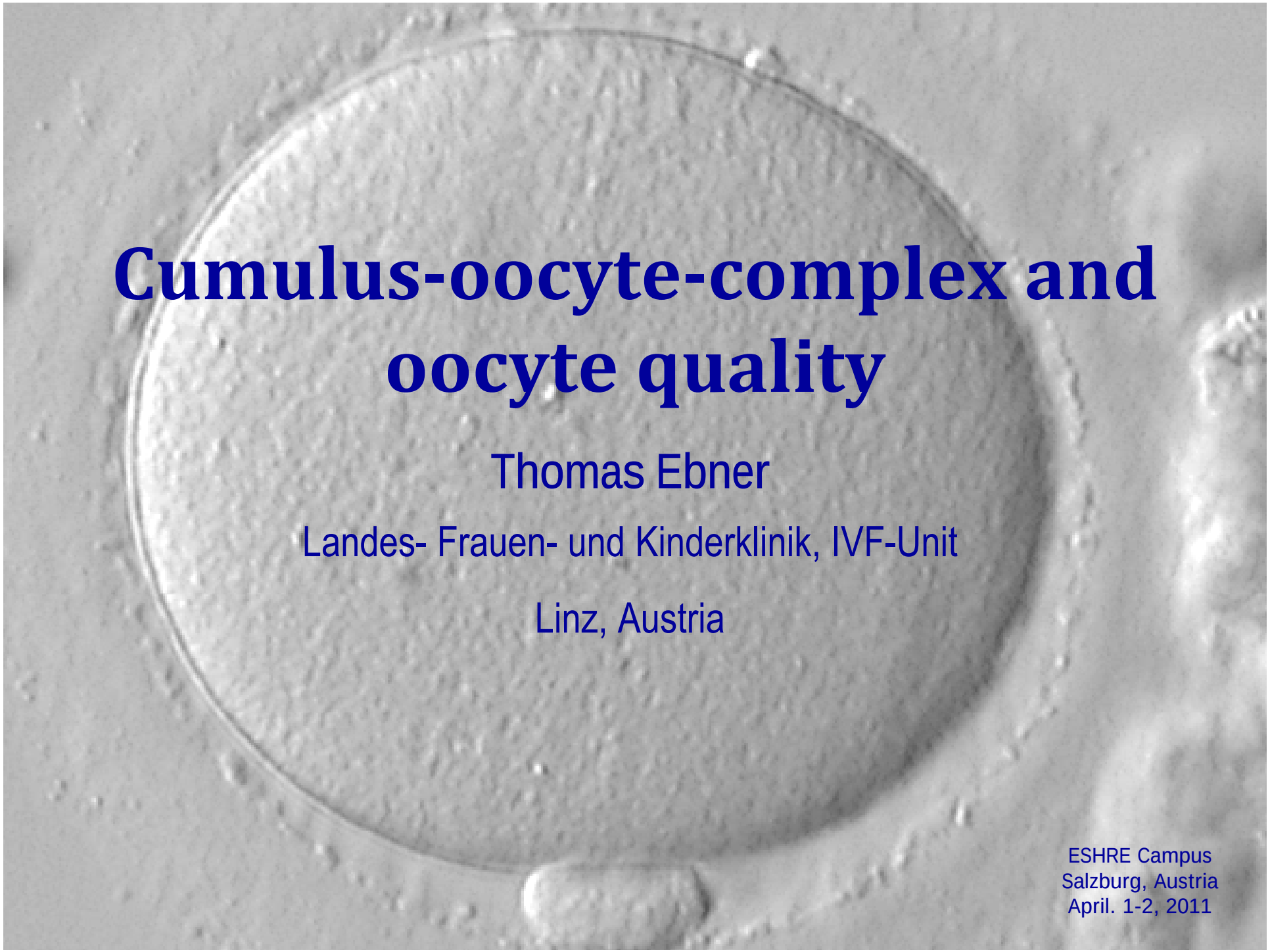
This speaker has NO conflict of interest



Cumulus-oocyte-complex and oocyte quality

Thomas Ebner

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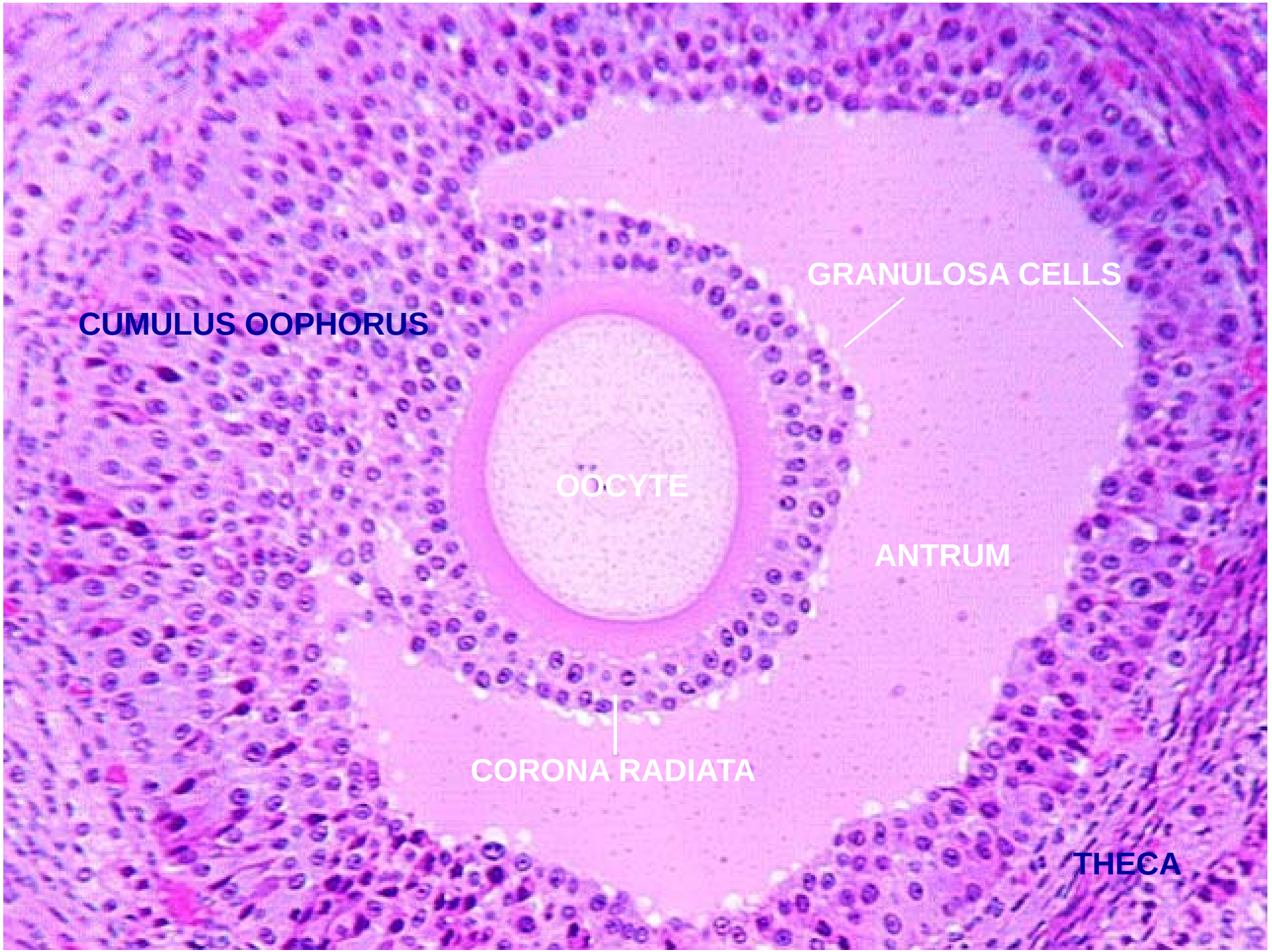
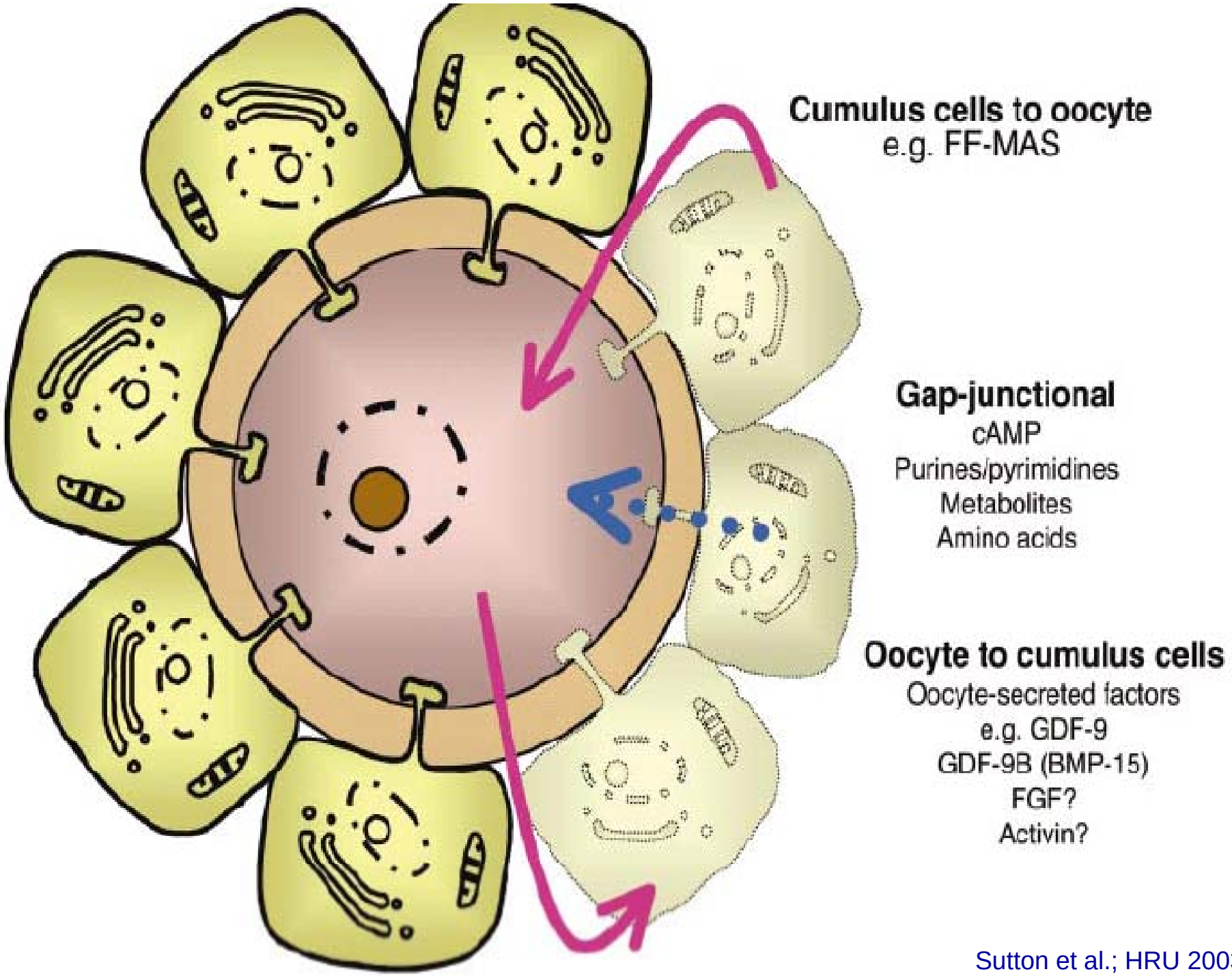
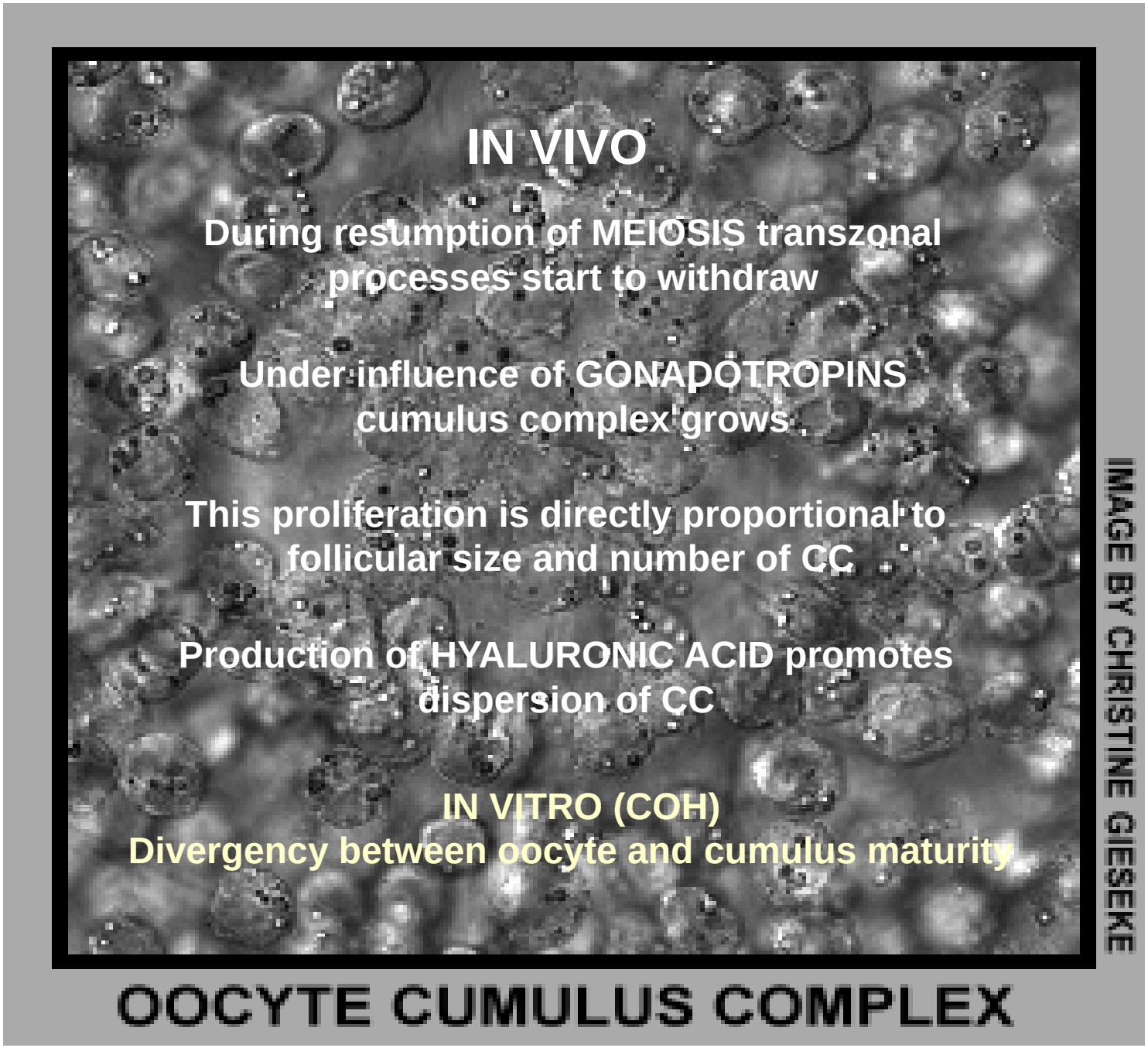
Salzburg, Austria
April. 1-2, 2011


