UNSW Embryology Course 2013

Human Gametogenesis and Fertilisation

Dr Steven Fleming
Honorary Associate
University of Sydney

Learning Objectives

- Meiosis
- Gametogenesis and aneuploidy
- Capacitation and hyperactivation
- Ovulation and oocyte maturation
- Fertilisation and polyspermy
- IUI, IVF, ICSI, PICSI and IMSI
- Assisted reproduction and society

Meiosis 1

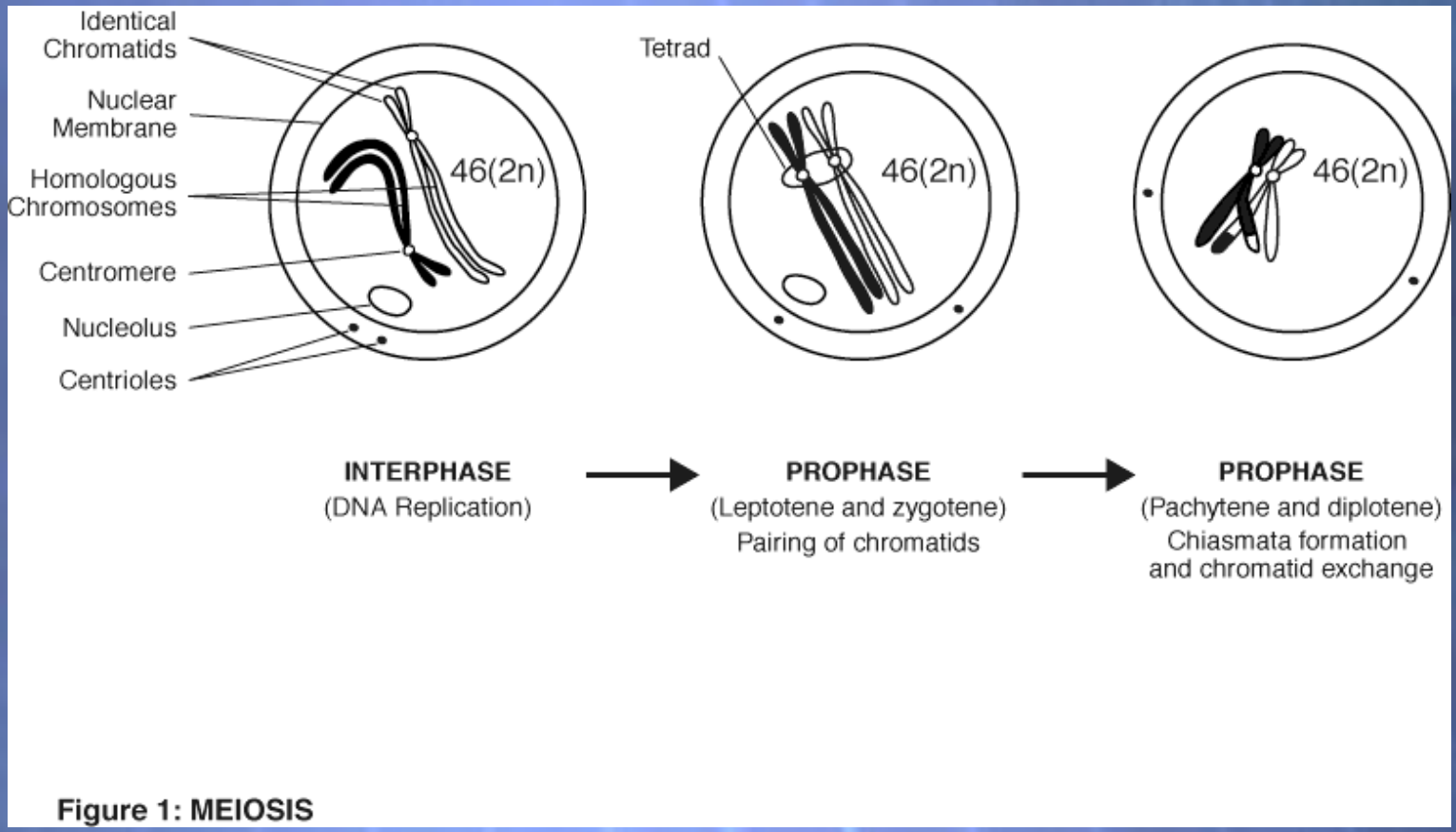


Figure 1: MEIOSIS

Elder, K. and Fleming, S. (2002) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

Meiosis 1 (continued)

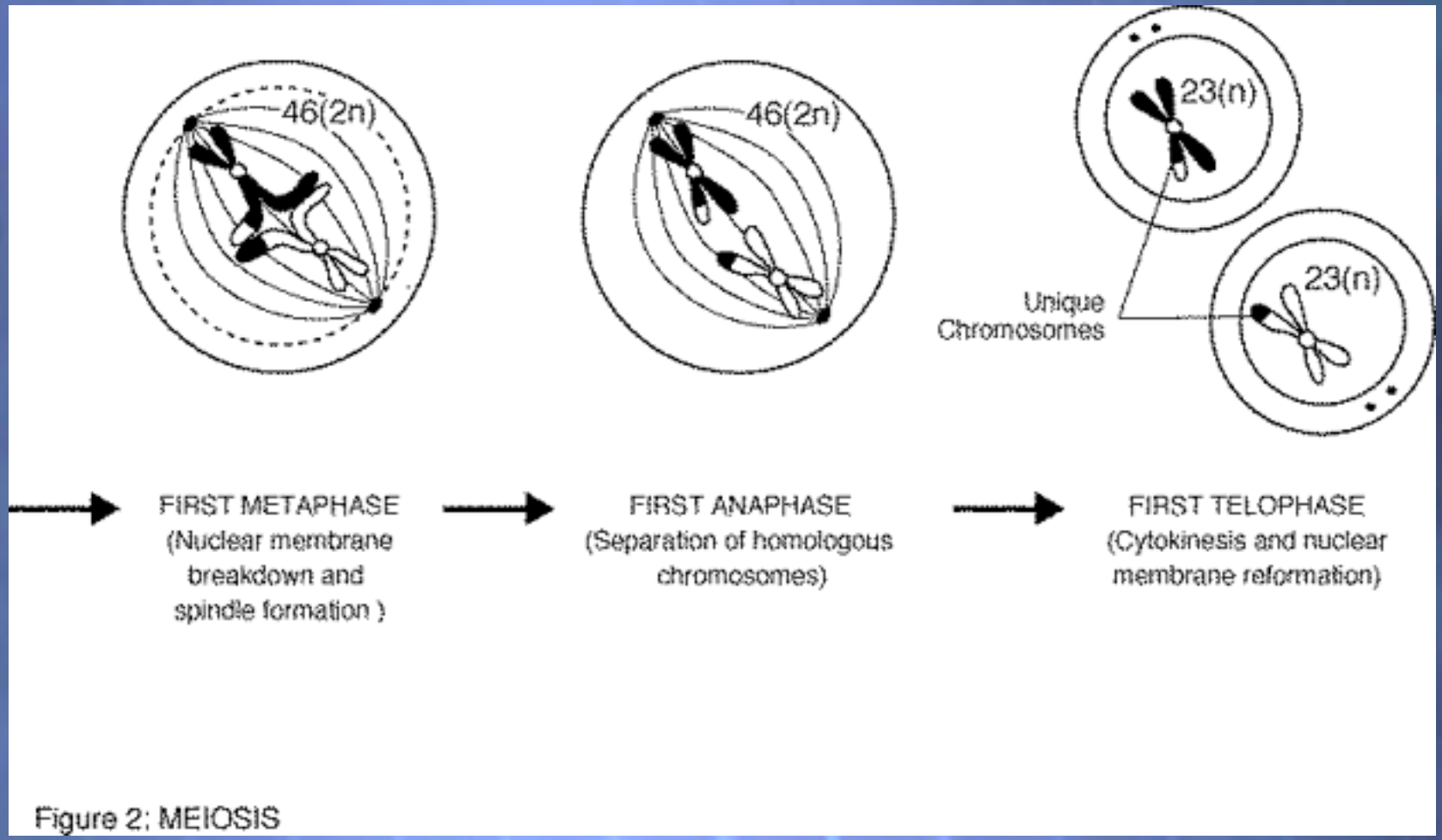


Figure 2: MEIOSIS

Elder, K. and Fleming, S. (2001) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

Meiosis 2

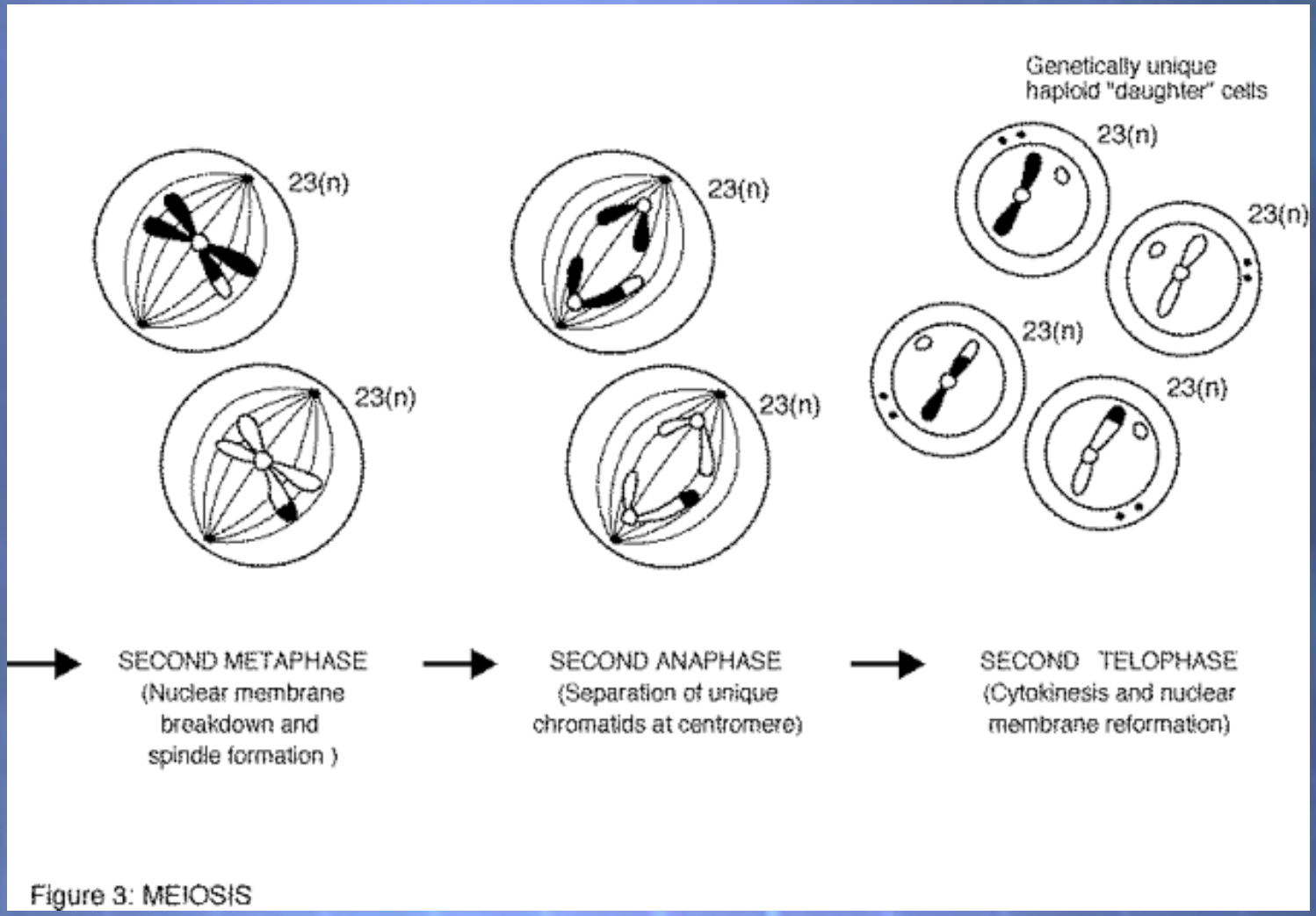


Figure 3: MEIOSIS

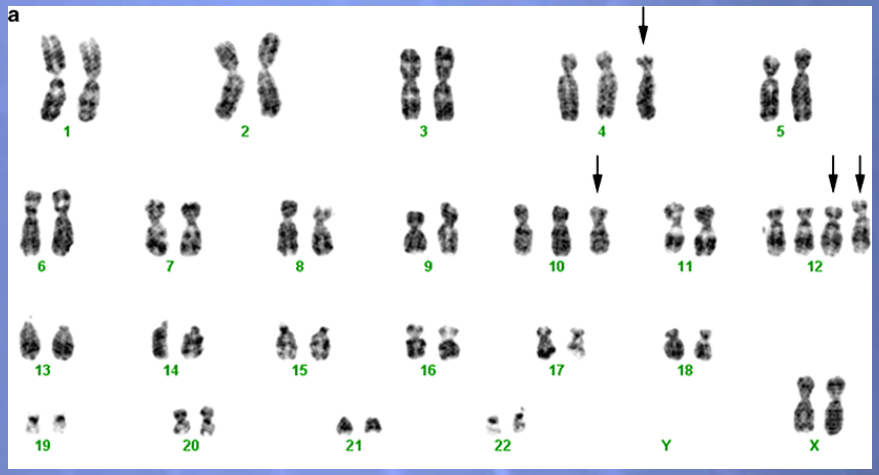
Elder, K. and Fleming, S. (2001) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

Why is meiosis a highly risky cell division?

Which aneuploidies result in subfertility or infertility?

Which aneuploidies increase with increased maternal age?

