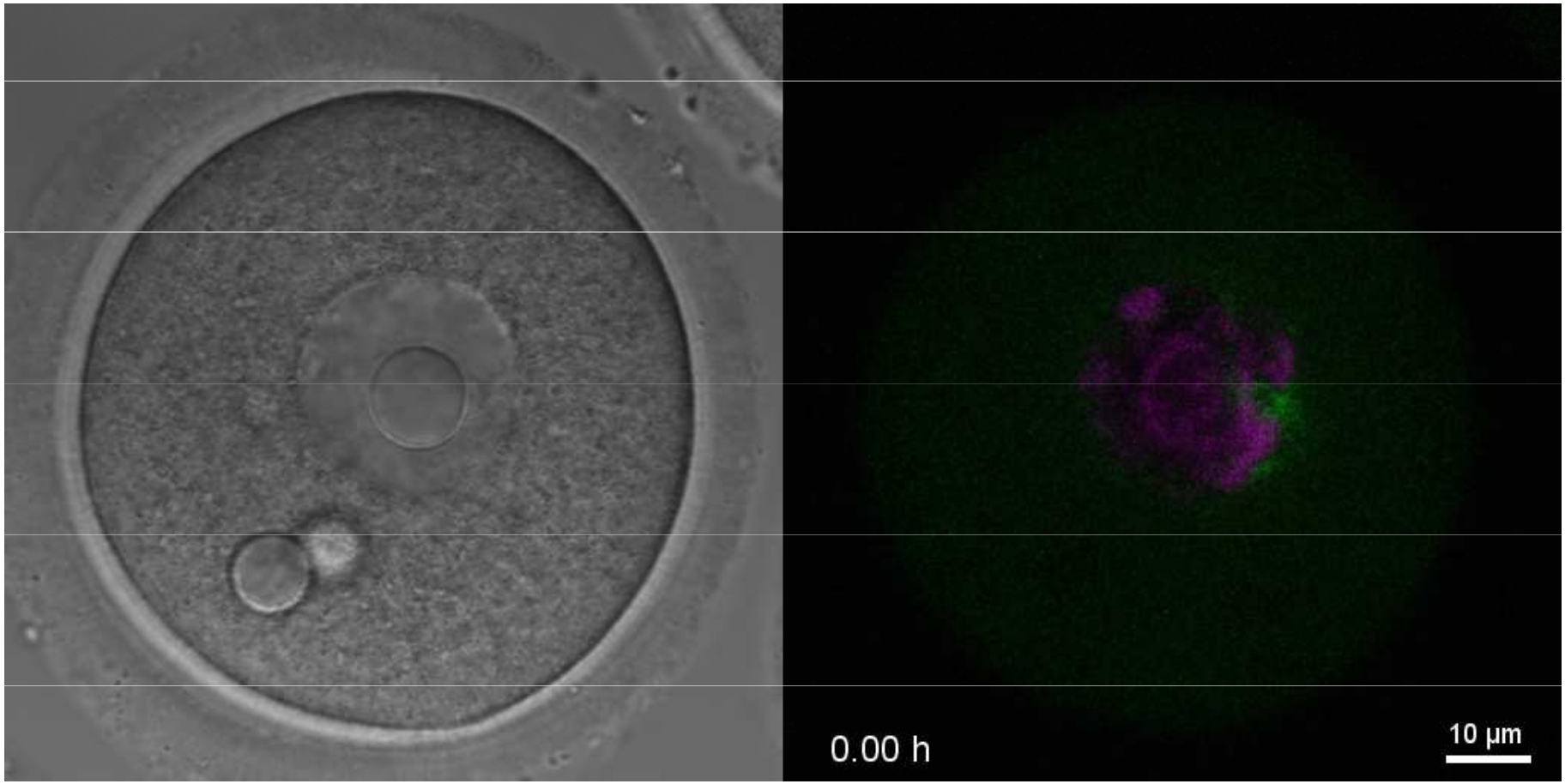
Schuh and Ellenberg, 2007


Cell

Self-Organization of MTOCs Replaces Centrosome Function during Acentrosomal Spindle Assembly in Live Mouse Oocytes

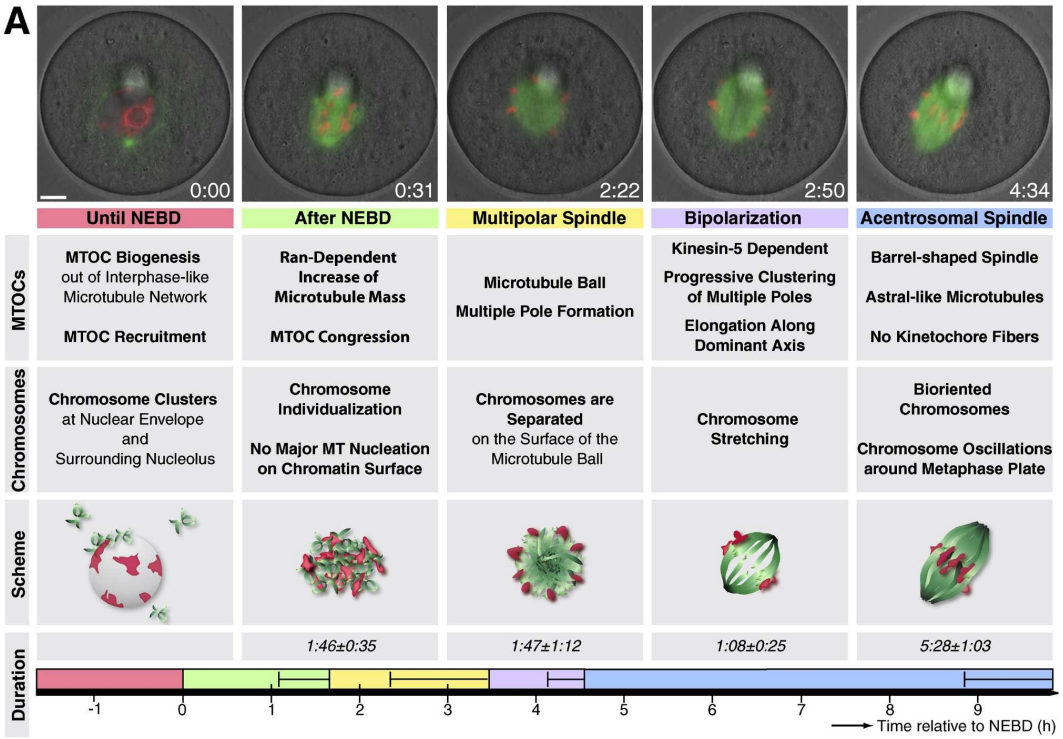
Melina Schuh[1] and Jan Ellenberg[1,*]
[1]Gene Expression Unit, European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, D-69117 Heidelberg, Germany
*Correspondence: jan.ellenberg@embl.de
DOI 10.1016/j.cell.2007.06.025


Melina Schuh



DNA microtubules

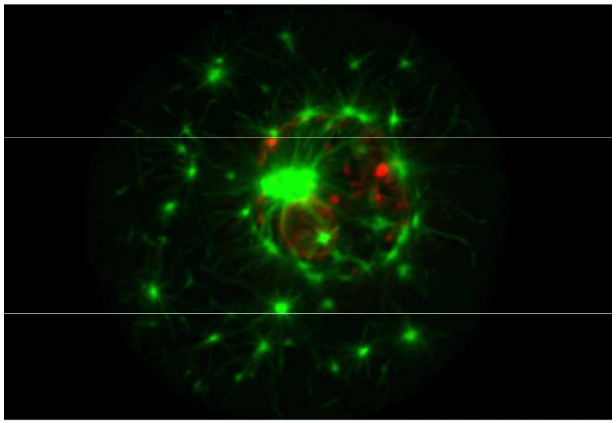
Acentrosomal spindle assembly in mouse oocytes



Schuh and Ellenberg, 2007

DNA

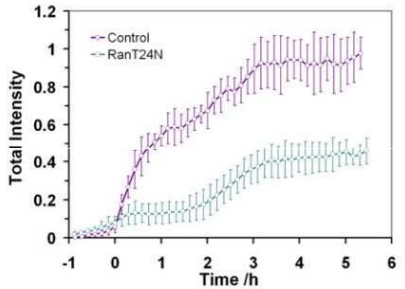
microtubules



Prophase microtubule network with low dynamics

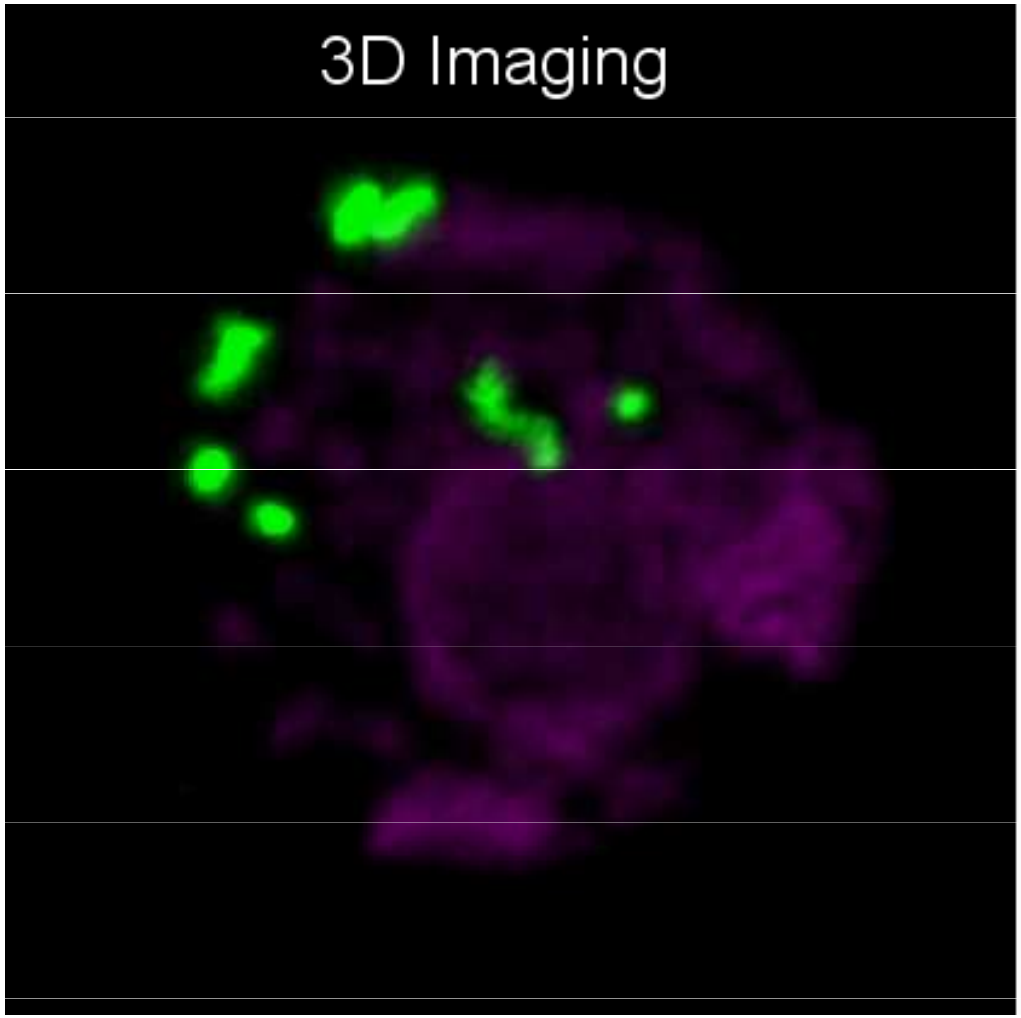
- MTOC consists of PCM proteins

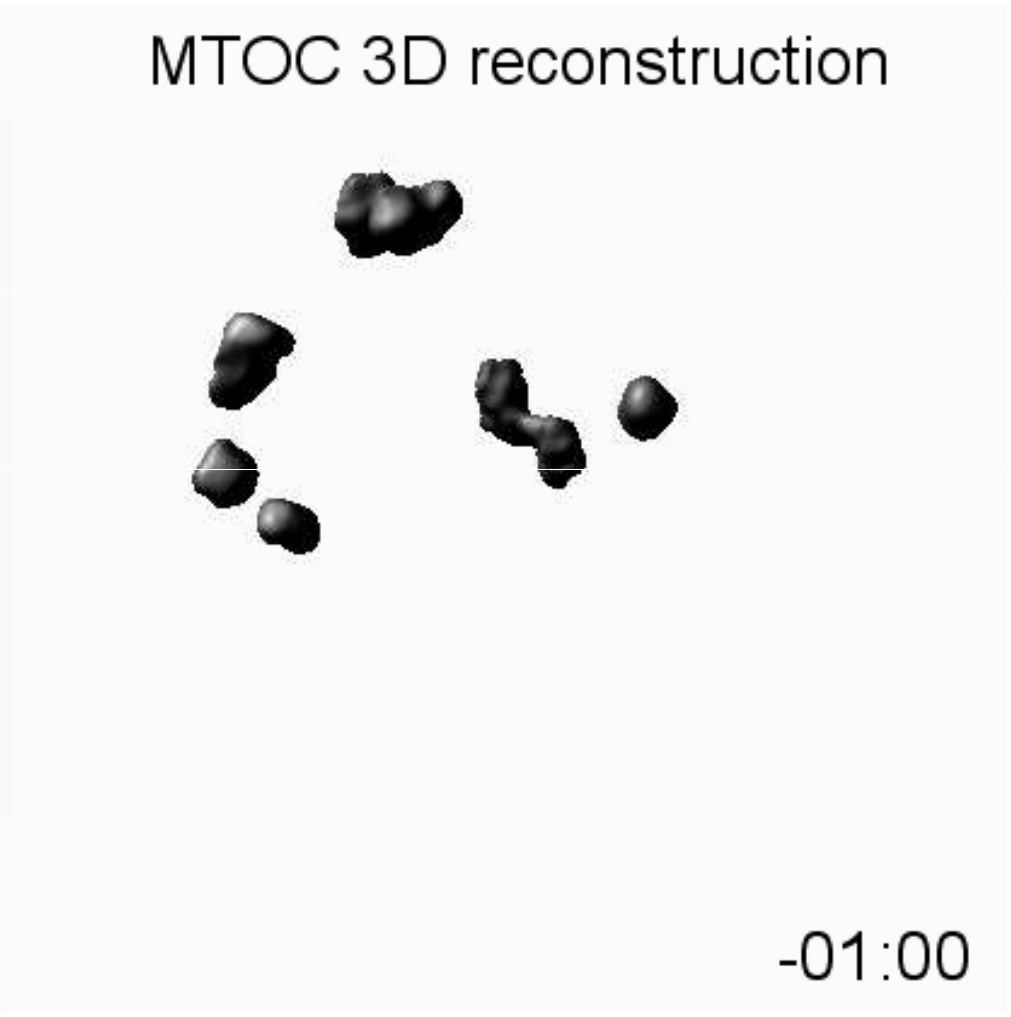
  (pericentrin, γ-tubulin, Cep192, Cep120, Cep 125, NEDD1,..)
- MTOCS cluster around nucleus before NEBD
- MTOC nucleate MT „ball“ which carries chromosomes on its surface
- MT mass elongates and chromosome congress
- chromosome alignment after spindle bipolarization
- spindle migration to the cortex
- Ran activity overdriven by coordinated action of MTOCS



Acentrosomal spindle assembly in mouse oocytes

Multiple acentriolar MTOCs converge at spindle poles and stabilize th

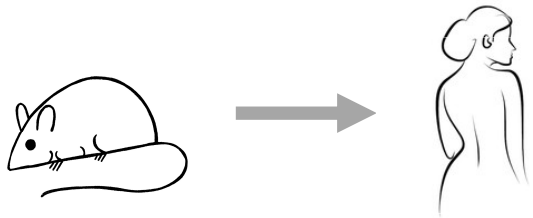




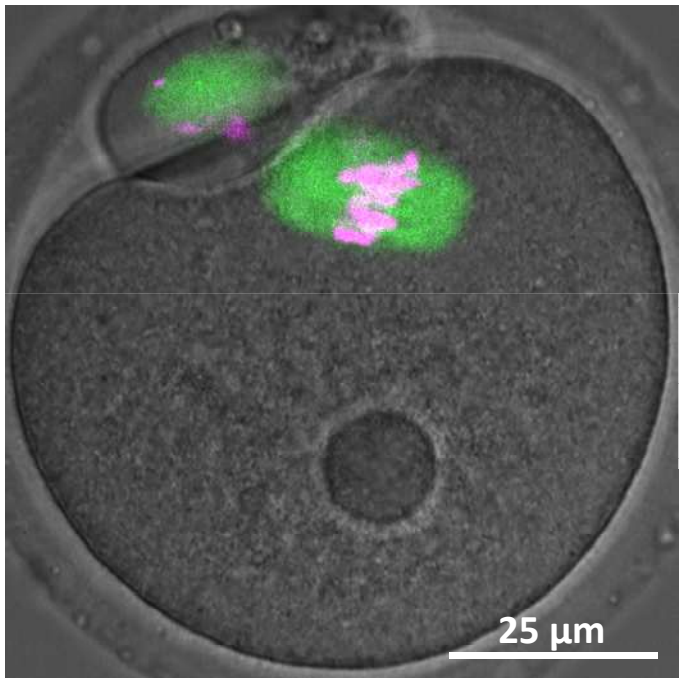
DNA
MTOCs

Clift and Schuh, 2015.