CUMULUS OOPHORUS
GRANULOSA CELLS
OOCYTE
ANTRUM
CORONA RADIATA
THECA



Sutton et al.; HRU 2003


IN VIVO
During resumption of MEIOSIS transzonal processes start to withdraw
Under influence of GONADOTROPINS cumulus complex grows
This proliferation is directly proportional to follicular size and number of CC
Production of HYALURONIC ACID promotes dispersion of CC
IN VITRO (COH)
Divergency between oocyte and cumulus maturity
IMAGE BY CHRISTINE GIESEKE
OOCYTE CUMULUS COMPLEX

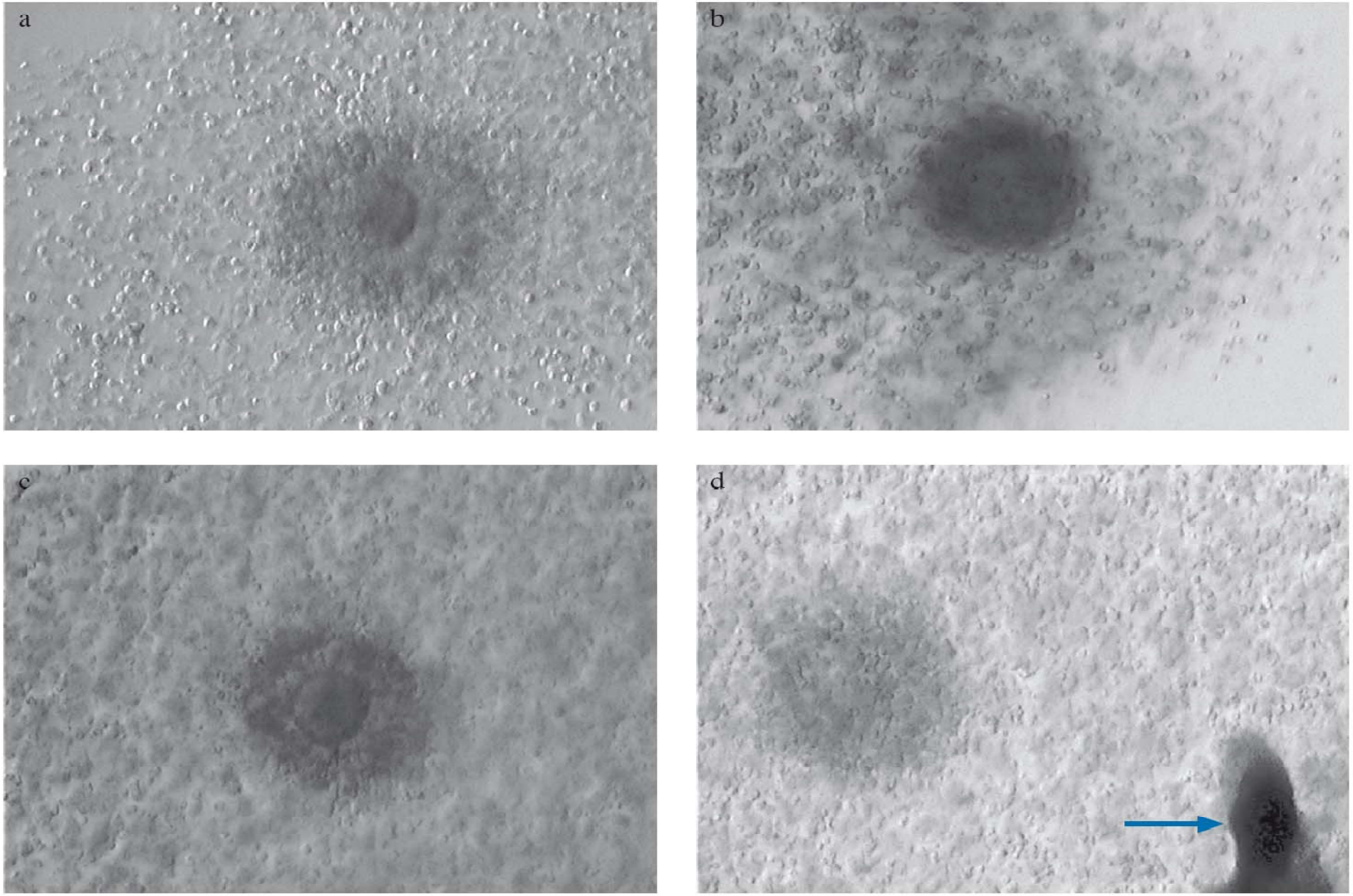
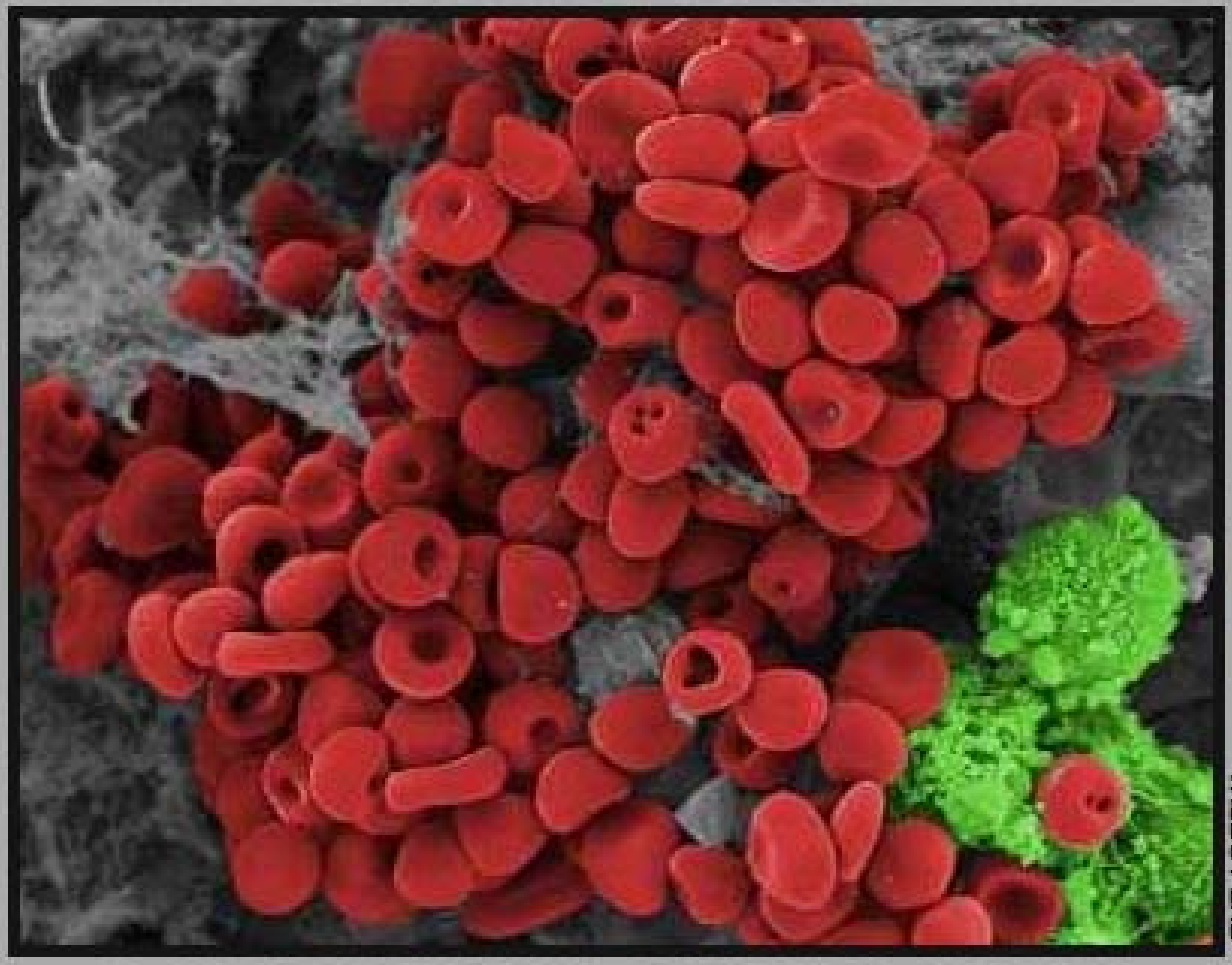
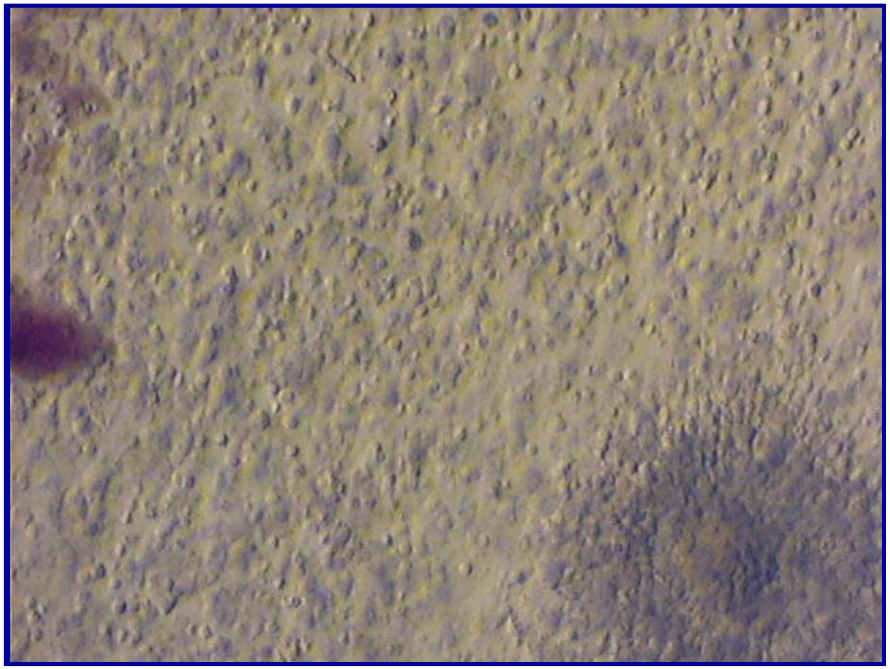
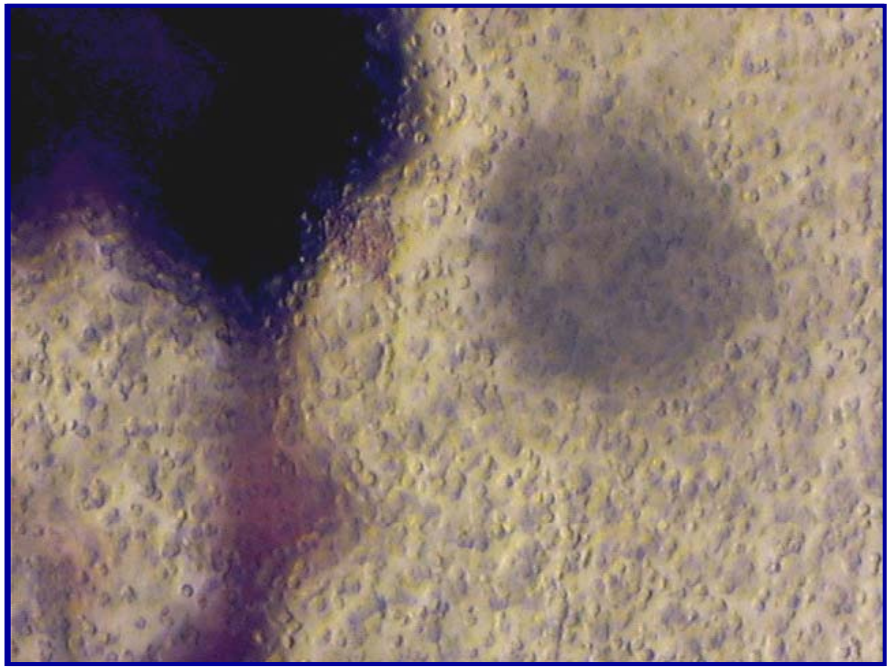


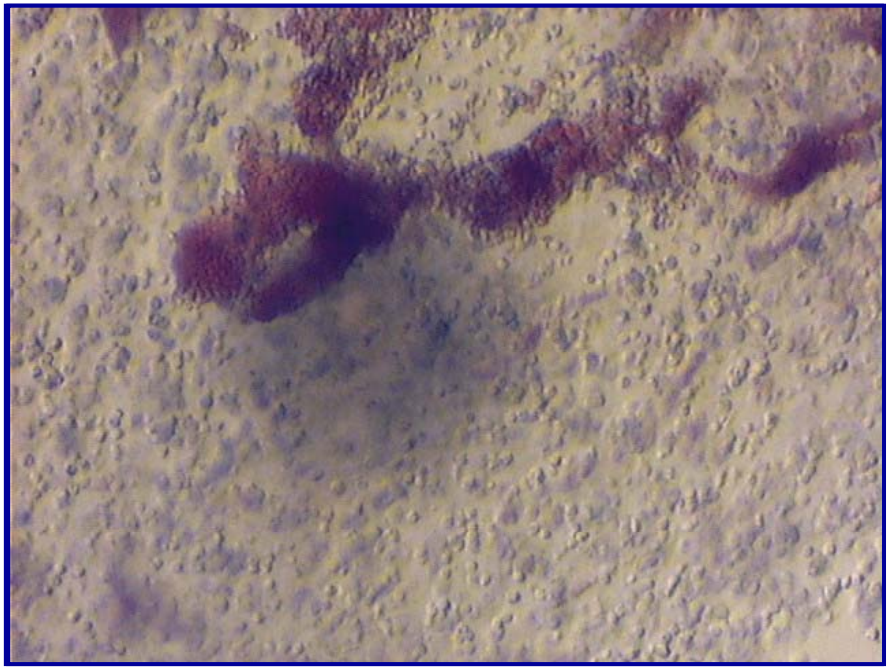
Figure 1. Grading of cumulus–oocyte complexes according to the expansion of corona radiata and cumulus matrix (×40). (**a**) Grade 1 (suspected mature): fluffy and radiant corona and cumulus with visible oocyte; (**b**) Grade 2: dense corona (oocyte hardly visible) but fluffy cumulus; (**c**) Grade 3: radiant corona (oocyte visible) and rather dense cumulus; (**d**) Grade 4 (suspected immature): dense corona and cumulus without visible oocyte with blood clot (arrow).

