The work of a fertility specialist

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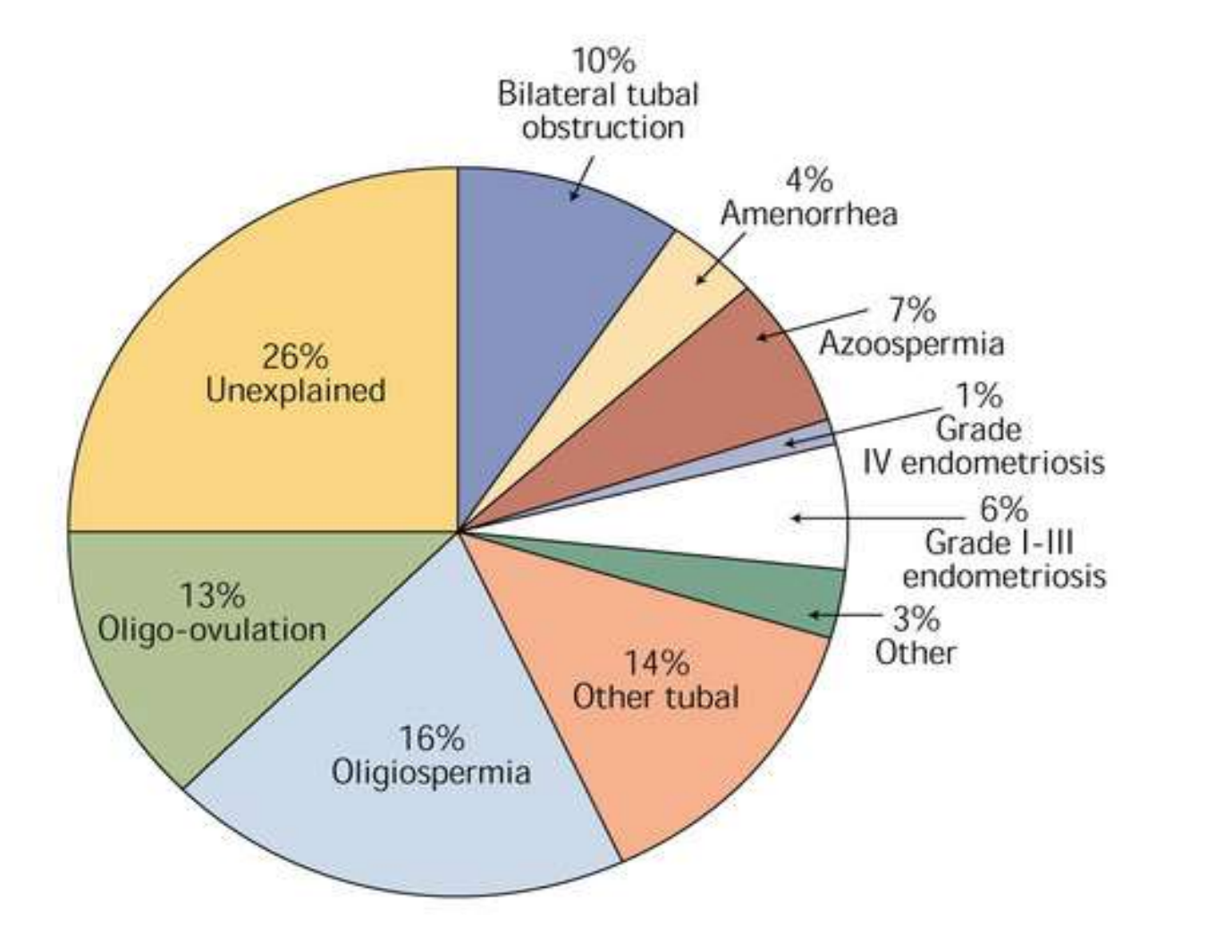
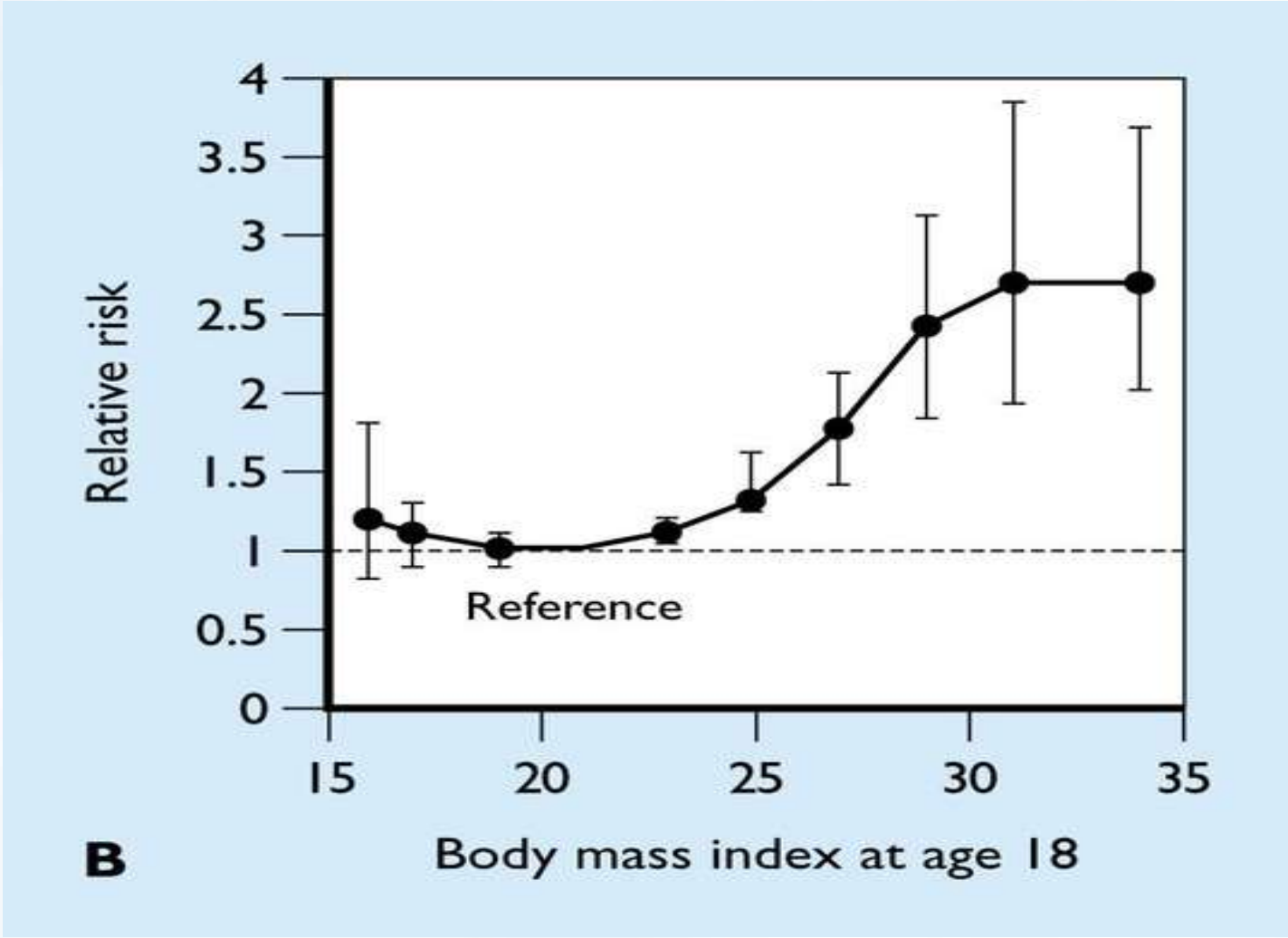
Scope of work

- Evaluation and diagnosis of the infertile couple
- Controlled ovarian hyper-stimulation and oocyte retrieval
- Sperm preparation and oocyte insemination
- Assessment of fertilisation and embryo culture
- Pre-implantation genetic screening and diagnosis
- Embryo transfer and cryopreservation

Diagnostic assignment and cause of infertility



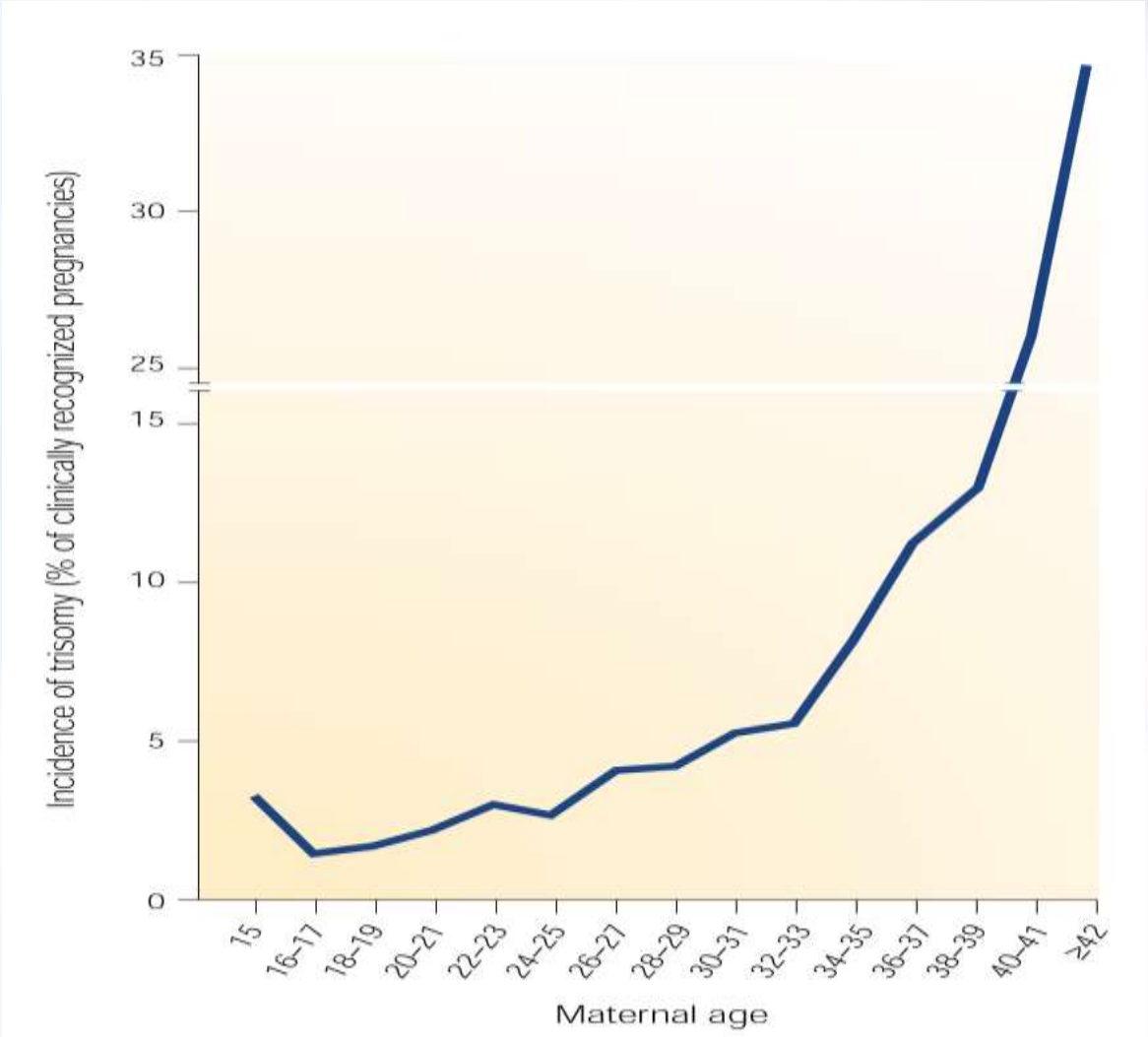
Obesity and infertility in women



Median Age for mothers (Australia)