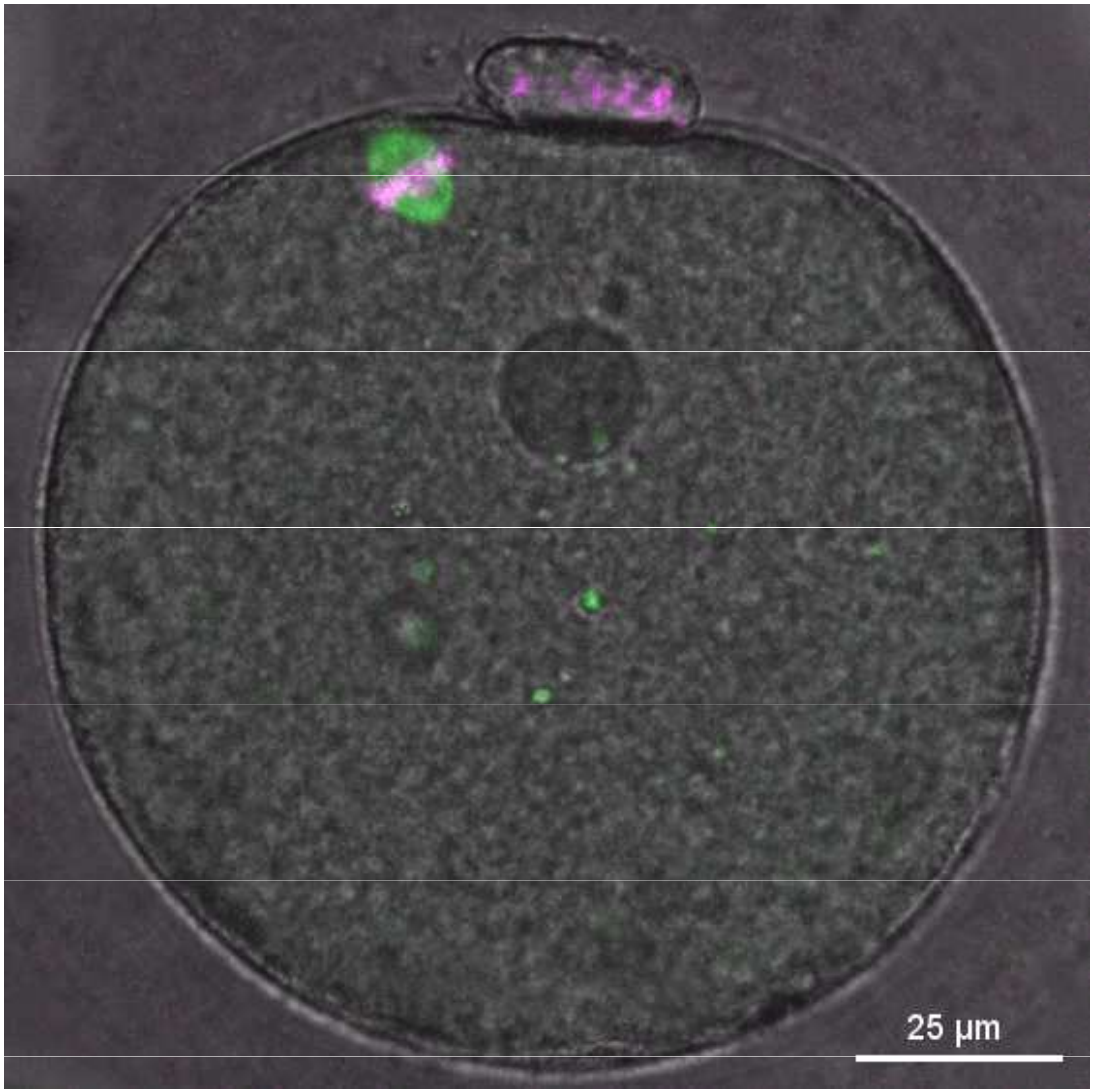
From mice to human



Mouse oocyte

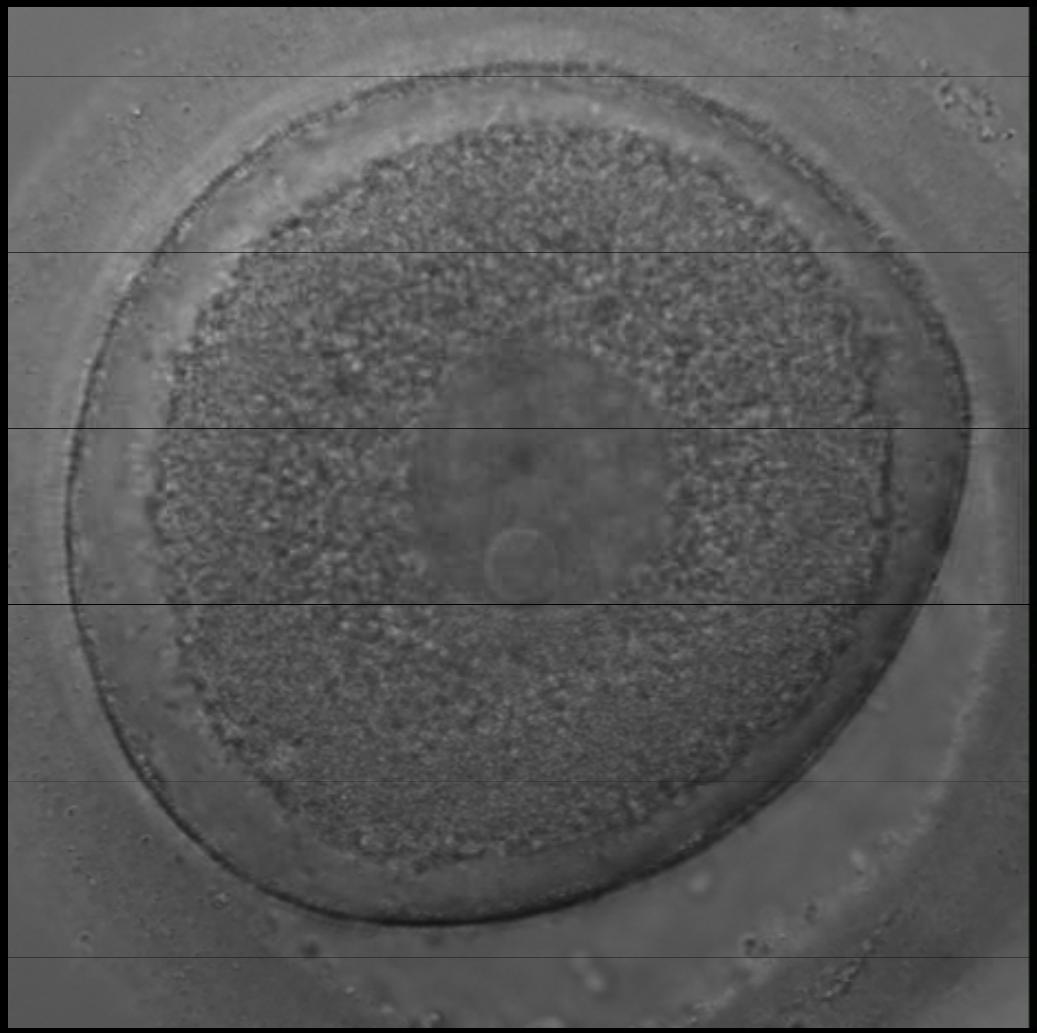


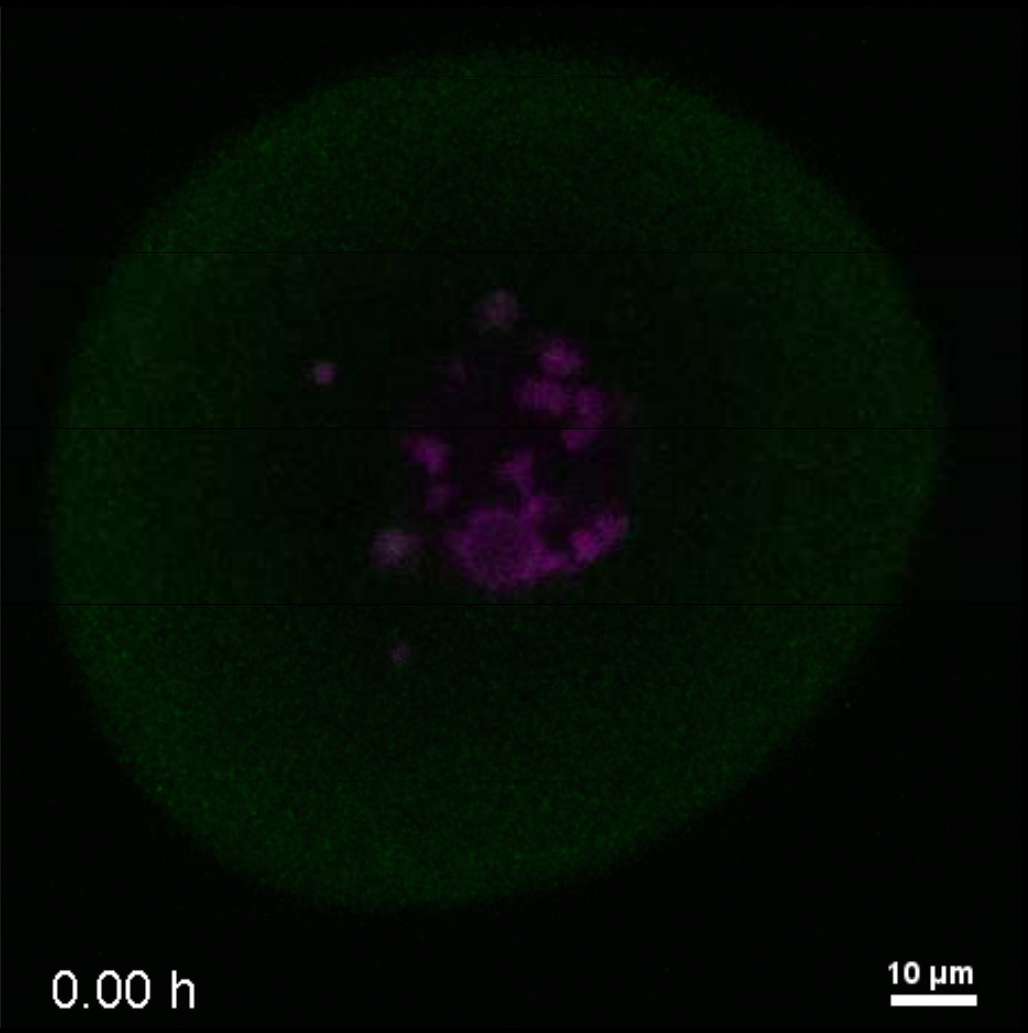
Human oocyte



Chromosomes
Microtubules

Acentrosomal spindle assembly in human oocytes



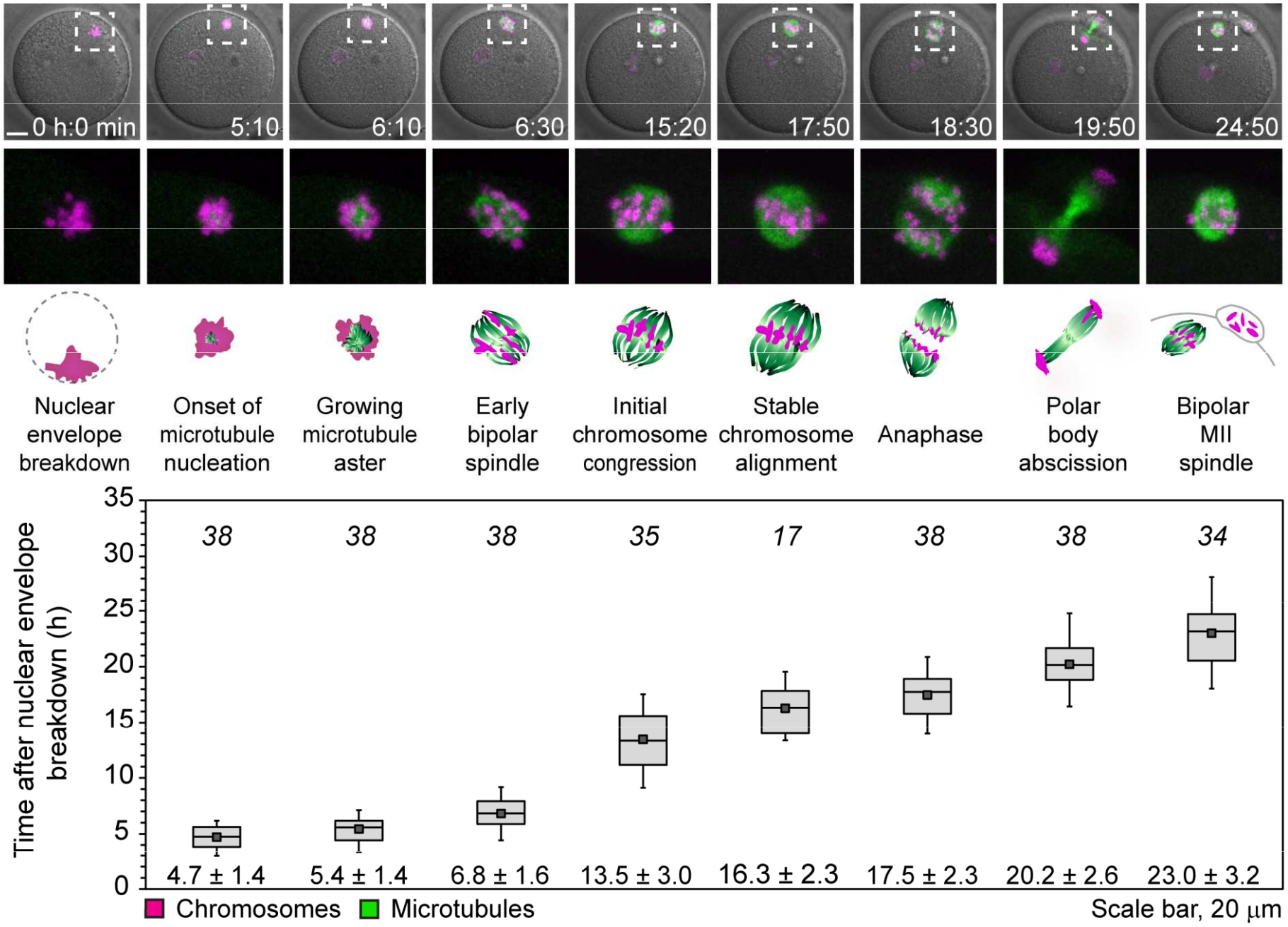


DIC (transmitted light)

Chromosomes (H2B-mRFP)
Microtubules (MAP4-EGFP)

Holubcova et al. Science 2015.

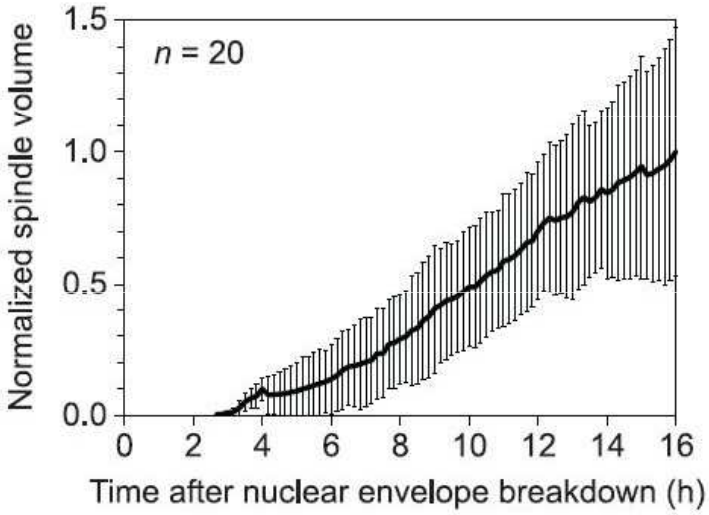
Acentrosomal spindle assembly in human oocytes

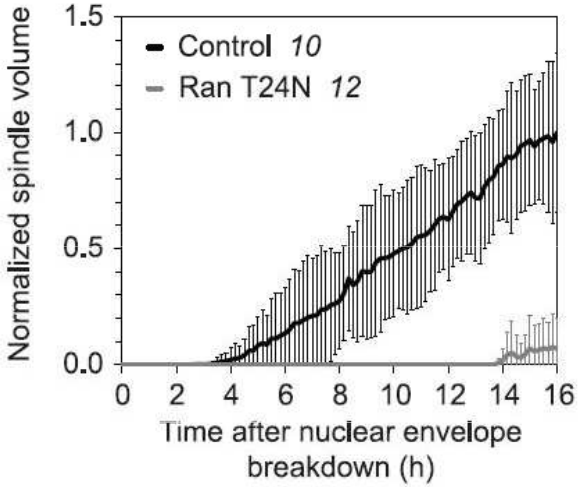


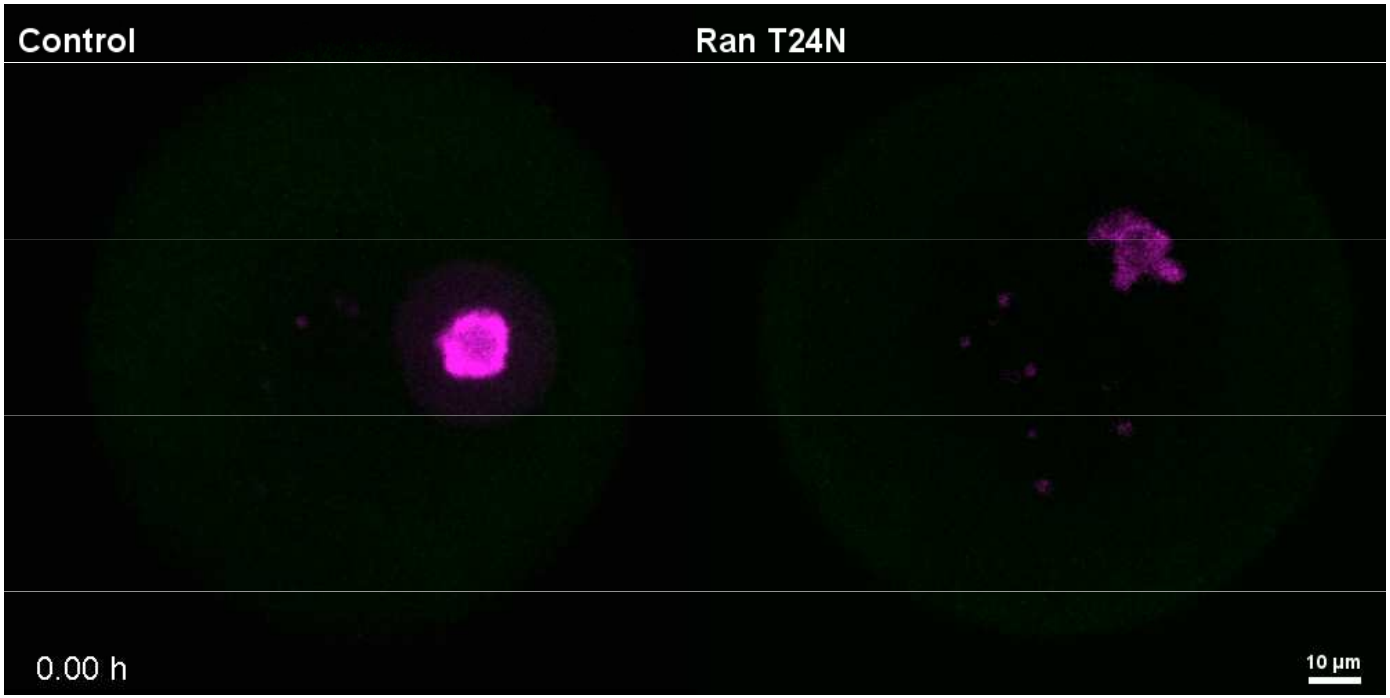
Holubcova et al. Science 2015.

Acentrosomal spindle assembly in human oocytes

- human oocytes assemble a meiotic spindle independently of either centrosomes or other MTOCs
- spindle assembly is mediated by chromosomes and the small guanosine triphosphatase **Ran**
- spindle assembly is unusually long,requiring ~16 hours



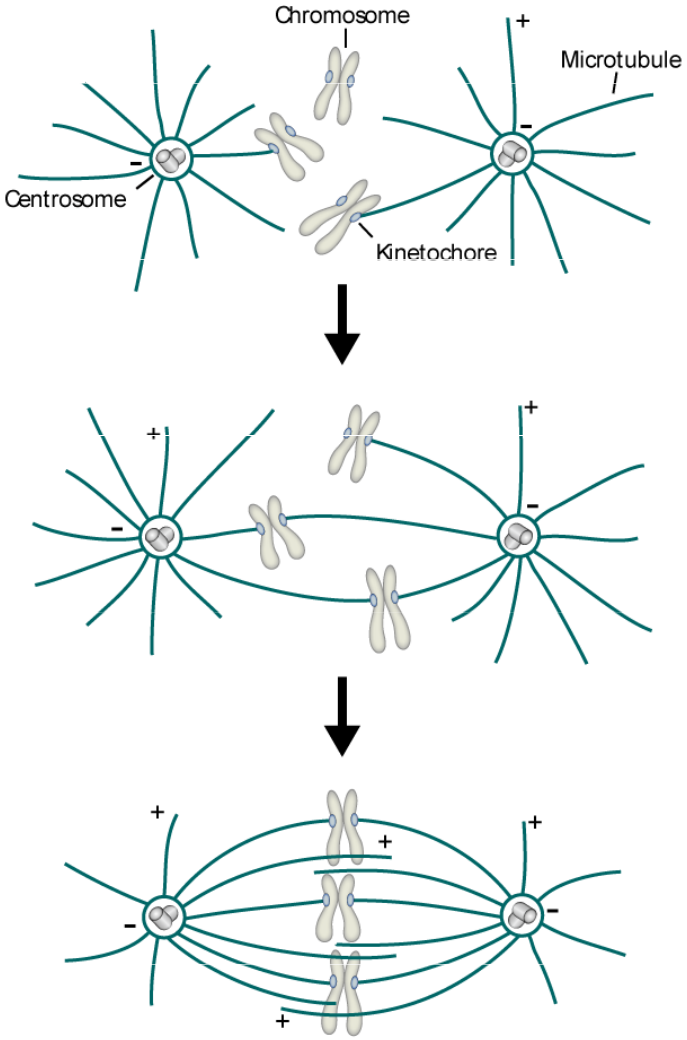


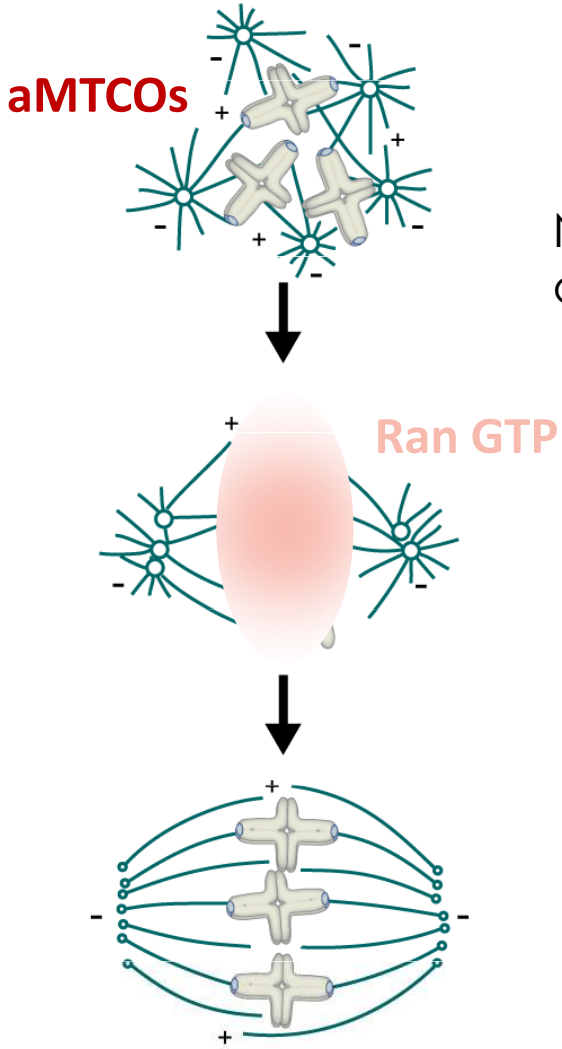


Holubcova et al. Science 2015.