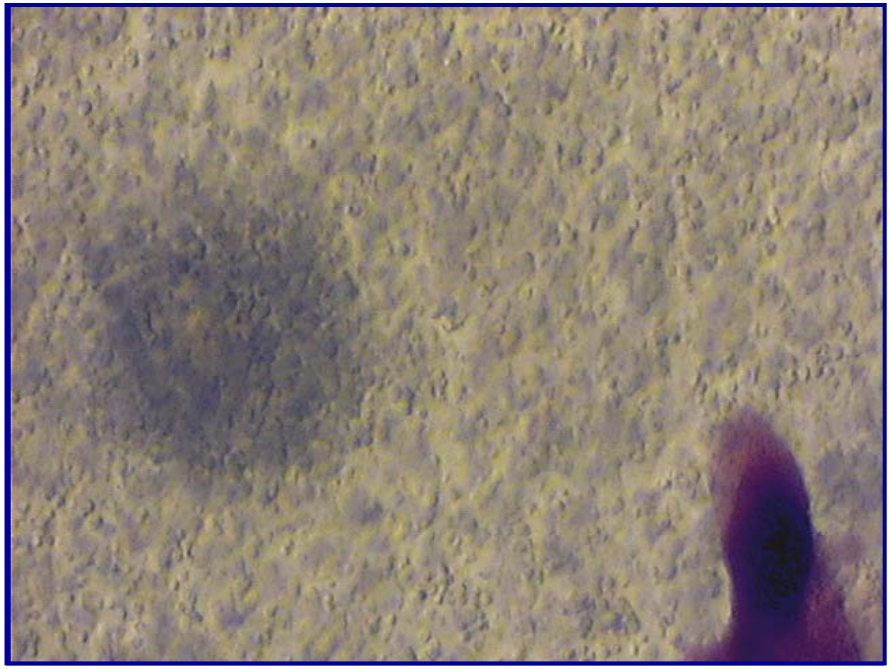


BLOOD CLOT IN OOCYTE CUMULUS COMPLEX









RBM Online - Vol 16 No 6. 2008 801-807 Reproductive BioMedicine Online; www.rbmonline.com/Article/3237 on web 14 April 2008

Article

Blood clots in the cumulus–oocyte complex predict poor oocyte quality and post-fertilization development

Table 1. Relationship between cumulus–oocyte complex morphology and maturity of the corresponding gamete evaluated within half an hour after collection.

COC morphology group	n	*Metaphase II*	*Metaphase I*	*Prophase I*	*No oocyte or empty zona pellucida*
Group 1	421	375 (89.1)	27 (6.4)	10 (2.4)	9 (2.1)
Group 2	47	24 (51.1)	10 (21.3)	10 (21.3)	3 (6.4)
Group 3	21	15 (71.4)	4 (19.0)	1 (4.8)	1 (4.8)
Group 4	46	12 (26.1)	9 (19.6)	22 (47.8)	3 (6.5)
Groups 1–4 (without blood)	515	426[a] (82.7)	50 (9.7)	43 (8.4)	16[b] (3.1)
Groups 1–4 (with blood)	97	70[a] (72.2)	9 (9.3)	9 (9.3)	9[b] (9.3)

Values in parentheses are percentages; values with the same superscript letter are significantly different.
[a]$P < 0.05$; [b]$P < 0.01$.

Table 2. Correlation between cumulus–oocyte complex morphology and quality of the corresponding metaphase II gamete evaluated immediately after denudation.

Gamete parameter	*Group 1*	*Group2*	*Group 3*	*Group 4*	*Groups 1–4 with blood*
Total	375	24	15	12	70
Normal	243 (64.8)	11 (45.8)	10 (66.6)	9 (75.0)	36 (51.4)[c]
Cytoplasmic anomalies					
sER	9 (2.4)	0 (0.0)	1 (6.7)	0 (0.0)	1 (1.4)
Vacuoles	22 (5.9)	2 (8.3)	3 (20)	0 (0.0)	4 (5.7)
Central granulation	31 (8.3)	5 (20.8)	0 (0.0)	1 (8.3)	17 (24.3)[d]
Incorporations[a]	43 (11.5)	4 (16.7)	1 (6.7)	0 (0.0)	3 (4.3)
Extracytoplasmic anomalies					
Ovoid shape	8 (2.1)	1 (4.2)	0 (0.0)	1 (8.3)	2 (2.9)
Discolouration	11 (2.9)	0 (0.0)	0 (0.0)	1 (8.3)	4 (5.7)
PVS anomalies[b]	8 (2.1)	1 (4.2)	0 (0.0)	0 (0.0)	3 (4.3)

Values in parentheses are percentages; PVS = perivitelline space; sER = aggregation of smooth endoplasmic reticulum. [a]Incorporations include refractile bodies; [b]PVS anomalies include giant first polar bodies; [c]$P < 0.05$ compared with pooled groups 1 4 without blood clots; [d]$P < 0.001$ compared with pooled groups 1 4 without blood clots.

Explanation?

Where do blood clots come from?

Artefact due to punction (Daya et al., 1990)

BUT:
- only a limited number of COCs showing blood clots stem from bloody follicular fluid
- observed relationship between blodd in COC and oocyte quality

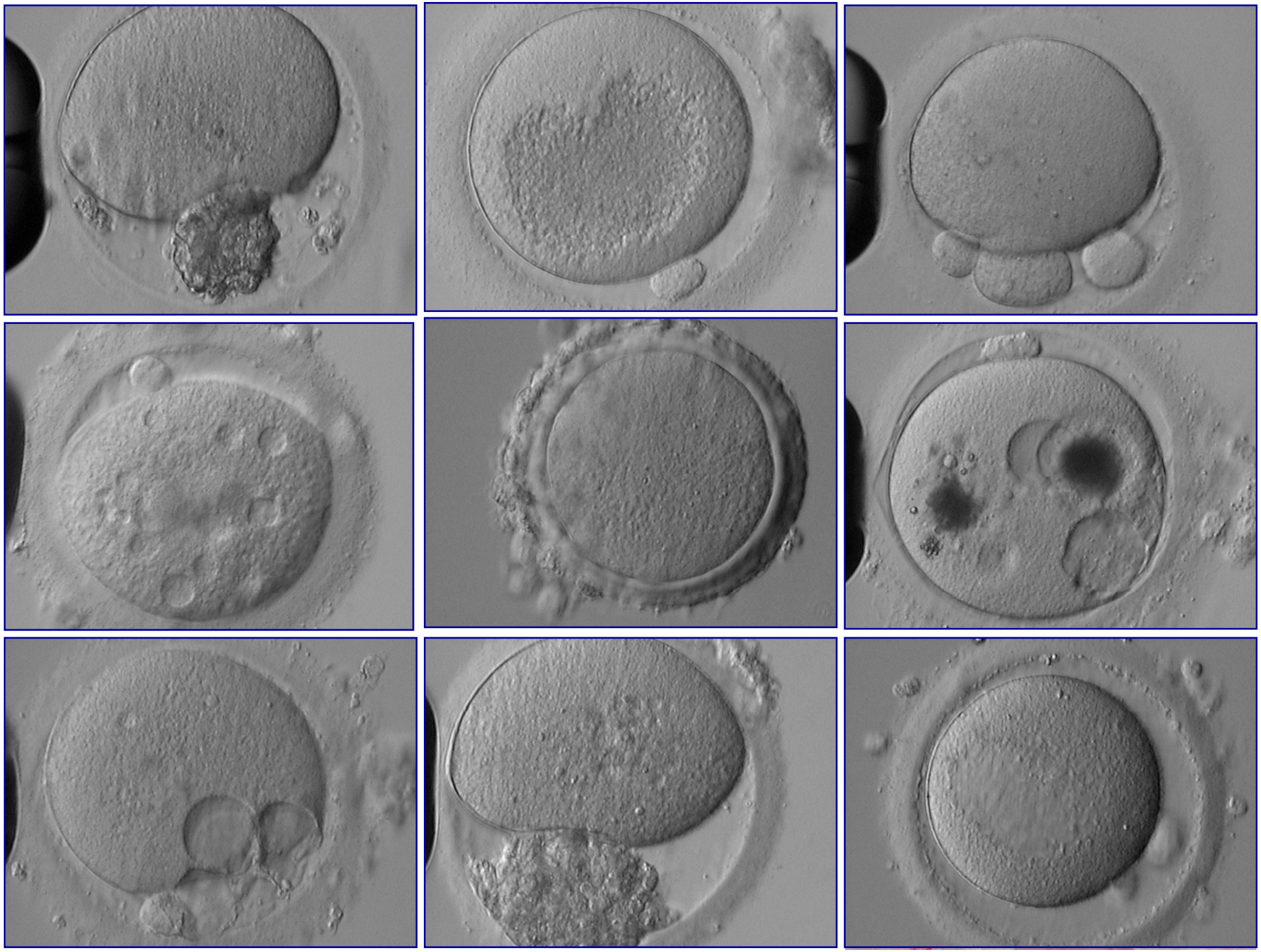
How could blood clots act?

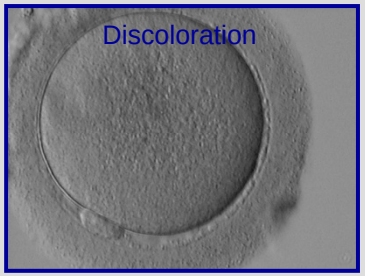
Sterical problems in IVF
pH und Temperaturschwankungen (Daya et al., 1990)
Production of ROS

Conclusion

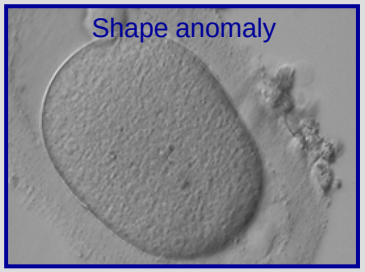
Oocytes are damaged a priori
Indication of suboptimal follicle
Removal of blood clot useless, but separate cultivation

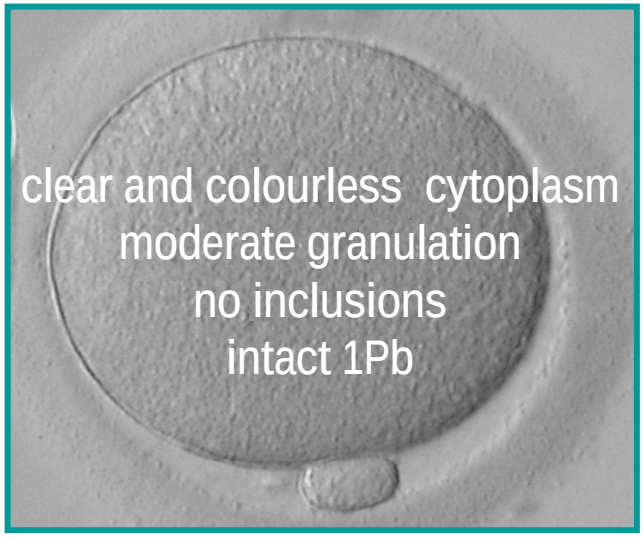
William HARVEY:
Exercitationes de Generatione Animalium (1651)