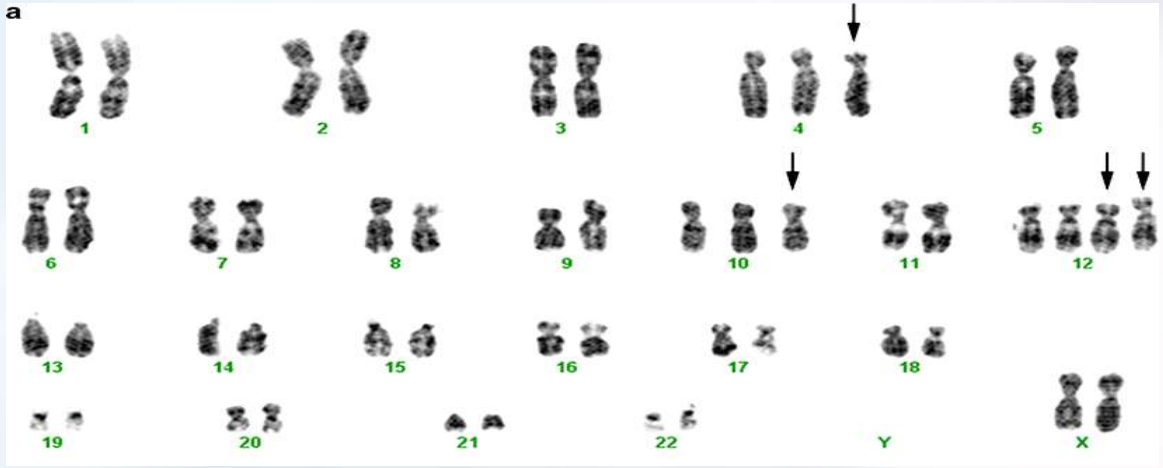
ABS (2007) Births. 3301.0

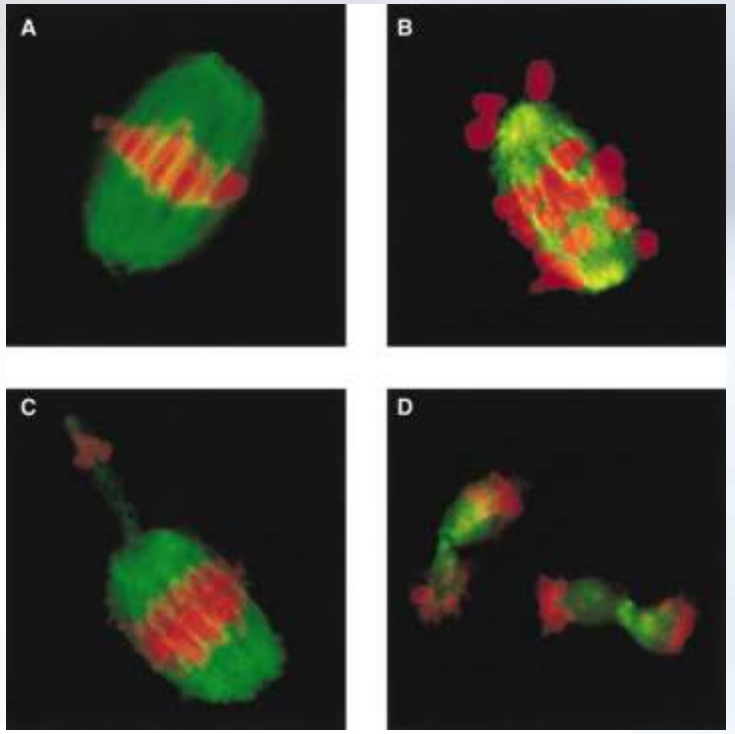
Aneuploidy and age



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

Scale & origin of aneuploidy



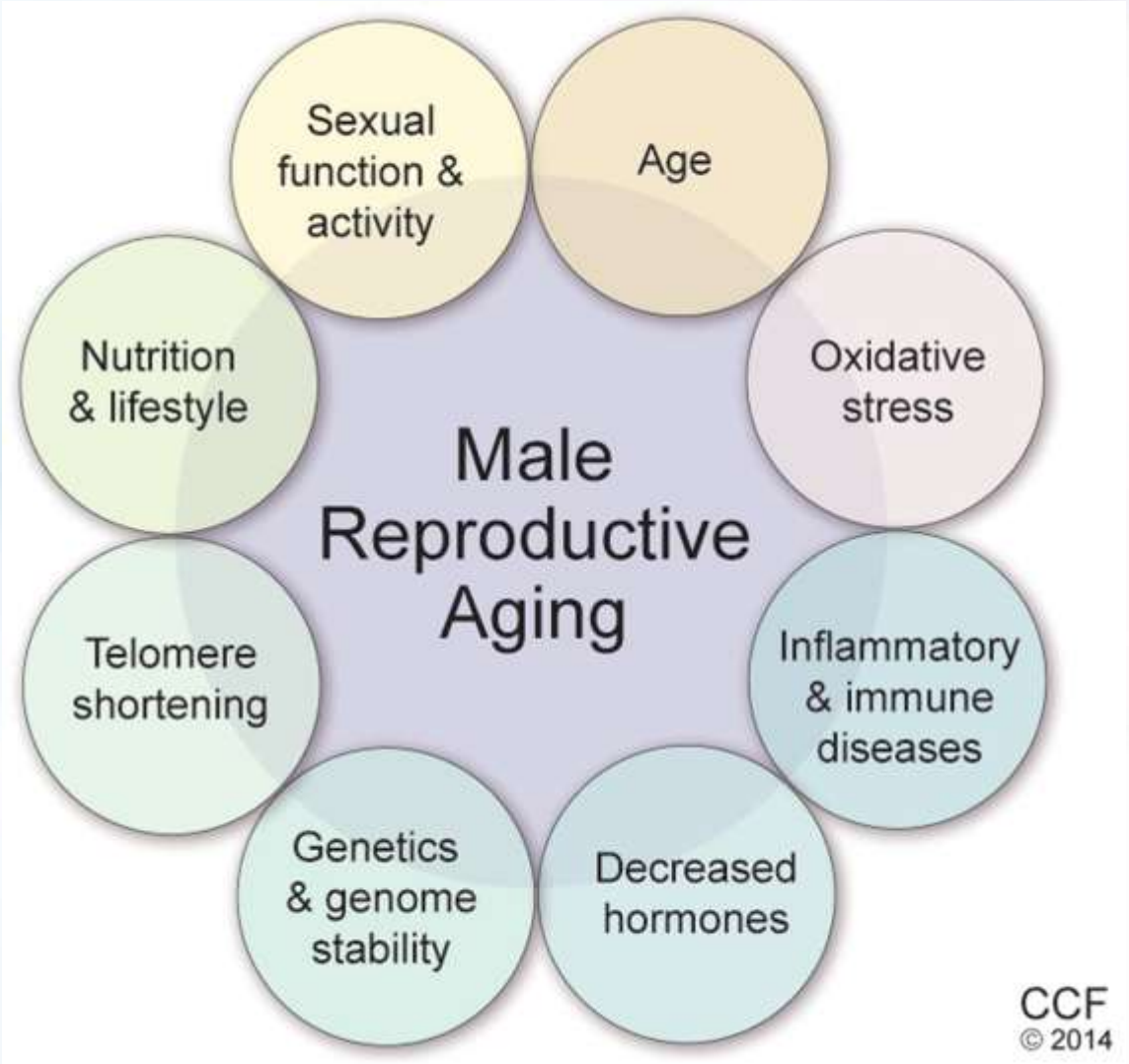


Incidence of aneuploidy during development

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

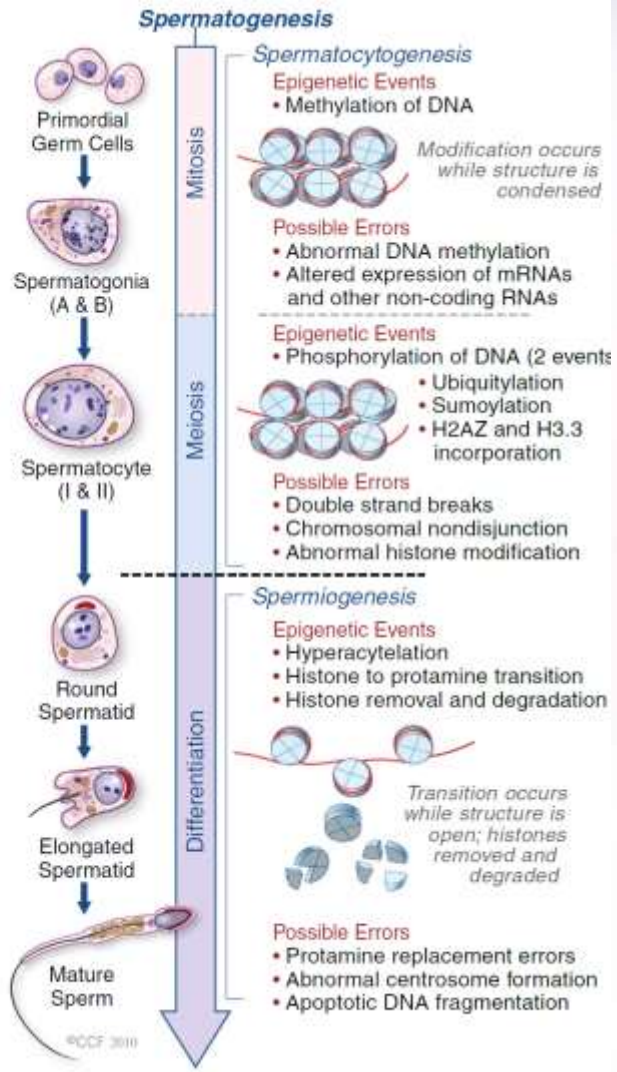
Effects of paternal age on sperm quality