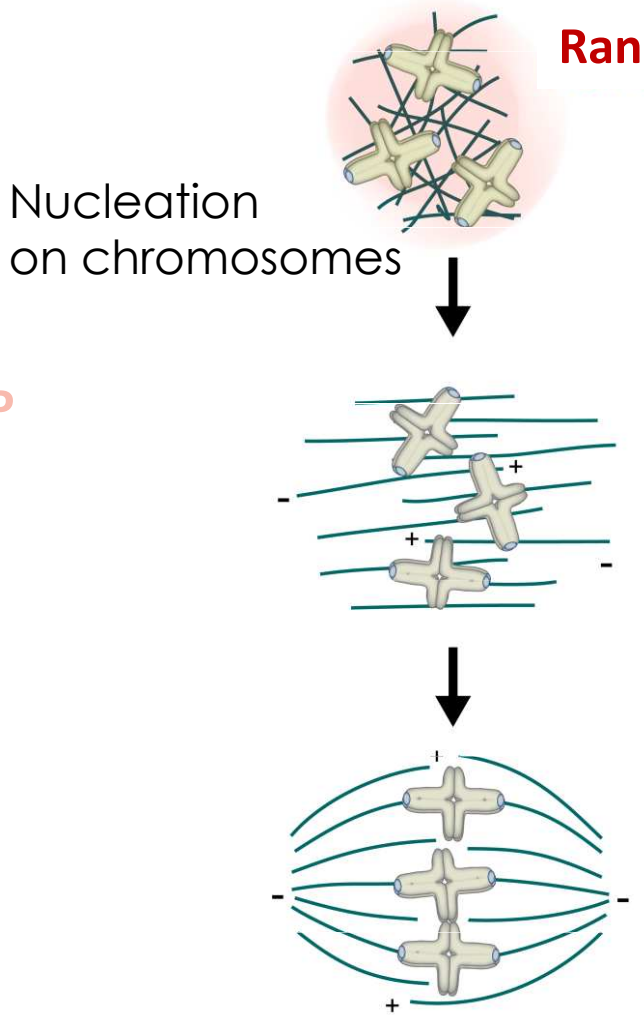
Spindle assembly strategies

Centrosomal Spindle *Mitotic cells*	Acentrosomal Spindle *Mouse Oocyte*	Acentrosomal Spindle *Human oocytes (and plant cells)*





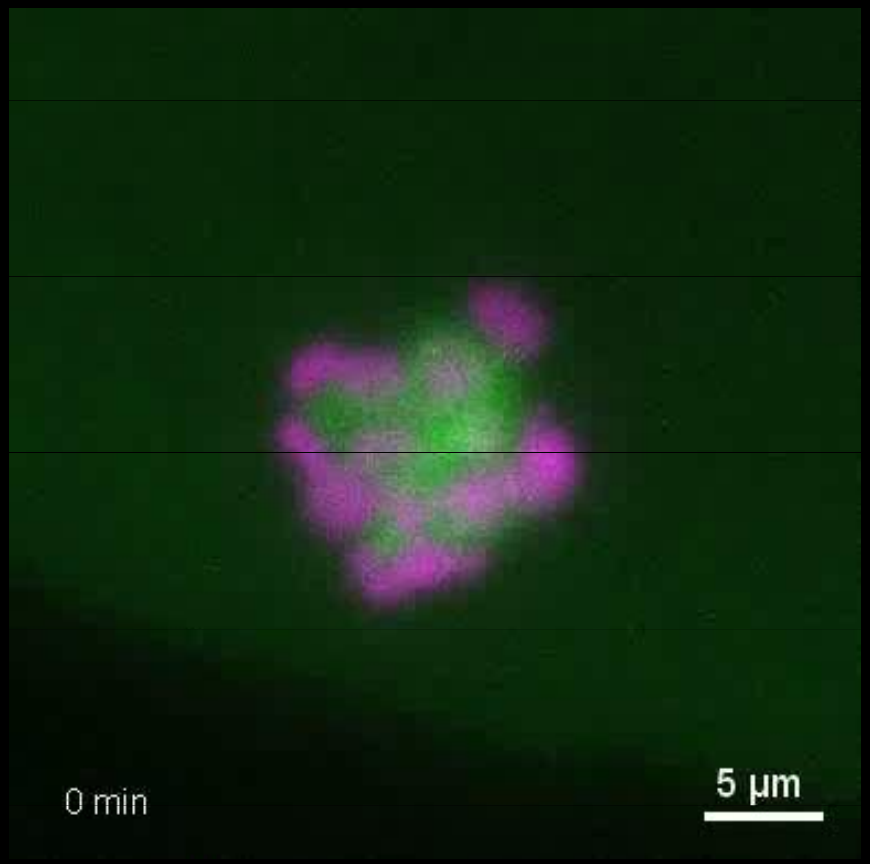
Schuh and Ellenberg, Cell 2007.



Holubcova et al., Science 2015.

Human oocyte spindle is unstable

Moderate spindle instability

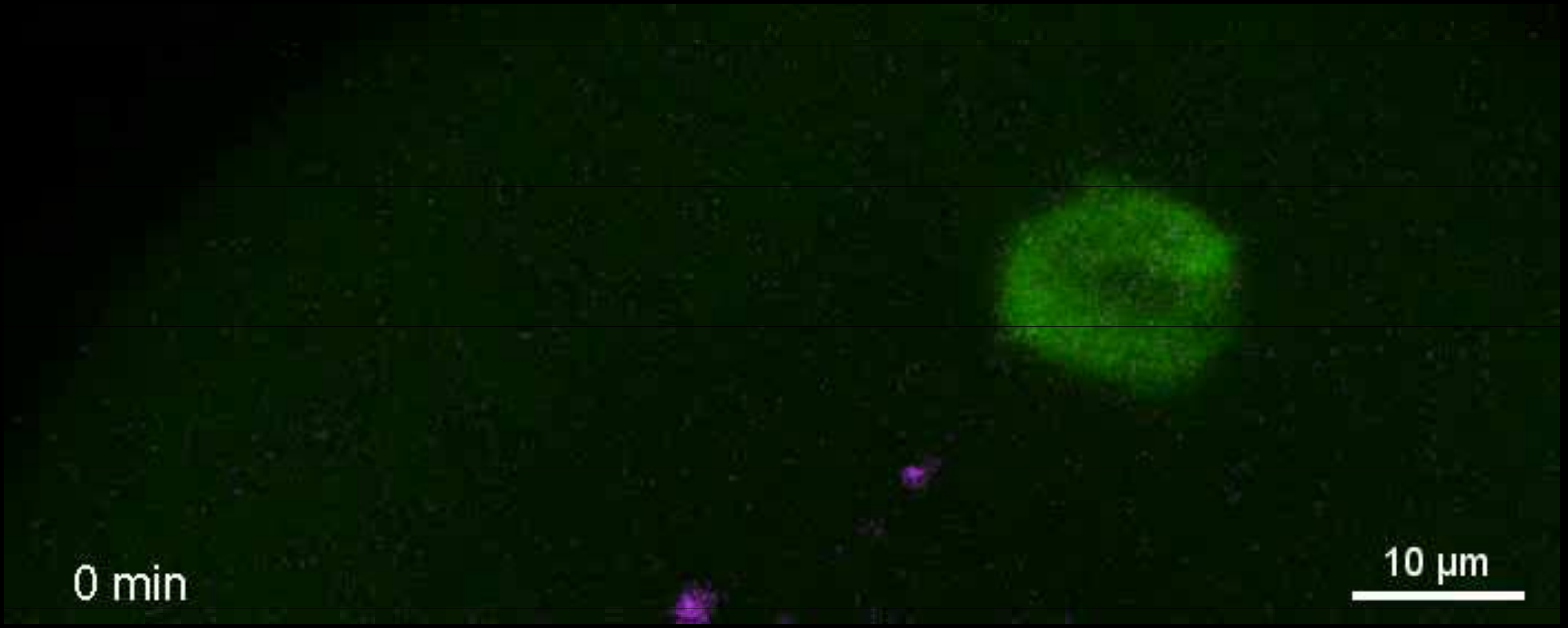


Chromosomes (H2B-mRFP)
Microtubules (MAP4-EGFP)

Holubcova et al. Science 2015.

Human oocyte spindle is unstable

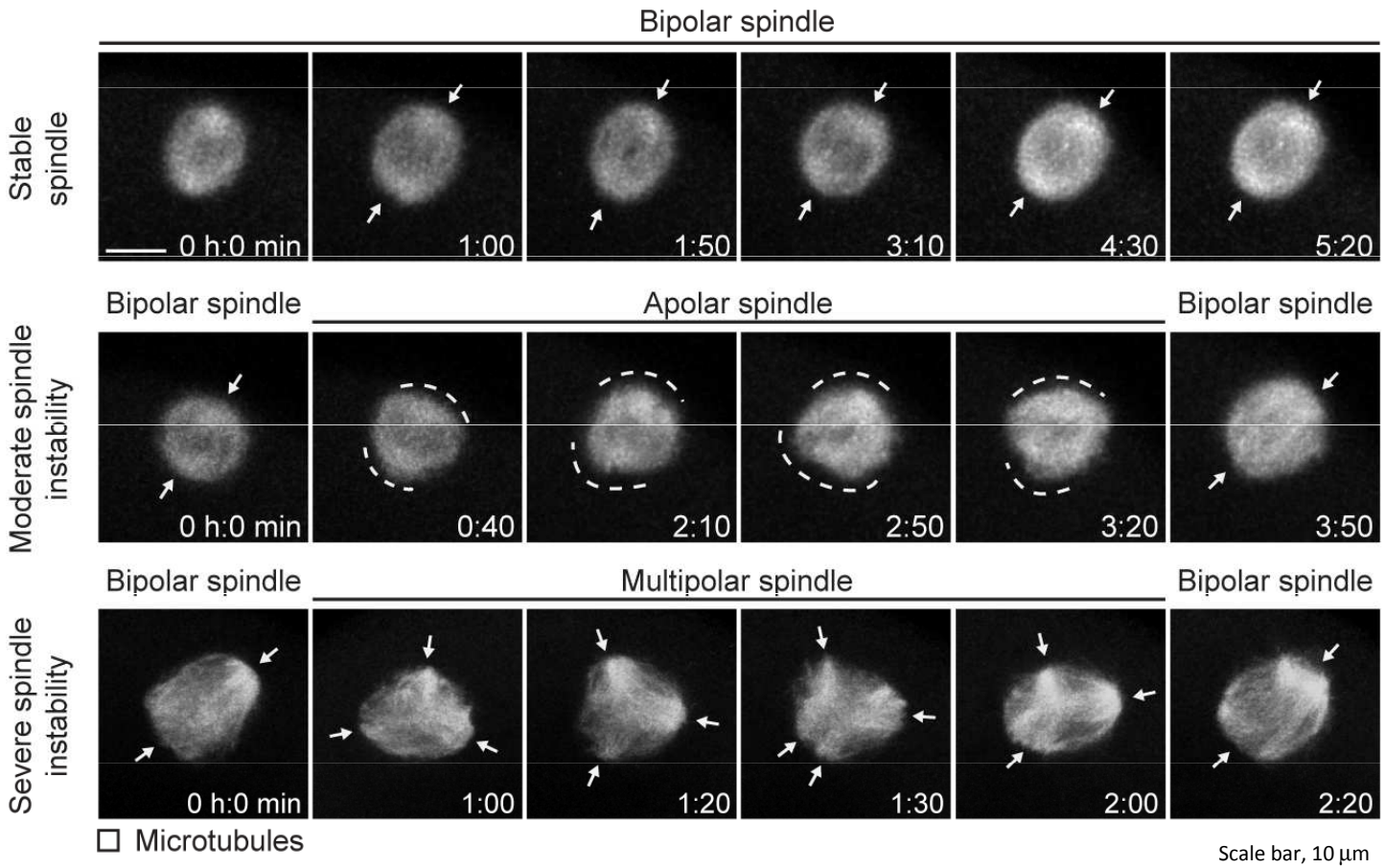
Severe spindle instability

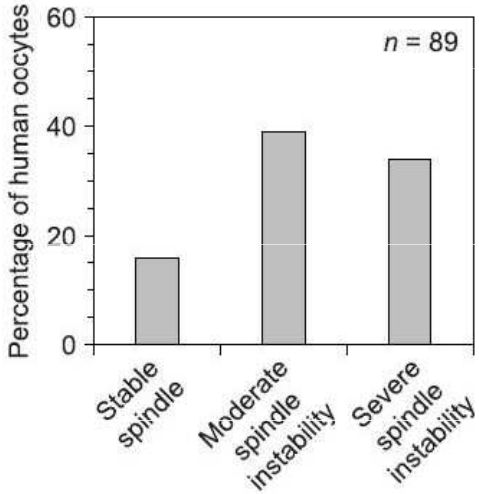


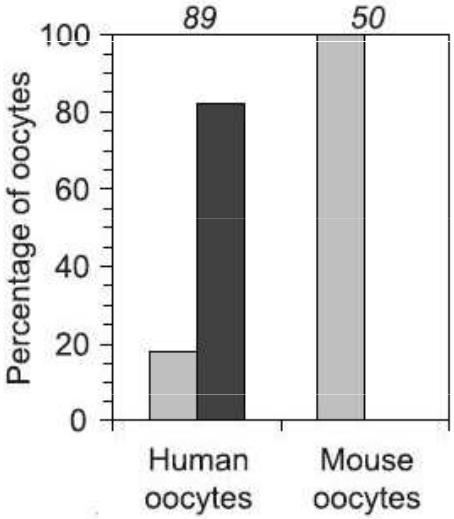
Chromosomes (H2B-mRFP)
Microtubules (MAP4-EGFP)

Holubcova et al. Science 2015.

Human oocyte spindle is unstable







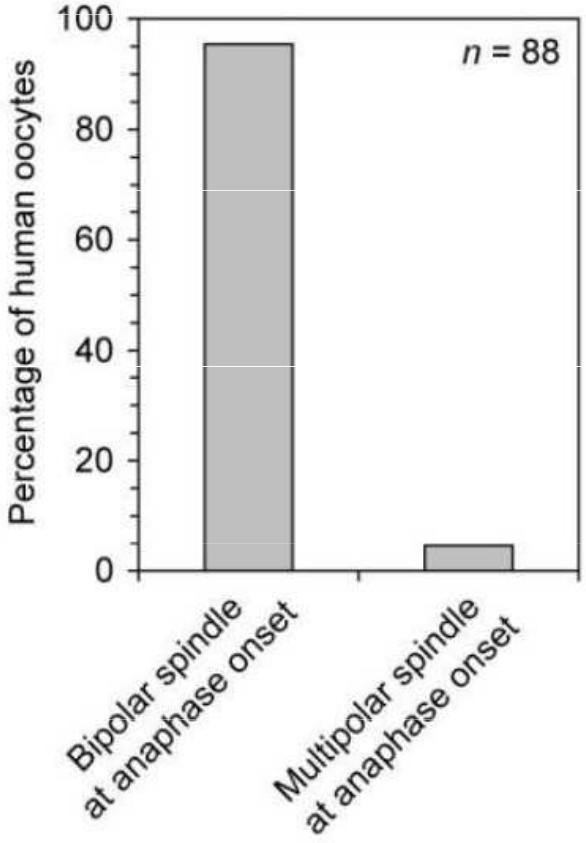
- **Prolonged spindle instability was observed in ~80% of human oocytes* but no mouse oocytes**

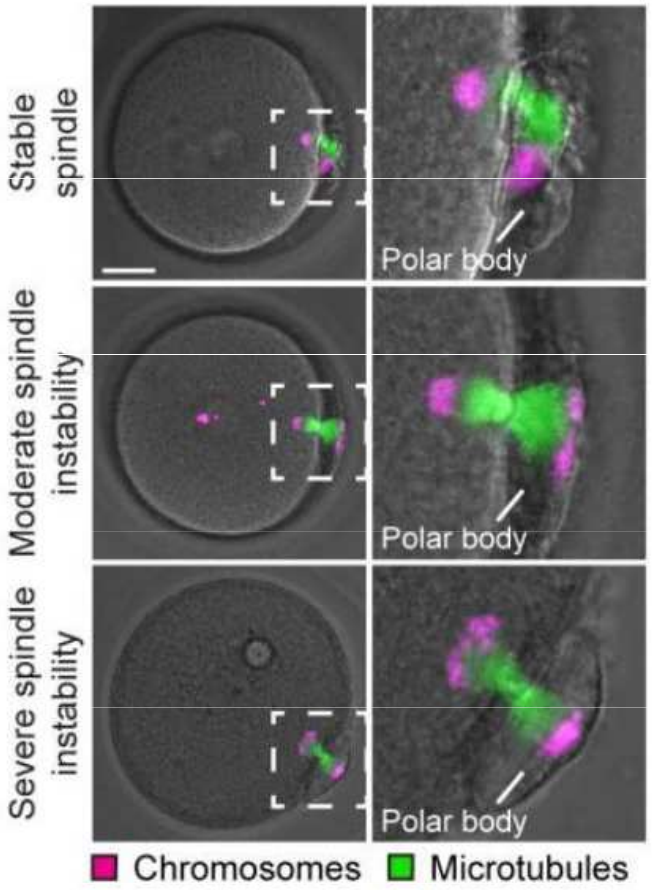
*Surplus oocytes from stimulated IVF cycles matured *in vitro !*

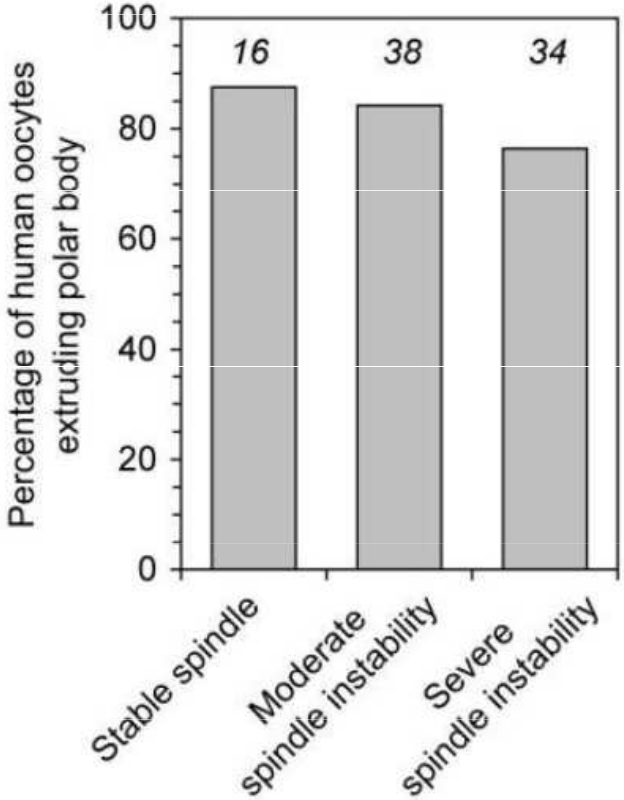
Holubcova et al. Science 2015.