Aneuploidy: the scale of the problem

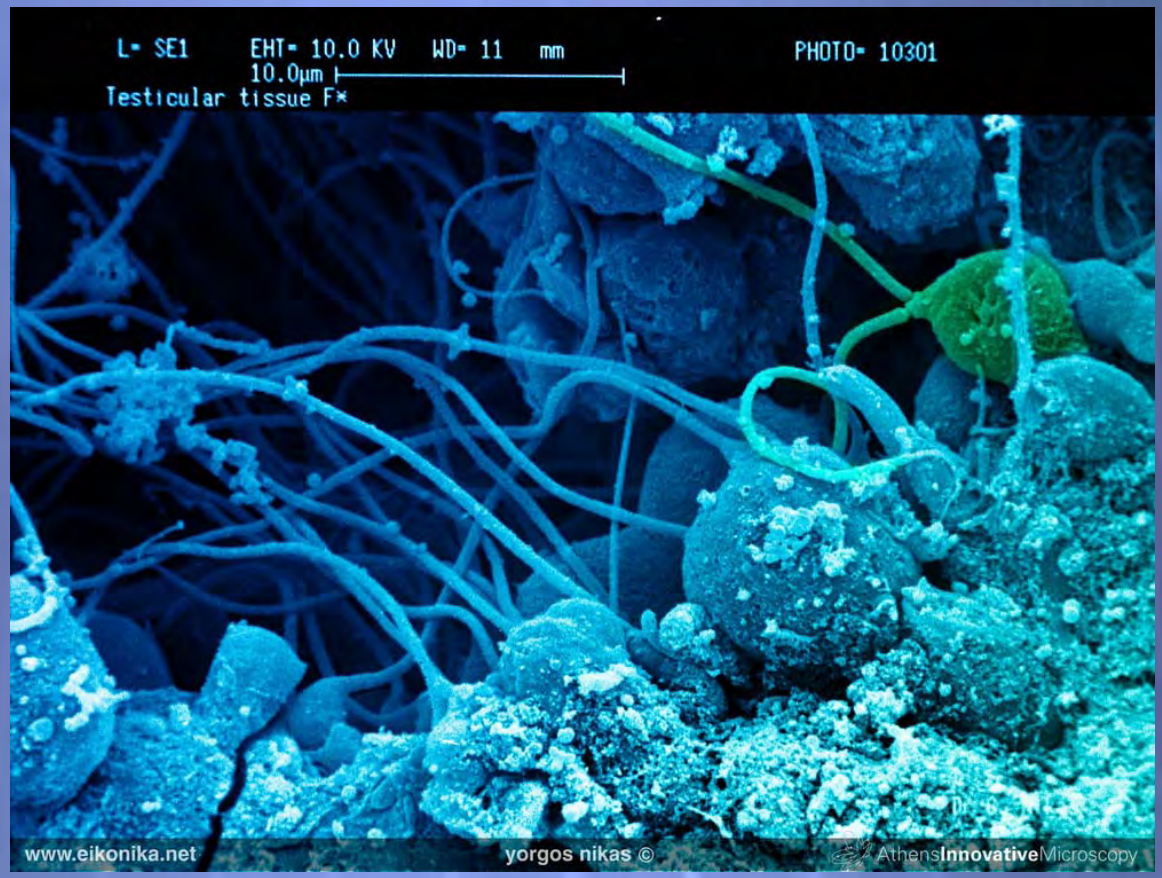


Incidence of aneuploidy during development

Gestation (weeks)			*0* — *6–8* — *20* — *40*				
	Sperm	**Oocytes**	**Pre-implantation embryos**	**Pre-clinical abortions**	**Spontaneous abortions**	**Stillbirths**	**Livebirths**
Incidence of aneuploidy	1–2%	~20%	~20%	?	35%	4%	0.3%
Most common aneuploidies	Various	Various	Various	?	45,X; +16; +21; +22	+13; +18; +21	+13; +18; +21 XXX; XXY; XYY

Hunt and Hassold (2001) Nature Reviews Genetics, 2 280-291

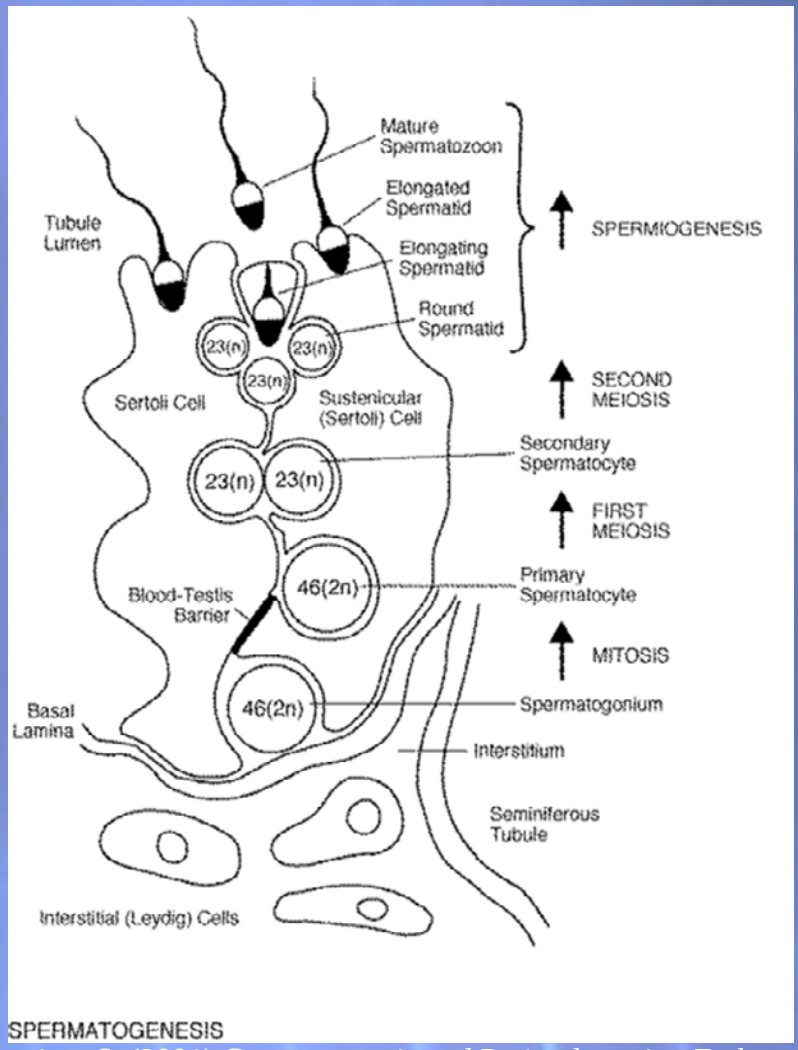
Spermatozoa within the seminiferous tubule



©Yorgos Nikas

Eikonica
Athens
Greece

Spermatogenesis



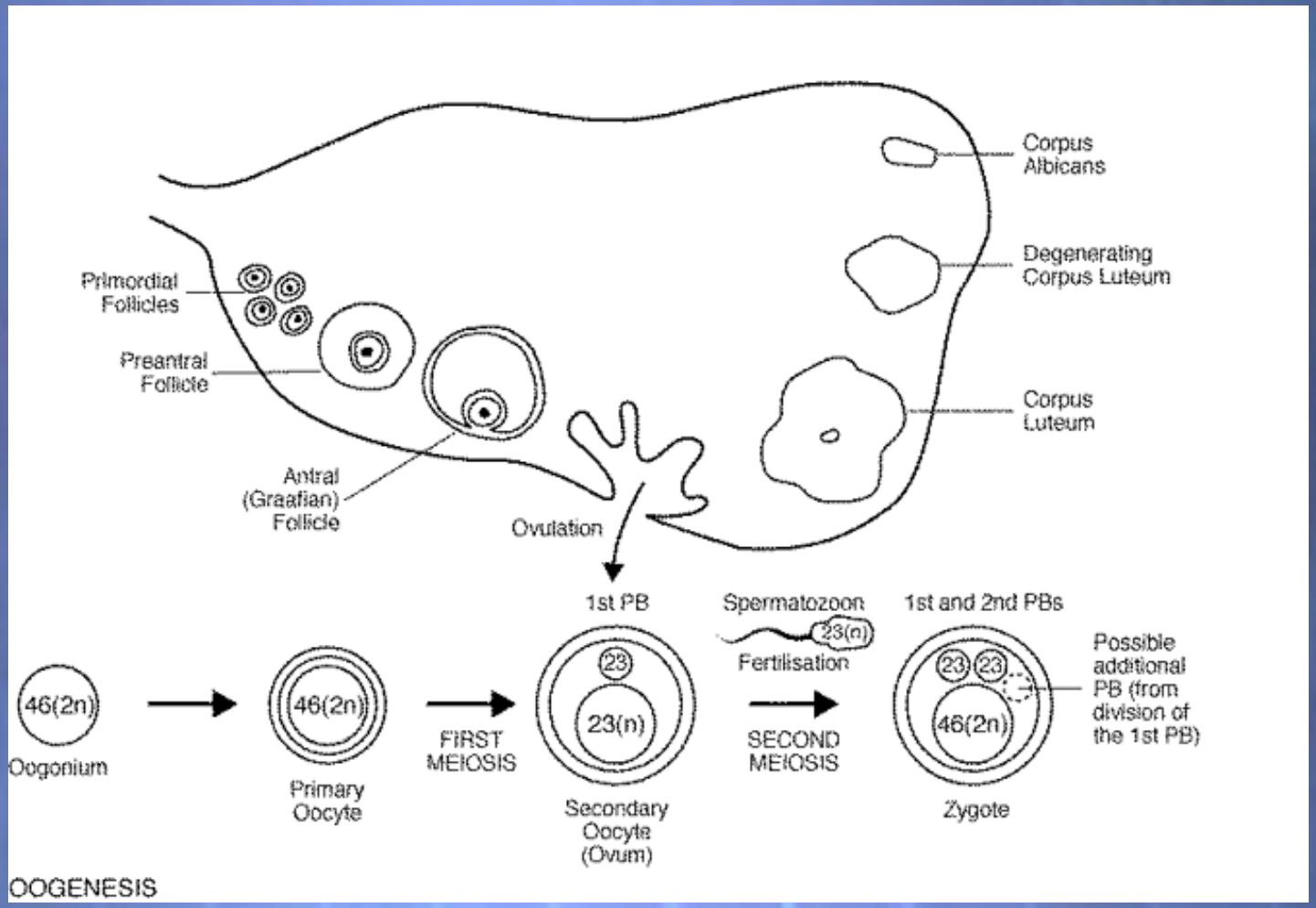
SPERMATOGENESIS

Elder, K. and Fleming, S. (2001) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

In what ways does meiosis differ during oogenesis?

What are the mechanisms underlying these differences?

Folliculogenesis/Oogenesis



Elder, K. and Fleming, S. (2001) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

In Vitro Maturation

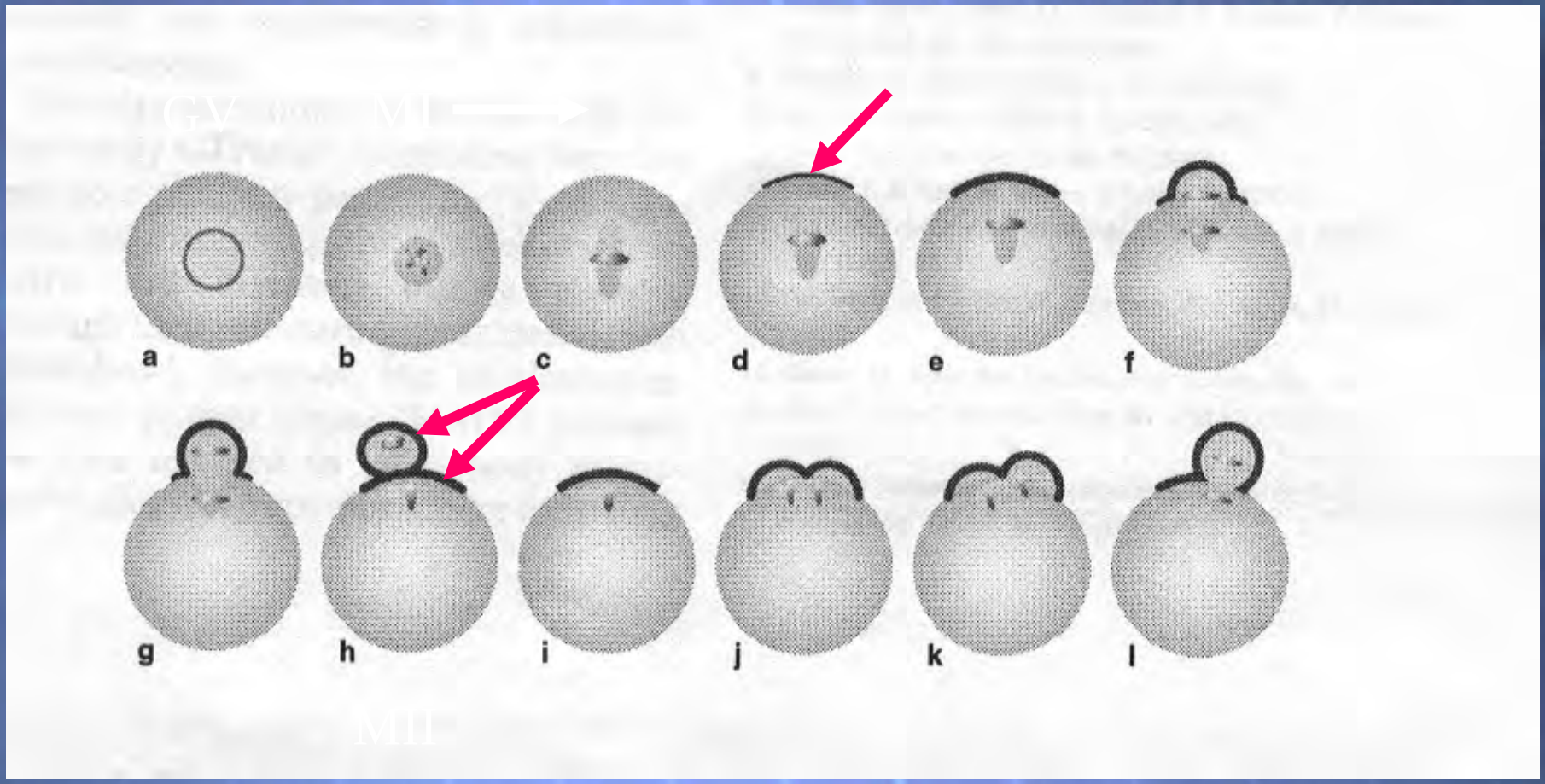
Prophase I

Metaphase I

Metaphase II

24-48 hours

Actin-rich domain during PB formation in mouse oocyte:



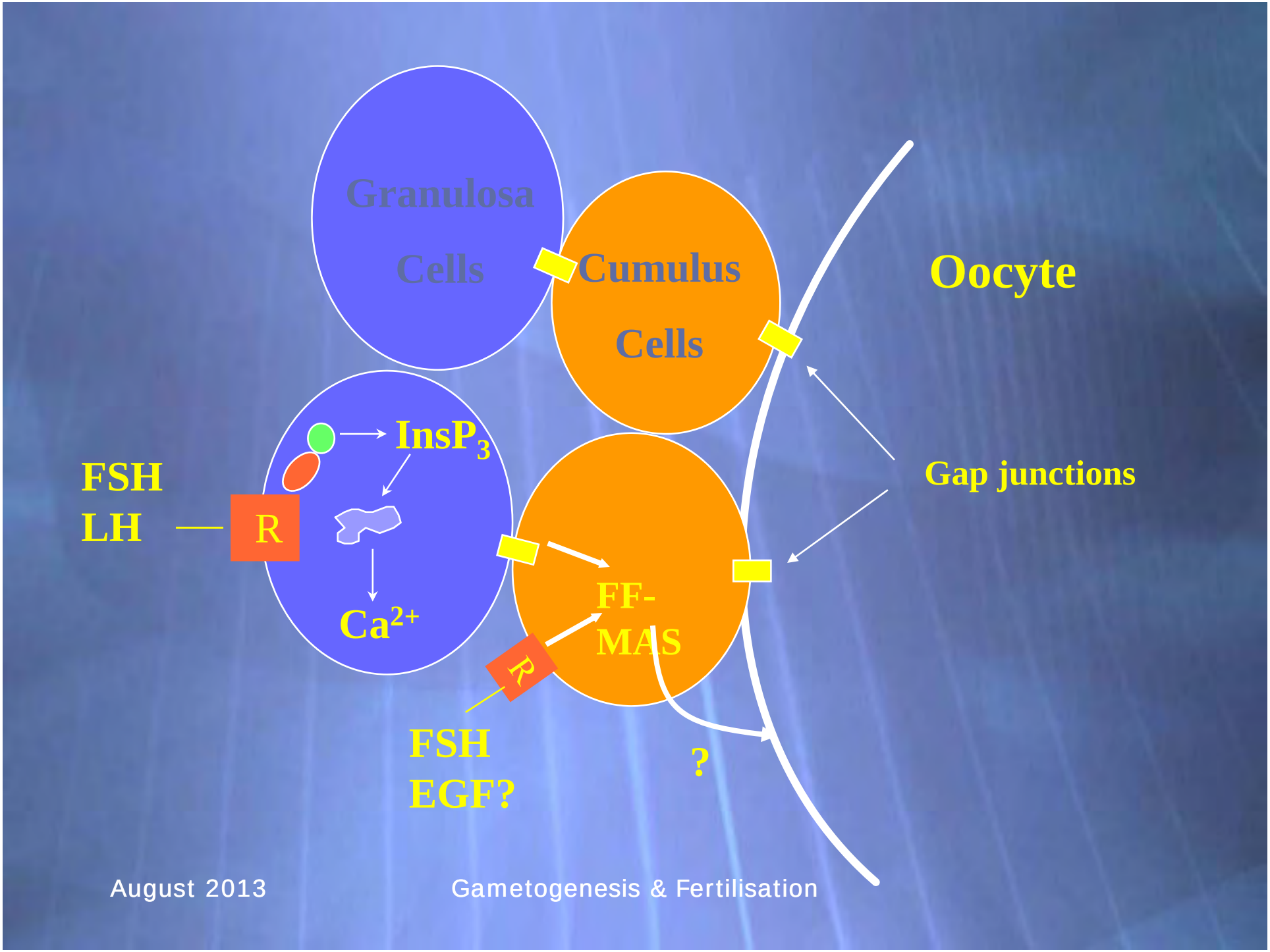
Maro and Verlhac, 2002 Nature Cell Biology 4, 281-3

Why is the timing of fertilisation regulated?

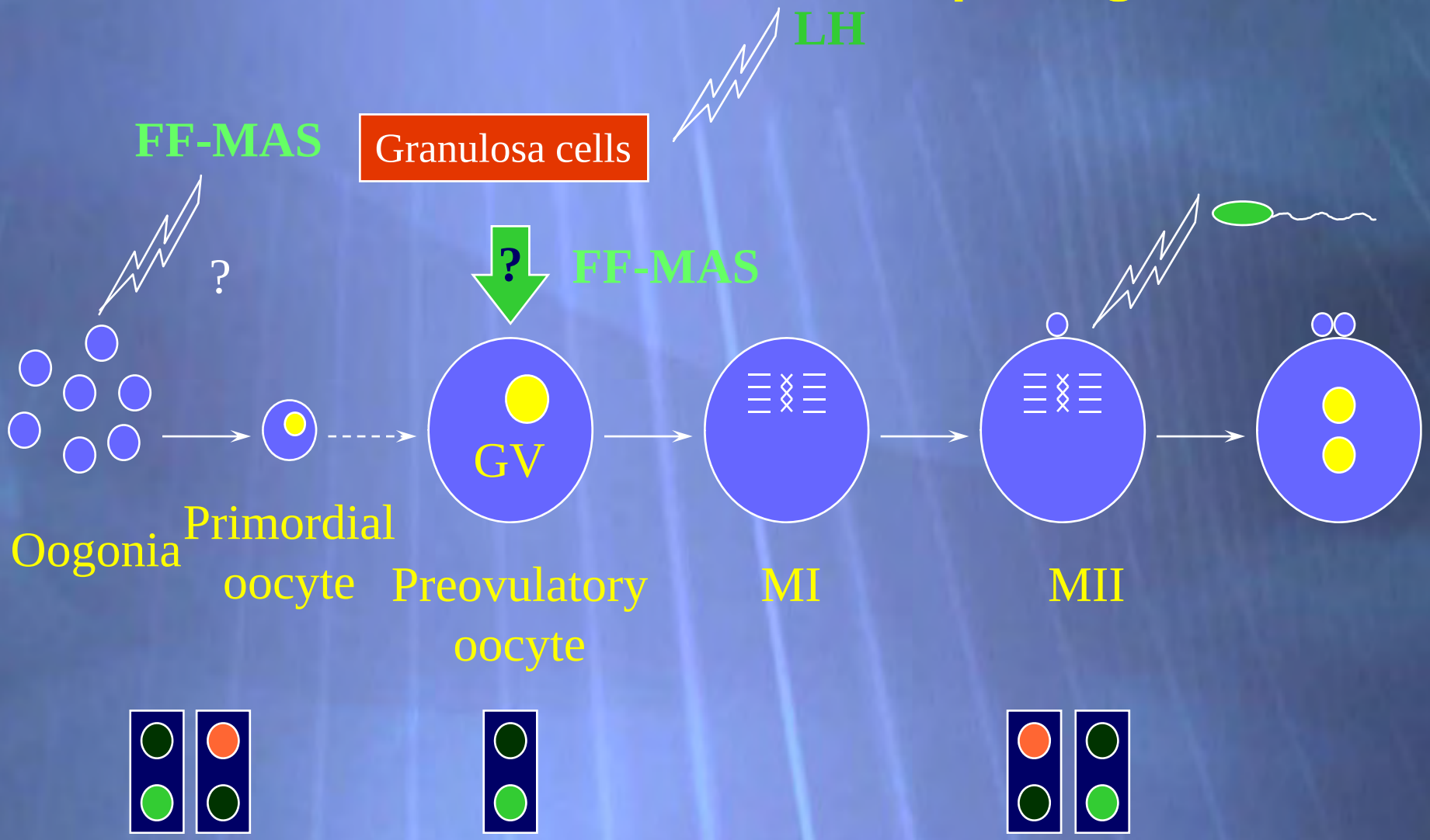
What are the mechanisms in the male and female that regulate fertilisation?

Graafian follicle

The follicle maintains the oocyte under meiotic arrest


Granulosa Cells
Cumulus Cells
Oocyte
InsP3
FSH
LH
R
Ca2+
Gap junctions
FF-MAS
R
FSH
EGF?
?

Meiotic start and stop signals

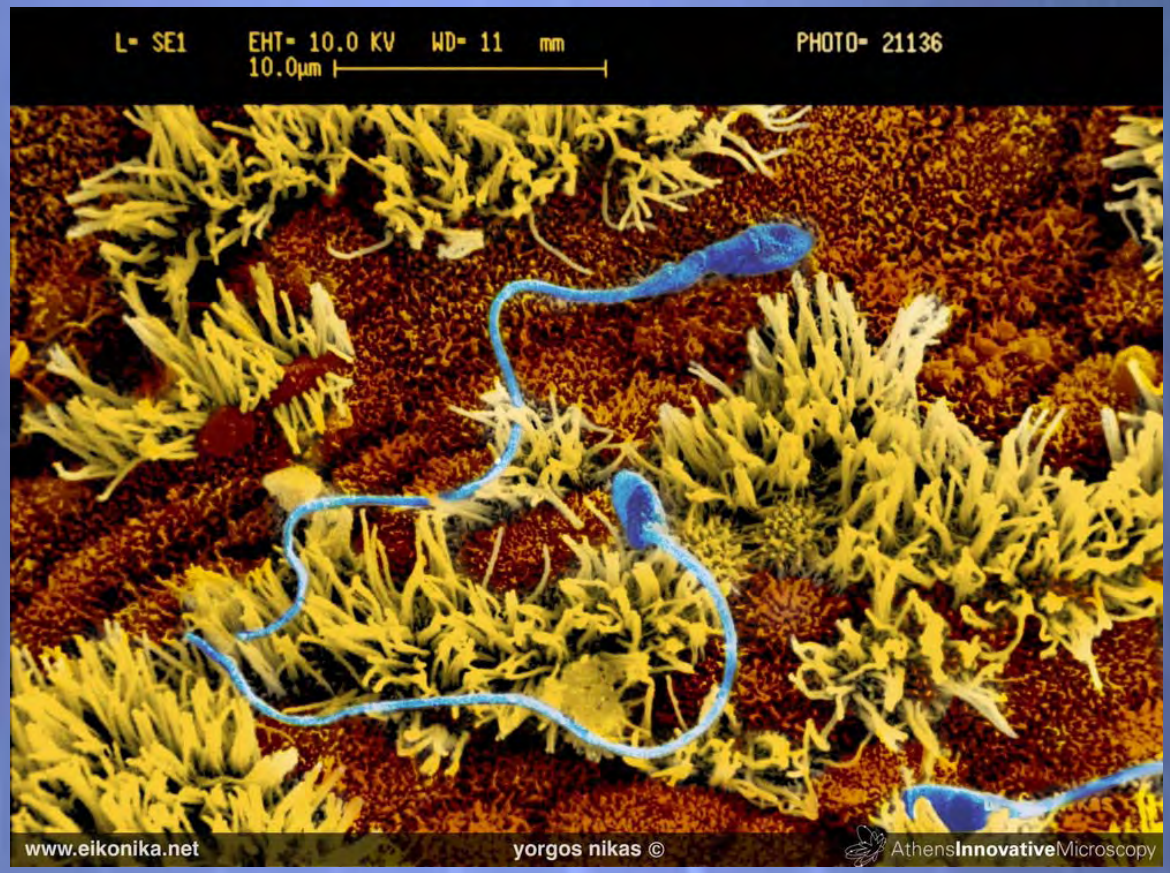


Elder, K. and Fleming, S. (2001) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

The pathway to fertilisation

- Capacitation
- Hyperactivation
- Oocyte maturation
- Sperm-egg recognition
- The acrosome reaction
- Sperm-egg fusion
- Oocyte activation
- Pronuclei formation
- Syngamy

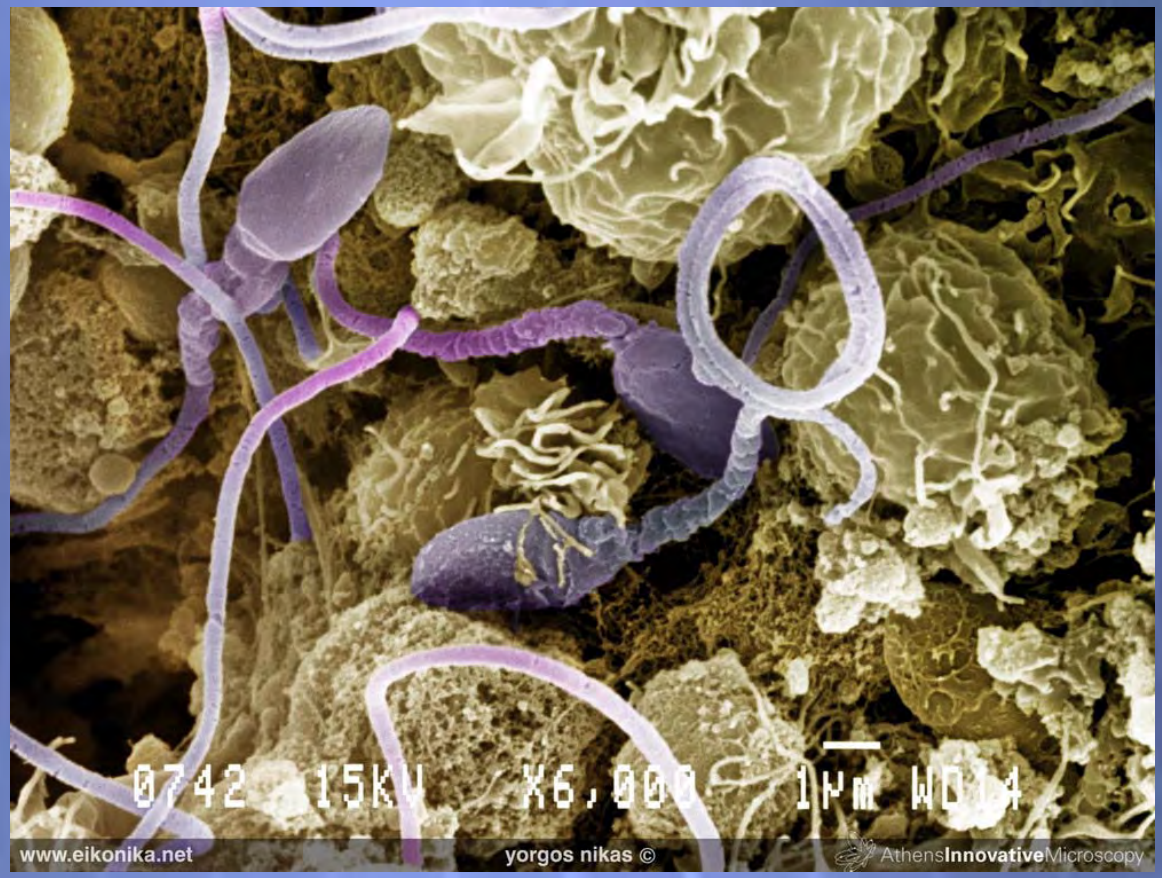
Spermatozoa traversing ciliated epithelium