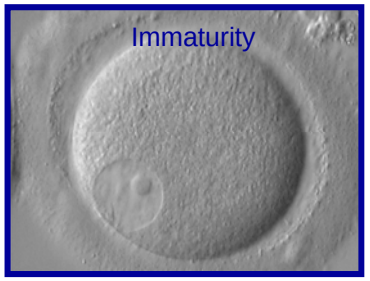


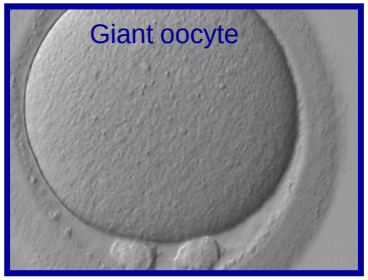


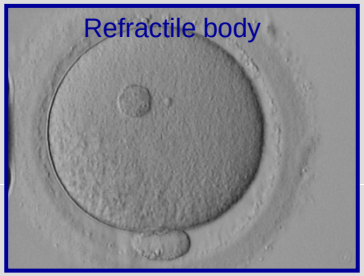
EXTRACYTOPLASMIC ANOMALIES

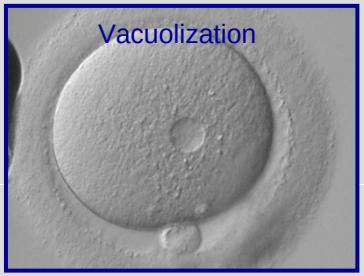






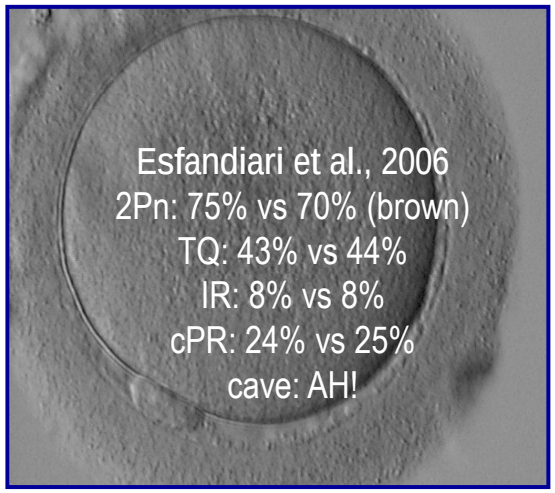


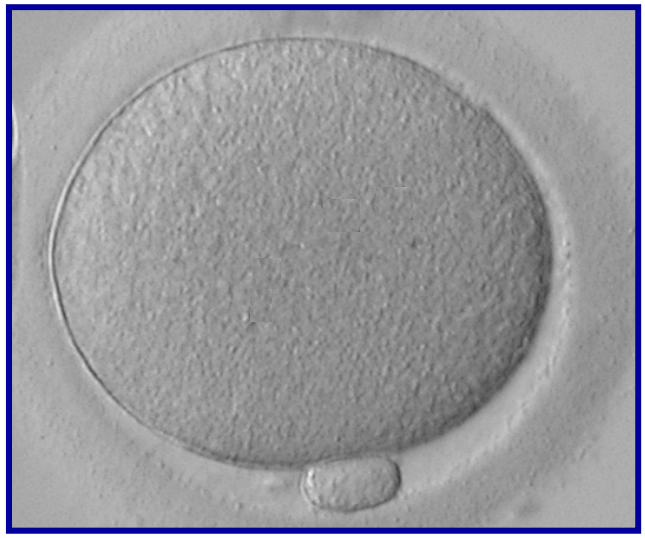


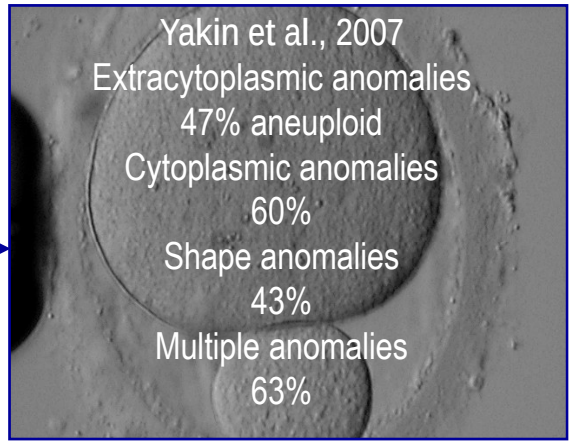


INTRACYTOPLASMIC ANOMALIES

Possible influences on oocyte quality





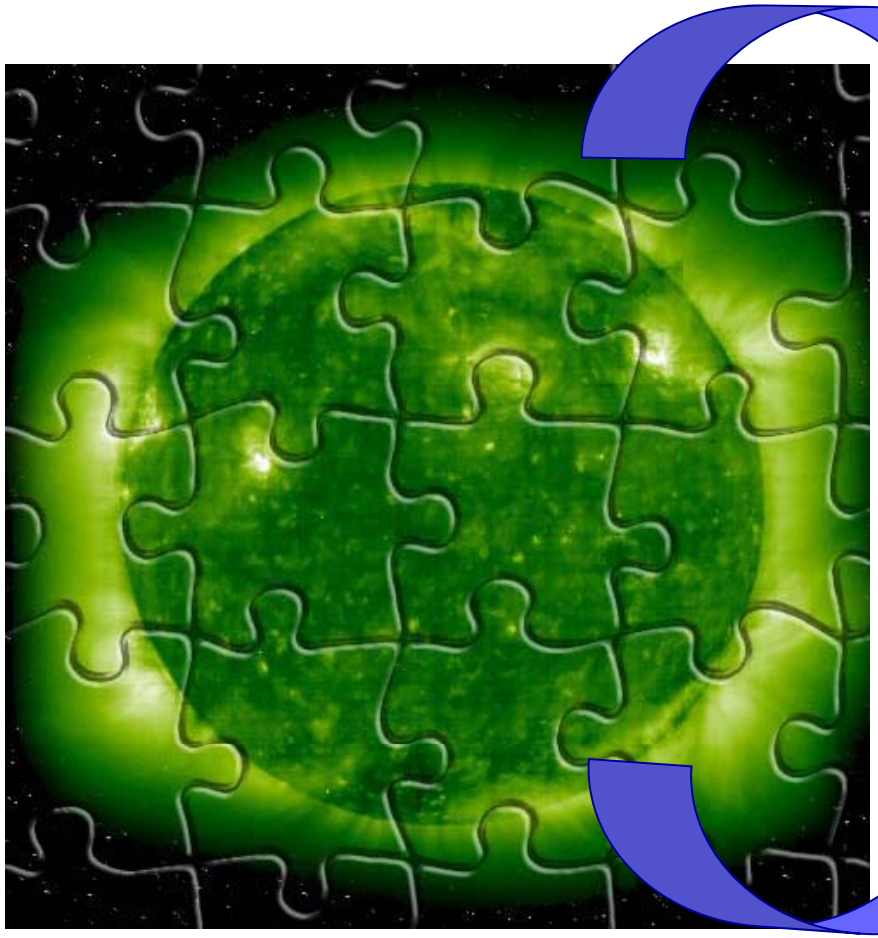


VASCULARIZATION/O_2/ATP
follicular fluid/ cumulus cells

-shape (Ebner et al.,2008a)
-zona pellucida BRF (Ebner et al., 2009)

- sER (Otsuki et al., 2004; Ebner et al., 2006)
- CG (Ebner et al., 2006; 2008b)
- PVS granules (Hassan-Ali et al., 1998)
- zona pellucida BRF (Ebner et al., 2009)

Oocyte maturation



Processes that prepare the egg for activation and fertilization

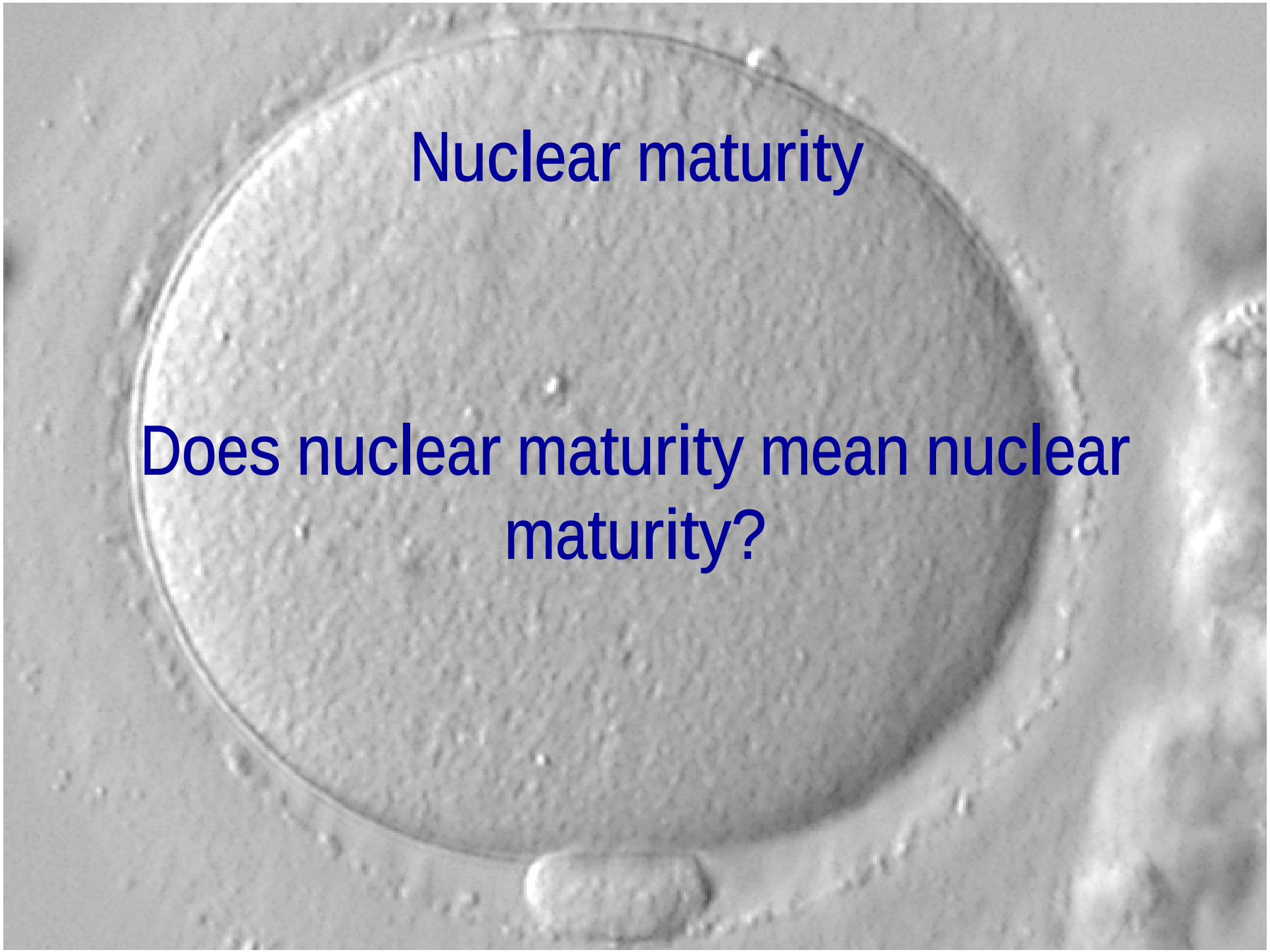
Ca^{2+} release
Glutathione production
Competence for exocytosis

Resumption of meiosis and progression to metaphase II

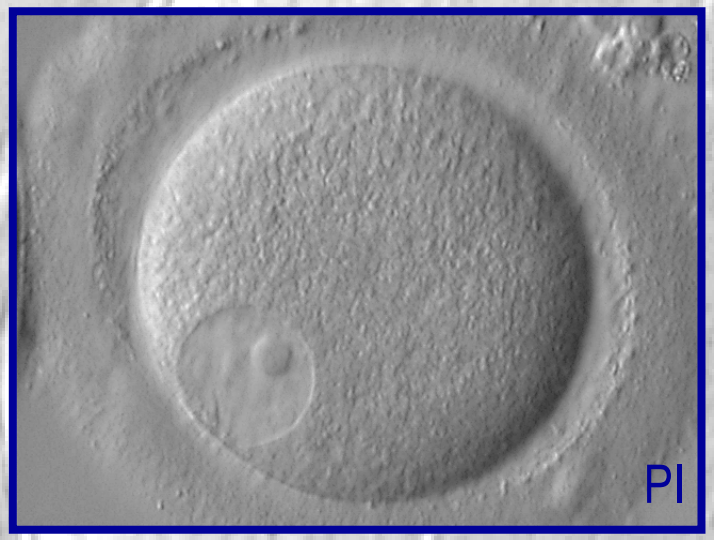
Purines and cAMP
vs
MPF and Ca^{2+}/IP3

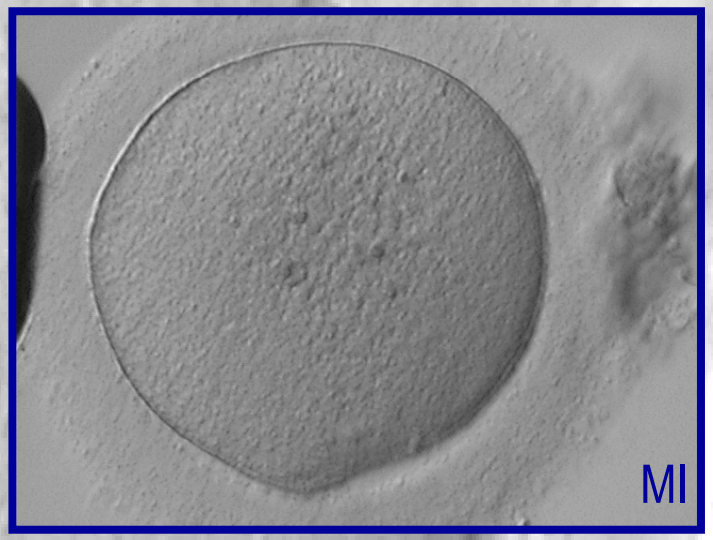
„IMMATURITY“
Desynchronization of nuclear and cytoplasmic maturation

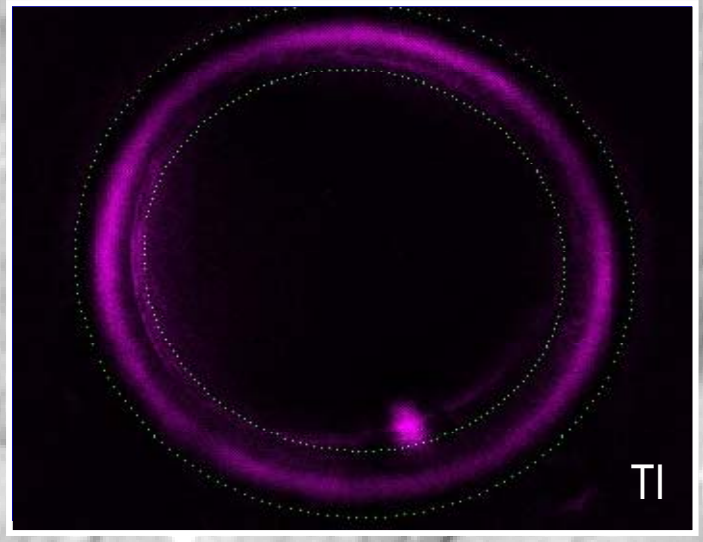
„OVERMATURITY“
Aged oocytes in vivo or in vitro


Nuclear maturity
Does nuclear maturity mean nuclear maturity?