Human oocyte spindle is unstable

- Majority of human oocytes recovered from spindle instability before anaphase and extruded a polar body



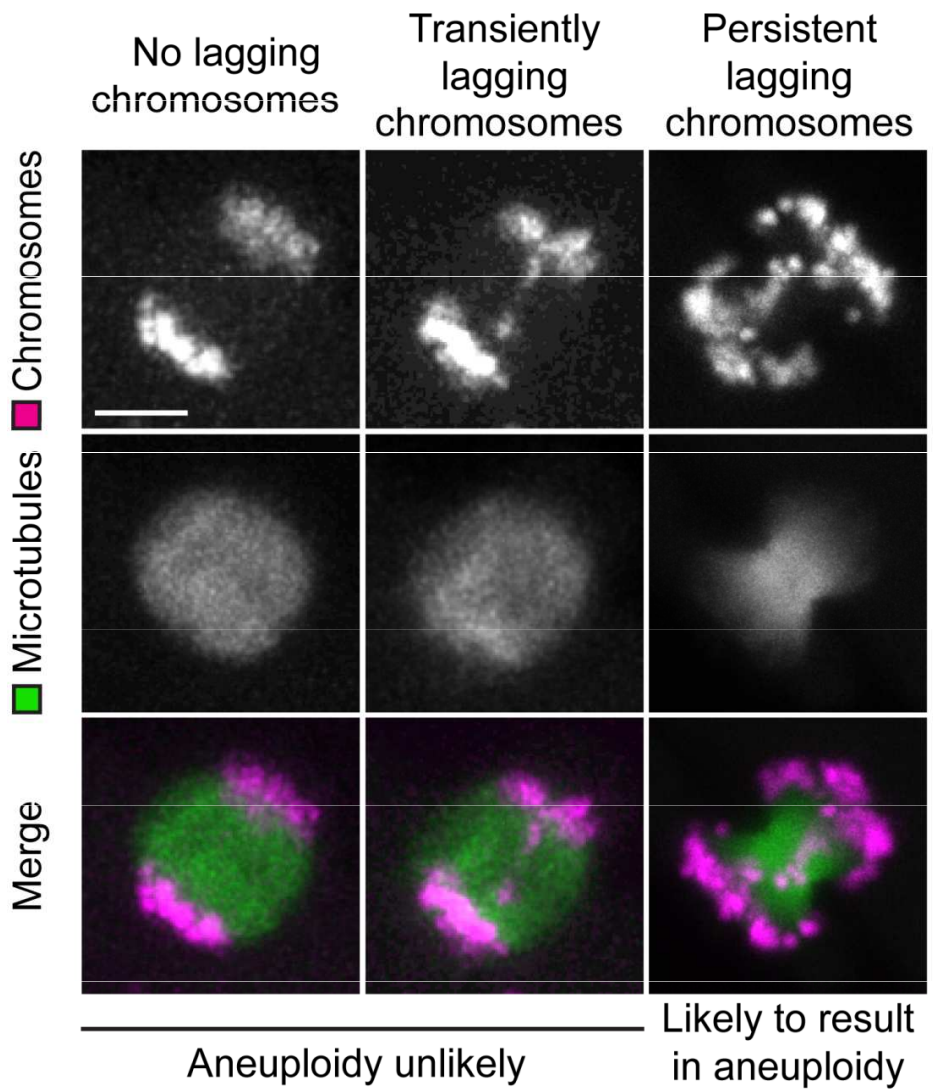




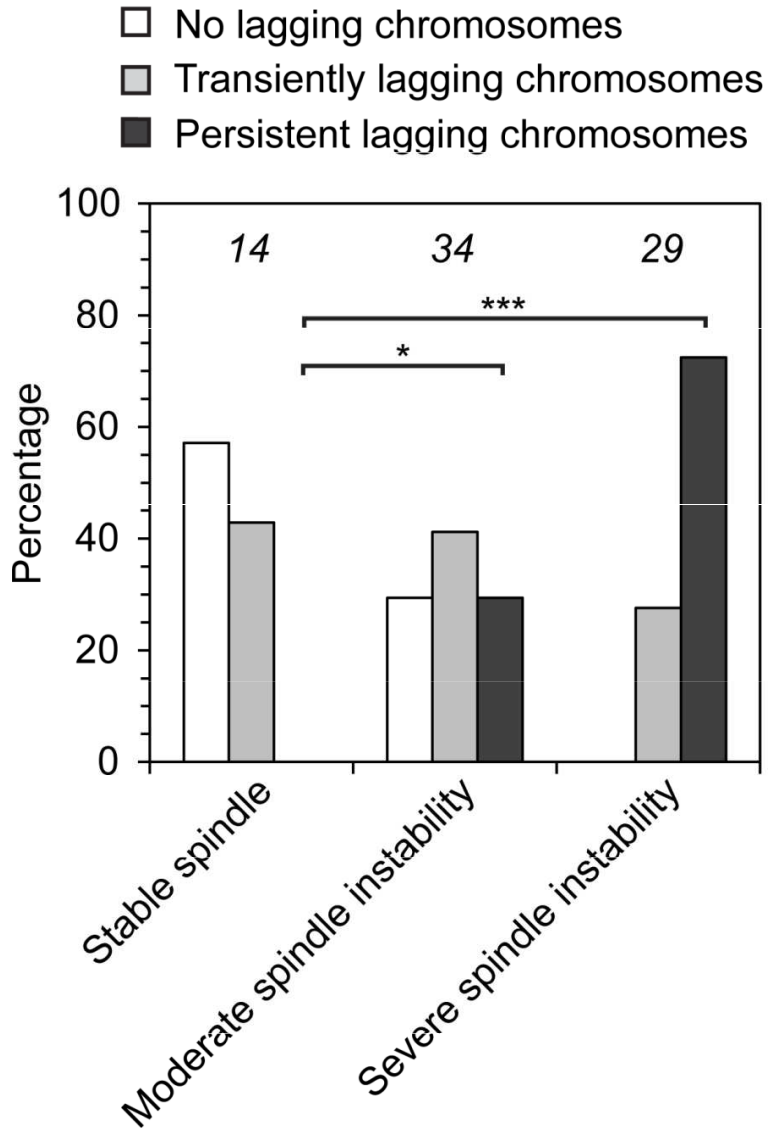
Holubcova et al. Science 2015.

Spindle instability favours chromosome missegregation

❖ **Spindle instability correlates with chromosome segregation errors**



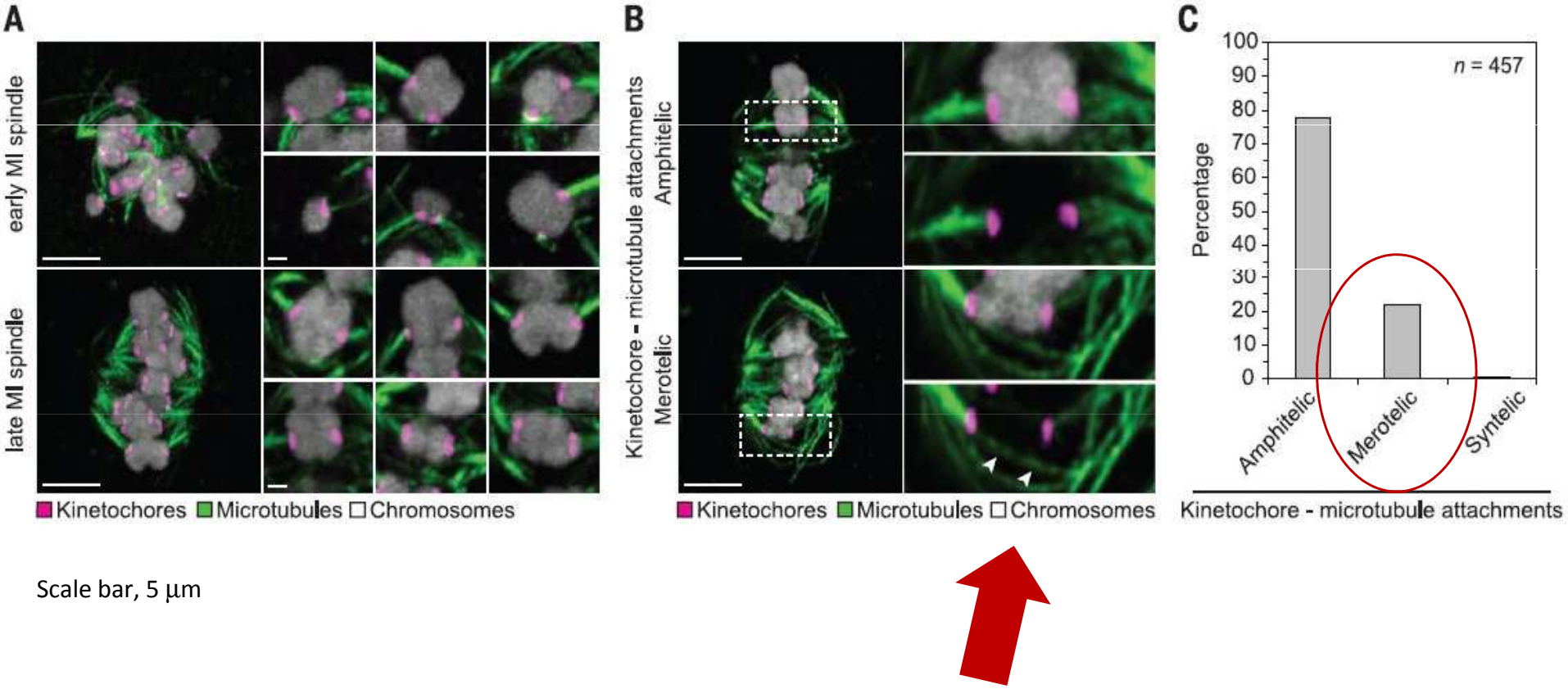
Scale bar, 10 µm



Holubcova et al. Science 2015.

Spindle instability favours chromosome missegregation

- **Correction of kinetochore-microtubule attachments is incomplete close to anaphase**

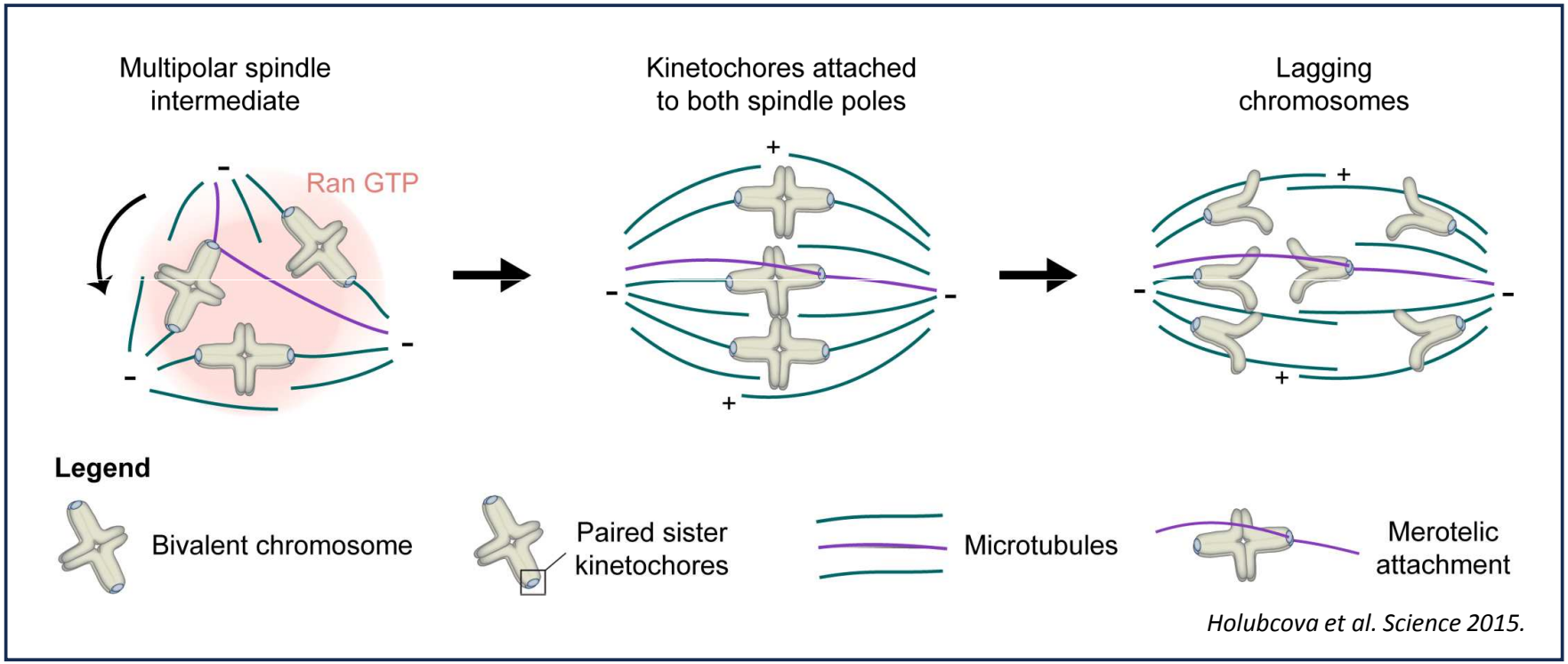


Scale bar, 5 µm

Holubcova et al. Science 2015.

Spindle instability favours chromosome missegregation

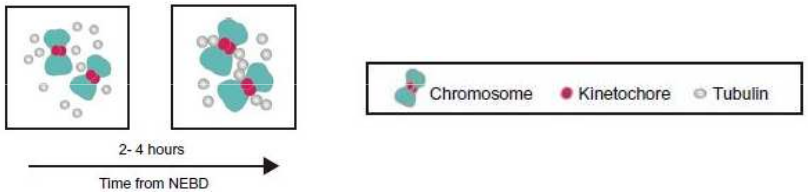
- **at the absence of centrosomes, human oocytes rely on MT nucleation from chromatin**
- **chromosome-mediated spindle assembly is slow process and formed spindle is inherently unstable**



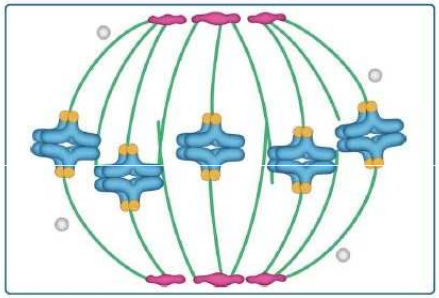
- **improper microtubule-kinetochore attachments established during spindle build-up and remodelling persist to anaphase causing chromosome lagging that is likely to result in aneuploidy**

Acentrosomal spindle assembly

- MT nucleation initiated at kinetochores



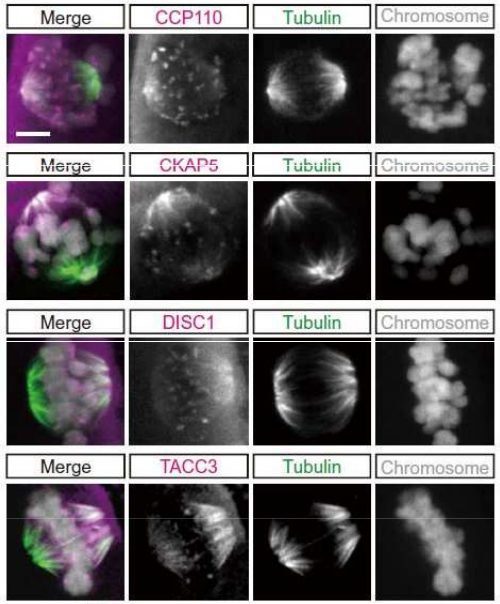
- molecular composition of human oocyte spindle



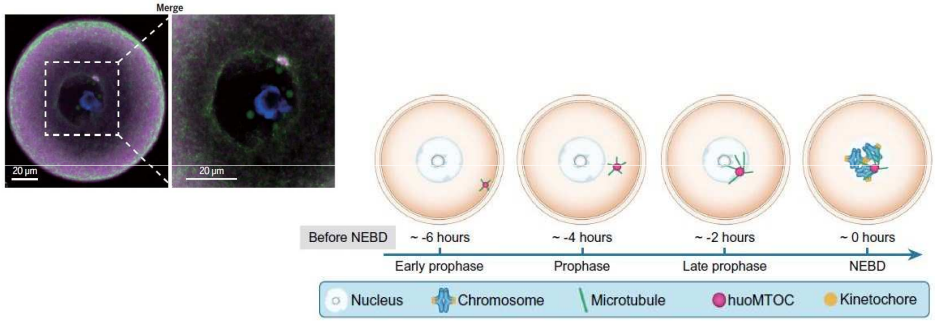
Spindle microtubules					
AKAP450	AURKA	BUGZ	CETN2	CETN3	CENPJ
CEP120	CEP192	CEP250	CLIP1	CLTC	DCTN1
DCTN2	DLGAP5	DYNLT1	GTSE1	HAUS4	HAUS6
HAUS8	HMMR	KANSL3	KIF11	KIF20A	KIZ
LIS1	MYO10	NDE1	NDEL1	NEK2	NUSAP1
PCM1	PLK1	PLK4	PRC1	TPX2	TUBG1

Kinetochores and spindle microtubules			
CCP110	CKAP5	DISC1	TACC3

Spindle poles	Spindle periphery
NUMA1 KIF2A	HOOK3



- putative human specific MT nucleators (huMTOC)?



Wu et al., Science 2022

RESEARCH

RESEARCH ARTICLE SUMMARY

HUMAN FERTILITY

The mechanism of acentrosomal spindle assembly in human oocytes

Tianyu Wu†, Jie Dong†, Jing Fu†, Yanping Kuang†, Biaobang Chen, Hao Gu, Yuxi Luo, Ruihuan Gu, Meiling Zhang, Wen Li, Xi Dong, Xiaoxi Sun*, Qing Sang*, Lei Wang*

Wu et al., Science 2024

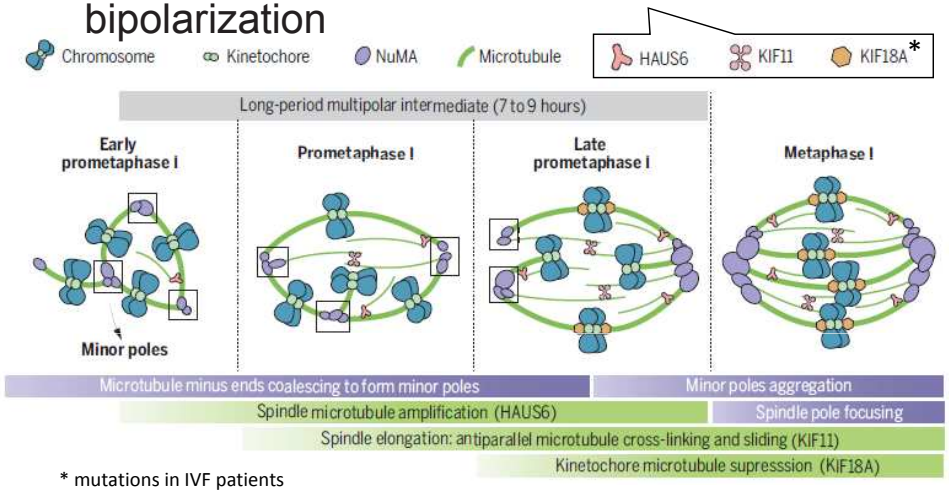
RESEARCH ARTICLE SUMMARY

REPRODUCTION

Mechanisms of minor pole–mediated spindle bipolarization in human oocytes

Tianyu Wu†, Yuxi Luo†, Meiling Zhang†, Biaobang Chen†, Xingzhu Du, Hao Gu, Siyuan Xie, Zhiqi Pan, Ran Yu, Ruiqi Hai, Xiangli Niu, Guimin Hao, Liping Jin, Juanzi Shi, Xiaoxi Sun, Yanping Kuang, Wen Li*, Qing Sang*, Lei Wang*

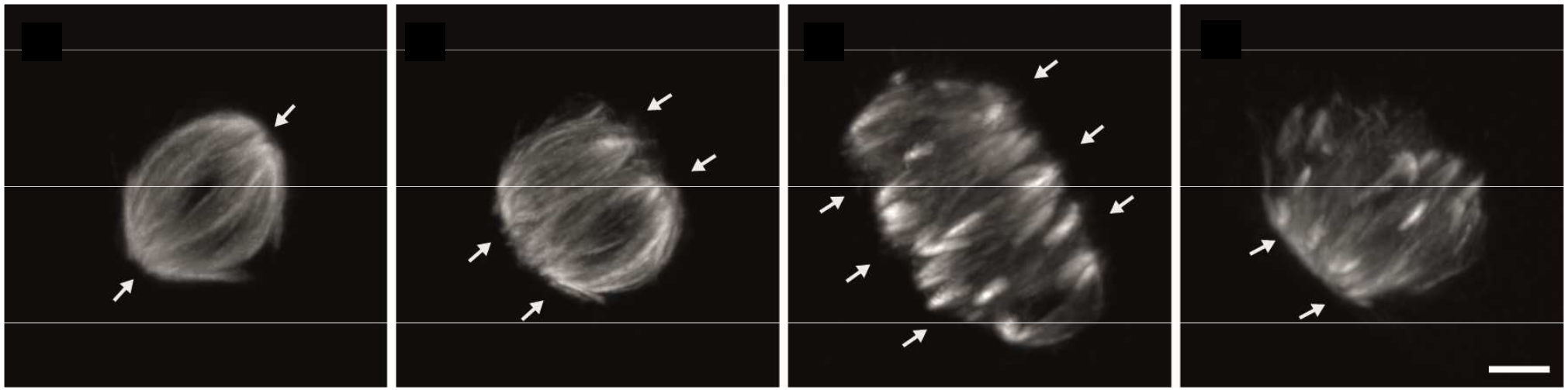
- nascent MT (-)ends coalesce into minor spindle poles which later aggregate to generate opposite spindle poles
- MT amplification, cross-linking and sliding that is required for spindle elongation and bipolarization



* mutations in IVF patients

Human oocyte spindle poles are not stabilized

❖ Established spindle poles in human oocytes are prone to loosening and disintegration



Focused spindle poles

Broad spindle poles

Loosen spindle poles

Disintegrated spindle pole

How are spindle poles organized at the absence of centrosomes?

Scale bar, 5 μm. Unpublished.