Yorgos Nikas

Eikonika
Athens
Greece

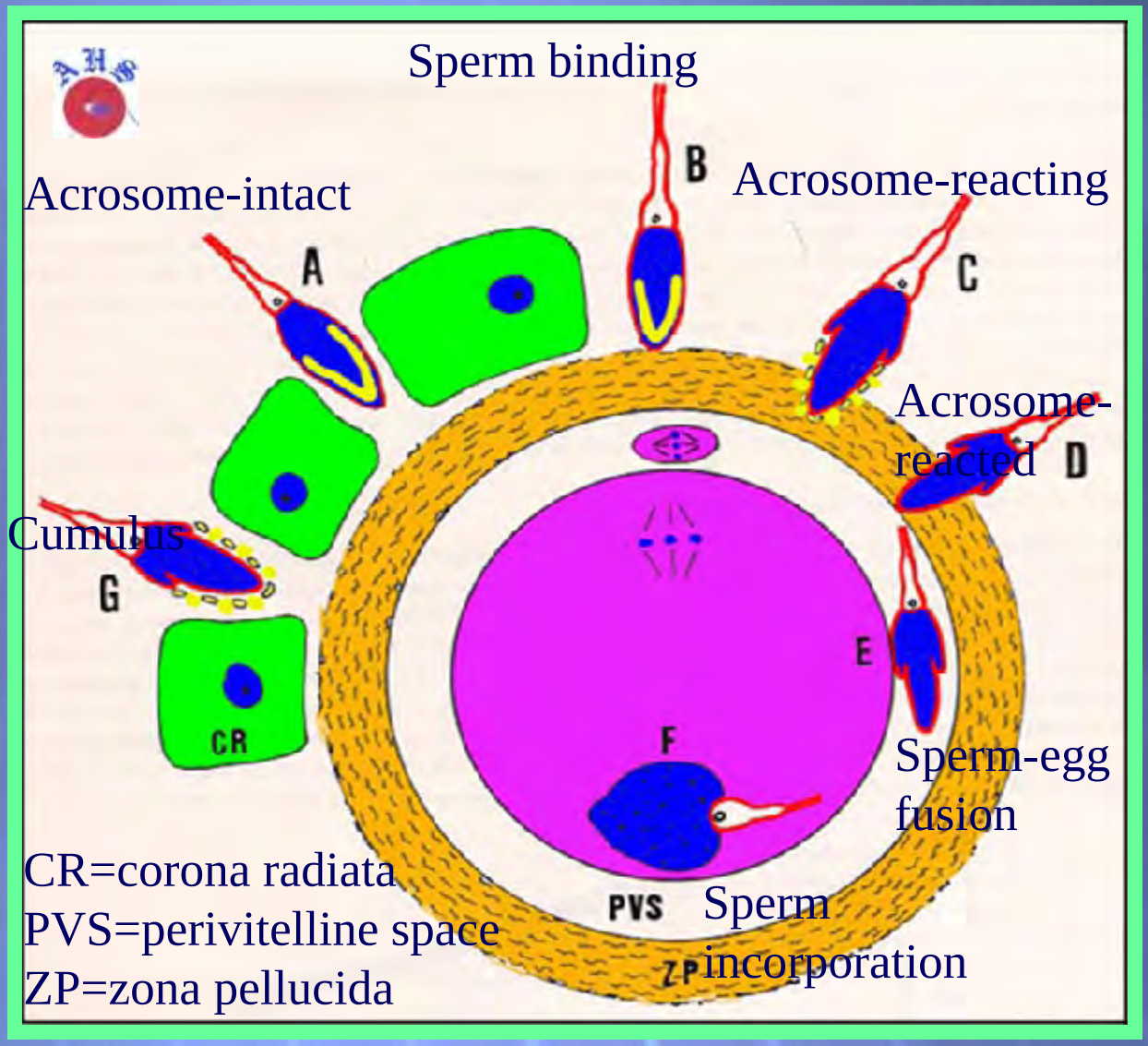
Acrosome reacted spermatozoa in cumulus



Yorgos Nikas

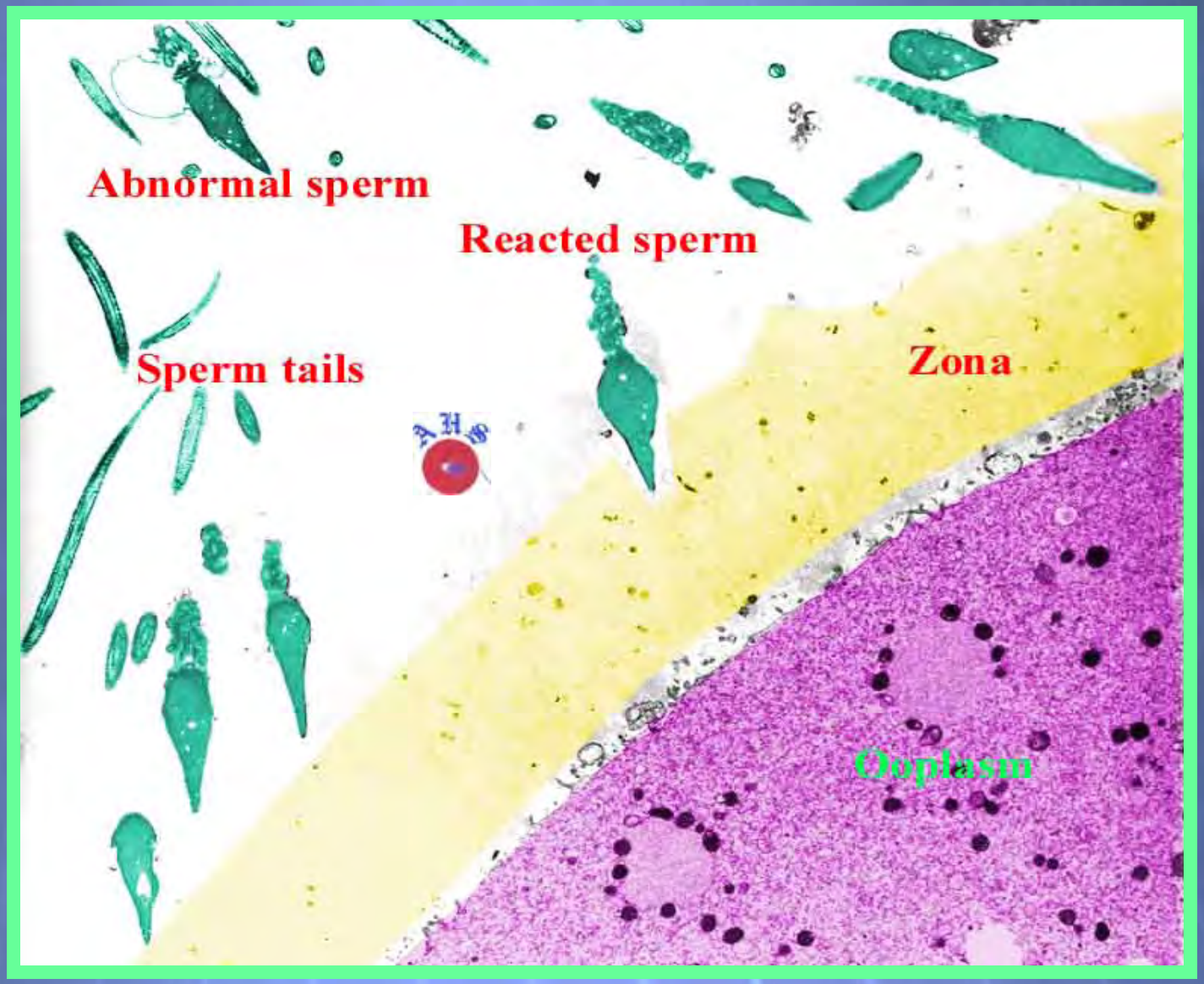
Eikonika
Athens
Greece

Sperm penetration of mature egg - Diagram

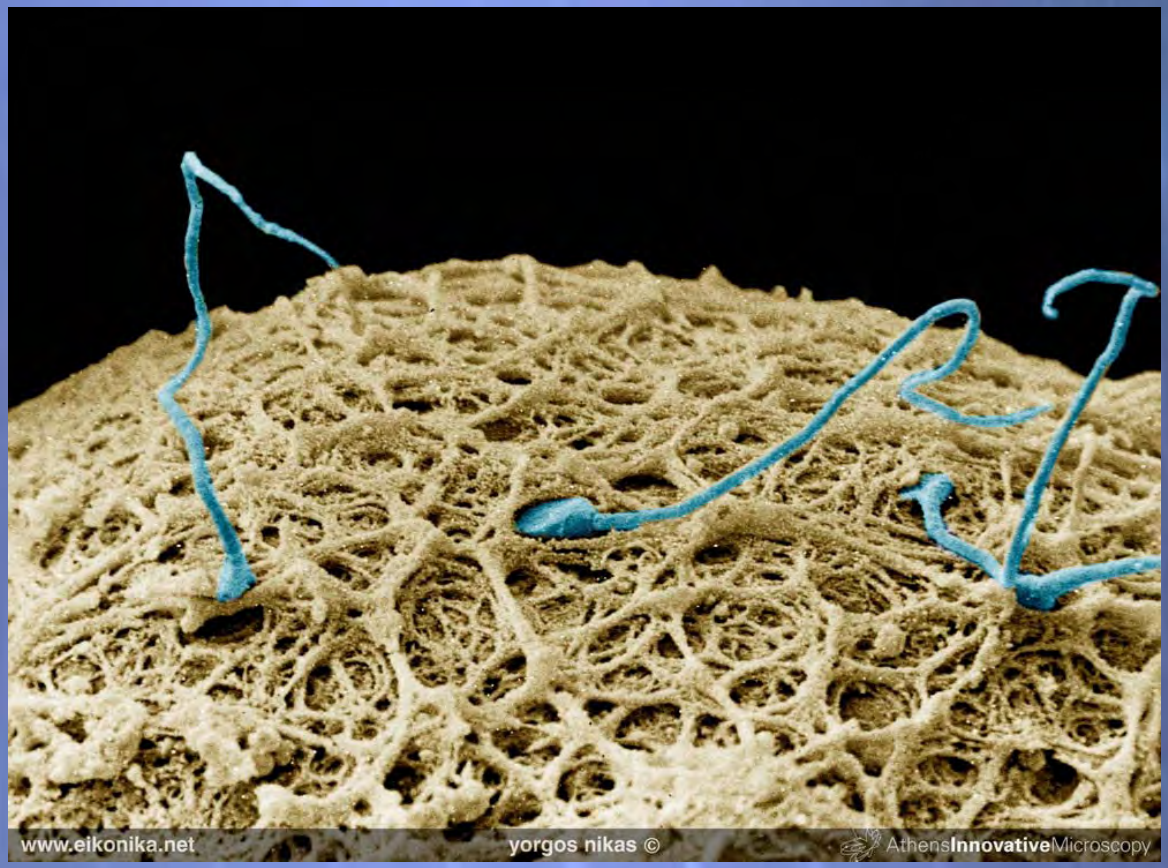


Sathananthan, H. (2008) Human fertilisation after IVF and ICSI in Textbook of Assisted Reproduction for Scientists in Reproductive Technology (eds S. Fleming and S. Cooke)

Sperm penetration of the zona - TEM



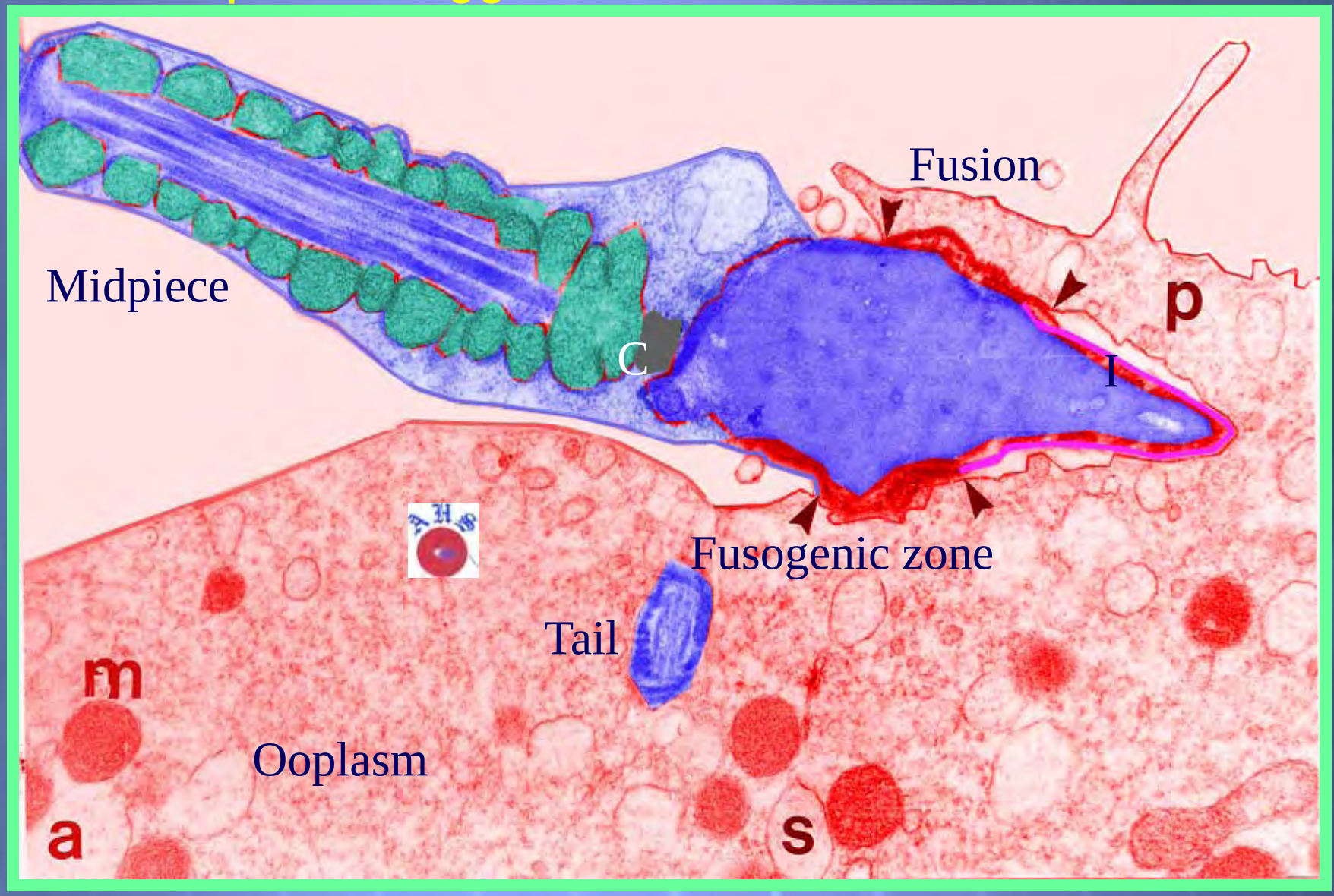
Spermatozoa penetrating the zona pellucida