Sharma *et al* (2015) *RBE* 13: 35

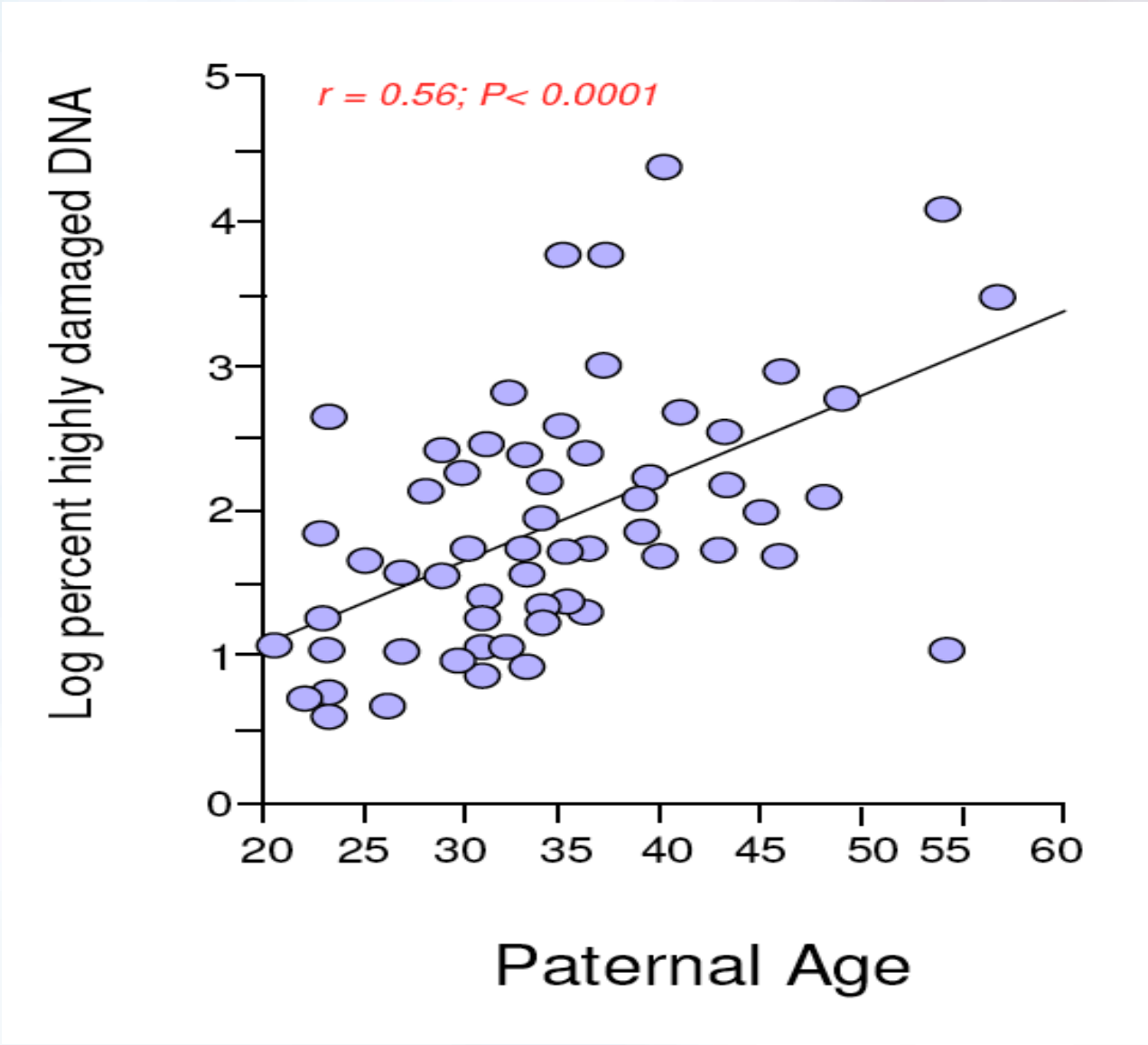
Epigenetic events during spermatogenesis

Fig. 1 Epigenetic events during spermatogenesis. In primodial germ cells (mitosis), DNA methylation occurs to set up the paternal specific imprints. Phosphorylation (in meiotic cell) occurs to assist in both recombination and XY body formation. Ubiquitylation, sumoylation and incorporation of H2AZ and H3.3 variants are all involved in XY body formation. Hyperacetylation occurs during spermiogenesis to assist in the Histone-Protamine exchange. Spermatocytogenesis can also give rise to chromosome non-disjunction during its meiosis I and II along with double strand breaks, abnormal histone modification and alteration in the expression on mRNA and other non-coding RNAs. DNA fragmentation is the consequence of apoptosis following double strand breaks or abnormal protamination during spermiogenesis



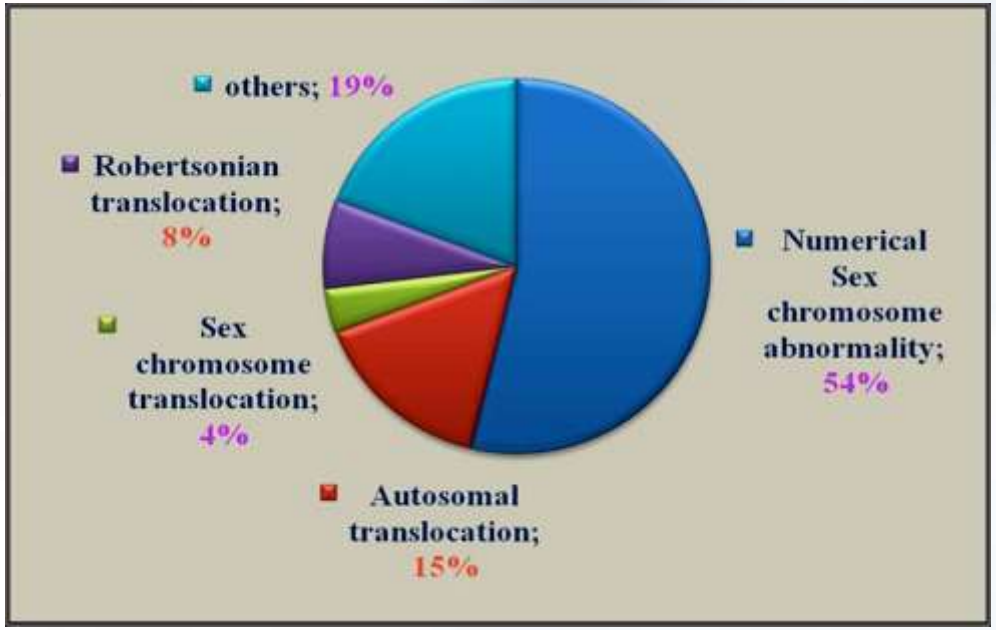
Dada *et al* (2012) *JARG* 29: 213-23

Sperm DNA damage with age

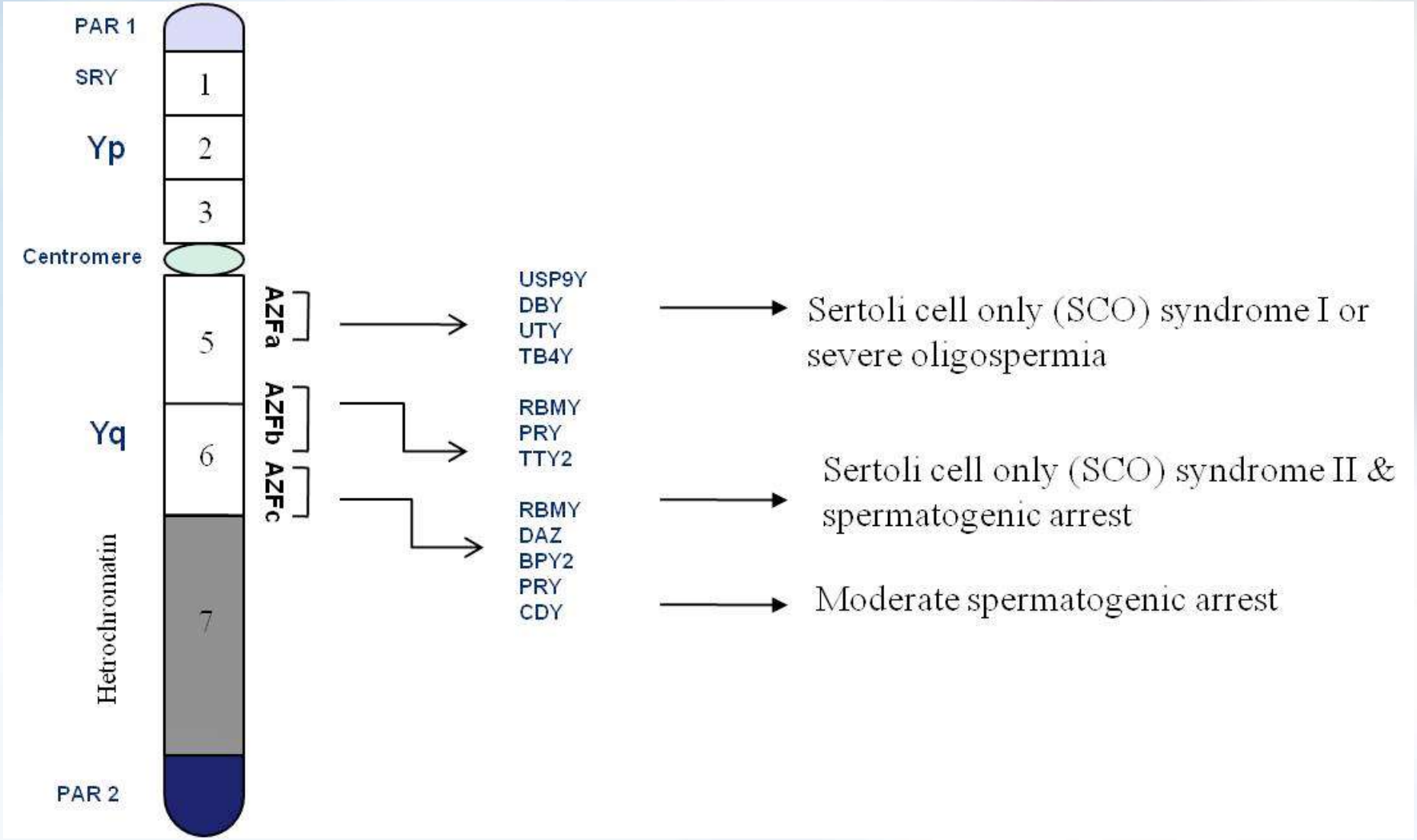


Genetic causes of male subfertility

- Chromosomal (eg. Klinefelter's XXY)
- Translocations & Meiotic errors
- Genome instability
- Point mutations
- Duplications & Deletions



Azoospermia Factor (AZF) regions



Dada *et al* (2011)
Open Reprod Sci J
3: 42-56

Laboratory investigations: Female

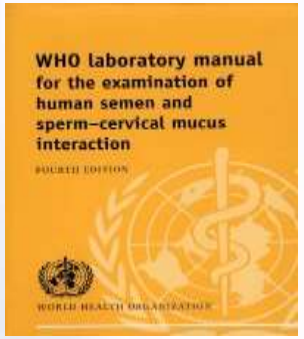
- FSH (Day 2)
- AMH
- Oestradiol
- Progesterone
- Prolactin
- Testosterone
- AsAb
- Kremer test

- Ovarian reserve
- Ovarian reserve
- Folliculogenesis
- Ovulation
- Prolactinaemia
- PCOS
- Immunoinfertility
- Cervical hostility

Laboratory investigations: Male

- Semen volume
- Semen pH
- Sperm density
- Sperm motility
- Sperm shape
- Agglutination
- Sperm DNA
- FSH
- Retrograde ejac’n
- Occlusions
- Spermatogenesis
- Sperm transport
- Sperm binding
- Sperm function
- Sperm quality
- Spermatogenesis

Functional evaluation of sperm populations – the semen analysis



Volume:	2.0	2.0	1.5 (1.4-1.7)
Concn (x10^6ml):	20	20	15 (12-16)
Total count(x10^6):	40	40	39 (33-46)
Prog Motility (%):	50	50	32 (31-34)
% normal morph:	30	-	4 (3.0-4.0)
% MAR:	20	50	<50

Predictive factors

Only TOTAL MOTILE COUNT (TMC)