Incidence of unstable acentrosomal spindles

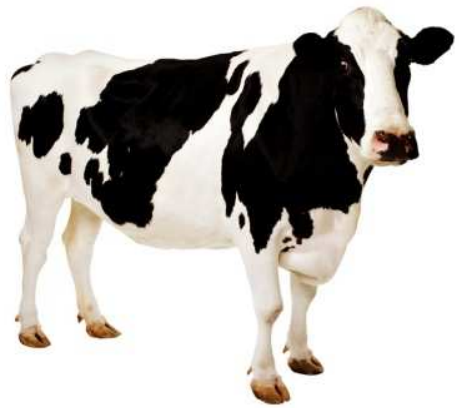
82 %

How are spindles in non-human mammalian oocytes stabilized

0 %

4.4 %

6%



Mammalian oocyte spindle pole organization

Science
AAAS

RESEARCH ARTICLE

OOCYTE DIVISION

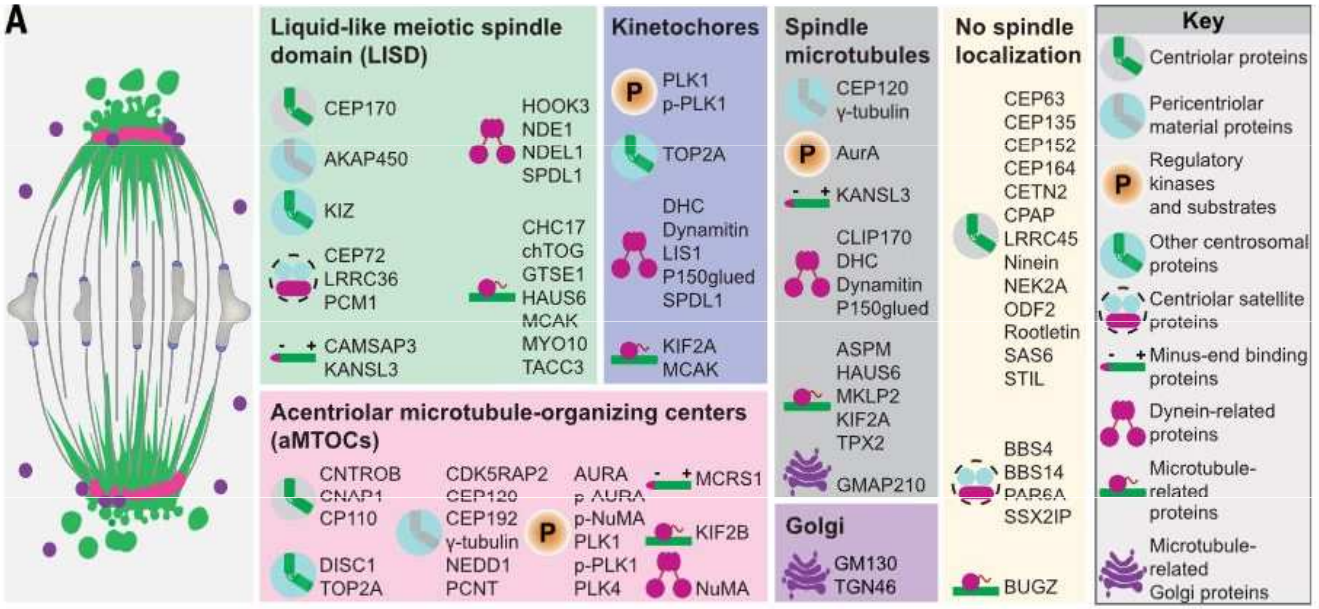

Chuh So

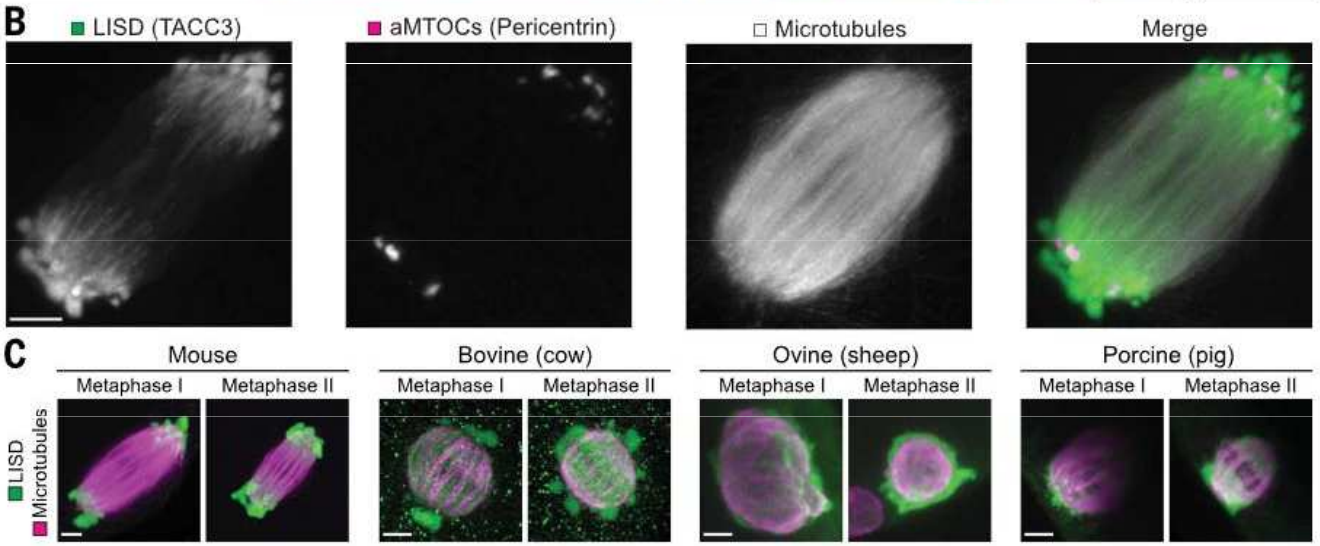

Melina Schuh

A liquid-like spindle domain promotes acentrosomal spindle assembly in mammalian oocytes

Chun So[1*], K. Bianka Seres[1,2,3*], Anna M. Steyer[4,5], Eike Mönnich[1], Dean Clift[2], Anastasija Pejkovska[1], Wiebke Möbius[4,5], Melina Schuh[1,2†]

- **Liquid-like meiotic spindle domain (LISD)**
  - localized at poles and permeates the MT mass of mammalian oocytes
  - selectively concentrates multiple centrosomal and MT-associated proteins
  - allows rapid diffision within the spindle volume
  - disruption of the LISD disperses spindle regulatory factors and leads to severe spindle assembly defects

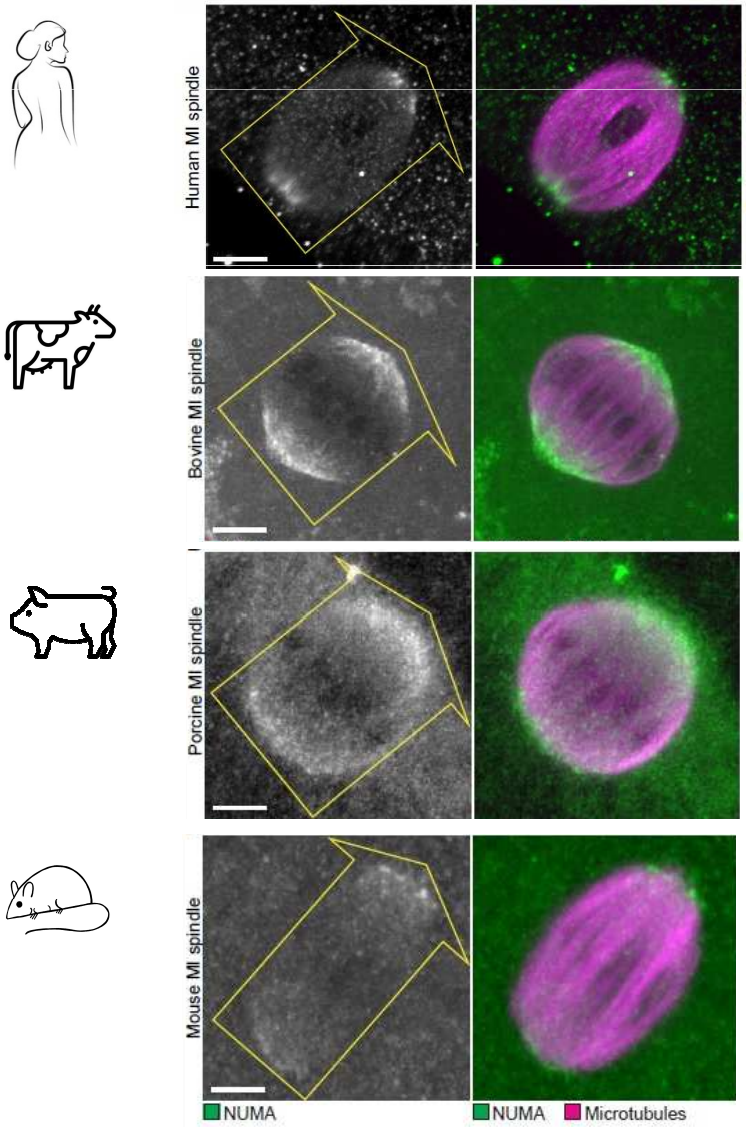




So et al., Science 2019

Human oocyte spindle pole organization

❖ **NuMA decorates spindle poles in mammalian oocytes**



RESEARCH ARTICLE

So et al., Science 2022

CELL BIOLOGY

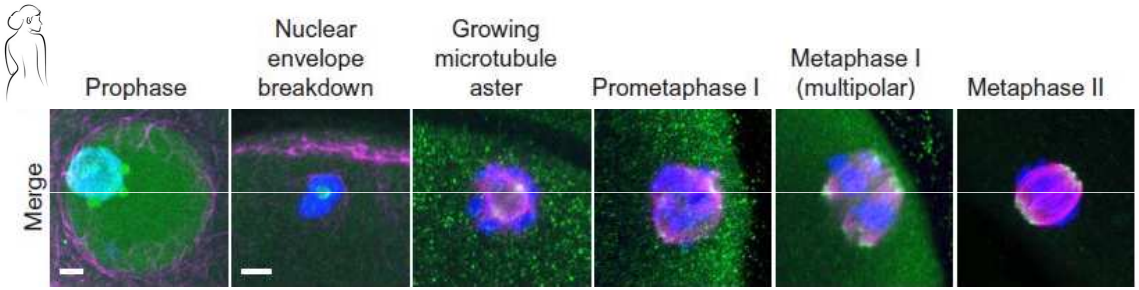
Mechanism of spindle pole organization and instability in human oocytes

Science AAAS

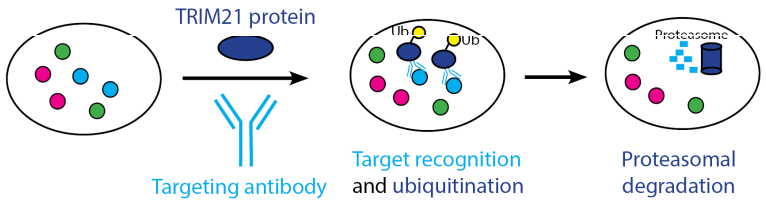
Chun So[1], Katerina Menelaou[1,2]†, Julia Uraji[1,2]†, Katarina Harasimov[1], Anna M. Steyer[3], K. Bianka Seres[1,2], Jonas Bucevičius[4], Gražvydas Lukinavičius[4], Wiebke Möbius[3,5], Claus Sibold[6], Andreas Tandler-Schneider[6], Heike Eckel[7], Rüdiger Moltrecht[7], Martyn Blayney[2], Kay Elder[2], Melina Schuh[1,5]*

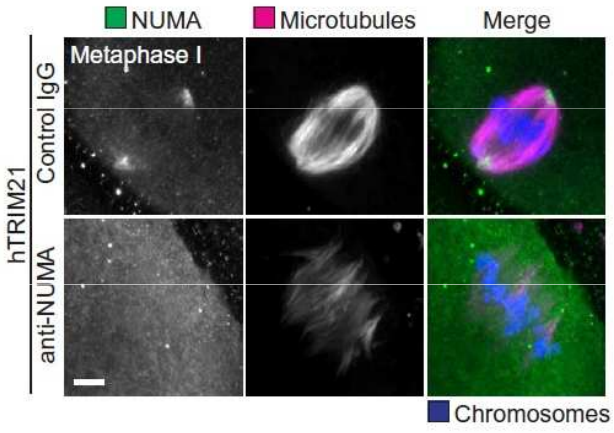
Chuh So

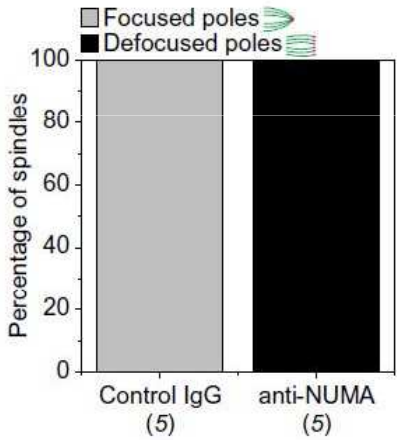
Melina Schuh



- NuMA is required for MT focusing at spindle poles in human oocytes

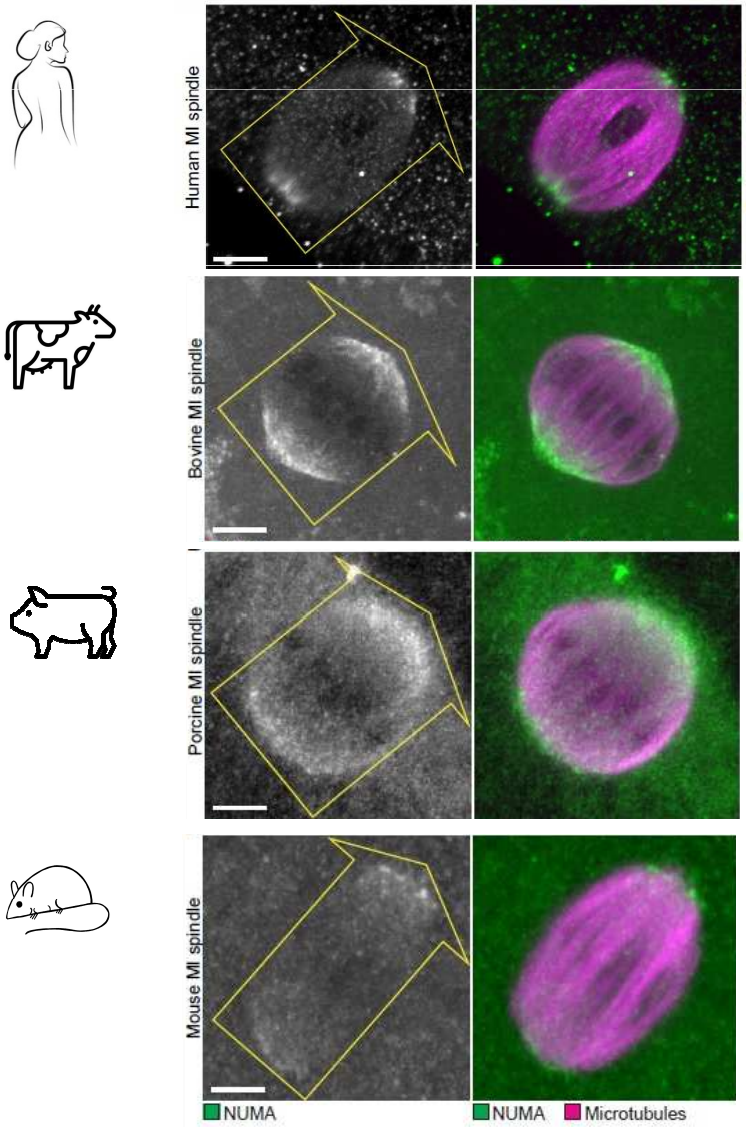






Human oocyte spindle pole organization

❖ NuMA decorates spindle poles in mammalian oocytes



RESEARCH ARTICLE

So et al., Science 2022

CELL BIOLOGY

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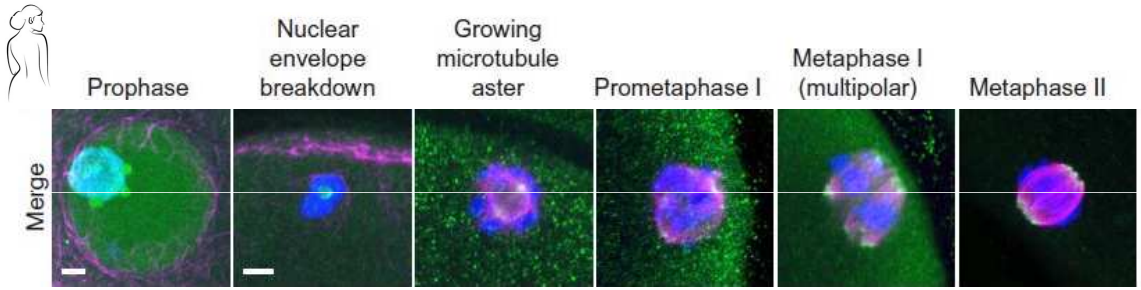
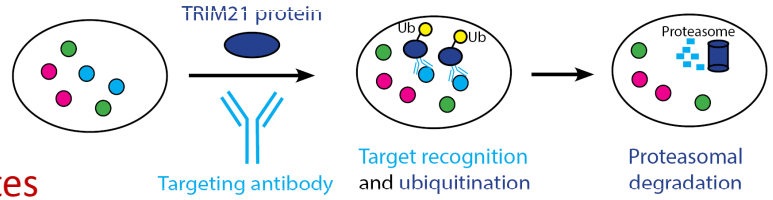
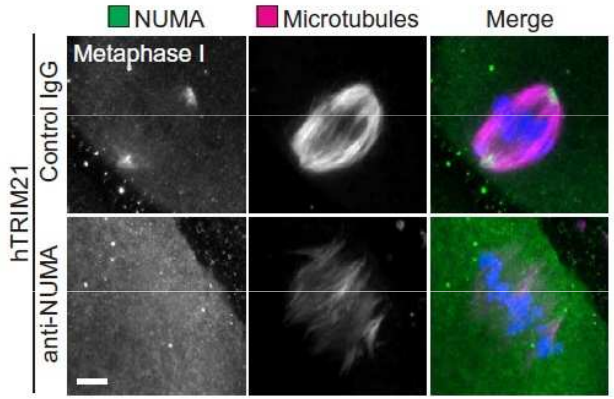
Chuh So

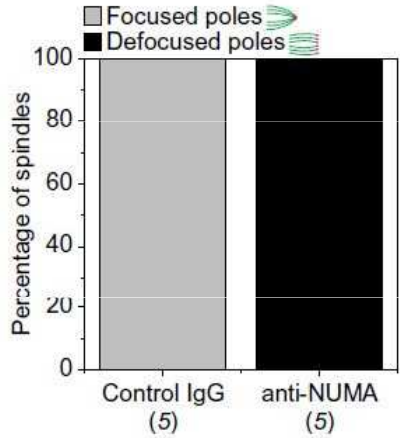
Melina Schuh



- acute depletion of NuMA → NuMA is required for MT focusing at spindle poles in human oocytes