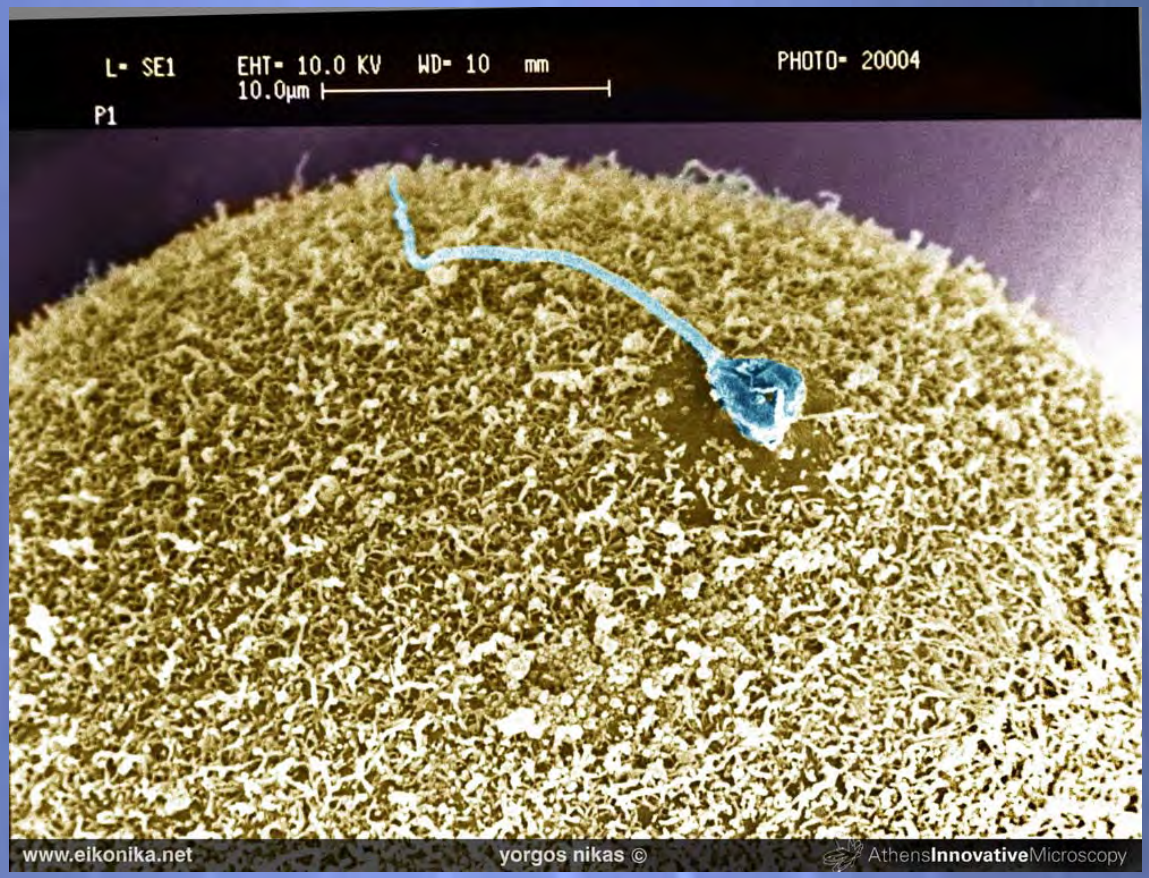
Yorgos Nikas

Eikonika
Athens
Greece

Sperm – egg membrane fusion - TEM



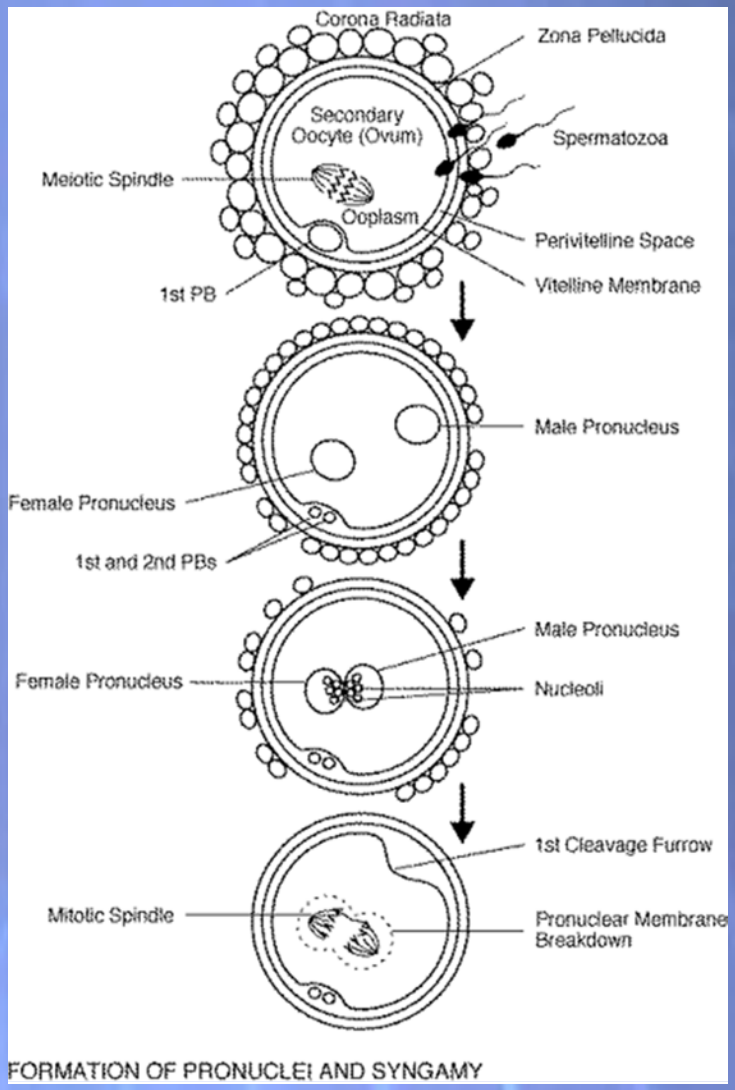
Spermatozoon binding to the oolemma



Yorgos Nikas

Eikonika
Athens
Greece

Formation of pronuclei and syngamy



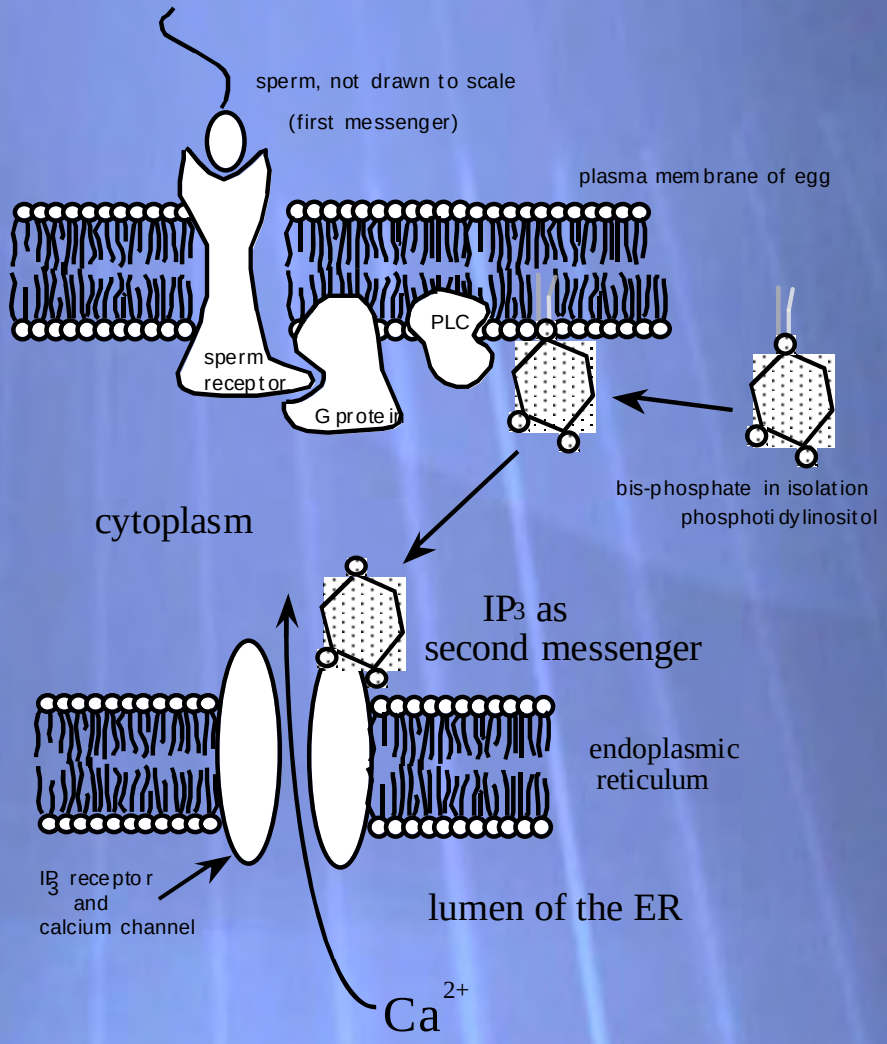
FORMATION OF PRONUCLEI AND SYNGAMY

Elder, K. and Fleming, S. (2001) Gametogenesis and Preimplantation Embryo Development in Preimplantation Genetic Diagnosis (eds J. C. Harper, J. D.A. Delhanty and A. H. Handyside)

How does the oocyte know it has been fertilised?

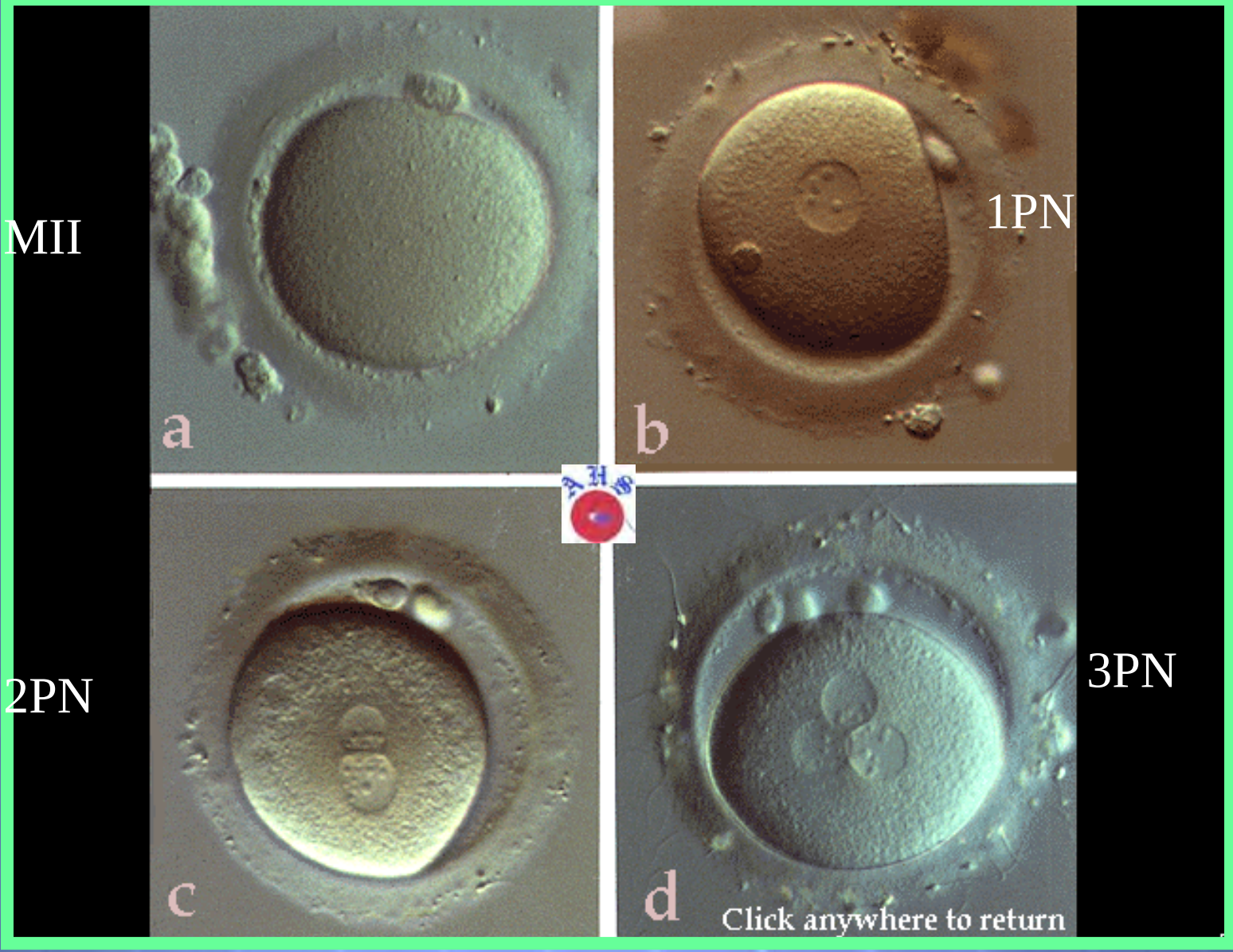
What is the mechanism by which the oocyte becomes activated?