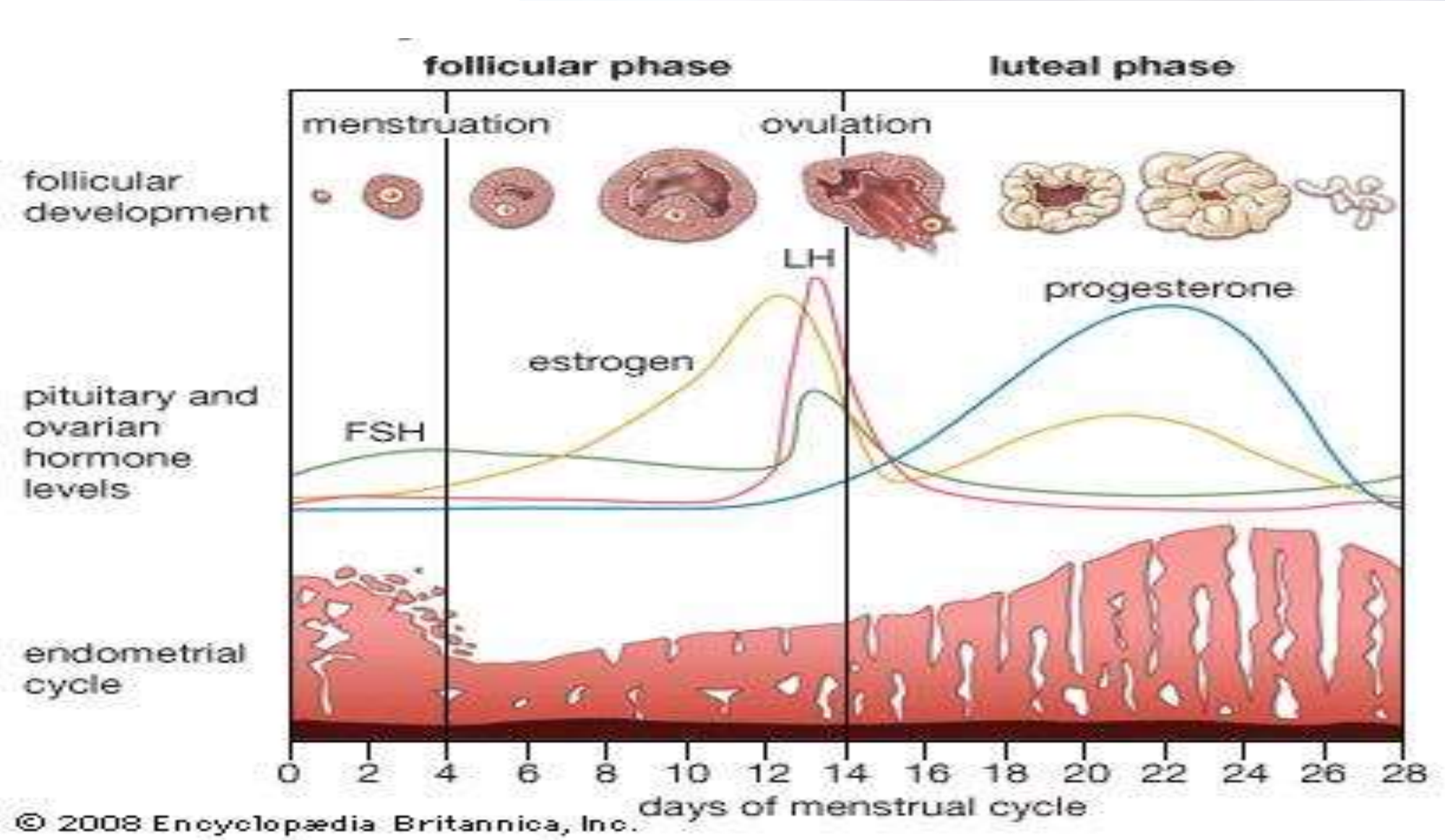
Small et al (1987) CMAJ 136, 829

Table I — Life-table estimates of 36-month cumulative pregnancy rates, by seminal variables, among 709 couples with no evident female infertility factor

Variable	Cumulative pregnancy rate, % (and standard error [SE])	p*
Sperm count, × 10^6/ml		NS
≤ 9 (n = 75)	30 (6)	
10–19 (n = 44)	42 (8)	
≥ 20 (n = 590)	50 (3)	
Seminal volume, ml		NS
1–2 (n = 229)	46 (5)	
3–9 (n = 480)	48 (3)	
% of progressively motile sperm		NS
≤ 25 (n = 107)	36 (7)	
26–50 (n = 175)	41 (5)	
51–100 (n = 427)	52 (3)	
% of sperm with abnormal morphologic features		NS
≤ 50 (n = 494)	51 (4)	
51–100 (n = 215)	39 (5)	
Total motile sperm count, × 10^6/ejaculate		0.001
≤ 9 (n = 83)	20 (6)	
10–19 (n = 43)	37 (9)	
≥ 20 (n = 583)	52 (2)	

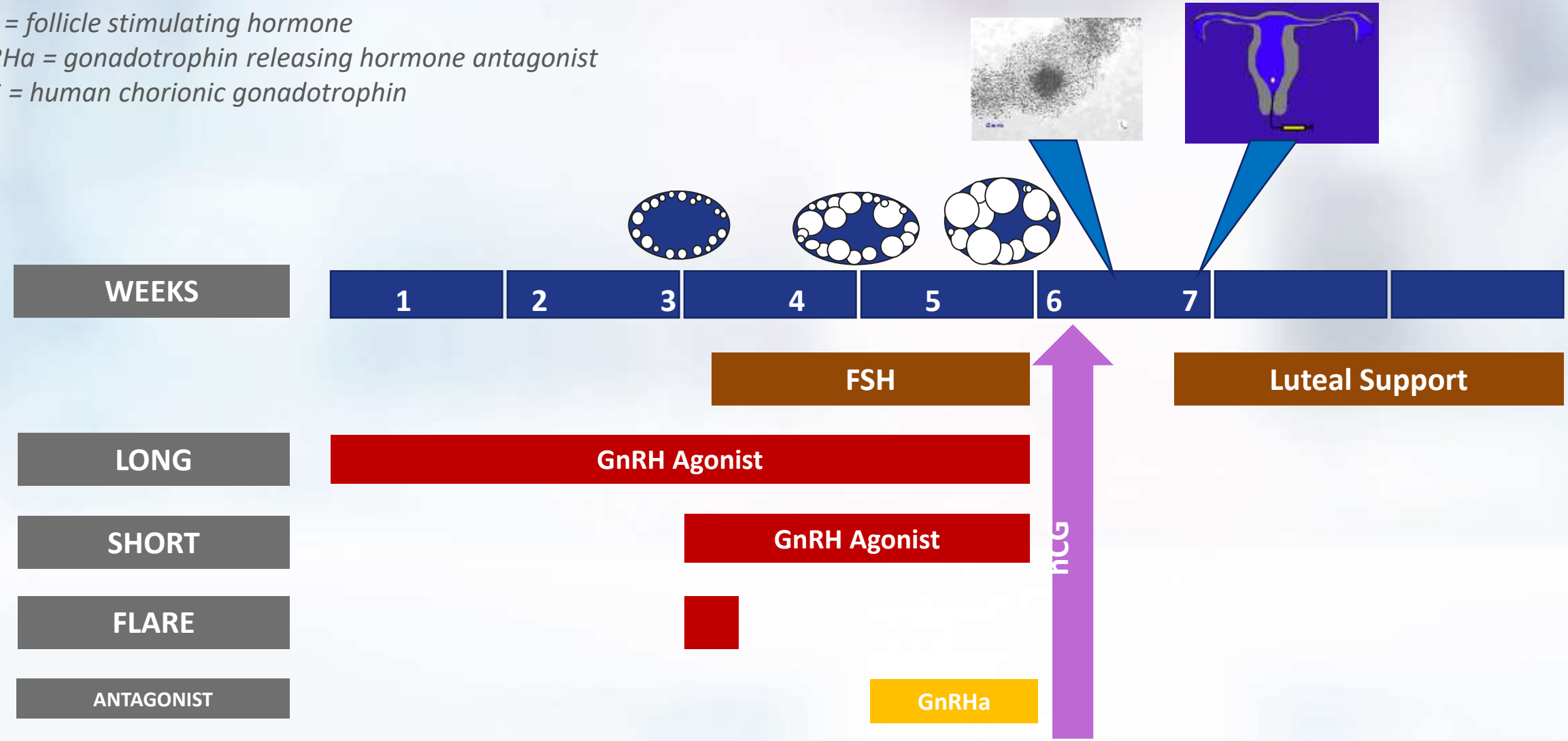
*NS = not significant.

Menstrual Cycle

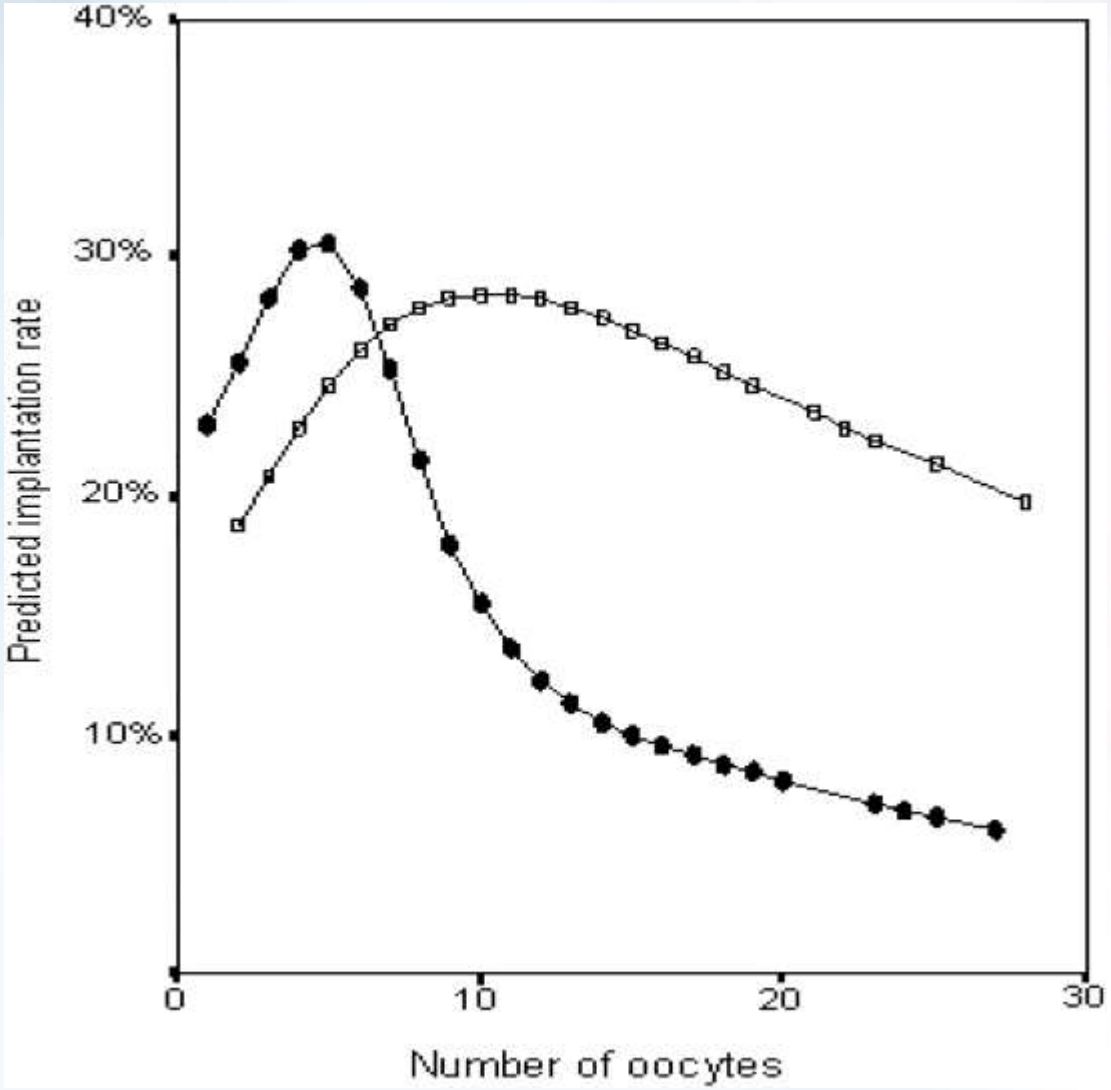


Ovarian Stimulation - regimens

FSH = follicle stimulating hormone
GnRHa = gonadotrophin releasing hormone antagonist
hCG = human chorionic gonadotrophin