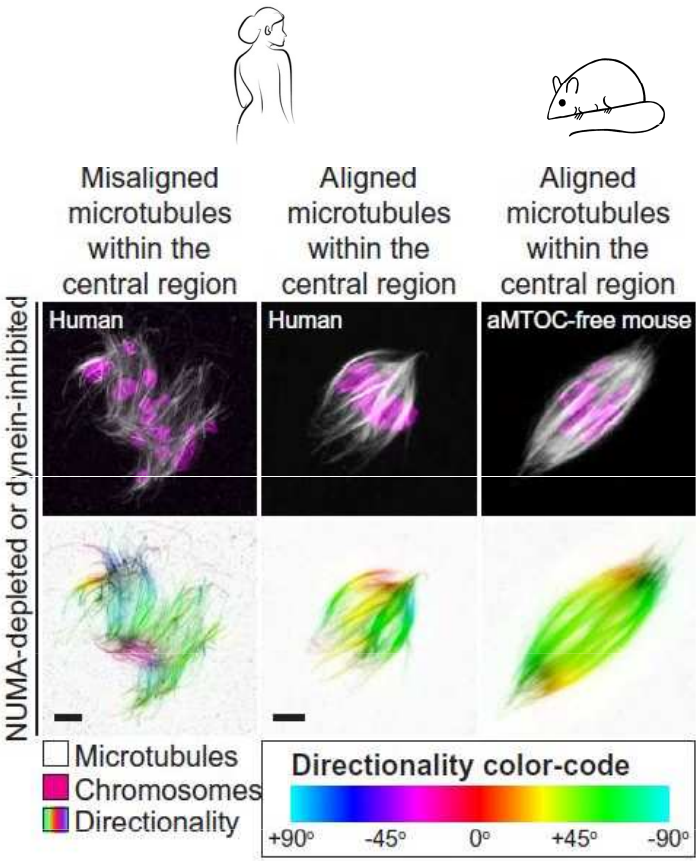


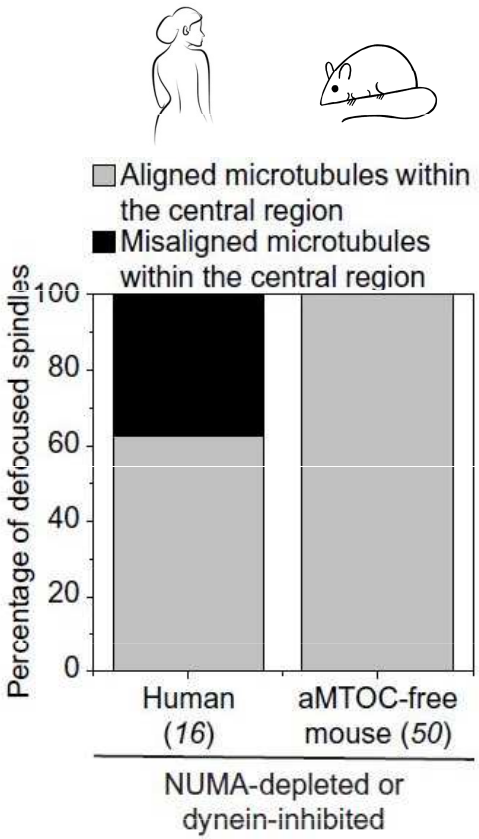


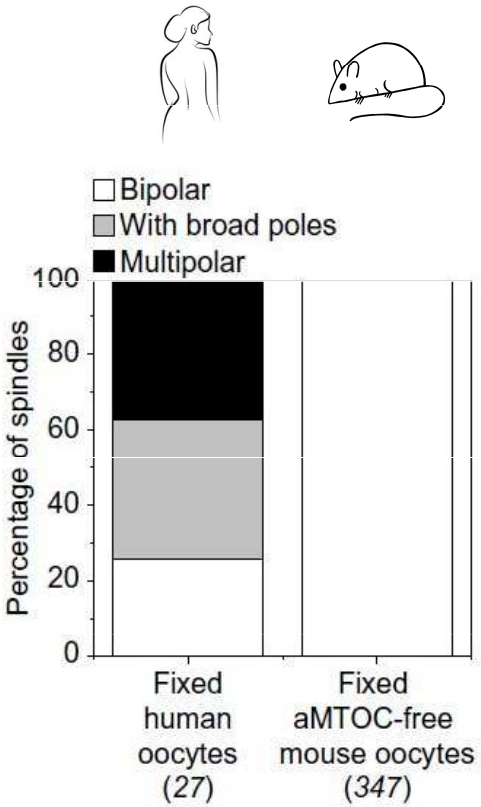


Search for oocyte spindle stabilizing factor

Misalignment of microtubules in **central** region of human oocyte spindle







Human oocytes must lack a stabilizing protein that protects mouse, porcine and bovine oocytes from spindle instability

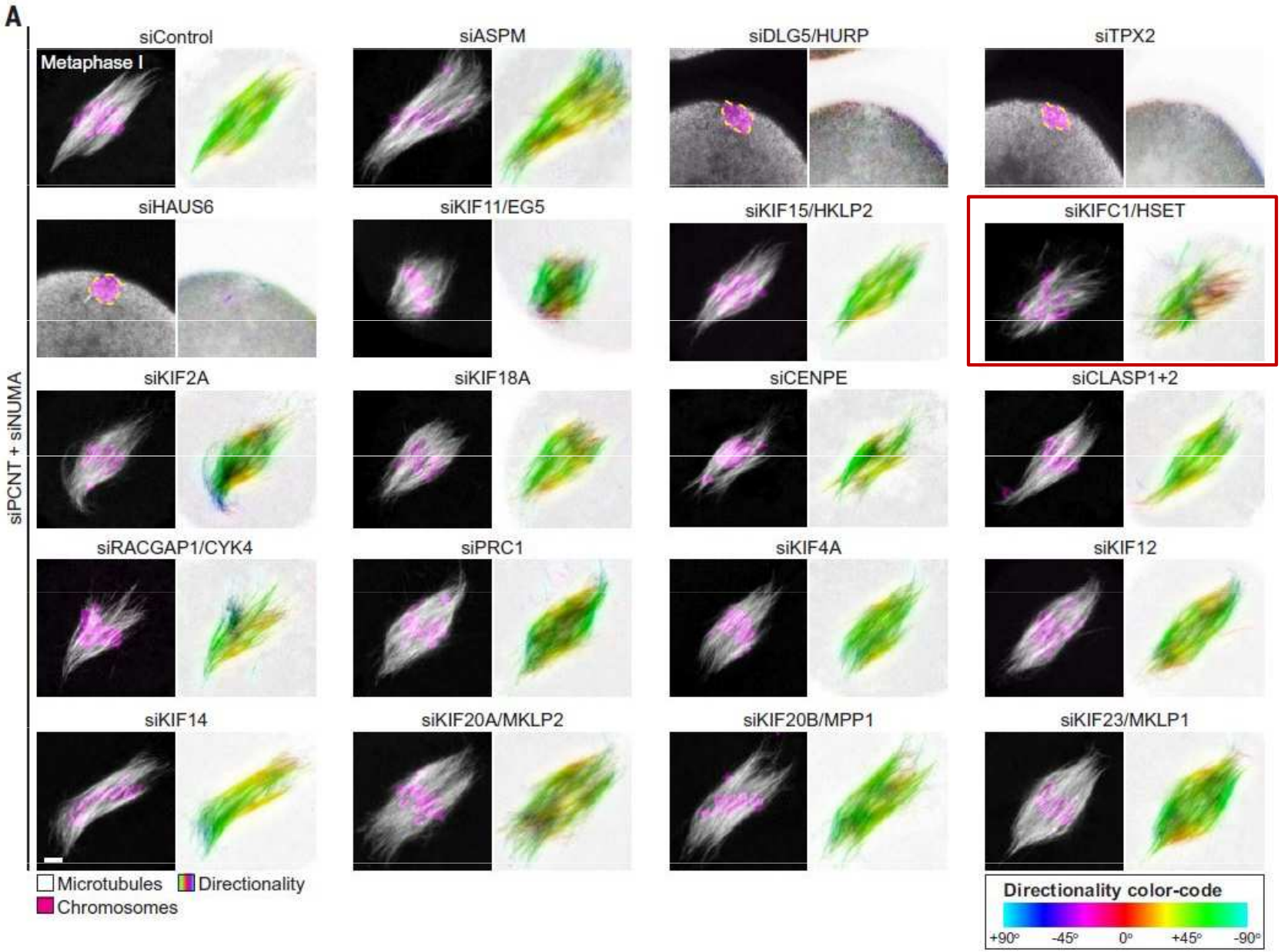
So et al., Science 2022

Search for oocyte spindle stabilizing factor

si RNA screen

co-depletion of NuMA and candidate spindle-associated proteins

phenotypes analyzed



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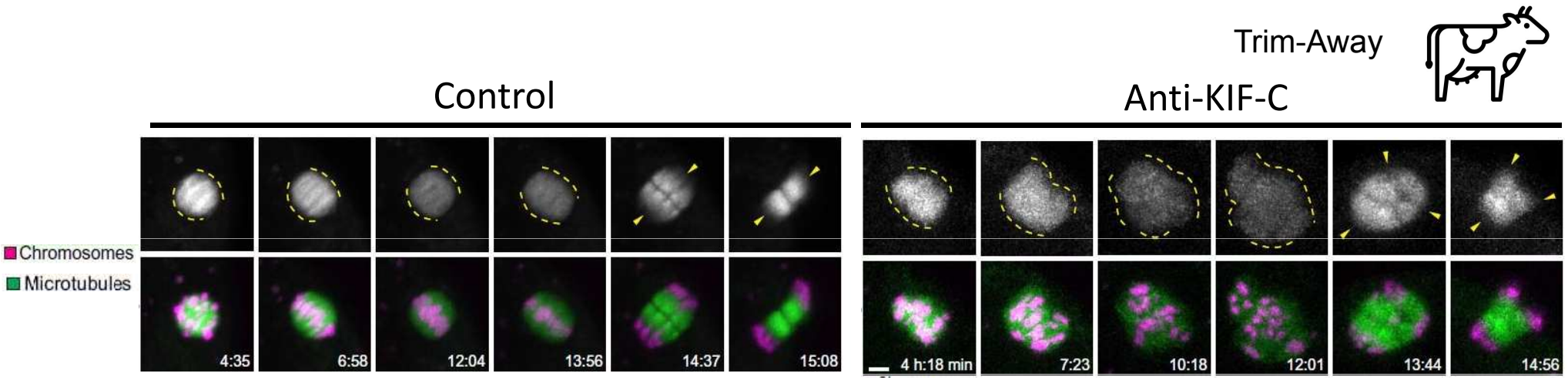
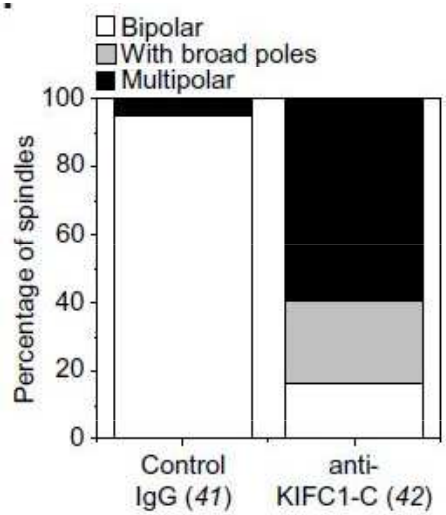
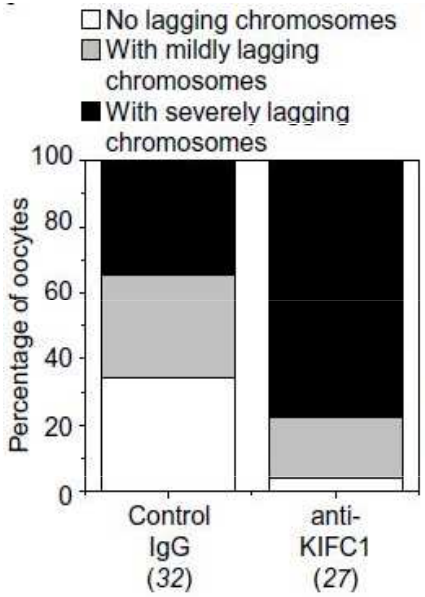
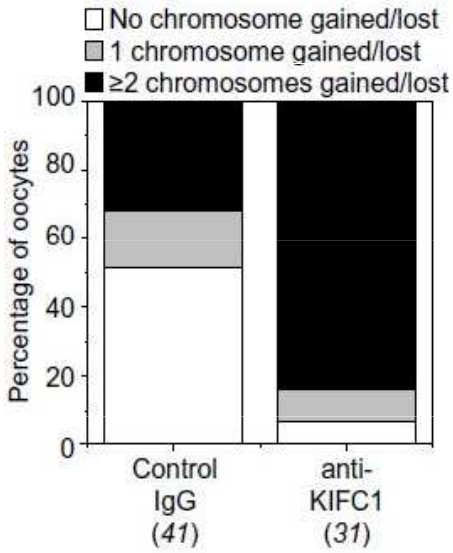
Search for oocyte spindle stabilizing factor







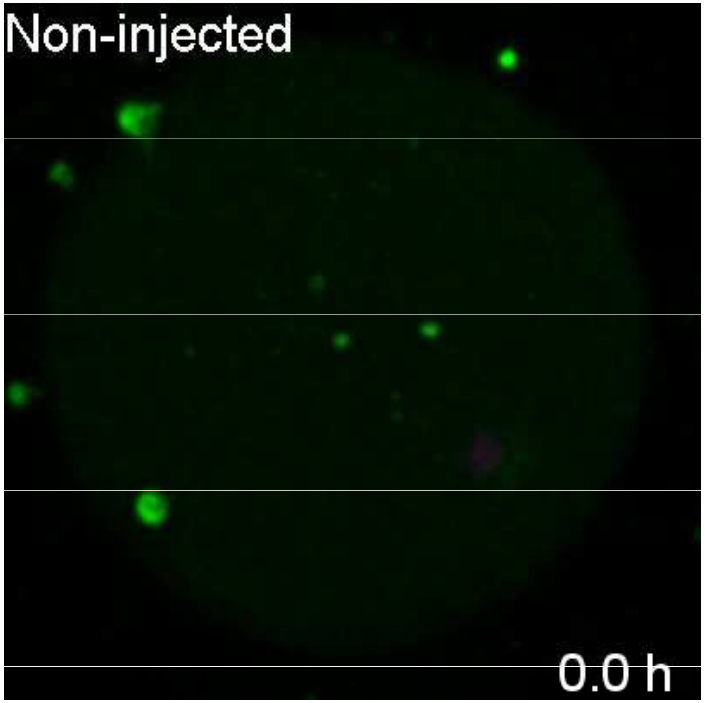


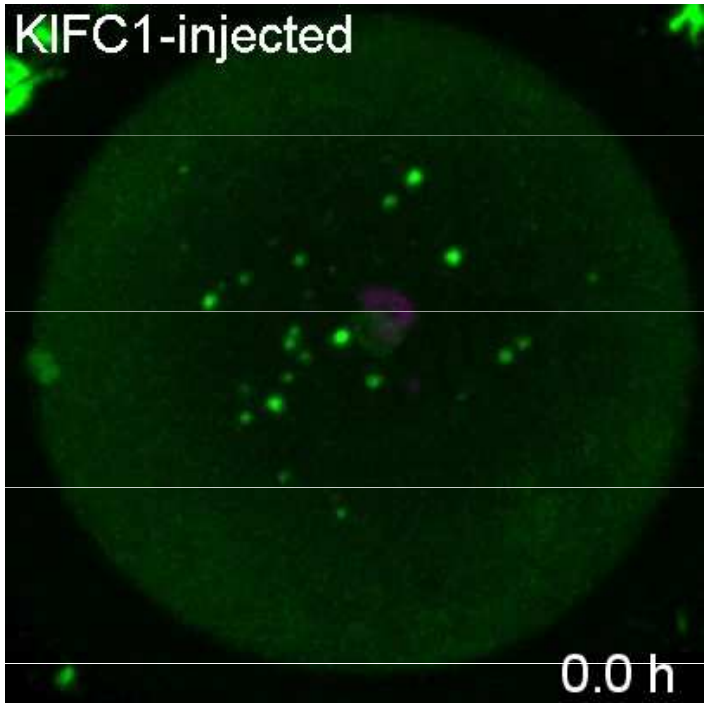
Depletion of KIFC1/HSET induces spindle instability and promotes aneuploidy in bovine oocytes

So et al., Science 2022

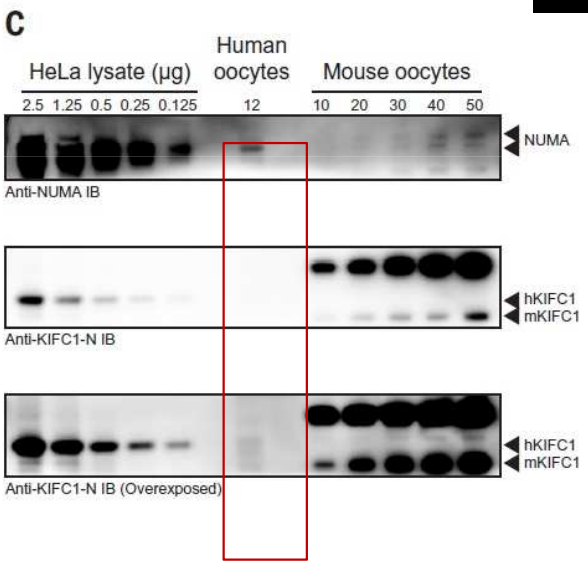
Search for oocyte spindle stabilizing factor

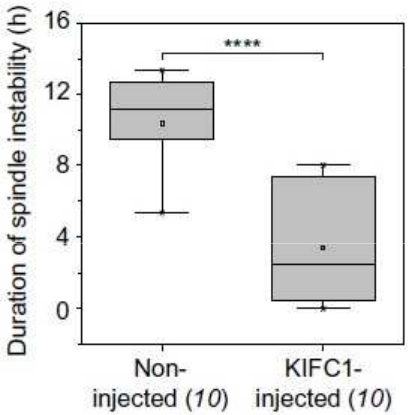
Chromosomes
Microtubules

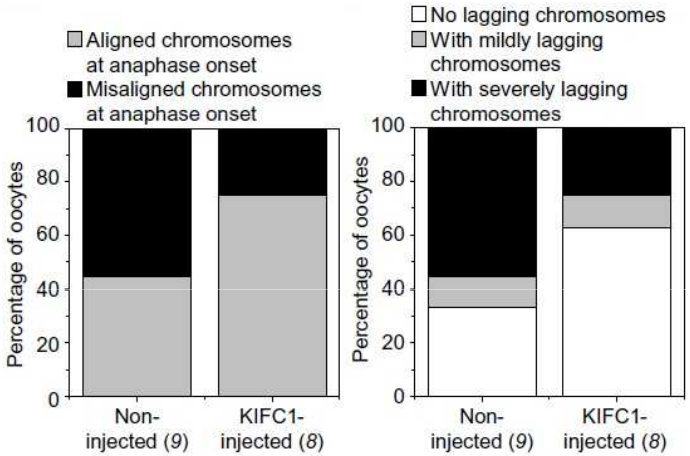




❖ Human oocytes are deficient in KIFC1





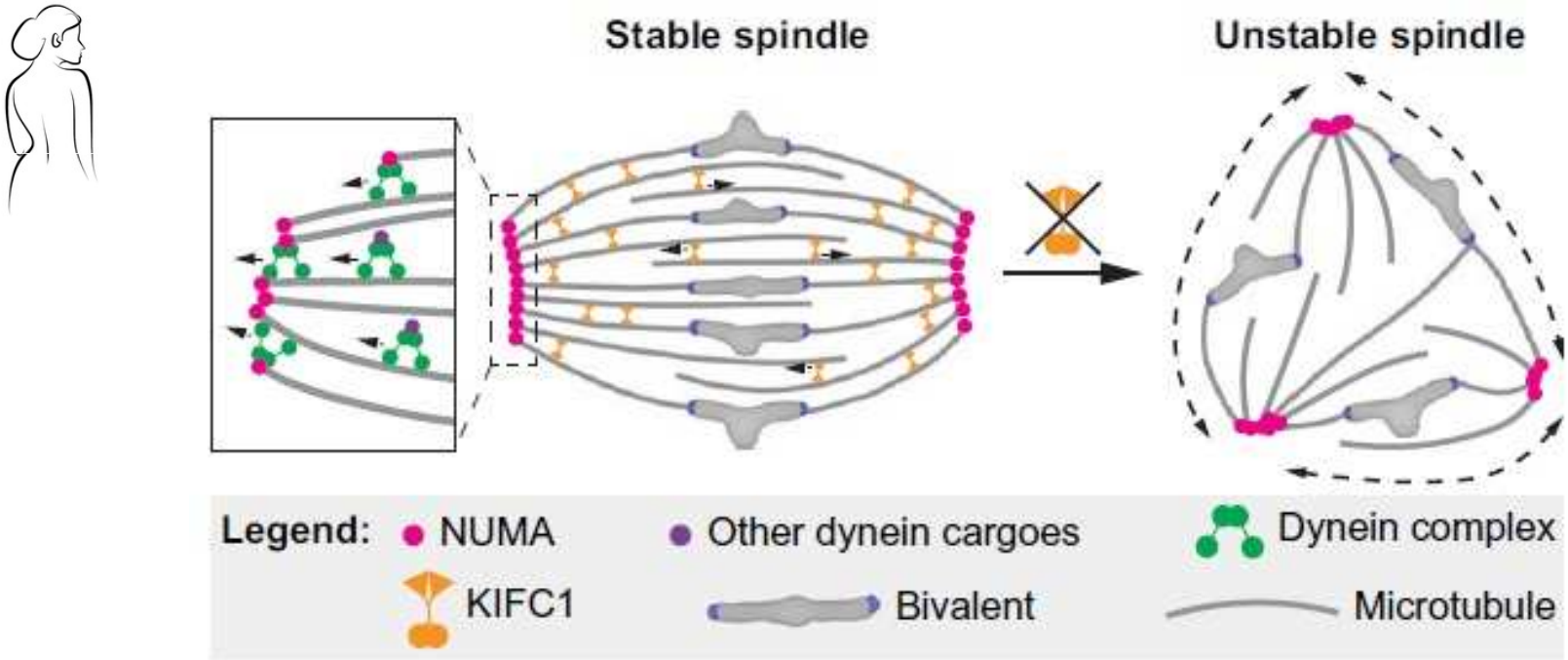


❖ Exogenous KIFC1 rescues spindle instability in human oocytes

So et al., Science 2022

Prevention of oocyte spindle instability

- KIFC1 ensures the spindle stability and prevents fragmentation of spindle poles by ensuring alignment of MT at central region and crosslinking MT minus ends at spindle poles