Role of second messengers in fertilisation



Adapted from *Developmental Biology* , third edition by Scott F. Gilbert

Mature and pronuclear eggs – Nomarski optics

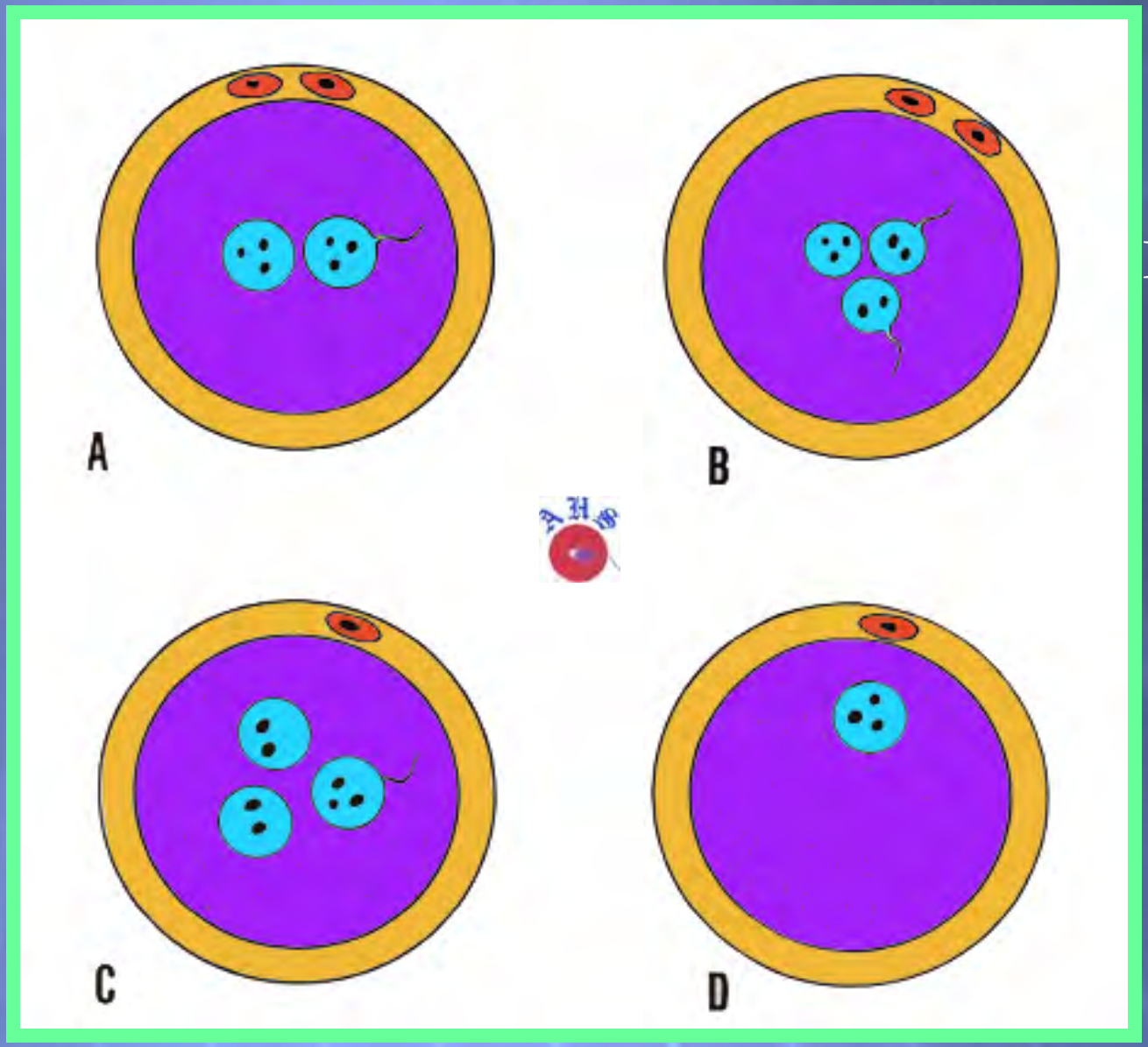


Courtesy Dr.D.Payne, Adelaide

Pronuclear ova - Diagram

Normal 2PN

Dispermy



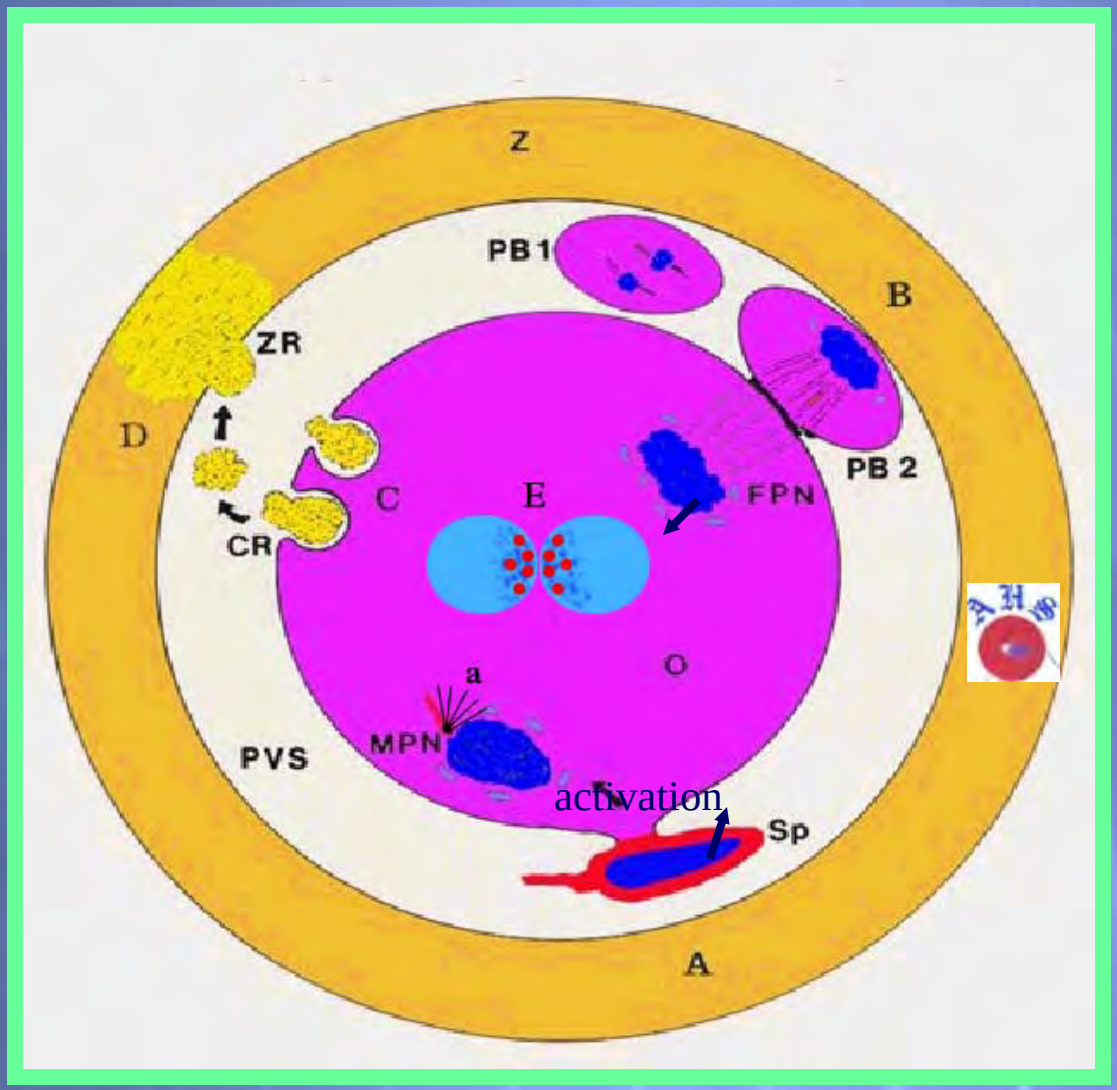
Digyny

Partheno-genesis

How does the oocyte prevent polyspermy?

How might polyspermy occur unwittingly in vitro?

Fertilisation events - Diagram



A: sperm-egg fusion

B: second maturation division

C: cortical reaction

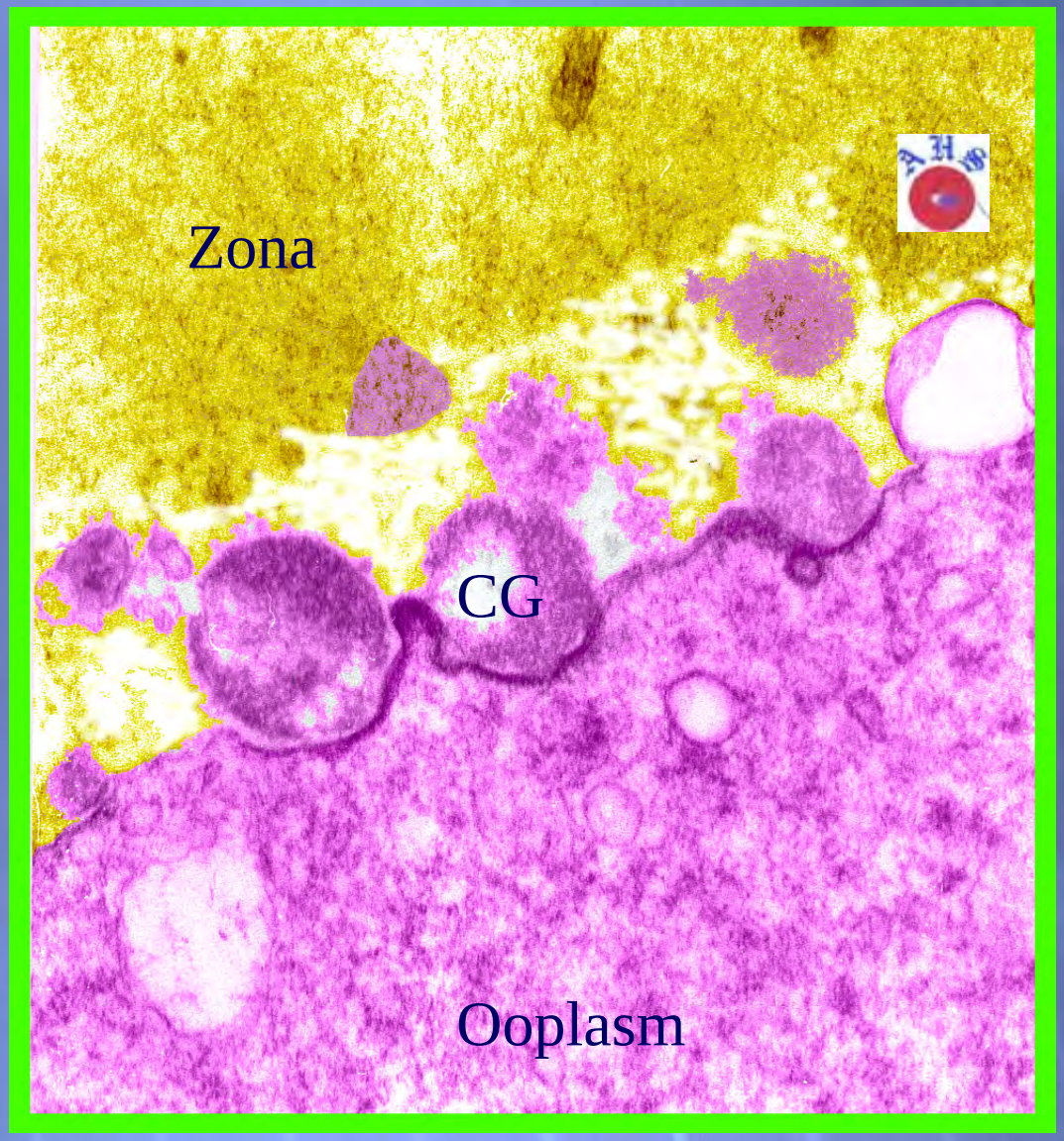
D: zona reaction

E: association of male & female pronuclei

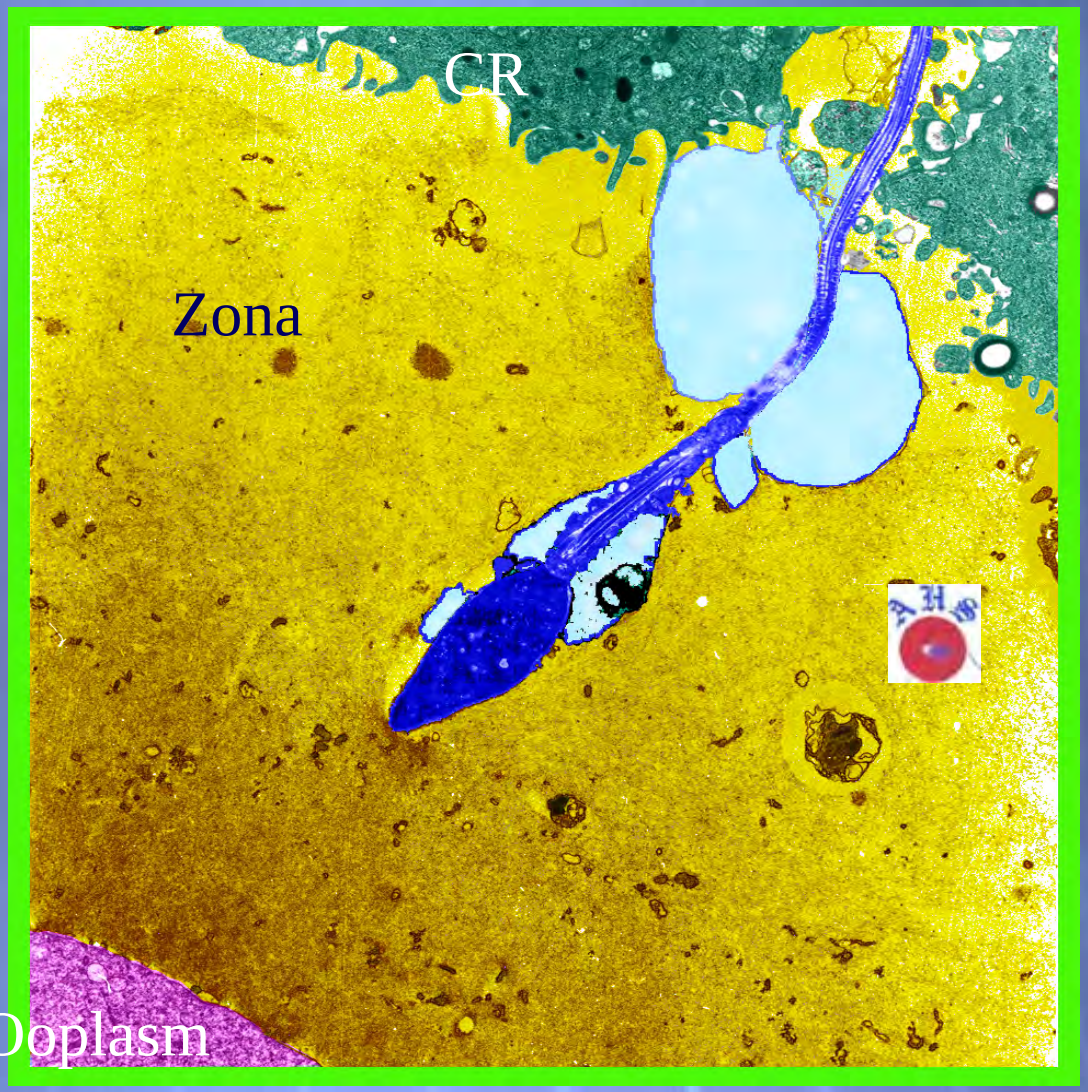
a: Sperm aster forms after sperm incorporation