Ovarian Stimulation - regimens



More oocytes does not always mean better results

Possibly optimised results with low numbers after mild stimulation

Potentially detrimental effects of supra-physiological levels of estrogens

Verberg *et al* (2009) *Hum Reprod Update* 15:5-12

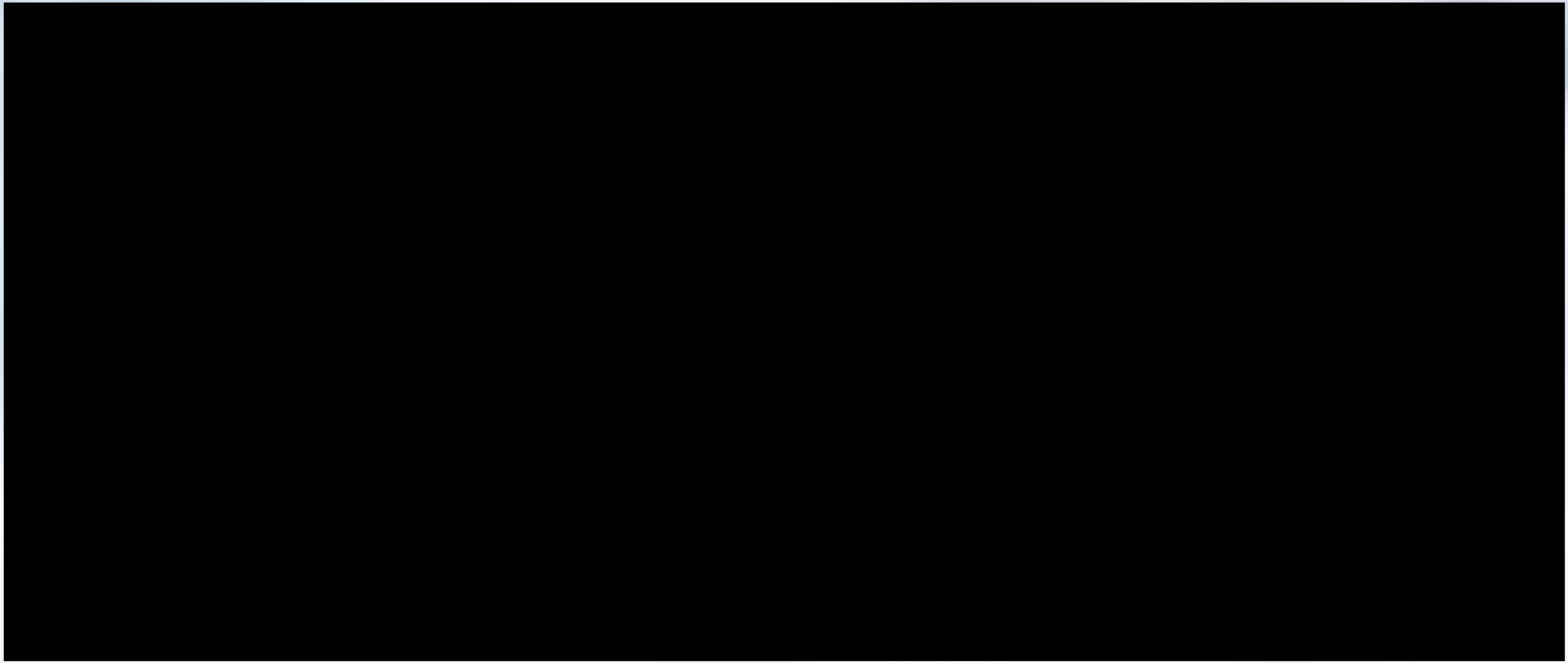
Oocyte Retrieval – Egg Collection

Heated & Adjustable Operating Table

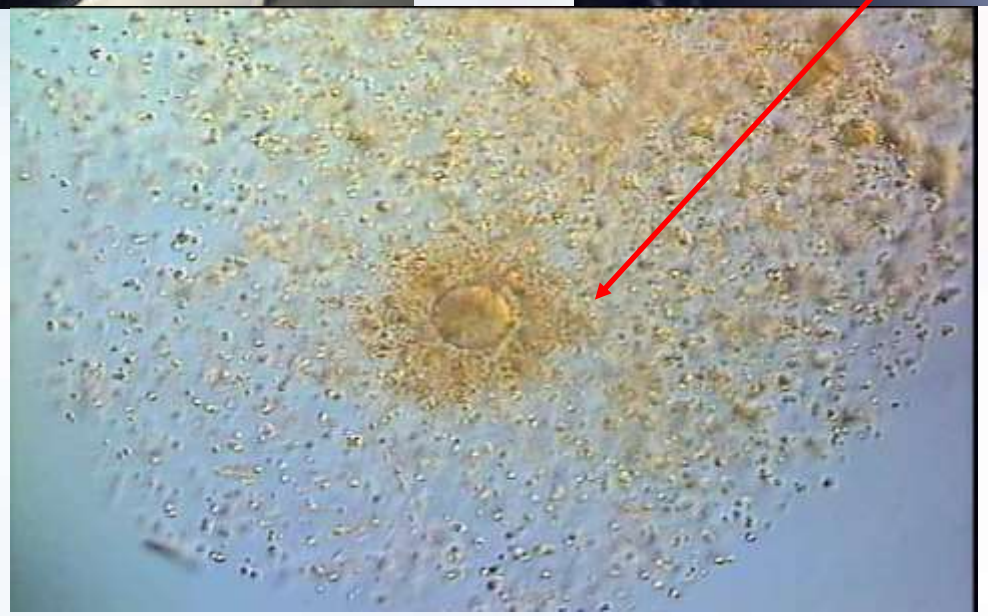
Single & Dual Lumen Needle Sets & Tube Heater

Digital Suction Pump System

Oocyte recovery procedure



Cumulus-Oocyte-Complexes in follicular fluid



Oocyte Maturational Stages

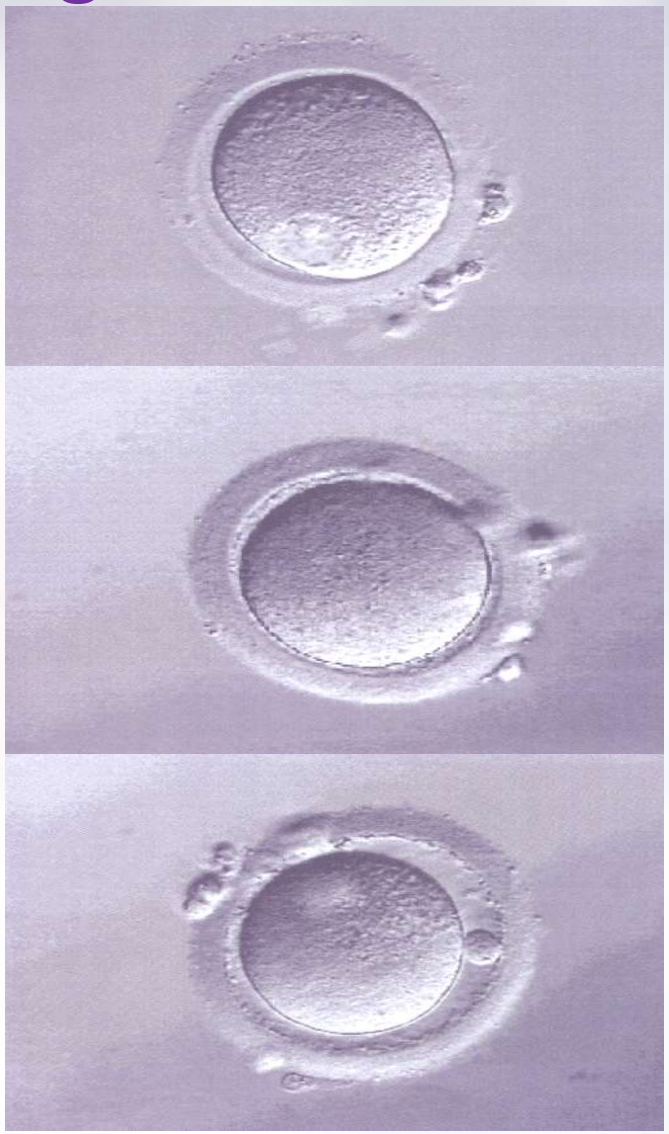
1. Germinal vesicle.
   Immature.

2. Oocyte in metaphase I (MI), the polar body (PB) not extruded.

   Might mature in Culture!

3. Oocyte in metaphase II (MII), the first PB is extruded.

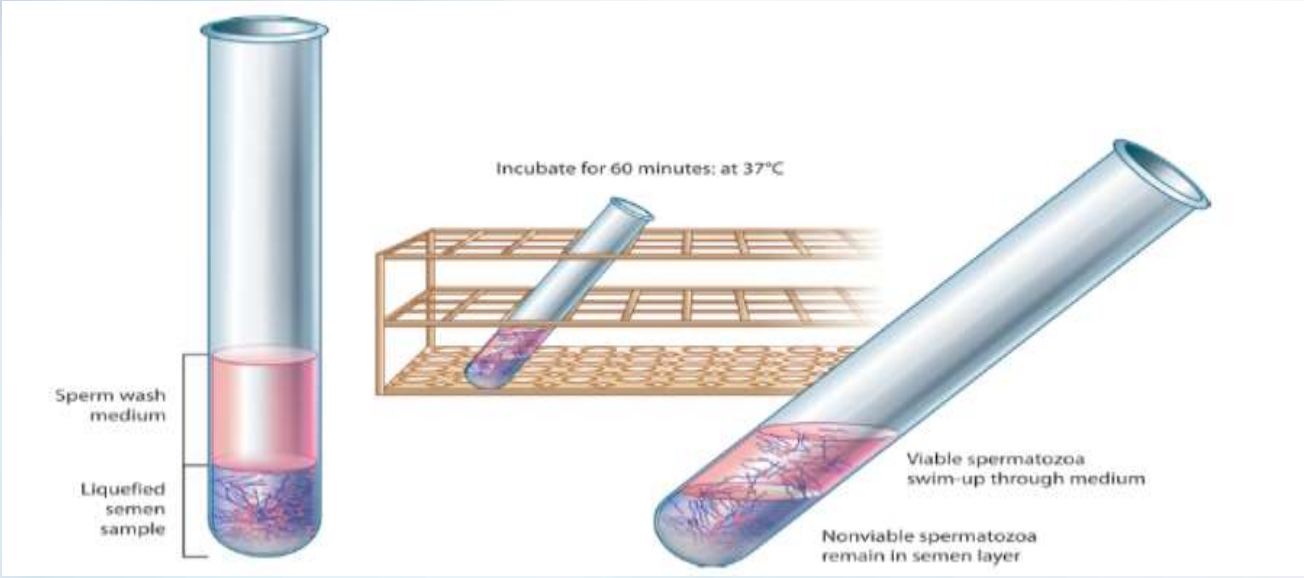
   Mature!