1. Nuclear maturity

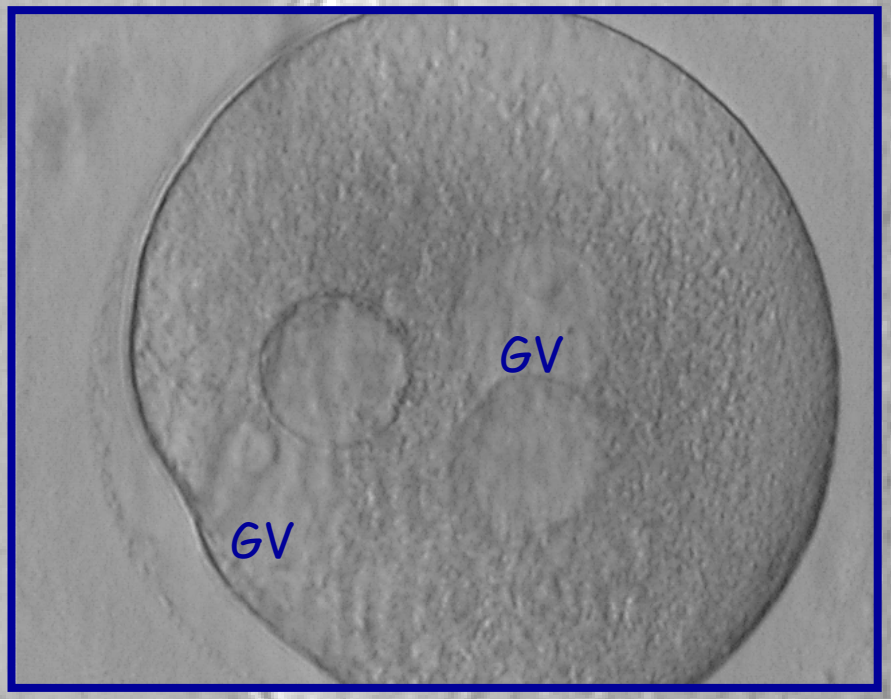


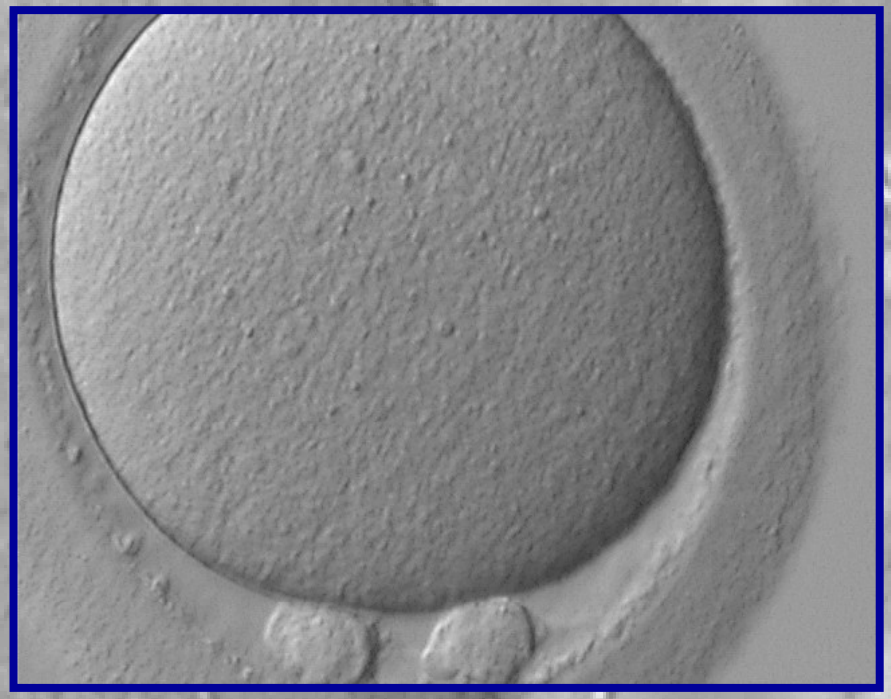




Giant oocyte

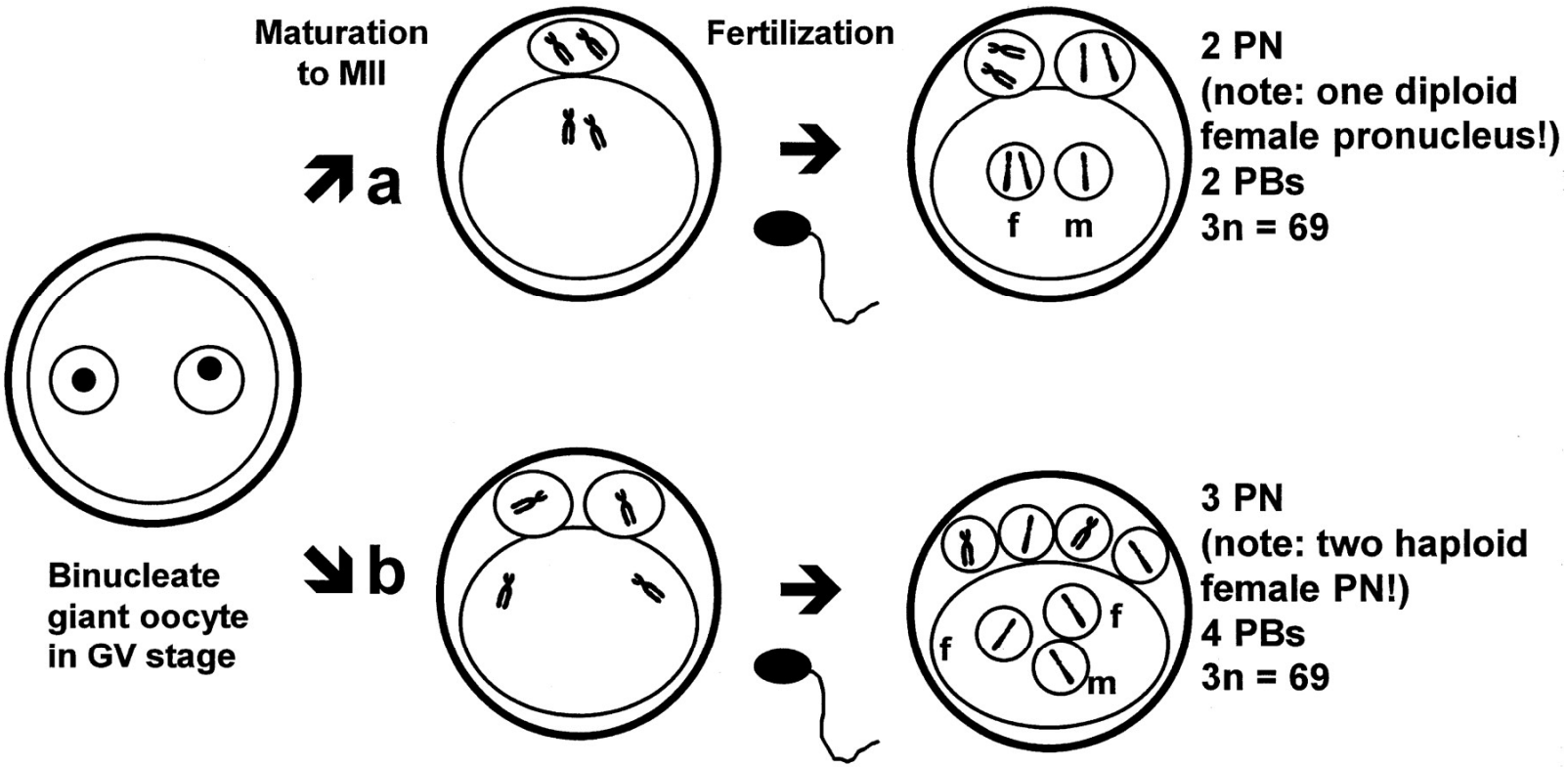
Balakier et al., HR 17, 2002
Rosenbusch et al., HR 17, 2002





Frequency approximately 0.3%
Mean diameter 200 µm (vs. 155 µm)
Contribution to digynic triploidy

Schematic representation of meiosis in giant oocytes



Rosenbusch et al., 2002

Oocyte diameter

- a critical oocyte size is necessary for resumption of meiosis (Otoi et al., 2000)
- size is determined by strong adhesion between oolemma and inner zona surface (Tartia et al., 2009)
- around ovulation GLYT1 is activated which mediates glycin accumulation which in turn acts as osmolyte and thus controls cell volume (Baltz and Tartia, 2009)

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TABLE 1

Comparison of the embryonic parameters of groups A, B, and C by the chi-square test.

Day	Parameter	Group A	Group B	Group C	*P* value: A versus B versus C
1	Number of oocytes	40	80	40	
	Mean oocyte diameter (μm)	<109.92[a]	109.92[a]–14.26[b]	>114.26[b]	
	Fertilized	33 (82.5%)	55 (68.75%)	30 (75%)	.2662
2	≥4 cells	12 (38.7%)	31 (56.4%)	15 (50%)	.2906
	Good quality	16 (51.6%)	22 (42.3%)	10 (34.5%)	.4051
3	≥8 cells	12 (57.1%)	16 (53.3%)	24 (77.4%)	.117
	Good quality	10 (47.6%)	9 (31%)	11 (52.4%)	.2689

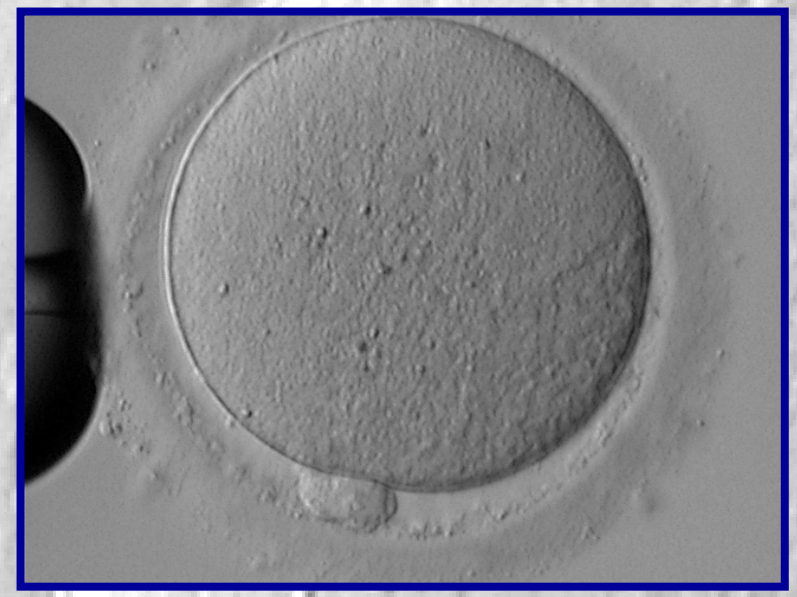
[a] 25th percentile.
[b] 75th percentile.

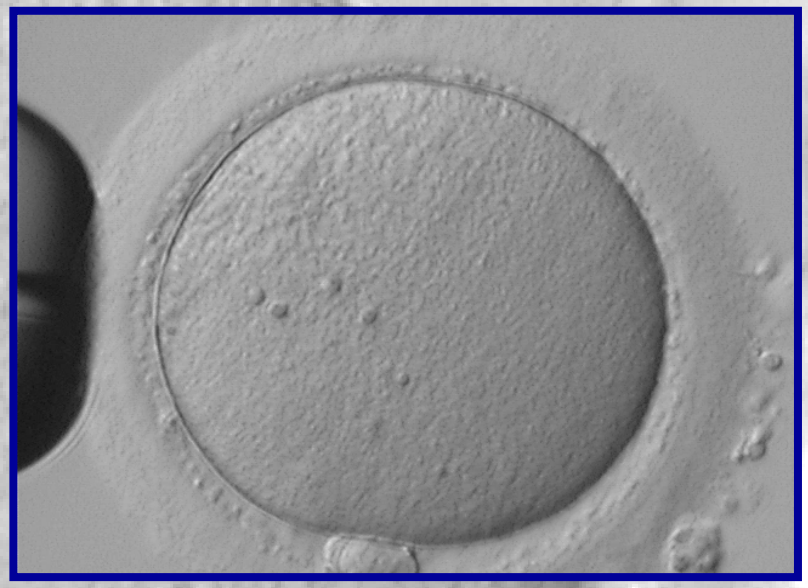
Romão. Oocyte diameter and embryo quality. Fertil Steril 2009.