Sathananthan, H. (2008) Human fertilisation after IVF and ICSI in Textbook of Assisted Reproduction for Scientists in Reproductive Technology (eds S. Fleming and S. Cooke)

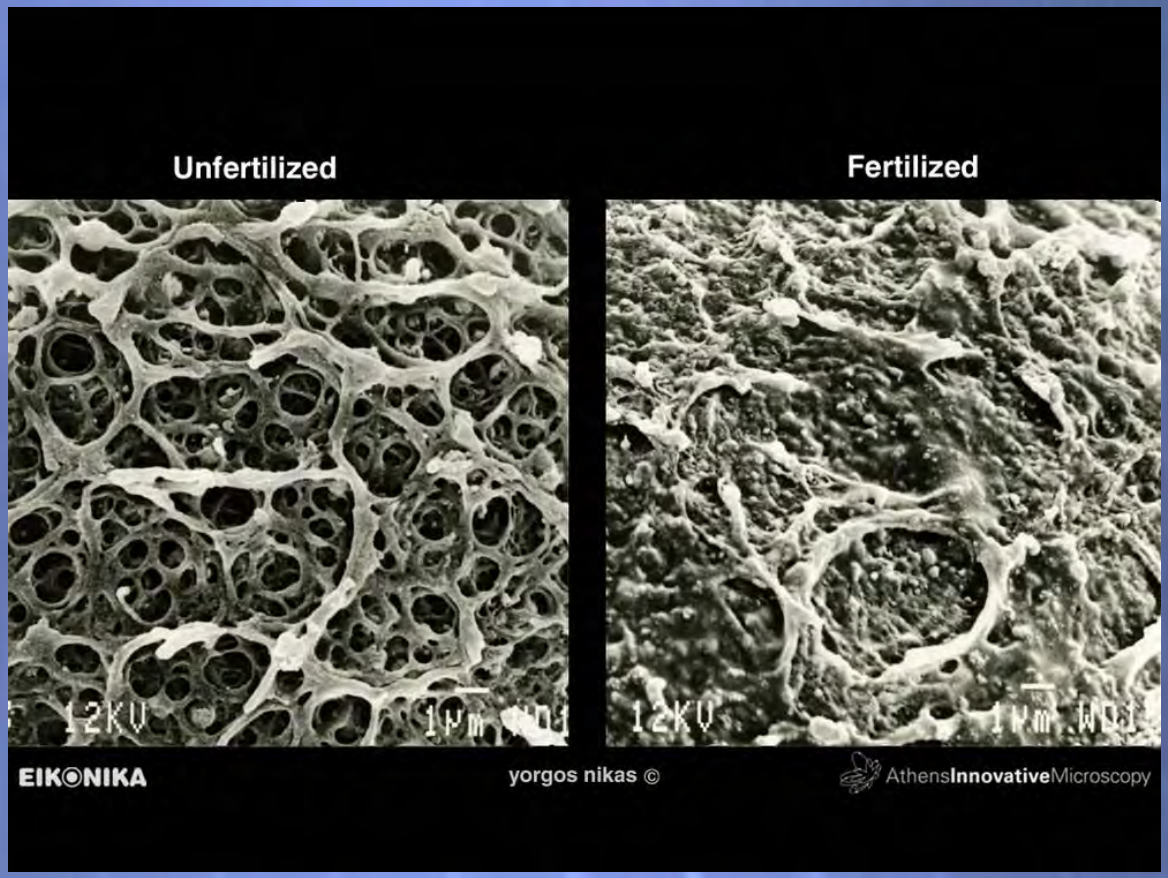
Cortical reaction at fertilisation - TEM



Block to polyspermy – nature's way - TEM



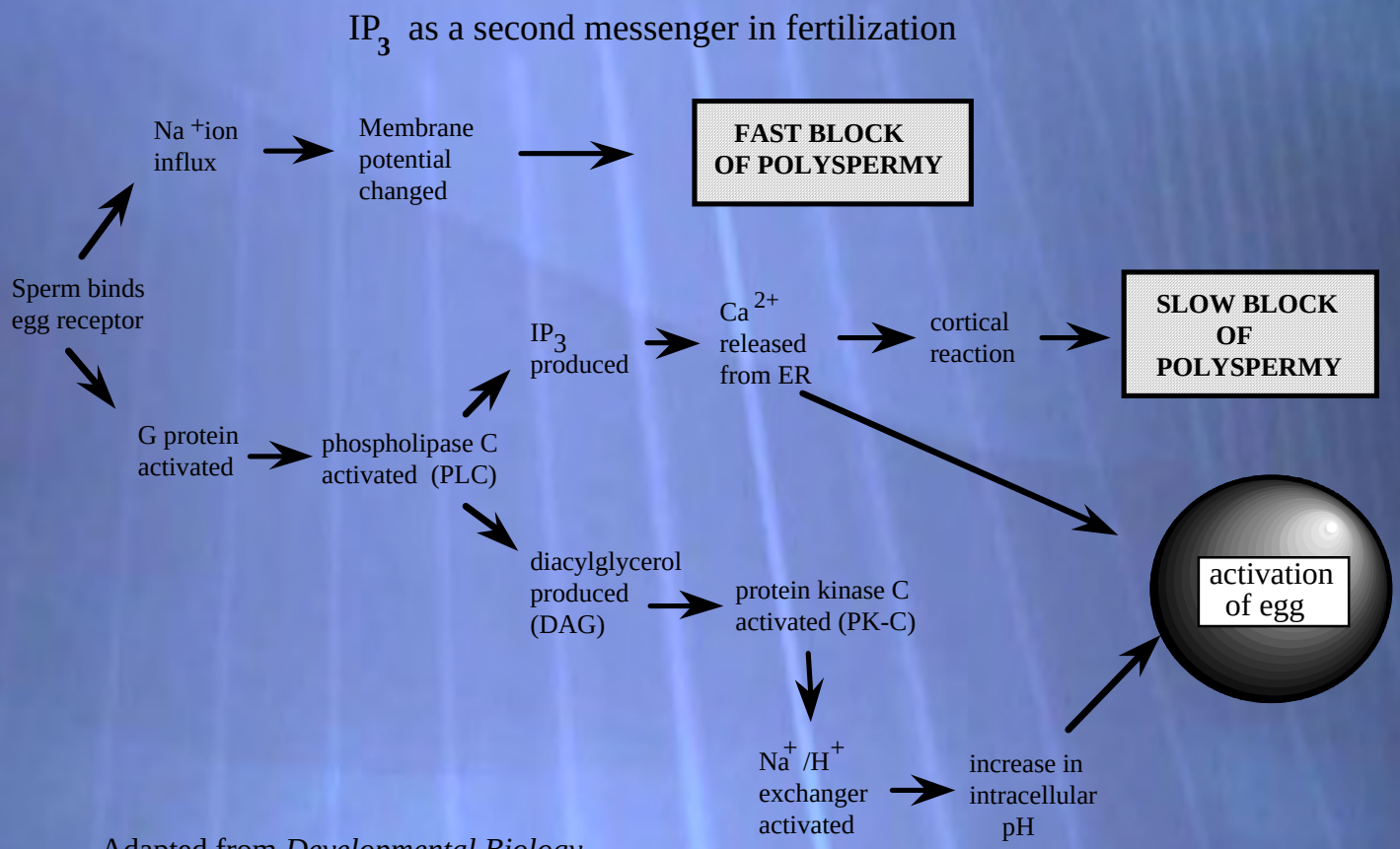
Zona morphology following fertilisation



Yorgos Nikas

Eikonika
Athens
Greece

Fast and slow blocks to polyspermy



Adapted from *Developmental Biology*, third edition by Scott F. Gilbert

Ovulation induction and Intrauterine insemination

- Patent fallopian tubes
- Ovulatory menstrual cycles
- Success rate = 10% - 15%
- Increases up to 4 attempts
- Total motile sperm count
  ≥ 10 million

In vitro fertilisation (IVF)

Success rate = 25% - 30%

What if the sperm concentration or sperm motility is too poor for IUI or IVF?

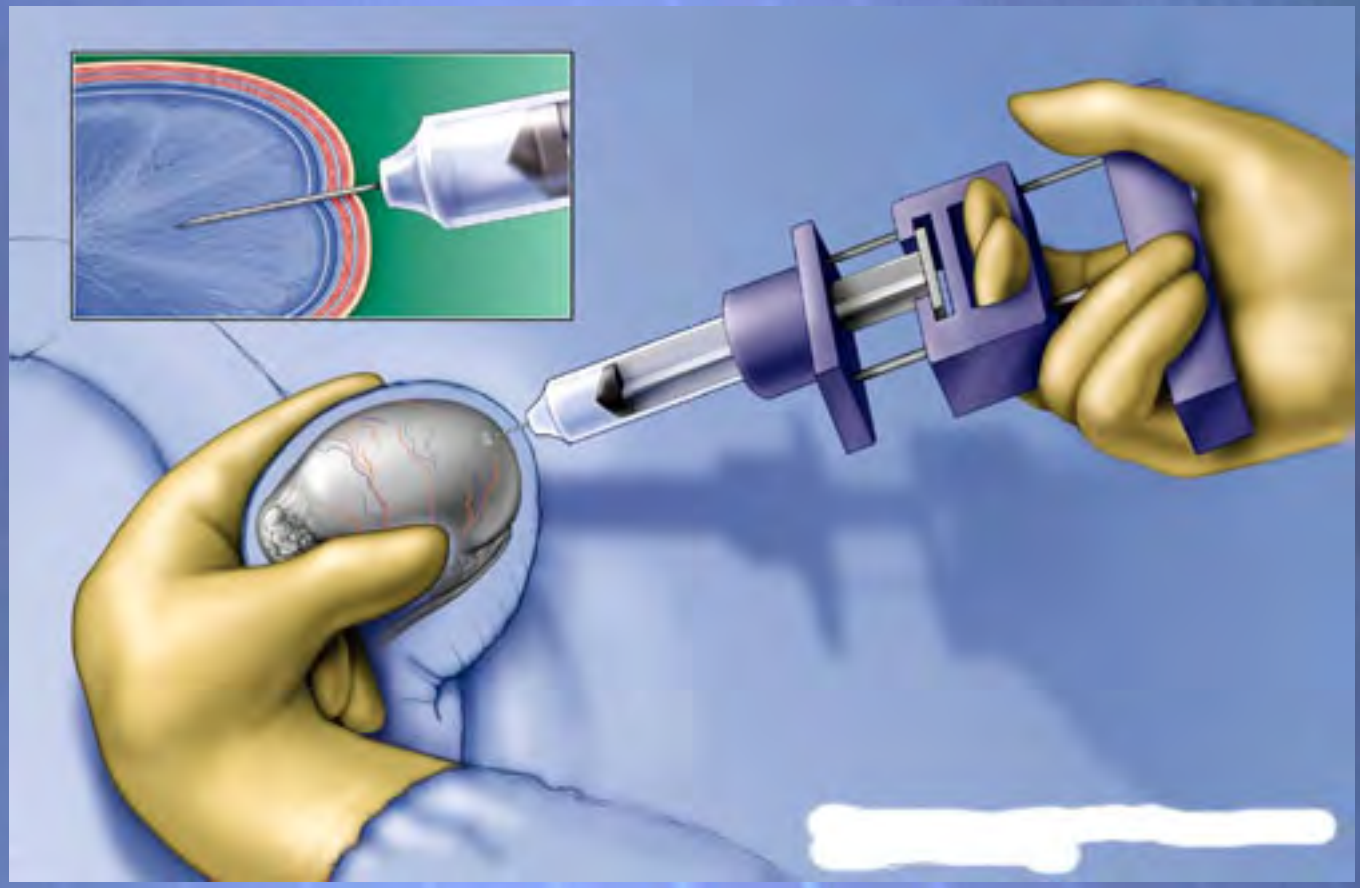
Intra-Cytoplasmic Sperm Injection (ICSI)

- Only one sperm required per egg
- Success rate = 25% - 30%

What if there are no sperm being ejaculated?