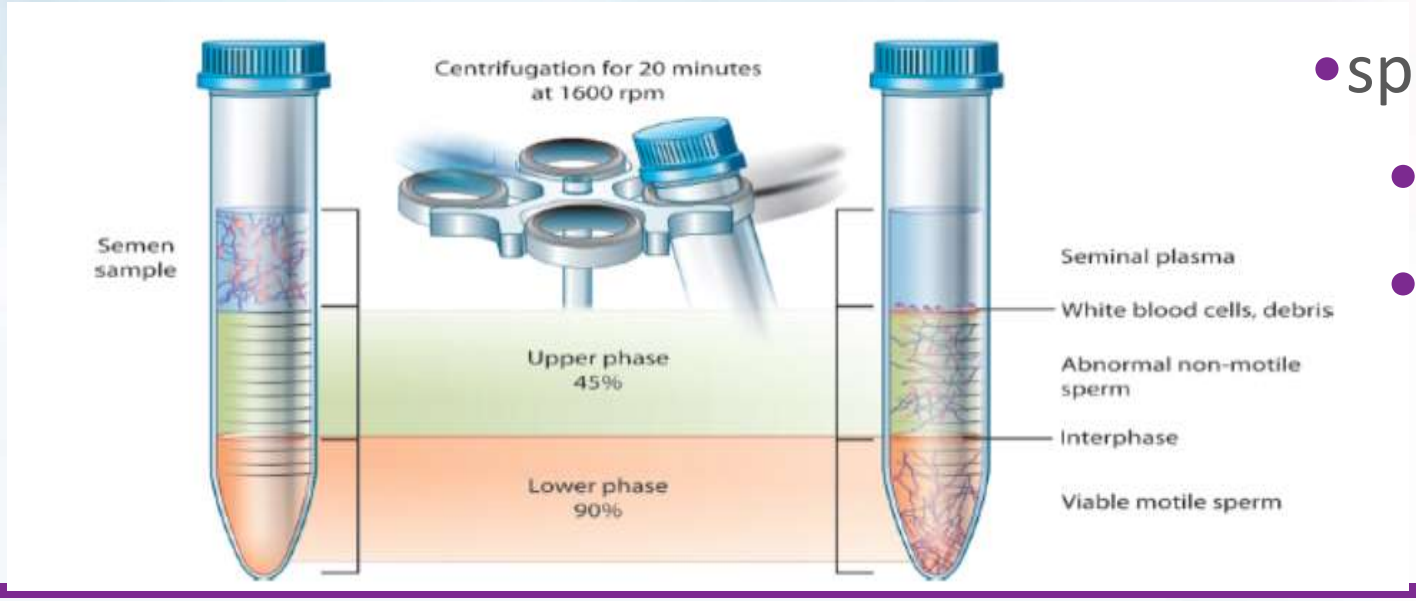


Sperm Preparation & Selection

- remove all seminal fluid
- remove all non-sperm cells
- isolate normal, motile sperm
- support capacitation
- reduce %DNA frag

Sperm Preparation & Selection





- vary time, speed (g) and/or gradient
  - (%, volume, number)
- reduced DNA fragmentation
- effects of temperature
  - pre-incubation
- sperm concentration
  - depends on use (IUI v IVF v ICSI)
  - 5 M/ml motile

Retrograde ejaculates

Development and in vitro testing of a new method of urine preparation for retrograde ejaculation; the Liverpool solution

Thomas R. Aust, M.B., Ch.B.,[a] Stephanie Brookes, B.Sc.,[a] Stephen A. Troup, Ph.D.,[a] William D. Fraser, M.D.,[b] and D. Iwan Lewis-Jones, M.D.[a]

Liverpool Solution

Dilution required: 1 in 8, 8.4% (wt/vol) sodium bicarbonate in sterile distilled water. This solution then is diluted 50:50 with normal saline.

APPENDIX TABLE 1

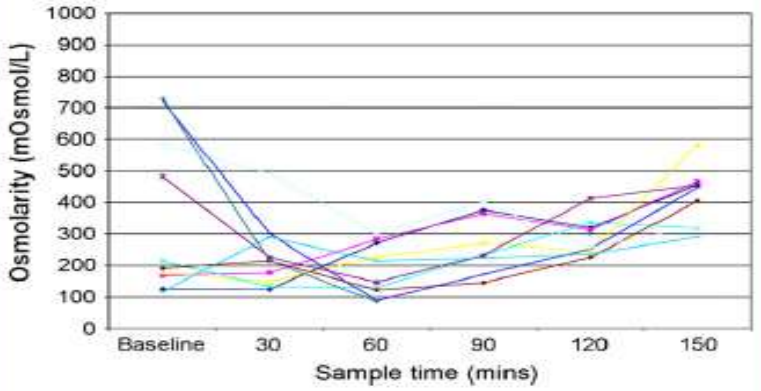
Liverpool solution drink volumes and other patient instructions.

Time from start (min)	Liverpool solution drink volume (mL)	Instruction
0	500	
30	250	
60	250	
90	250	Urinate to empty bladder completely
120	Nil	Ejaculate and void mixture

Aust. Retrograde ejaculation urine preparation. Fertil Steril 2008.

FIGURE 1

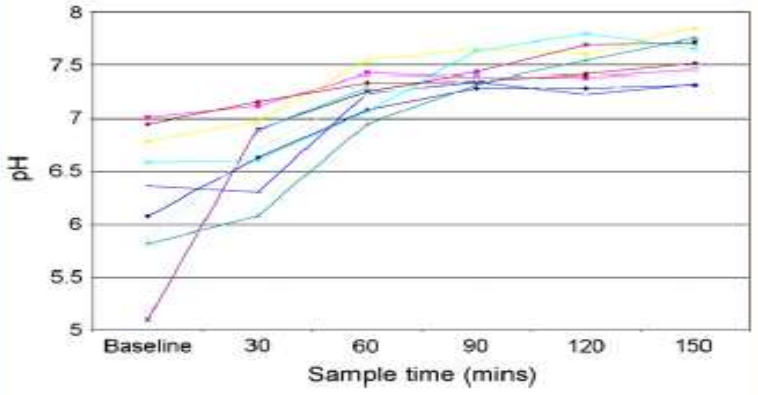
Change in urinary osmolarity in 10 subjects ingesting Liverpool solution.



Aust. Retrograde ejaculation urine preparation. Fertil Steril 2008.

FIGURE 2

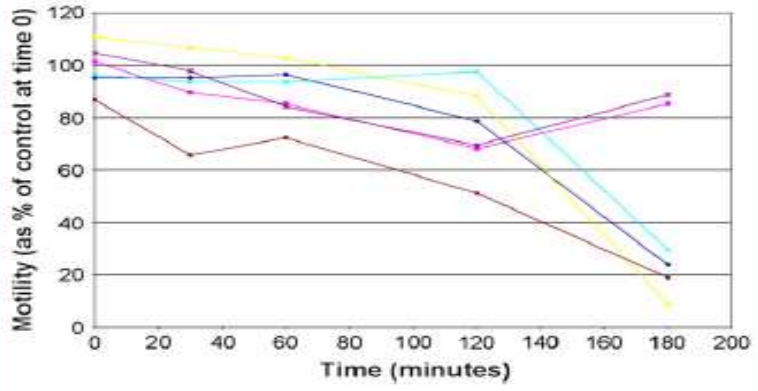
Change in urinary pH in 10 subjects ingesting Liverpool solution.



Aust. Retrograde ejaculation urine preparation. Fertil Steril 2008.

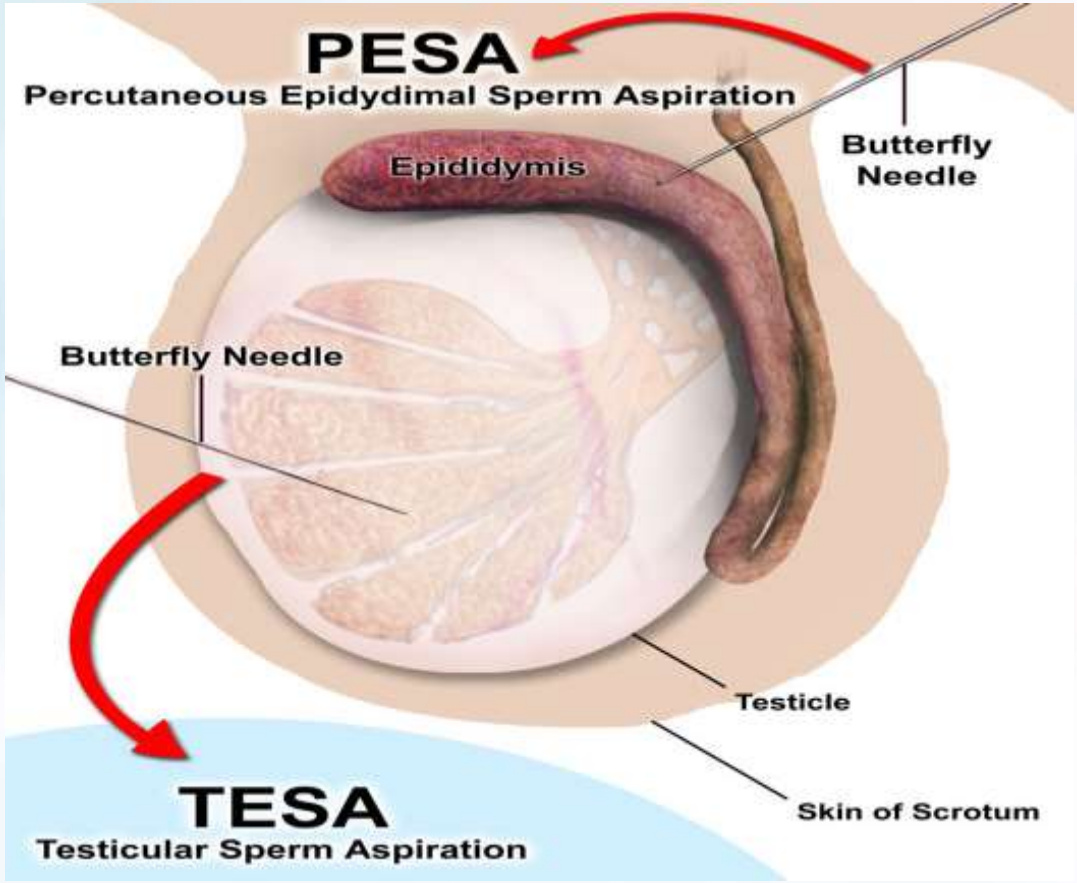
FIGURE 4

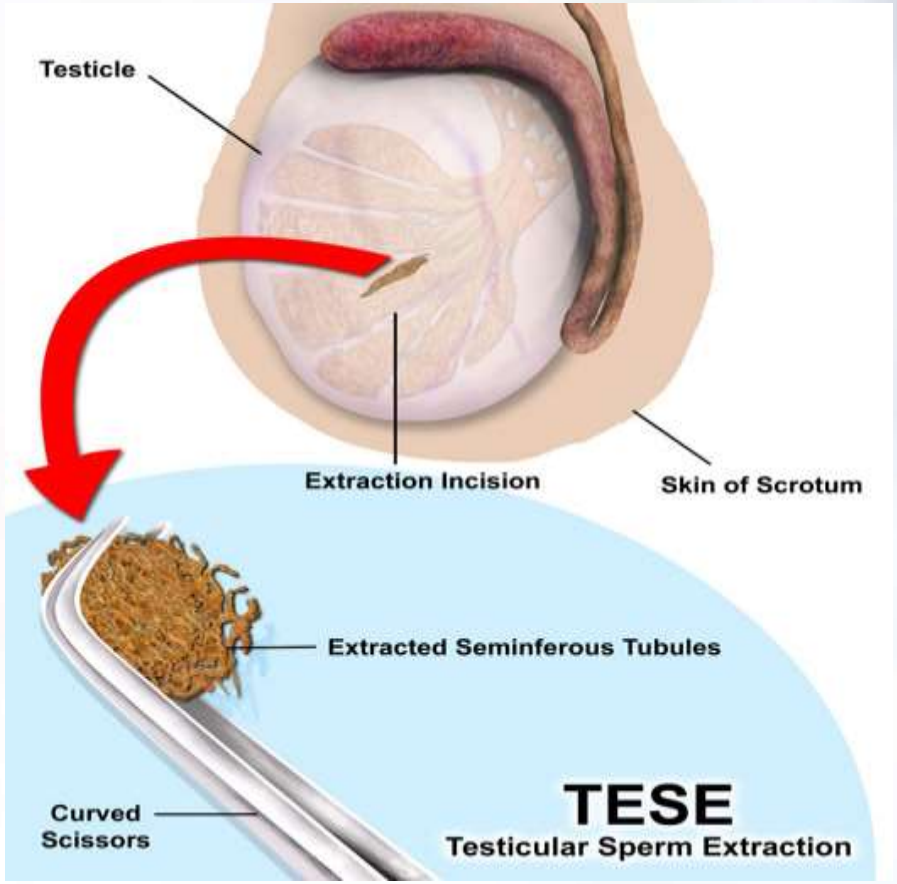
Change in progressive motility (grade A and B) with time in sperm exposed to prepared urine (n = 6).