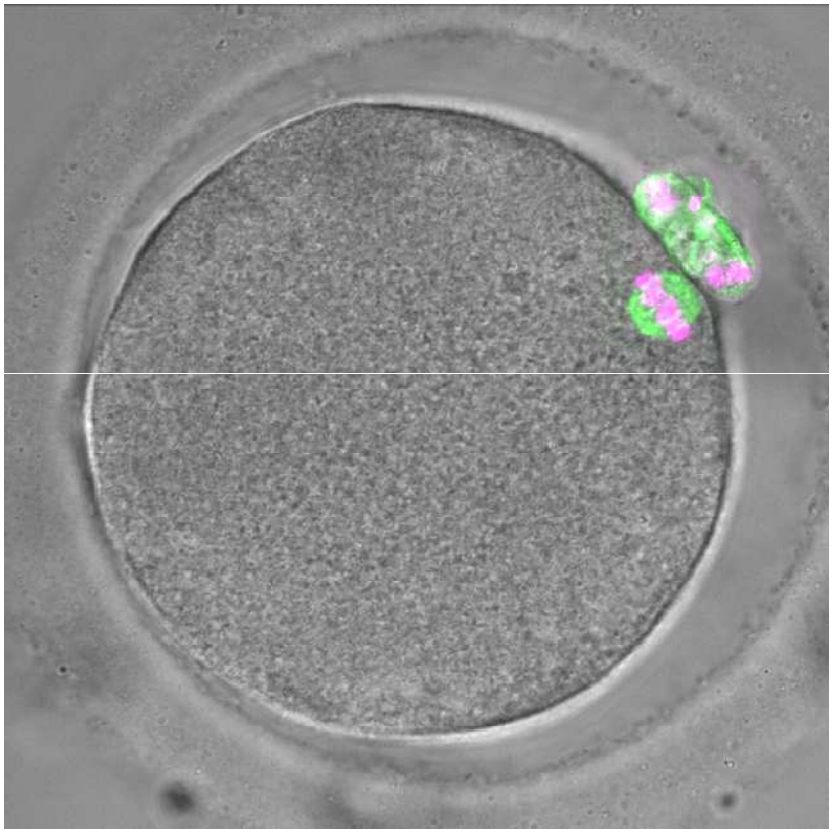
- NuMa organize acentrosomal spindle poles by ensuring coalescence of crosslinked MT-minus ends

So et al., Science 2022

Egg maturity

❖ **Mature egg**

= metaphase II arrested oocytes with PB extruded and chromosmes aligned in MI



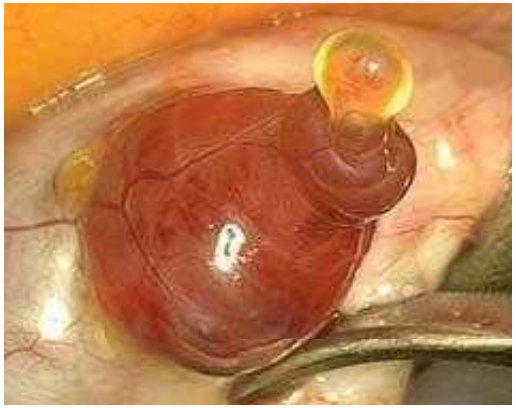
DNA, microtubules

In IVF practice,
all PB-displaying oocytes
are regarded as MIIs
and subjected to ICSI

Oocyte maturity

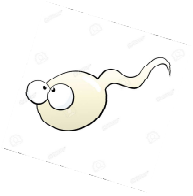
IN VIVO

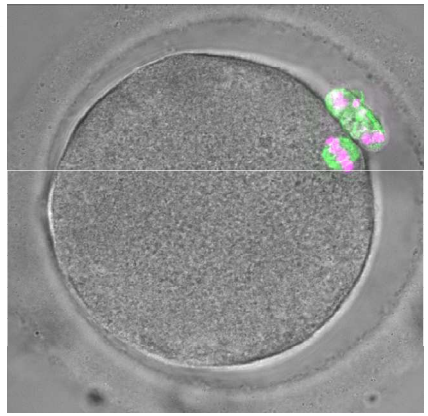
NATURAL CYCLE



OVULATION

SINGLE COC

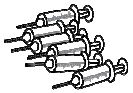




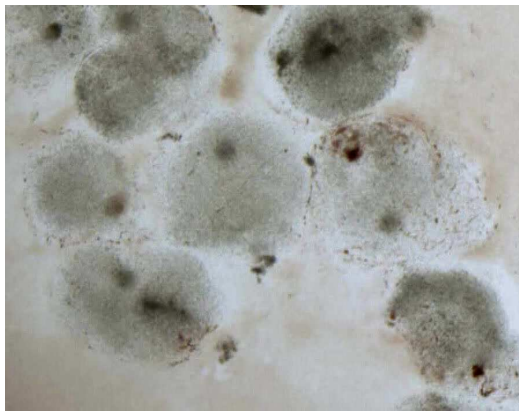
MATURE EGG

IN VITRO

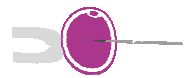
CONTGROLLED OVARIAN STIMULATION

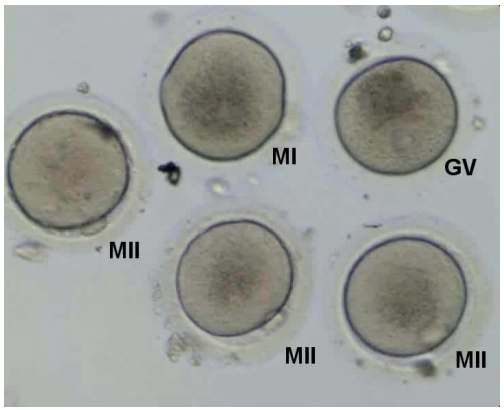


PREOVULATORY FOLLICLES



MULTIPLE COCS

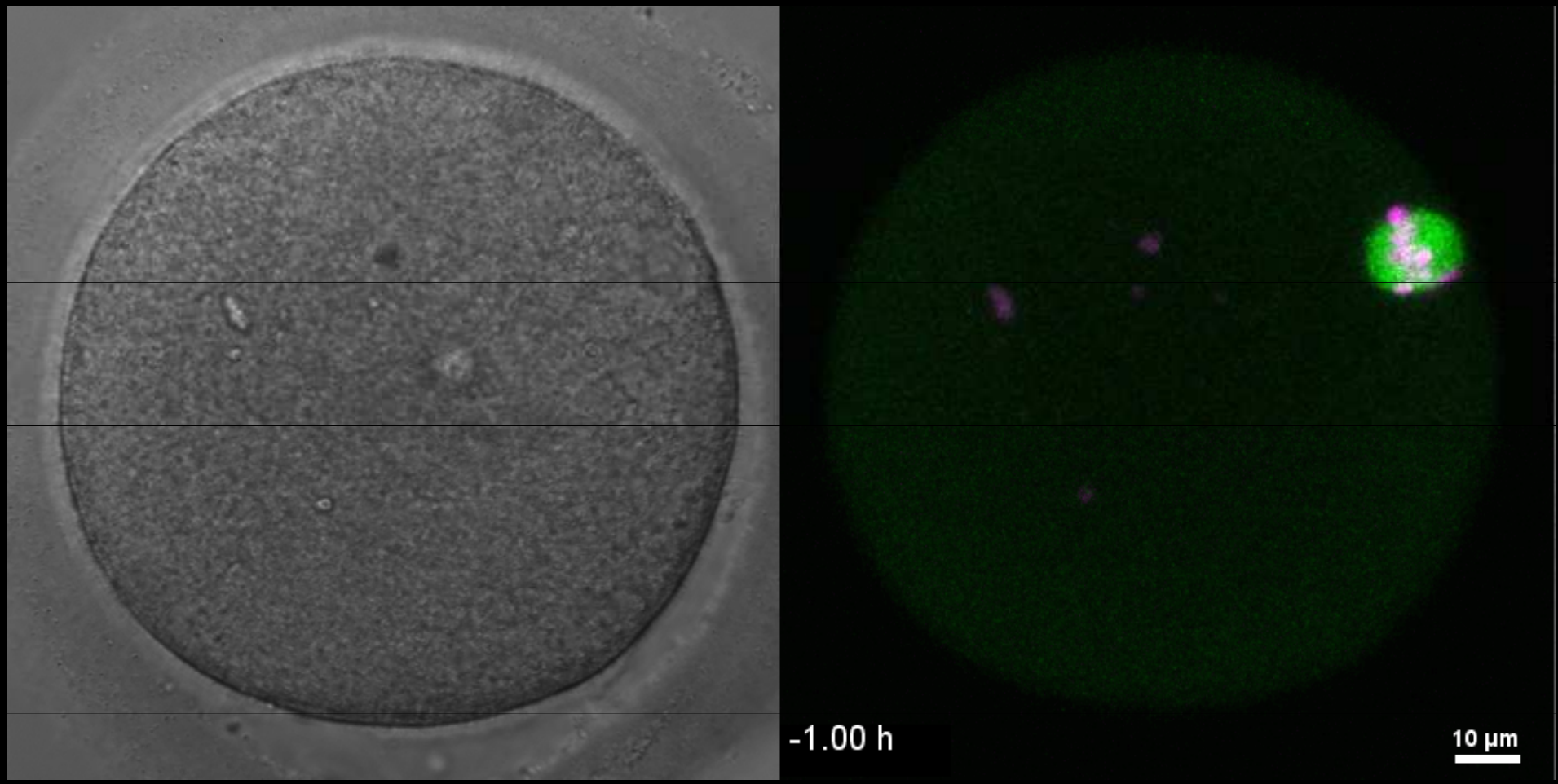




MATURE + IMMATURE OOCYTES

MI to MII transition and MII spindle assembly

- MII spindle formation is rapid compared to MI



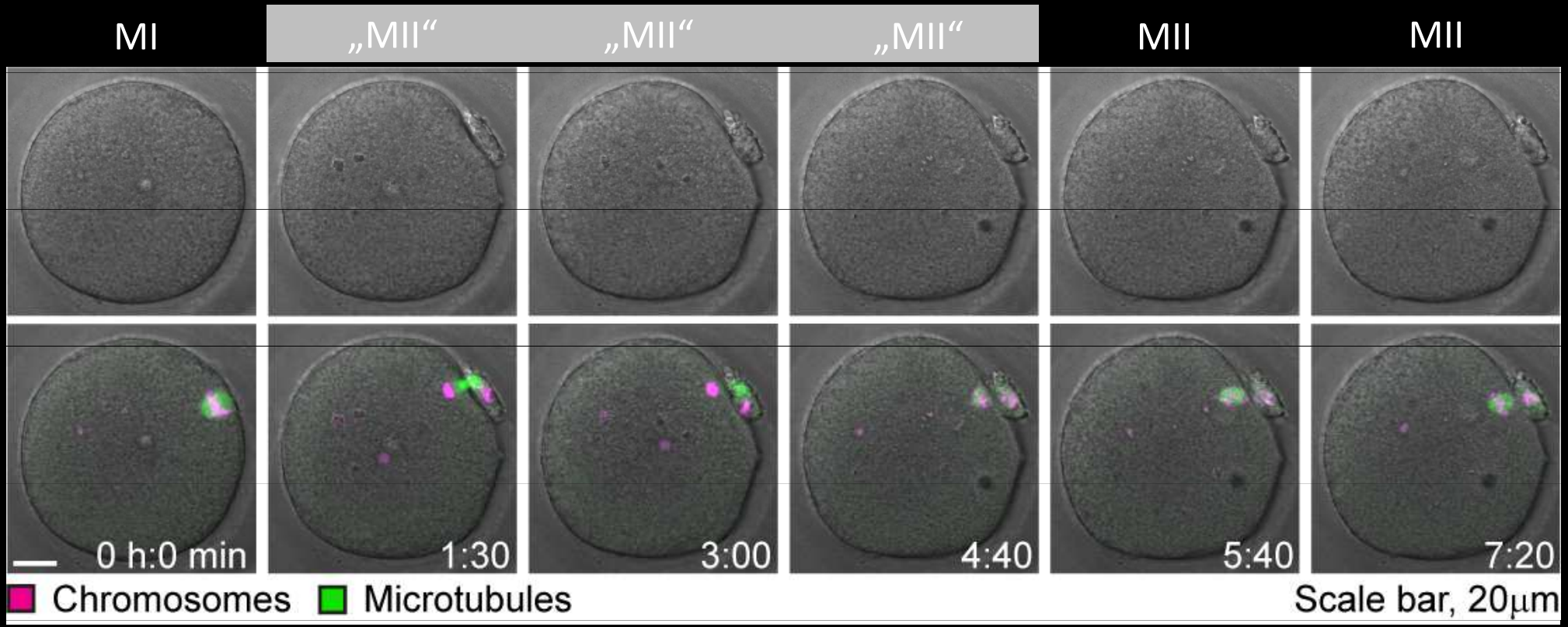
Chromosomes
(H2B-mRFP)
Microtubules
MAP4-EGFP)

- asynchrony between PB extrusion and MII arrest !

Holubcová et al Science 2015

MI to MII transition and MII spindle assembly

- Emergence of PB precedes MII arrest

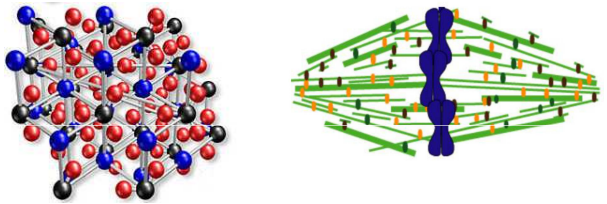


→ risk of untimely fertilization (ICSI)

Non-invasive spindle visualization

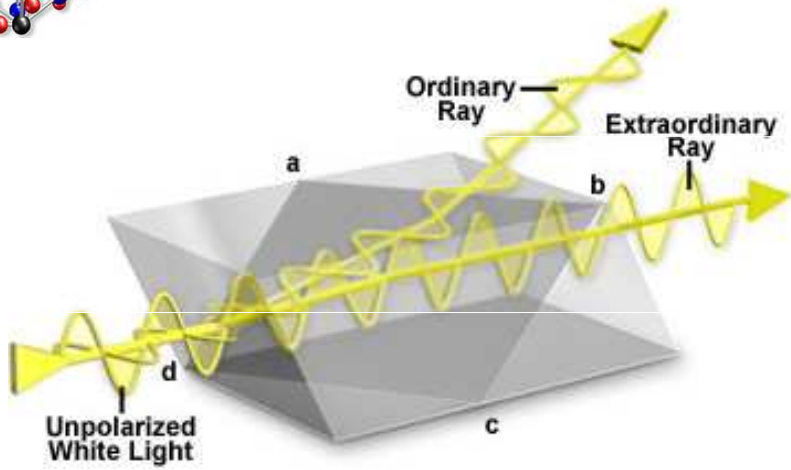
❖ Polarized Light Microscopy (PLM)

- based on interference of **polarized light** with anisotropic substances e.g. axial crystals, liquid crystals and oriented (bio)polymers

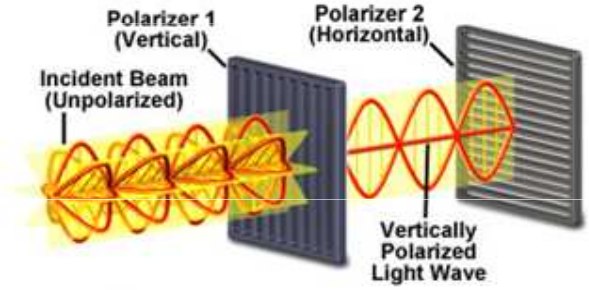


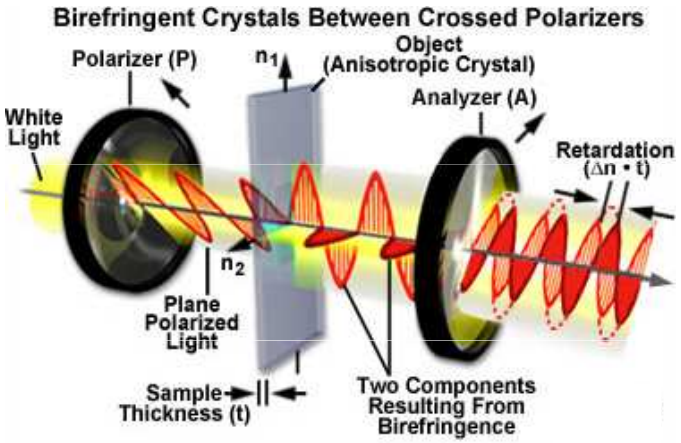
BIREFRINGENCE

- property of certain materials to split a light beam to two rays (ordinary/extraordinary)



- polarized light is refracted by these anizotropic materials and divided to separate components vibrating perpendicularly
- both polarized light ray then pass through the analyzer and the relative retardance of one ray to the other is calculated



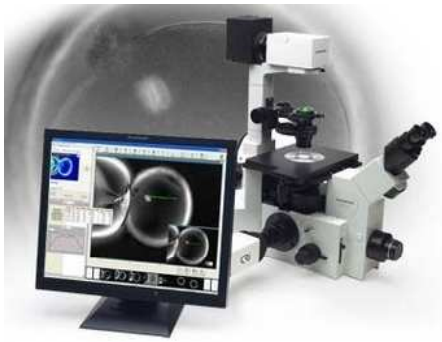


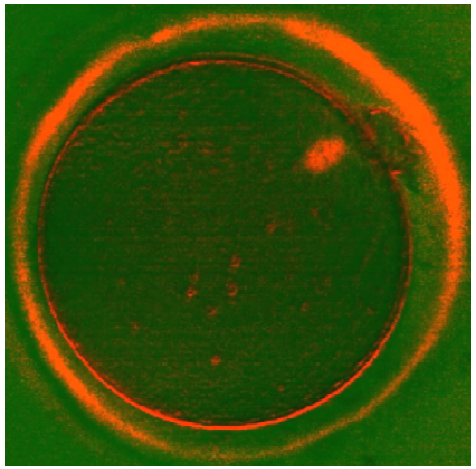
https://www.microscopyu.com/techniques/polarized-light/principles-of-birefringence

Non-invasive spindle visualization

❖ Polarized Light Microscopy (PLM)

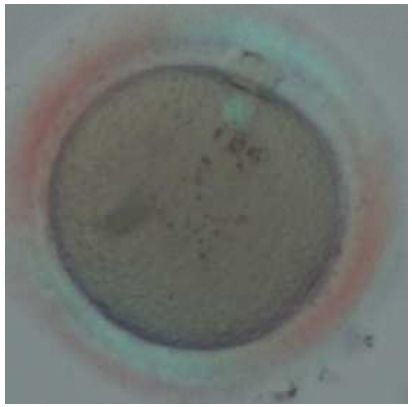
- enables **non-invasive** imaging of **birefringent** structures in living cells

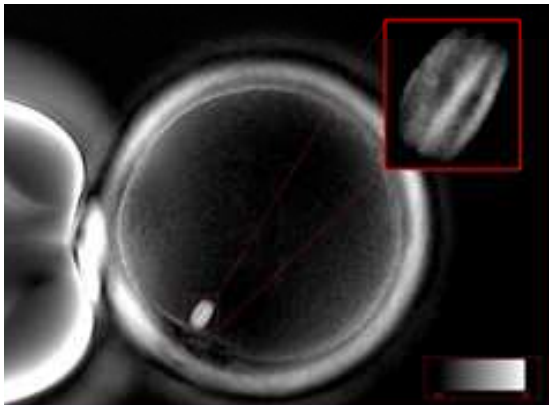




- presence and positioning of MII spindle
- pattern of zona pellucida

- presence of PLM-detectable MII spindle is a positive marker of egg´s fertilization and developmental competence





jove Journal of Visualized Experiments www.jove.com

Video Article

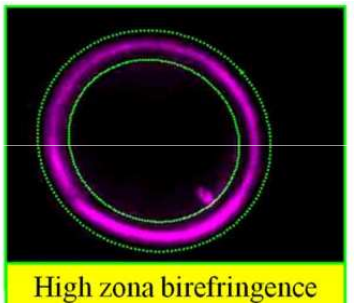
Human Egg Maturity Assessment and Its Clinical Application

Zuzana Holubcová[1,2], Drahomíra Kyjovská[1], Martina Martonová[1], Darja Páralová[1], Tereza Klenková[1], Soňa Kloudová[1]

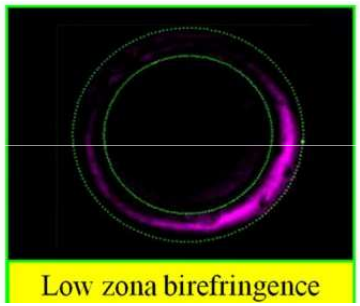
[1]Reprofit International, Clinic of Reproductive Medicine