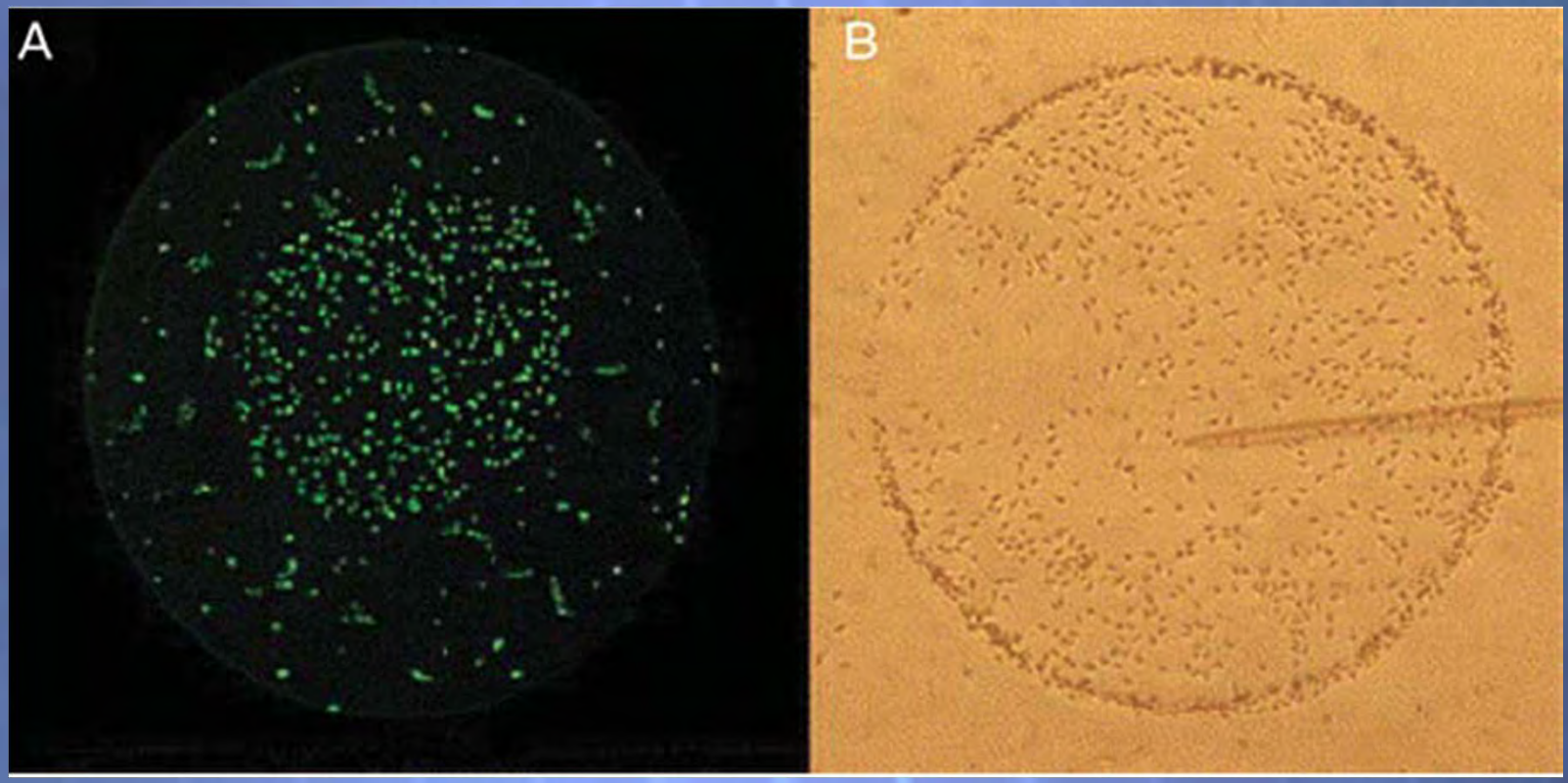
- Retrograde ejaculation?
- Genital tract obstruction?
- Very low sperm production?

Surgical Sperm Recovery



Testicular sperm extraction

PICSI

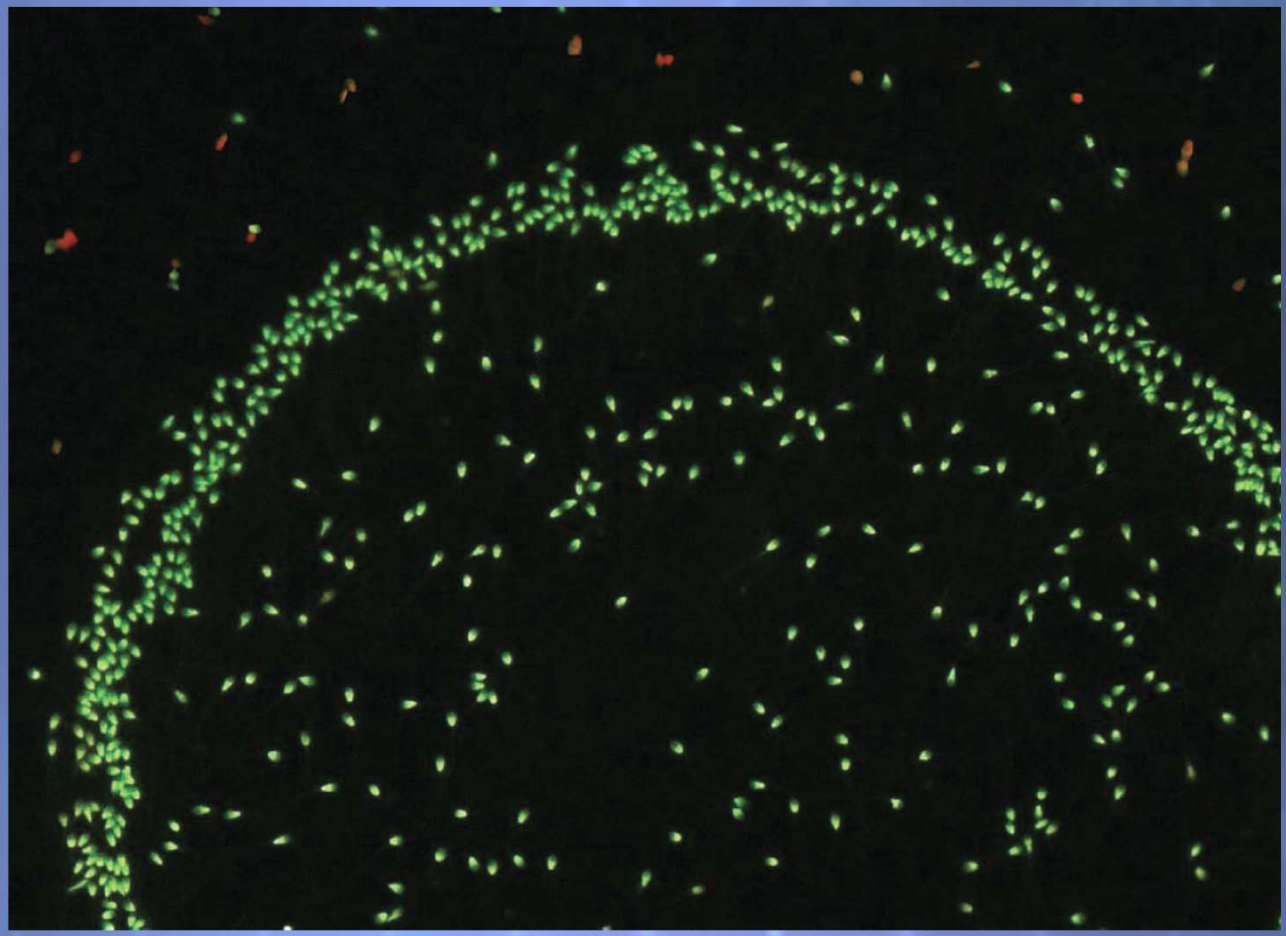


Jakab et al (2005) Fertil Steril 84: 1665-1673

Acridine Orange Fluorescence

- Fixation with methanol and glacial acetic acid (9:1)
- Staining with Acridine Orange solution for 10 minutes
- Metachromatic shift in fluorescence
  - Green (native, double-stranded DNA)
  - Red (single stranded DNA breaks)

HA selection of sperm



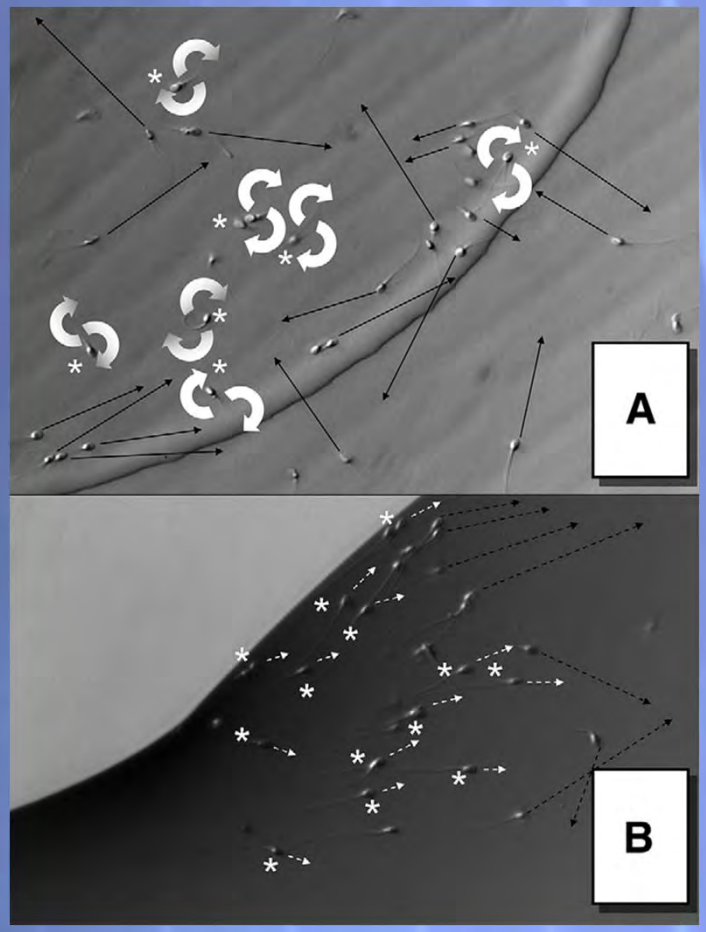
Yagci *et al* (2010) *J Androl* 31: 566-572

DNA integrity of HA selected spermatozoa

Variable	Mean ± SD (%)
Green sperm in semen	54.9 ± 2.0
Green sperm outside HA spot	56.1 ± 1.9
Green sperm inside HA spot	99.1 ± 0.2
Red sperm in semen	45.0 ± 1.9
Red sperm outside HA spot	43.9 ± 1.9
Red sperm inside HA spot	0.9 ± 1.9

Yagci *et al* (2010) *J Androl* 31: 566-572

PICSI vs Sperm Slow

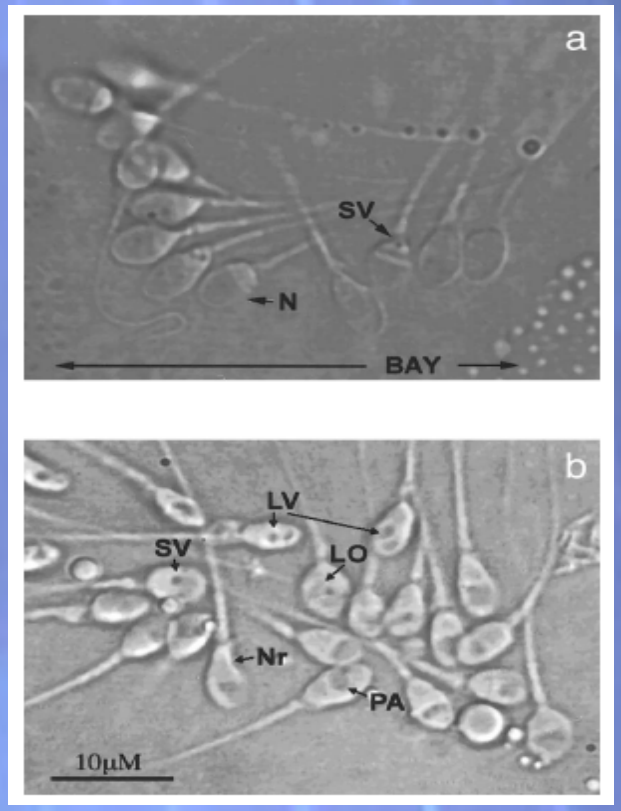


Mean ICSI procedure duration (s)

PICSI = 450.0 ± 30.5
SSlow = 284.1 ± 10.1
$p < 0.001$

Parmegiani *et al* (2012) *Fertil Steril*

Intracytoplasmic Morphologically Selected Sperm Injection (IMSI)



Bartoov *et al* (2002) *J Androl* 23: 1-8

RCT of ICSI vs IMSI

	Live birth per initiated cycle (%)			*Implantation rate (%)*		
	ICSI	*IMSI*	*P-value*	*ICSI*	*IMSI*	*P-value*
No male factor	20/42 (47.6)	24/49 (49.0)	NS	26/110 (23.6)	34/120 (28.3)	NS
Male factor	11/39 (28.2)	14/38 (36.8)	NS	16/105 (15.2)	32/108 (29.6)	0.01
Sperm count						
<1 million/ml	4/16 (25.0)	4/11 (36.4)	NS	7/43 (16.3)	11/31 (35.5)	NS
1–20 million/ml	7/22 (31.8)	10/27 (37.0)	NS	9/59 (15.3)	21/77 (27.3)	NS

Values are *n*/total (%).
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection; NS = not significant.

Balaban *et al* (2011) *RBM Online* 22: 472-476)

Outcome	ICSI	IMSI	P-value
Duration of ICSI procedure (min)	13.55 ± 5.43	20.54 ± 9.43	<0.001
2-pronuclei fertilization rate (%)	80.97 ± 15.06	81.60 ± 10.65	NS
Embryos with 4 blastomeres on day 2 post fertilization (%)	34.70 ± 21.88	30.43 ± 16.23	NS
Embryos with 8 blastomeres on day 3 post fertilization (%)	31.65 ± 17.21	33.61 ± 16.34	NS
Grade 1 and 2 embryos on transfer day (%)	4.84 (63.95)	5.01 (66.44)	NS
Mean no. of embryos transferred[a]	2.76 ± 0.46	2.72 ± 0.48	NS
Clinical pregnancy per initiated cycle (%)	36/81 (44.4)	47/87 (54.0)	NS
Live birth rate per initiated cycle (%)	31/81 (38.3)	38/87 (43.7)	NS
Implantation rate (%)	42/215 (19.5)	66/228 (28.9)	NS
Multiple pregnancy rate (%)	6/36 (16.7)	16/47 (34.0)	<0.001

Values are mean ± SD or *n* (%).
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection; NS = not significant.
[a]Six patients (three in each group) who did not undergo embryo transfer due to total fertilization failure (one in IMSI) or pending ovarian hyperstimulation syndrome (two in IMSI, three in conventional ICSI groups) are excluded from this analysis.

Balaban *et al* (2011) *RBM Online* 22: 472-476

ART, Ethics & Society

- Mitochondrial disease and ooplasmic transfer
- Haploidisation of somatic cells to derive gametes
- Microarray CGH and sperm selection