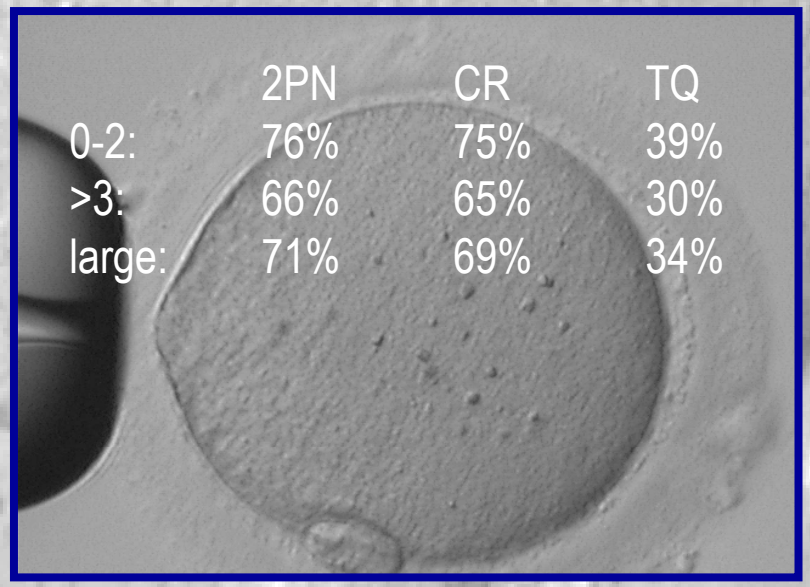
Romao et al., 2010

INTRACYTOPLASMIC ANOMALIES

Incorporations



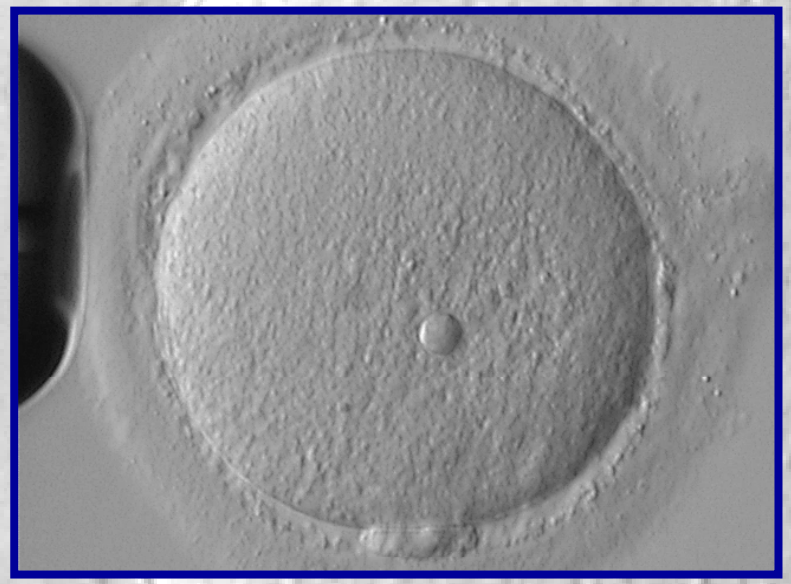


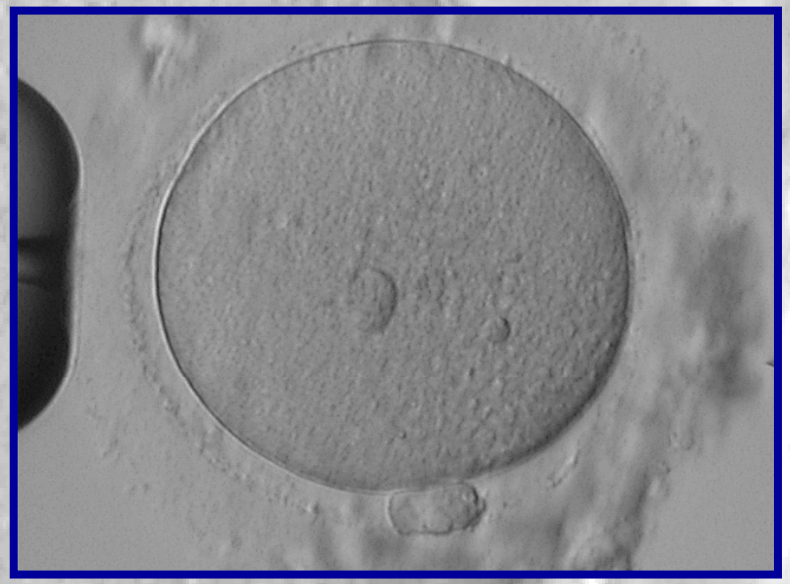


	2PN	CR	TQ
0-2:	76%	75%	39%
>3:	66%	65%	30%
large:	71%	69%	34%

Fancsovits et al., 2004

Refractile bodies



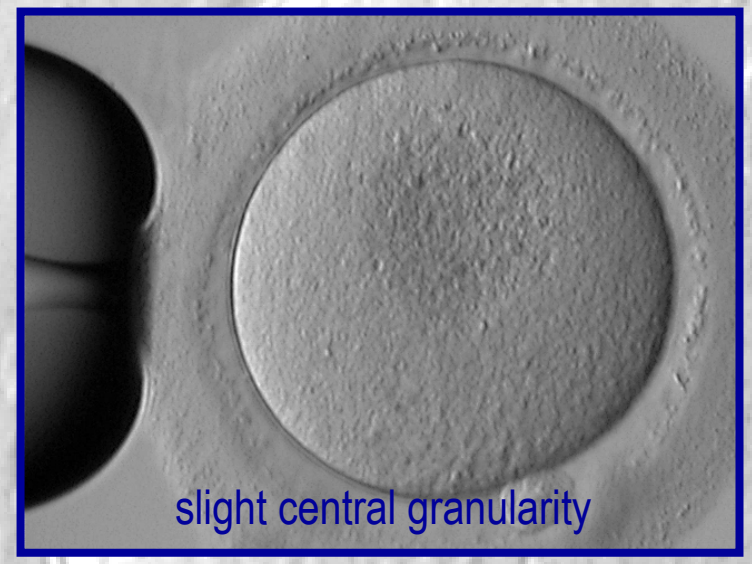


Viewed by transmitted electron microscopy, the refractile bodies showed the conventional morphology of lipofuscin inclusions and consisted of a mixture of lipids and dense granule materials

Larger lipofuscin inclusions (>5 μm) were associated with significantly reduced fertilization and unfavorable blastocyst development

Otsuki et al., 2007

Central granularity



slight central granularity

moderate central granularity

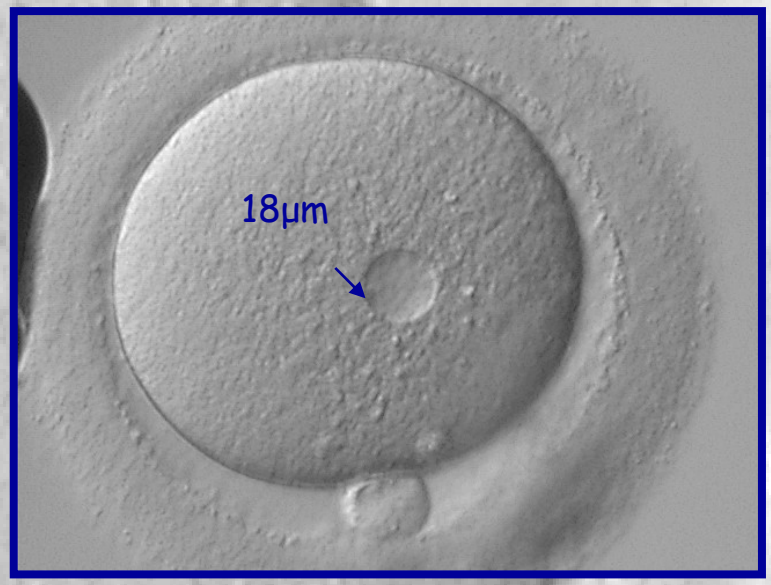
severe central granularity

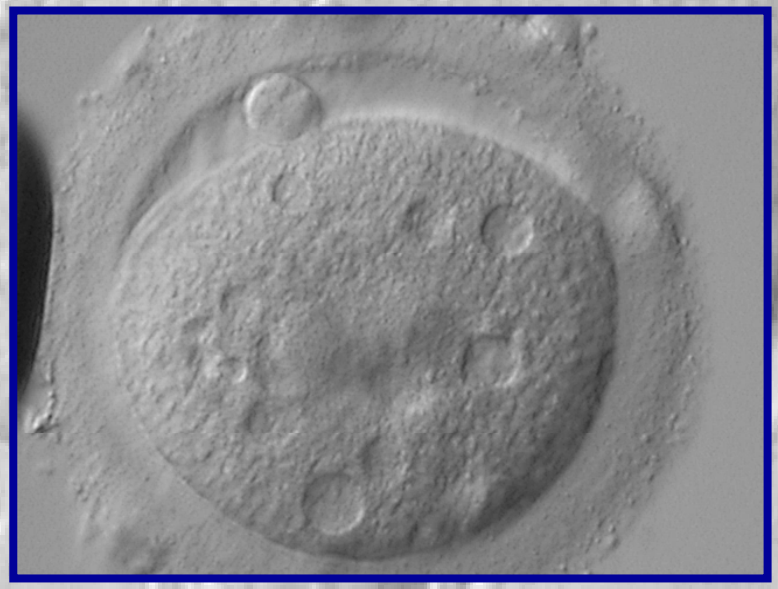
Relationship between central granularity and pregnancy outcome

- Anomaly was observed in 8% of the cycles (35% of the eggs were positive)
- Fertilization rate, embryo quality were inconspicuous
- Ongoing pregnancy rate was 12.8% (from slight form of CG), the implantaion rate 4.3%

Kahraman et al., 2000

Vacuolization



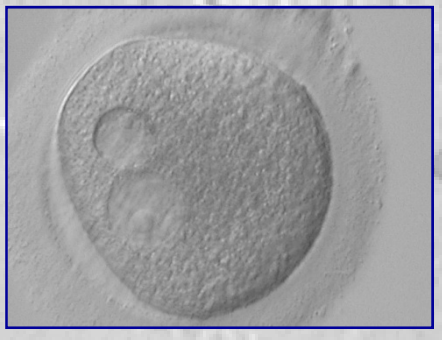


Formation of vacuoles

Vacuoles are membrane-bound cytoplasmical inclusions filled with fluid that is virtually identical with perivitelline fluid

Vacuoles can arise spontaneously around extrusion of the first polar body

Van Blerkom, 1990

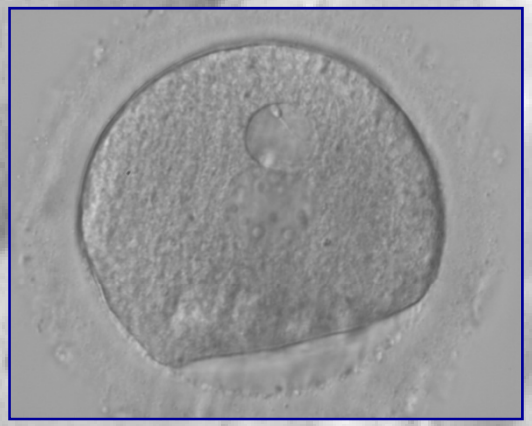


Vacuoles can form from preexisting vesicles derived from the ER or GA

El.Shafie et al., 2000

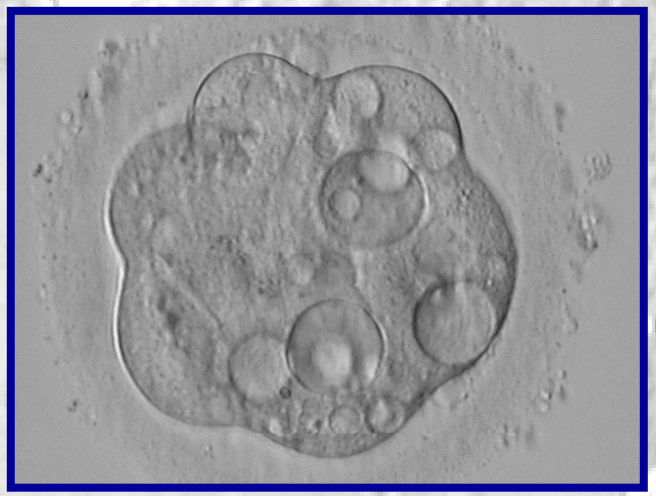
Vacuoles can be generated unintentionally by ICSI