[2]Department of Histology and Embryology, Faculty of Medicine, Masaryk University

https://www.youtube.com/watch?v=tlKo9nqmqGY



High zona birefringence

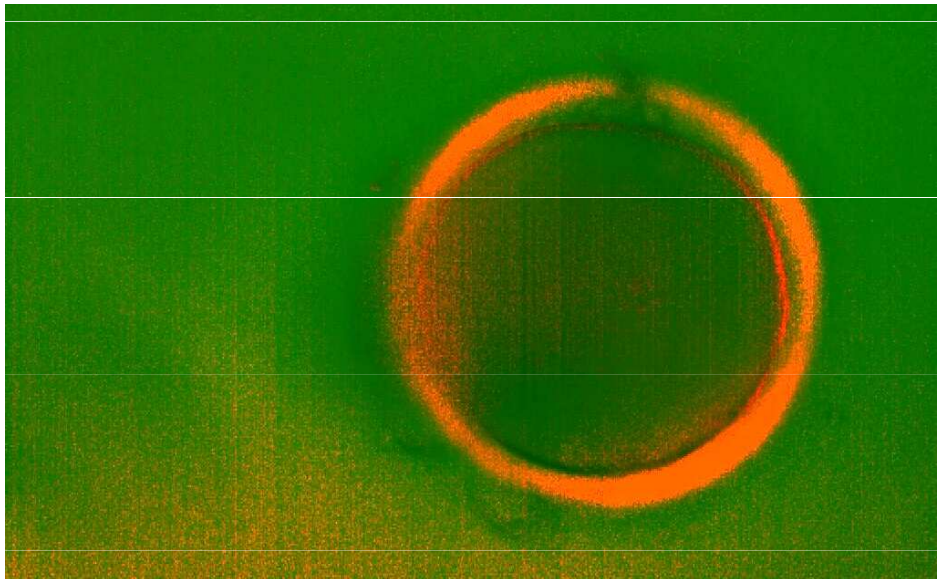


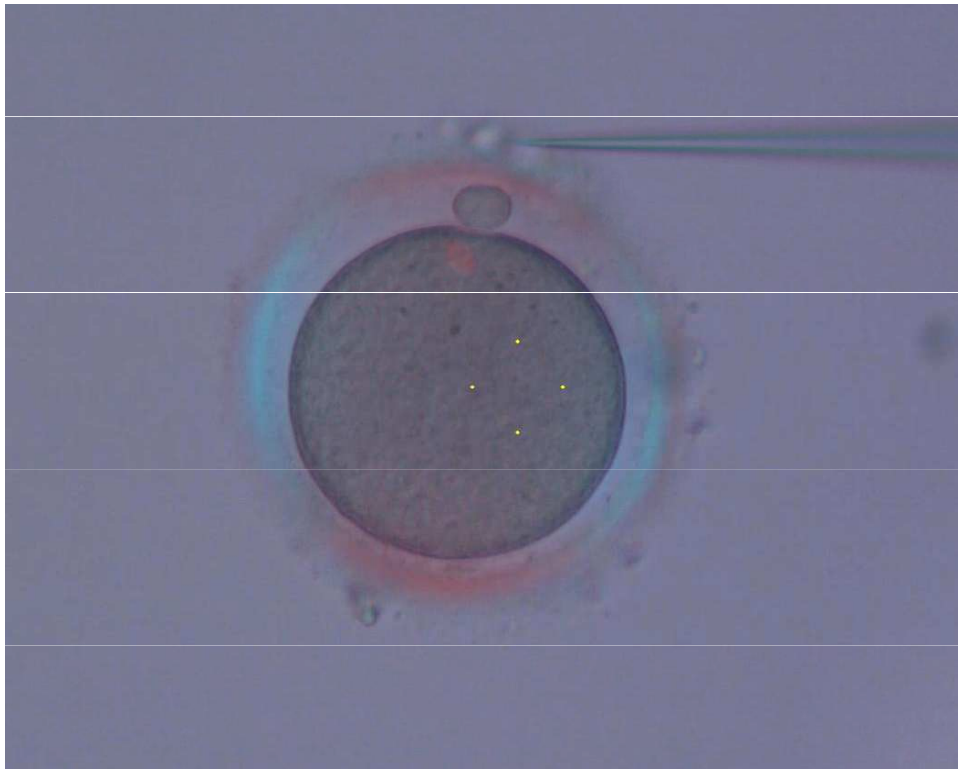
Low zona birefringence

Non-invasive spindle visualization

❖ Polarized Light Microscopy (PLM)

- PLM signal is orientation-dependent
- spindle imaging requires oocyte orientation



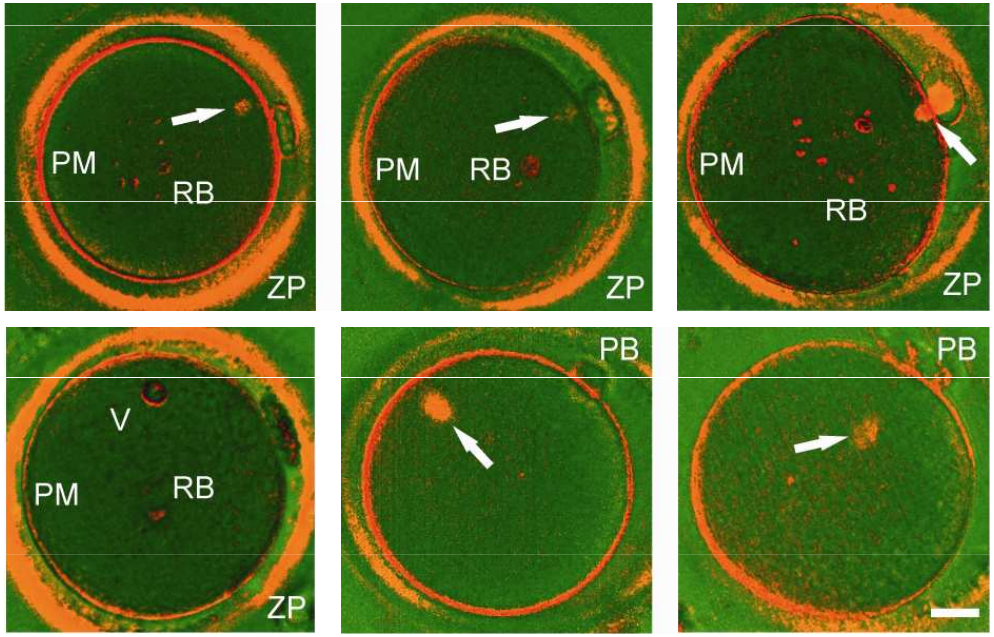


Holubcová et al, JoVE 2019

Non-invasive spindle visualization

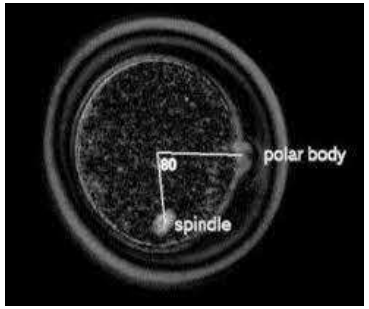
❖ Polarized Light Microscopy (PLM)

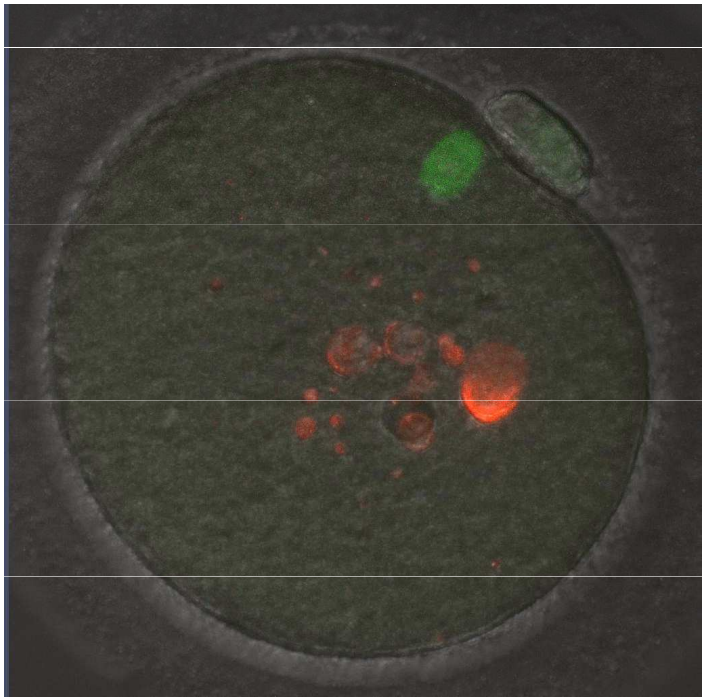
- birefringent structures in human oocytes



PM.... Plasma mebrane
ZP.....Zona pellucida
RB.....Refractile body
V.....Vacuole

- relative position of spindle and PB



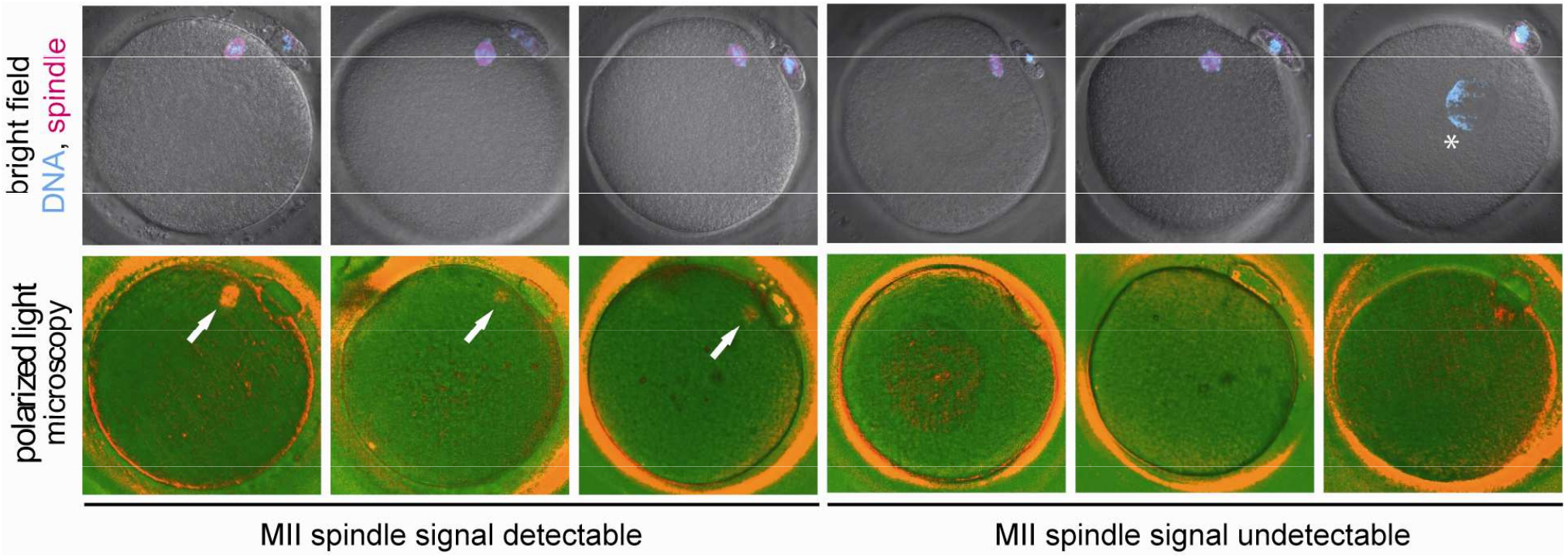


Holubcová et al, JoVE 2019

Non-invasive spindle visualization

❖ Polarized Light Microscopy (PLM)

- the strengh of the signal reflect the material ordering
- suffient mass of paralelely oriented MT required to produce noticible signal

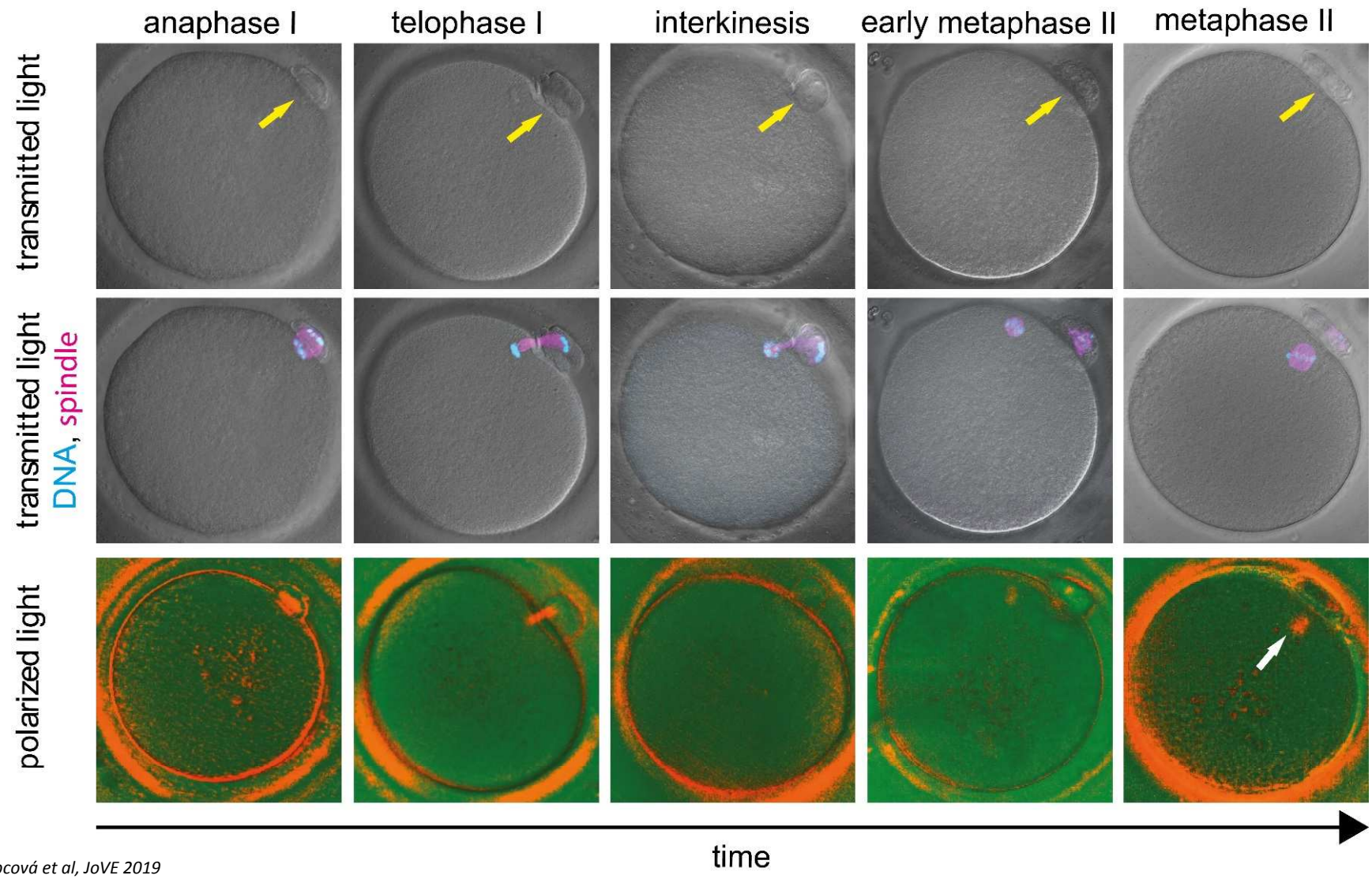


Holubcová et al, JoVE 2019

Non-invasive spindle visualization

❖ Polarized Light Microscopy (PLM)

- enables monitoring of MI/MII transition and ICSI time optimisation in clinical practice

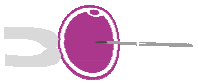


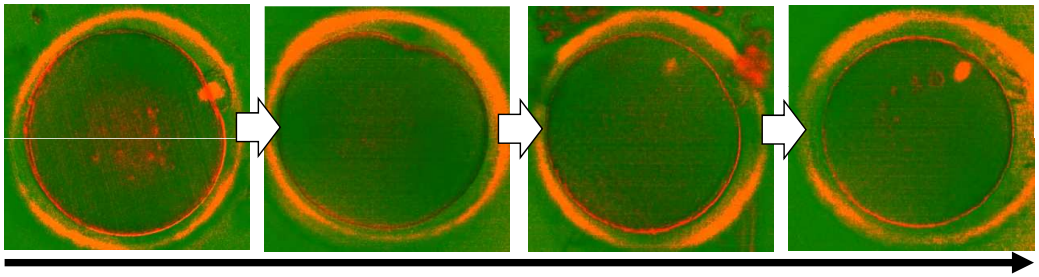
Holubcová et al, JoVE 2019

Non-invasive spindle visualization

❖ Polarized Light Microscopy (PLM)

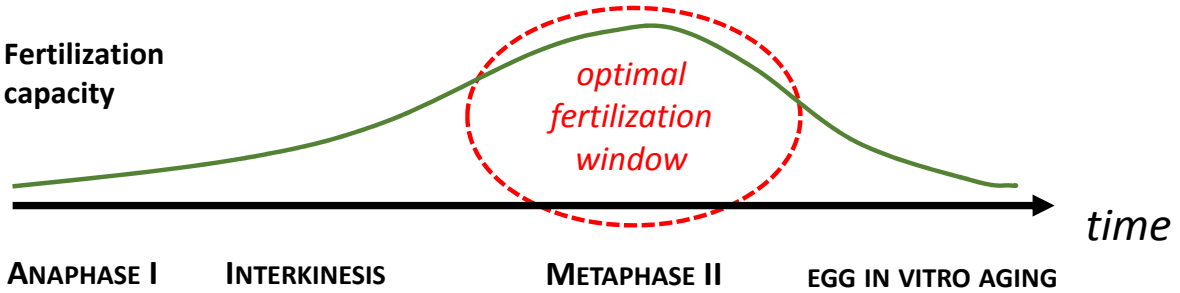
- enables monitoring of MI/MII transition and ICSI time optimisation in clinical practice

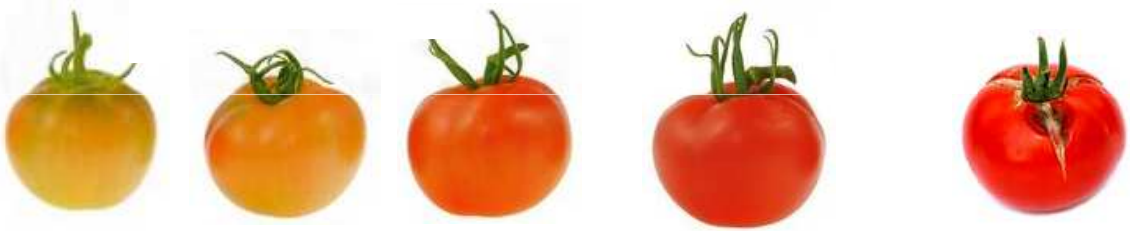




Postponing ICSI

time





Montag RBM Online 2006
Montag and van der Ven, RBM Online 2008
Holubcova et al, JARG 2019

Factor affecting human oocyte spindle stability in vitro

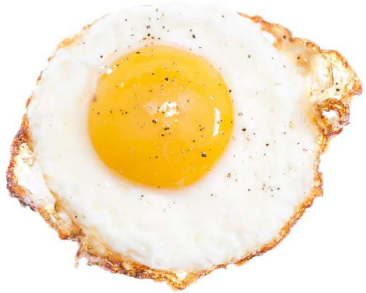
❖ MII spindle is sensitive

➢ **temperature**

- **optimal 37°C**

OVERHEATING

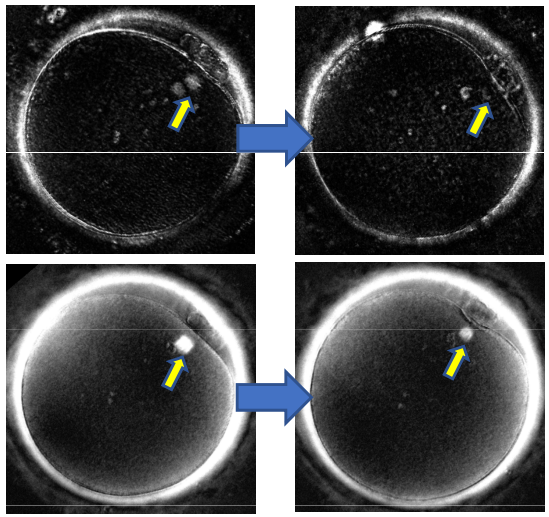
→ irreversible denaturation



COOLING

→ spindle desintegration

10 min RT



➢ **osmolarity alterations**

- avoid evaporation
- parafine/mineral oil overlay
- humid conditions

➢ **pH fluctulation**

- MOPS/HEPES buffered medium for work in ambient conditions

avoid excessive manipulation !