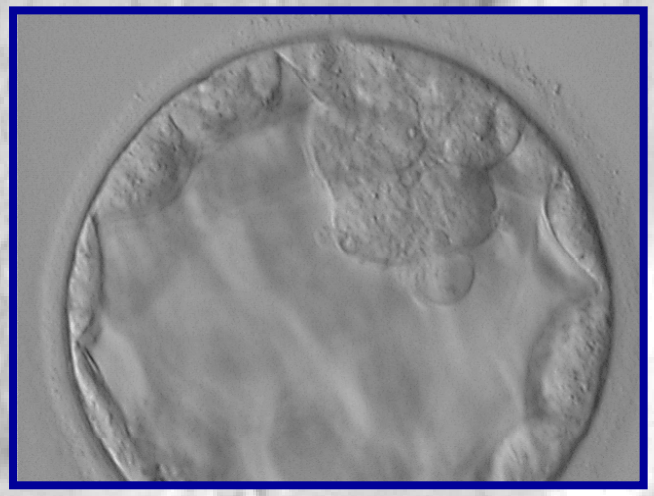
Ebner et al., 2005



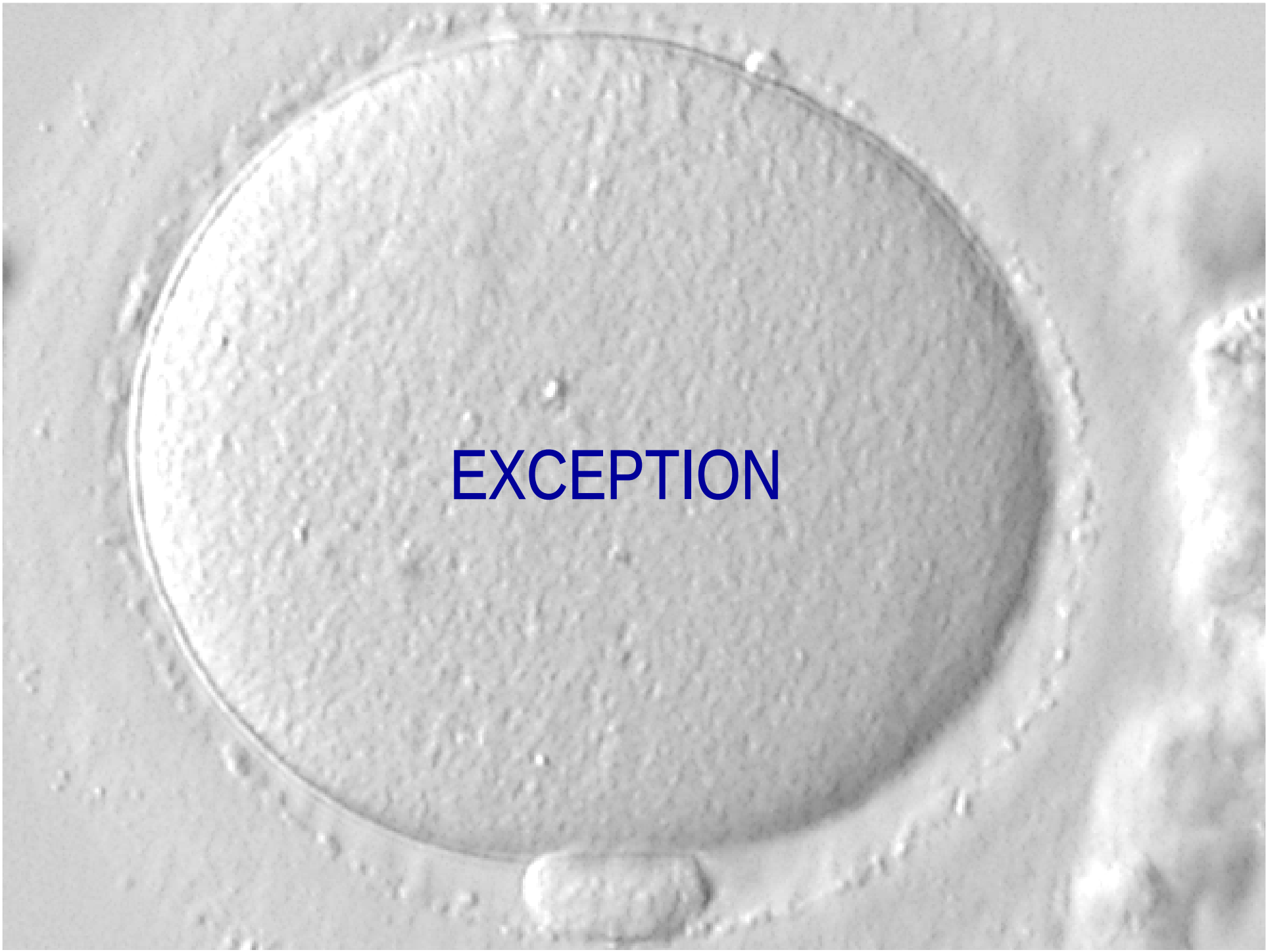
Occurence and developmental consequences of vacuoles

- 47 out of 1198 MII-oocytes showed at least one vacuole (3.9%)
- Fertilization rate was influenced negatively (48.9% vs 65.3%)
- A threshold was found above which fertilization did not occur (14 µm)
- Vacuolized oocytes had a blastocyst formation rate of only 12.5% compared to unaffected gametes (48.6%) ($p<0.05$)

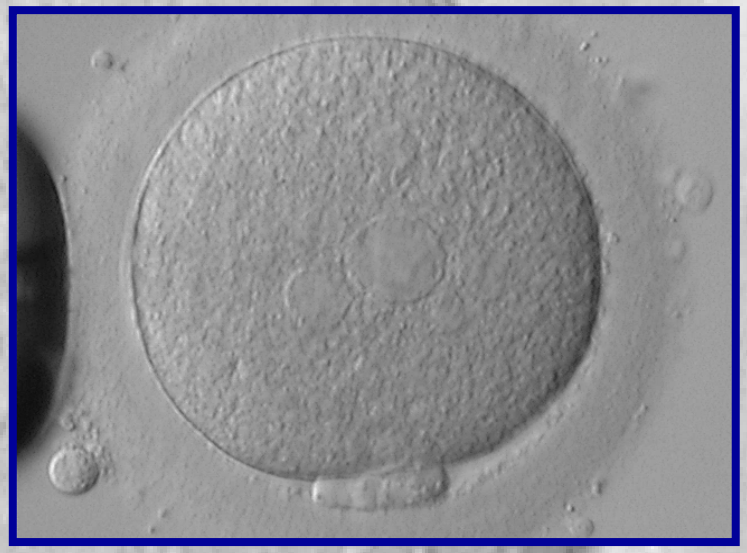


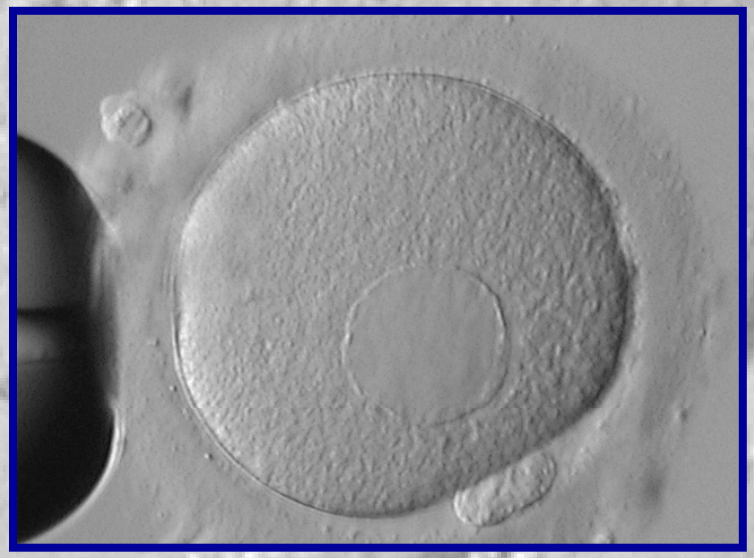


Ebner et al., 2005


EXCEPTION

Aggregation of smooth endoplasmic reticulum

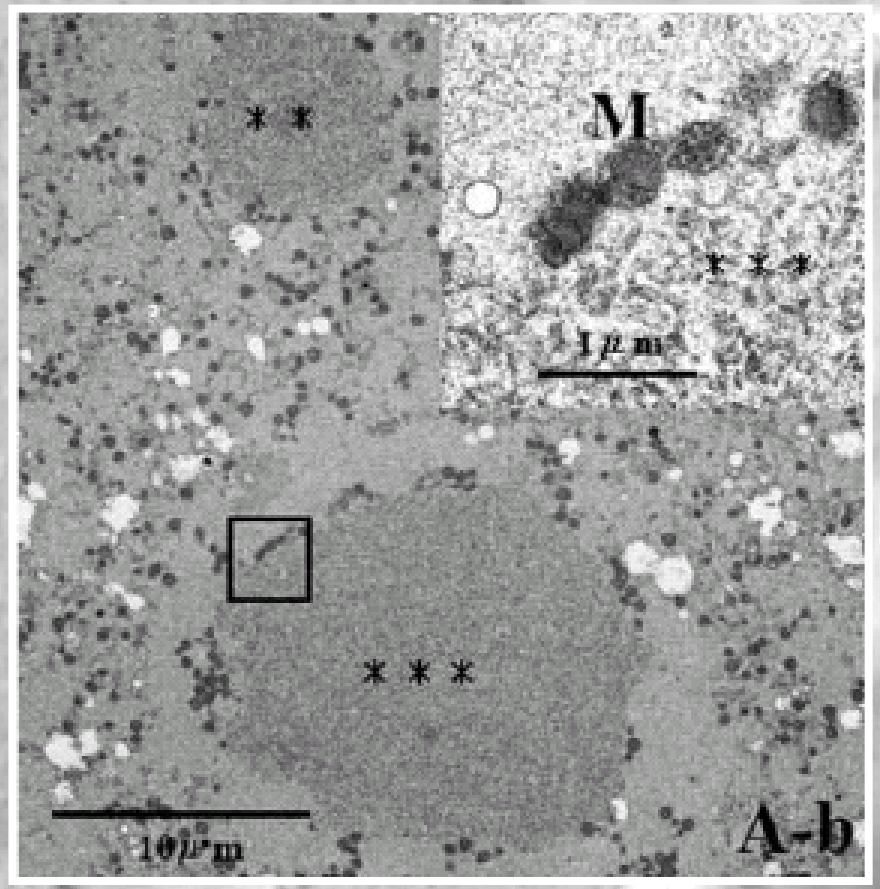




Relationship between sER clusters and outcome

Ebner et al., RBM 16, 2008; Otsuki et al., HR 19, 2004

- 6.2 to 9.4% of the cycles affected
- To our experience less than 2% of oocytes are affected (25% in pos cycles)
- Only MII oocytes
- Normal fertilization if rupture of sERC is avoided
- At lightmicroscopical level not all sERCs can be seen (2-9µm)!
- Blastocyst formation was 18%



Otsuki et al., 2004; Ebner et al., 2008

Relationship between sER clusters and outcome

- No relation to stimulation protocol, age, endometriosis but to E_2, dose of gonadothrophins, duration of COH
- sERC presence resulted in a desastrous outcome
  - IR, PR — no difference
  - Biochemical pregnancies — 58% vs 22% (P<0.01)
  - Take-home baby rate — 42% vs 78.% (p<0.001)
  - Increase in obstetric problems (33% vs. 5%) and lower birth weight (2500g vs. 3100g)
  - 2/6 stillbirths (not to forget one Beckwith-Wiedemann syndrome in the Otsuki paper)

Ebner et al., 2008

CASE REPORT

Smooth endoplasmic reticulum aggregations in all retrieved oocytes causing recurrent multiple anomalies: case report

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[a] Ankara Private IVF Center, Ankara, Turkey; [b] Present address: Department of Obstetrics and Gynecology, Ufuk University Faculty of Medicine, Ankara, Turkey; and [c] Department of Obstetrics and Gynecology, Gazi University Faculty of Medicine, Ankara, Turkey

At 36 weeks' gestation the patient was delivered by cesarean section. The male newborn was depressed at birth and was taken to the neonatal intensive care unit. Follow-up evaluation of the baby showed patent ductus arteriosus, disgenesis of the cerebral sulcus, variation of septum pellicidum, and closed external meatus of the right ear. Moreover, ultrasonography showed infantile polycystic kidney. Neonatal infection developed and antibiotic supplementation was begun. Days later, respiratory depression required mechanic ventilation. On postnatal day 14 the baby died of cardiopulmonary arrest.

OOCYTE AGING

IN VIVO or IN VITRO acquired cellular, molecular, biochemical, morphological and epigenetic changes of gametes closely associated with poor developmental potential and recuced fecundity

- Decreased Fertilization
- Polyspermy
- Digyny
- Parthenogenesis
- Chromosomal disorders
- Retarded development

Aged oocytes: morphological changes

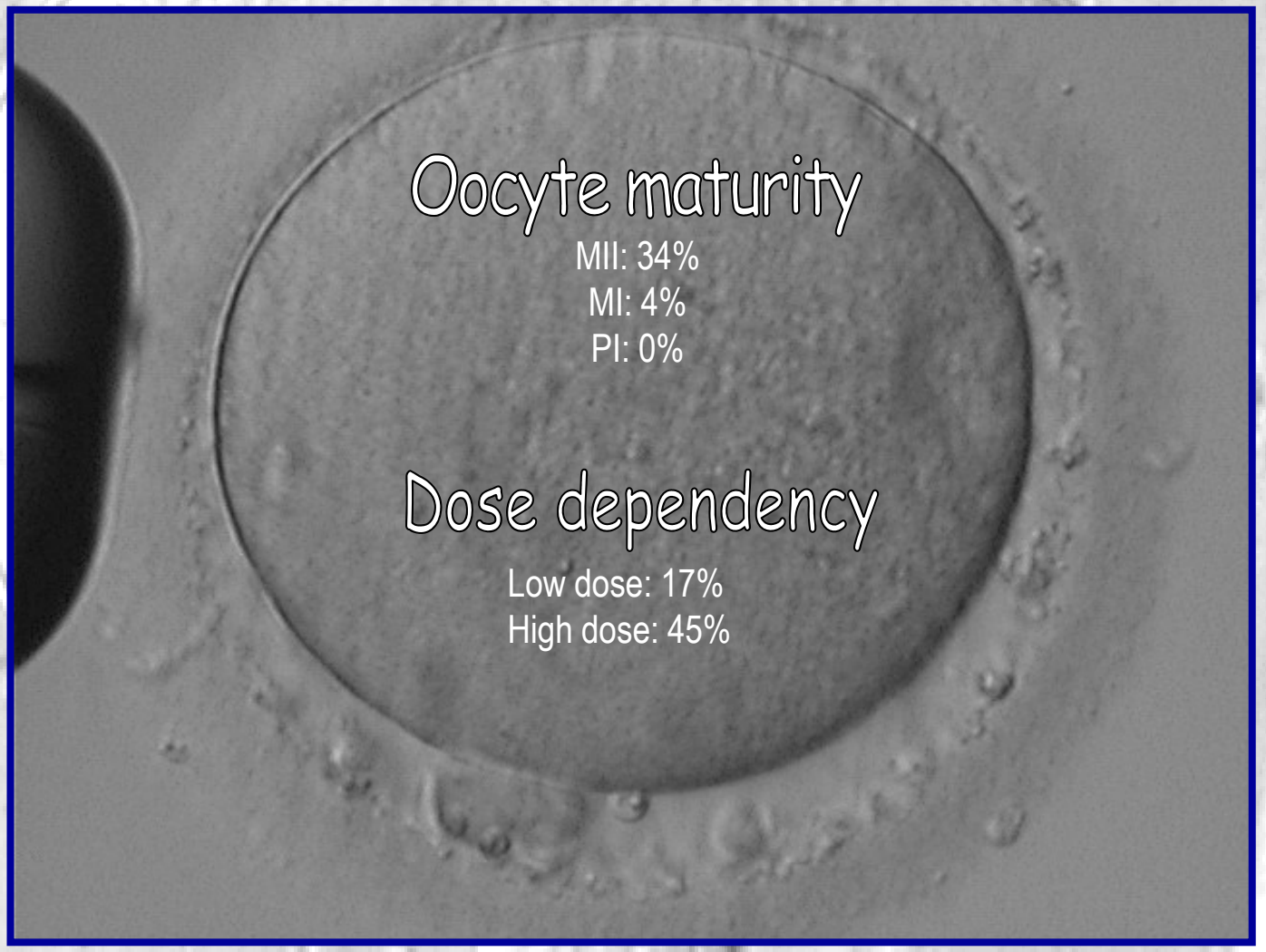
Table I Changes in morphology and cell biology during mammalian oocyte aging

	Fresh oocytes	Aged oocytes	Reference
PM	Microvilli extensions display intact structure	Microvilli extensions display structural alterations and are budded off into the PVS	Kim *et al.* (1996); Longo (1974); Pickering *et al.* (1988); Szollosi (1971); Webb *et al.* (1986)
Zona pellucida	Zona pellucida appears as a granulofibrillar, interconnected reticulum with pores	Zona pellucida displays a 'cobblestone' appearance and becomes harden	Goud *et al.* (2005b); Longo (1981); Miao *et al.* (2005); Xu *et al.* (1997)
PVS	Small	Large	Miao *et al.* (2004)
CG	CGs are densely populated in a line just beneath the oolemma, with a typical normal CG-free domain above the meiotic apparatus	CGs undergo migration and partial exocytosis	Dodson *et al.* (1989); Goud *et al.* (2005b); Gulyas (1979); Longo (1974); Szollosi (1971); Xu *et al.* (1997)
Microfilament	A thick microfilament domain exists in the oocyte cortex	Disrupted or lost	Kim *et al.* (1996)
Spindle	Spindles display vertical orientation to the oolemma and each pole is associated with a ring of centrosome proteins	Spindles become elongated and/or smaller and few microtubular foci are detectable at the cortex	Eichenlaub-Ritter *et al.* (1986); Eichenlaub-Ritter *et al.* (1988); Goud *et al.* (2004); Longo (1974); Meyer and Longo (1979); Segers *et al.* (2008); Slozina *et al.* (1990); Wang *et al.* (2001)
Chromosomes	Chromosomes are intact and arranged symmetrically on the metaphase plate	Chromosomes display PCS and are scattered throughout the degenerating spindle and some chromosomes show centripetal migration, dispersion, decondensation and formation of a single chromatin mass	Eichenlaub-Ritter *et al.* (1988); Mailhes *et al.* (1998); Rodman (1971); Steuerwald *et al.* (2005); Szollosi (1971); Van Wissen *et al.* (1991); Zenzes and Casper (1992)
Mitochondria	Mitochondria are intact	Membrane potential decrease and mitochondrial matrix swell	Wilding *et al.* (2001)
PB1	PB1 is intact and adjacent to the MII spindle	PB1 degenerates and deviates from the MII spindle	Miao *et al.* (2004)

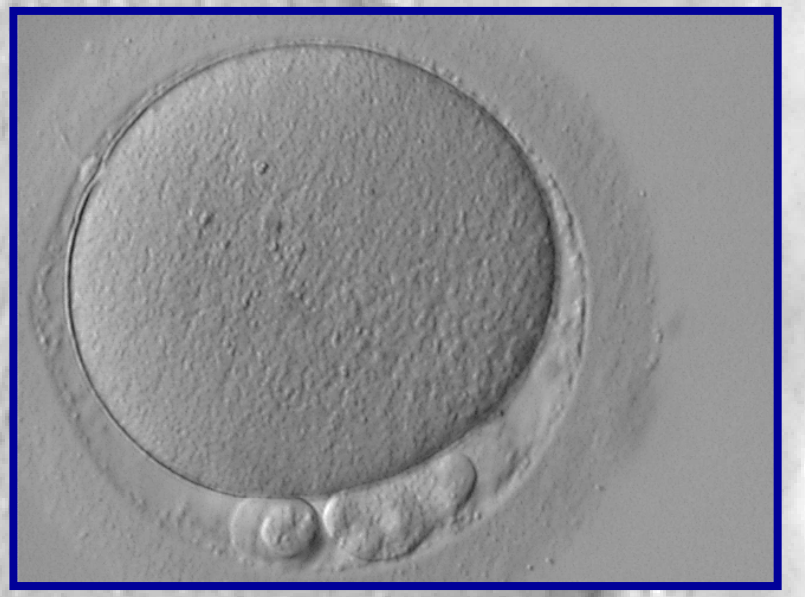
Miao et al., 2009

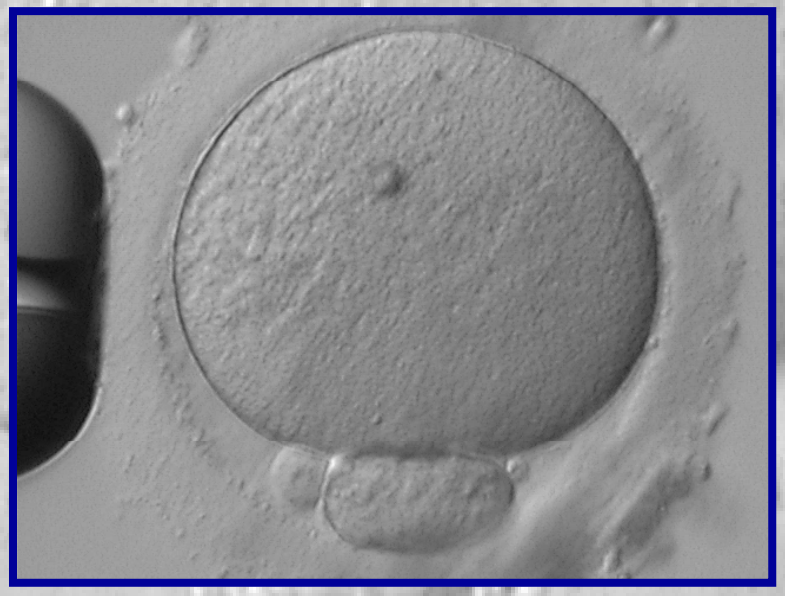
Perivitelline space granularity

Hassan-Ali, HR 13, 1998



First polar body morphology

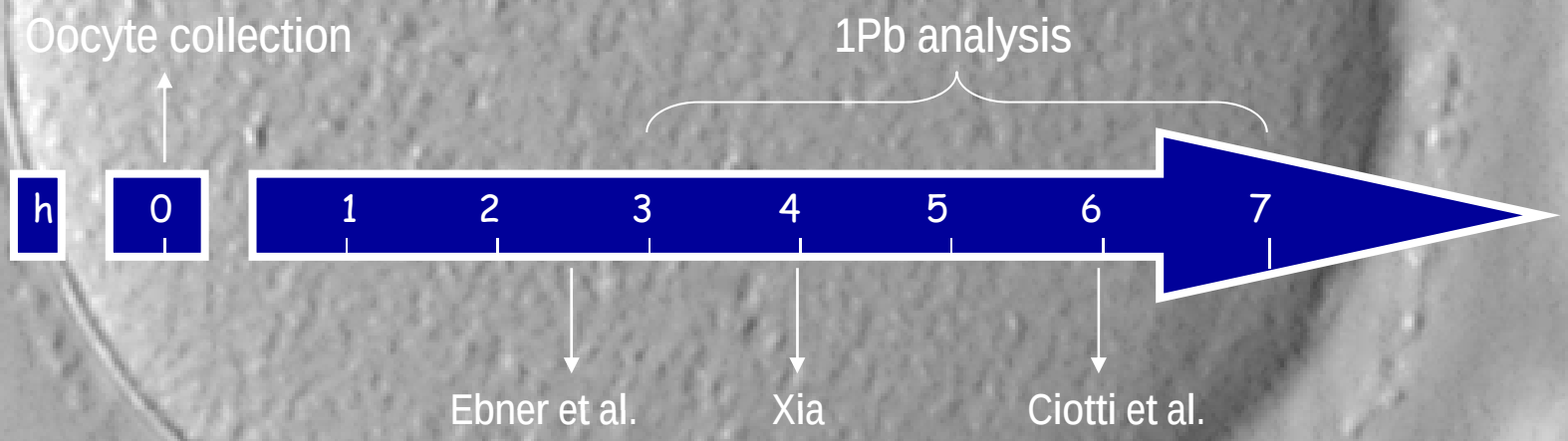




First polar body and outcome

Xia, HR 12, 1997
Ebner et al., F&St 1999, HR 2000, HR 2002
Ciotti et al., HR 19, 2004

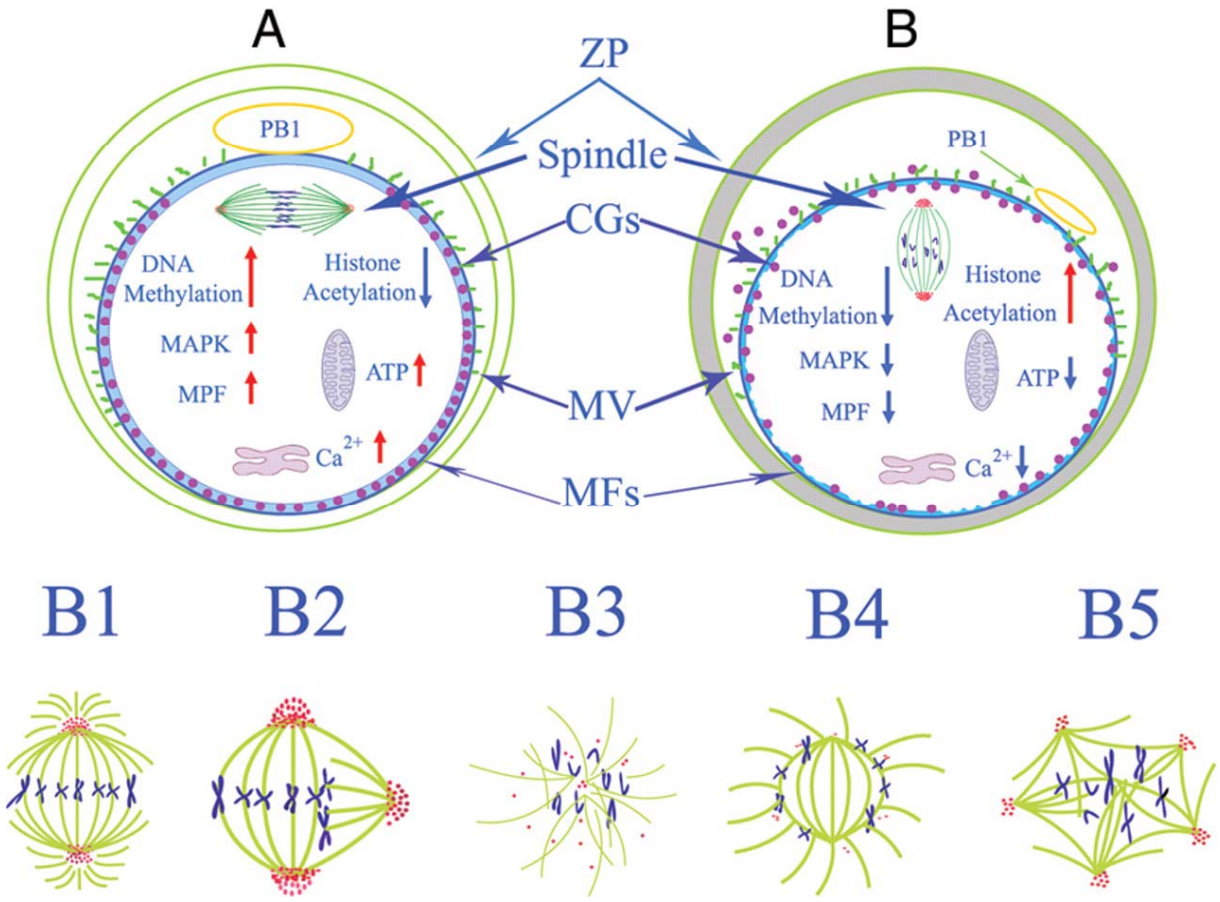
Since first polar body has a short half-life time it is thought to be an indicator of postovulatory age of the oocyte



Morphology of polar body has nothing to do with chromosomal situation of the gamete

Aged oocytes:

cellular, biochemical and epigenetic aspects



Miao et al., 2009

Factors affecting oocyte aging

Table II **Effects of various environmental factors on oocyte aging**

Aging environment	Effects	References
Temperature	Fertilization of room-temperature-aged (27°C) oocytes results in mouse full-term births. Oocytes aged in a refrigerator (4°C) or incubator (37°C) loses the developmental potential	Lei *et al.* (2008a); Lei *et al.* (2008b); Wakayama *et al.* (2004)
In vivo and *in vitro*	NO: similar morphological alterations and cytoskeletal organization	Longo (1980); Miao *et al.* (2005); Webb *et al.* (1986)
	YES: oocytes aged *in vivo* display a larger spindle and microtubule asters. Spindles in oocytes aged *in vitro* are close to the PM and display different orientations. *In vitro* culture retards oocyte aging	Abbott *et al.* (1998); Adenot *et al.* (1997)
CC	Accelerate oocyte aging by secreting a soluble APF into the medium	Miao *et al.* (2005); Qiao *et al.* (2008)
ROS	Superoxide induces oocyte zona pellucida hardening, ooplasmic microtubule dynamics increase and major CGs losses. H_2O_2 renders fresh oocytes resistant to aging but enhances the further aging in aged oocytes. Low levels of HOCl induce the aging of fresh and aged oocytes, while higher concentrations of HOCl compromise oocyte viability	Goud *et al.* (2008)

Miao et al., 2009

Conclusion

- The developmental fate of an oocyte is strongly dependant on the quality of the follicle (O_2, apoptosis)
- Controlled ovarian hyperstimulation recruits follicles of different qualities
- Either nuclear or cytoplasmic maturation may be affected both of which can influence oocyte morphology
- Oocyte aging is underestimated
- Potential negative predictors are aggregation of sER, vacuolization, dense central granulation and undetectable meiotic spindles

Conclusion II

In the context of oocyte morphology and outcome Van Blerkom and Henry (1992) suggested a interesting hypothesis

DYSMORPHISM

EARLY in maturation ⟷ LATE in maturation

↓ aneuploidies

GIANT OOCYTES
CENTRAL GRANULATION
MEIOTIC SPINDLE

↓ developmental failure

AGGREGATION of sER
VACUOLIZATION

Thanks for your

attention!