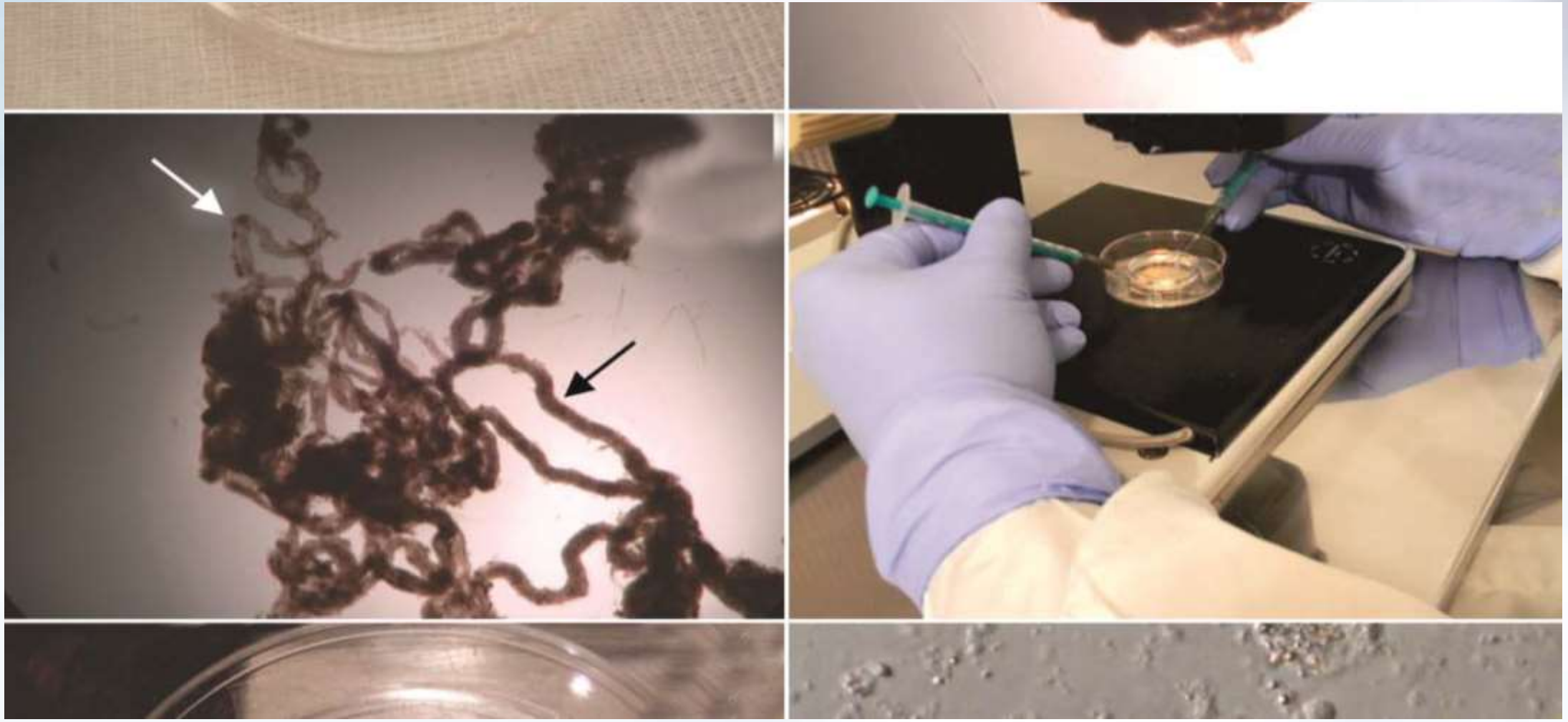
Aust. Retrograde ejaculation urine preparation. Fertil Steril 2008.

Surgical sperm retrieval

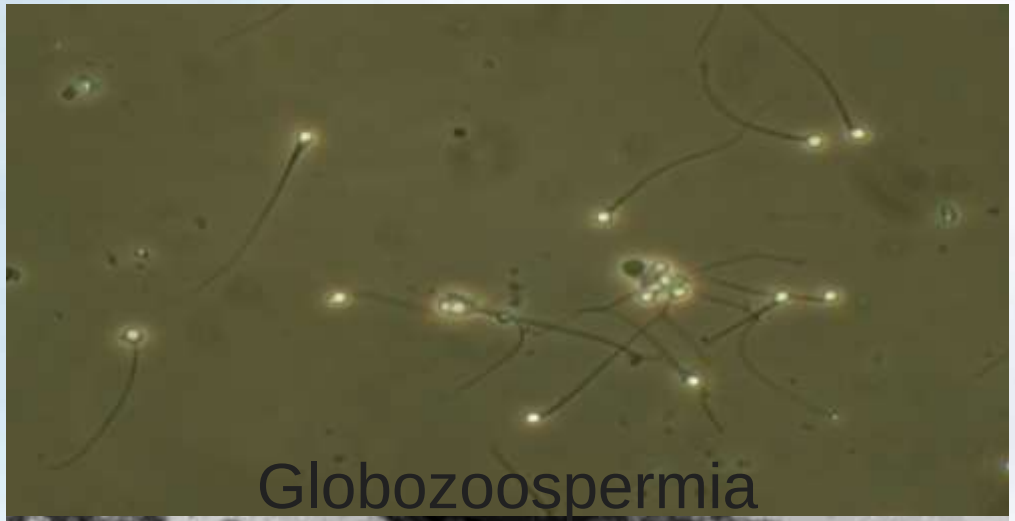




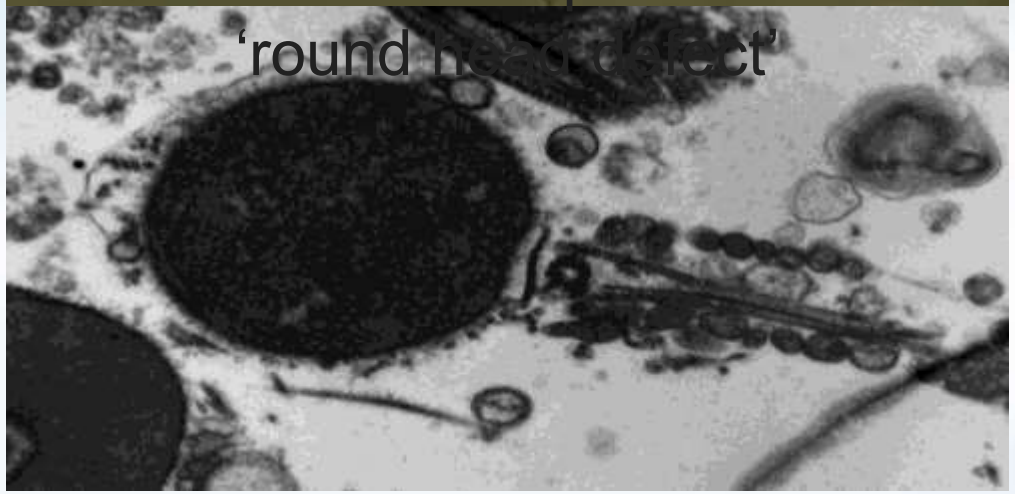
Mechanical dissection



Sterilising sperm defects



Globozoospermia



‘round head defect’

Stump tail defect

Specialised fertilisation media

Higher glucose supports

- sperm maturation

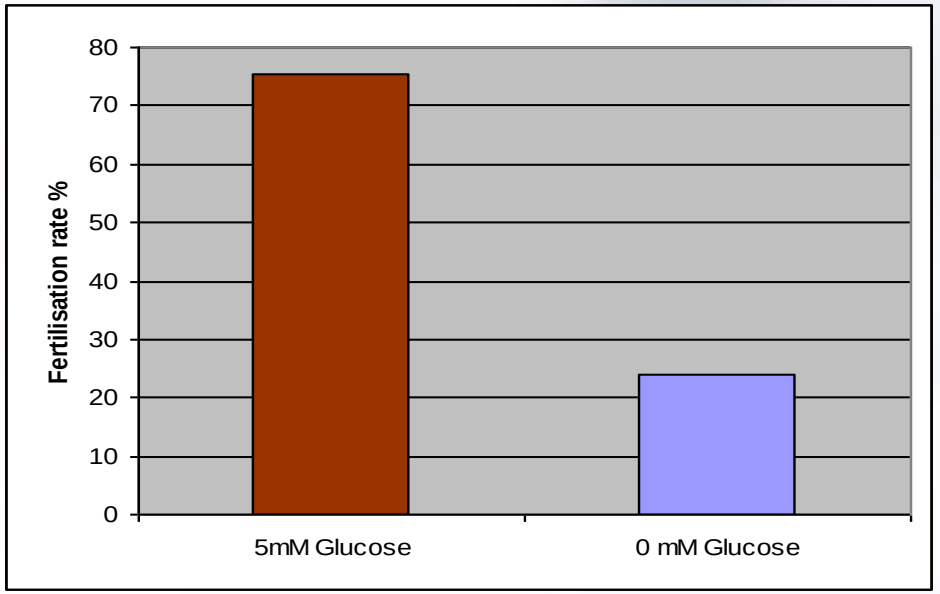
- sperm function:

  -capacitation

  -binding

  -acrosome reaction

  -fertilisation



Mahadevan 1997

Sequential v Single-step Culture Media

PHASE 1

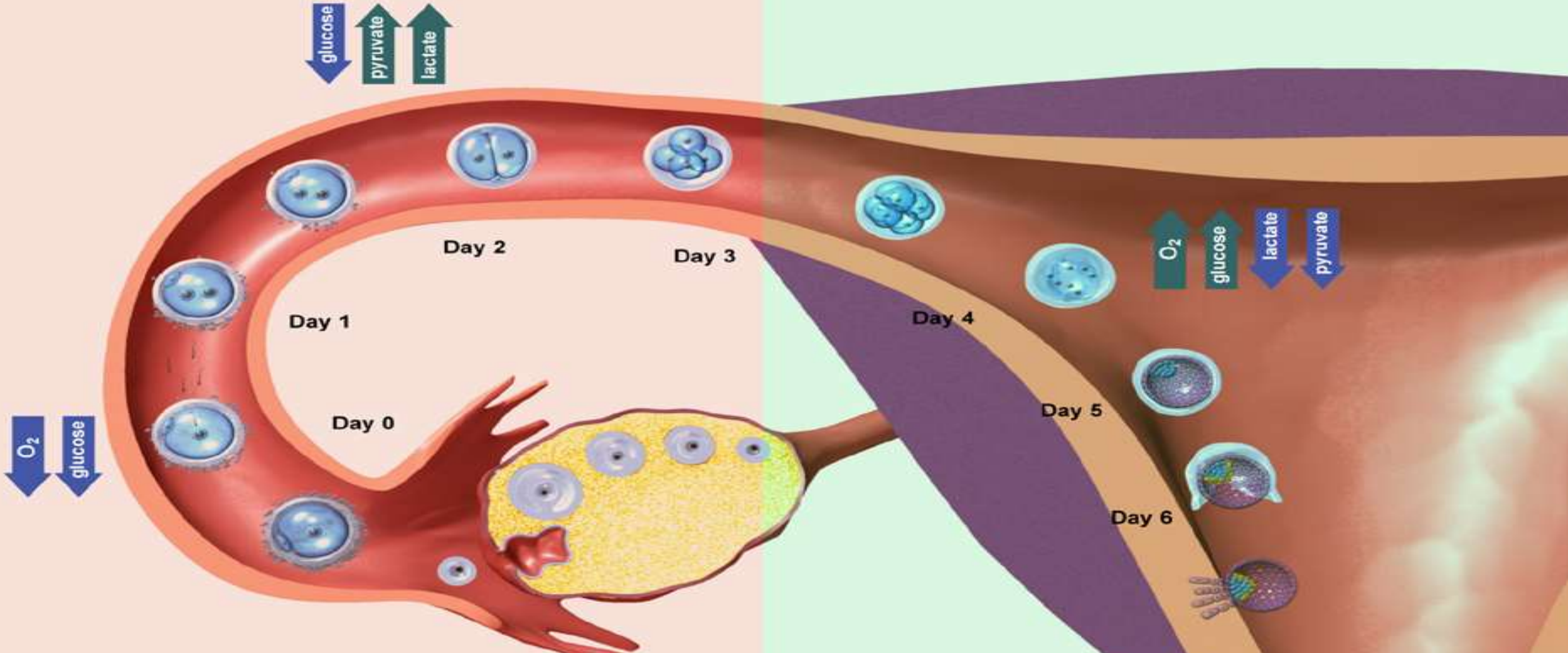
Embryo genome is inactive

Low metabolism, development controlled by maternal gene transcripts

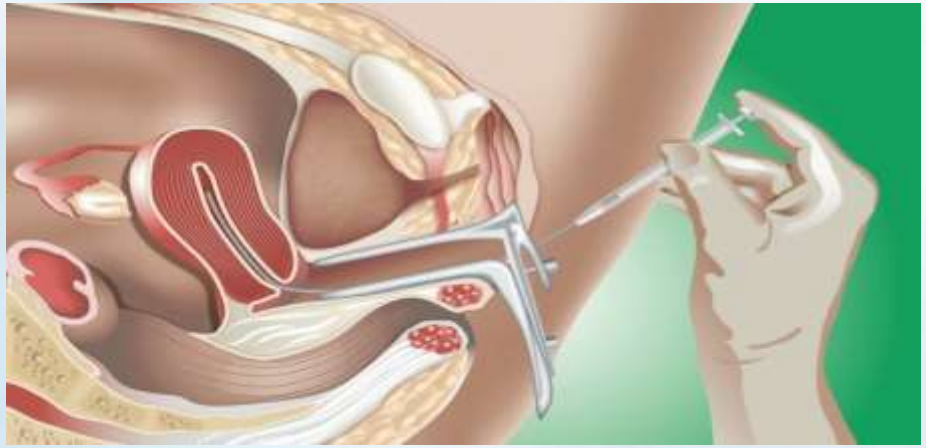
PHASE 2

Embryo genome is activated

Metabolism rises. Proteins, many growth factors and receptors are produced.

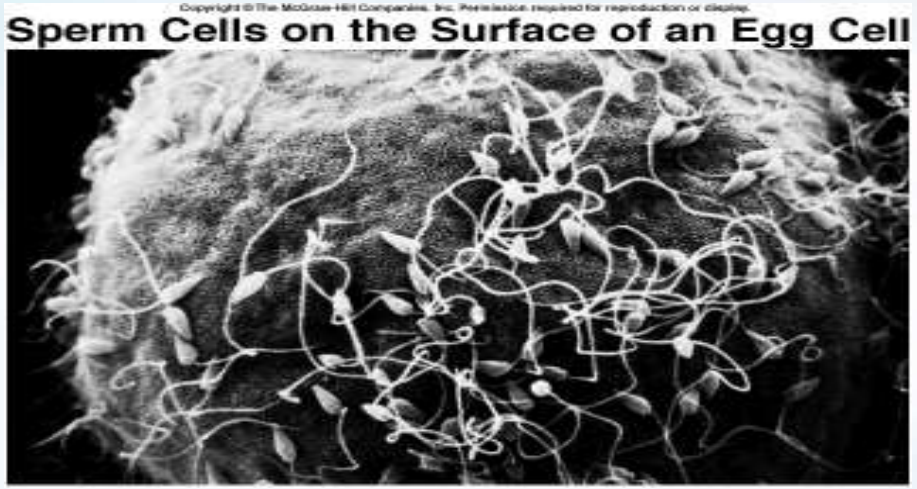


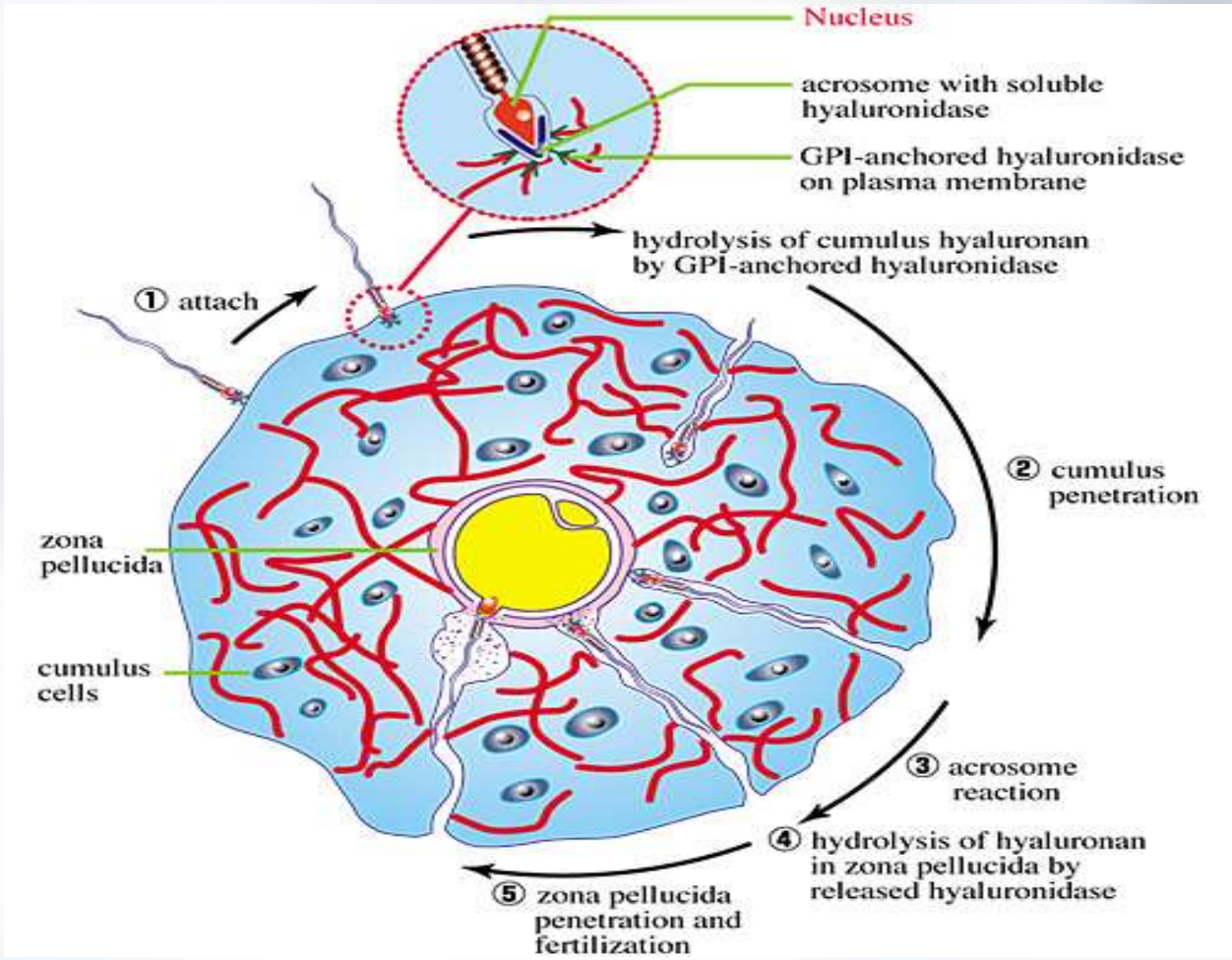
Intrauterine insemination (IUI)



- often the first step in ART
- prepared sperm injected into the uterine cavity
- 3-6 cycles of IUI often attempted before other more invasive fertility treatment
- with or without ovarian stimulation

IVF - Insemination

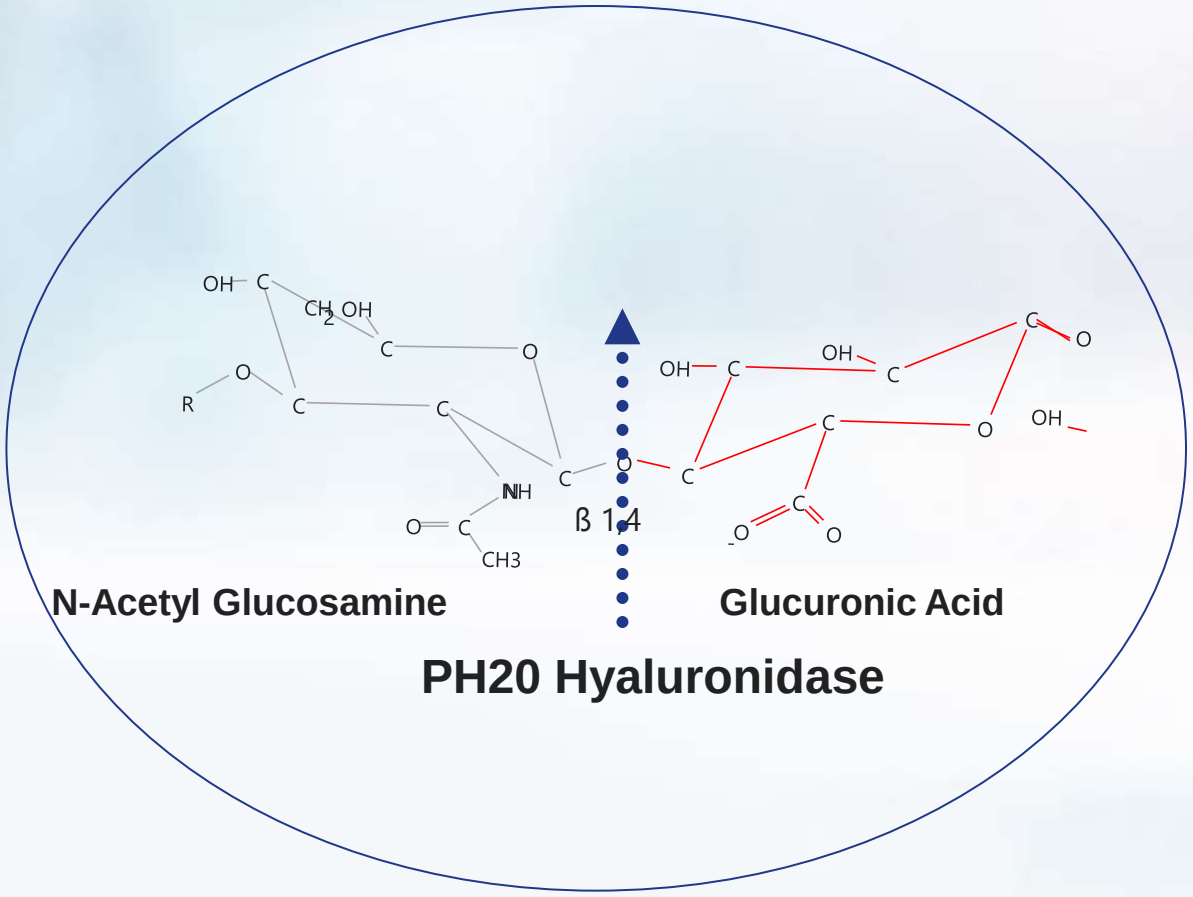




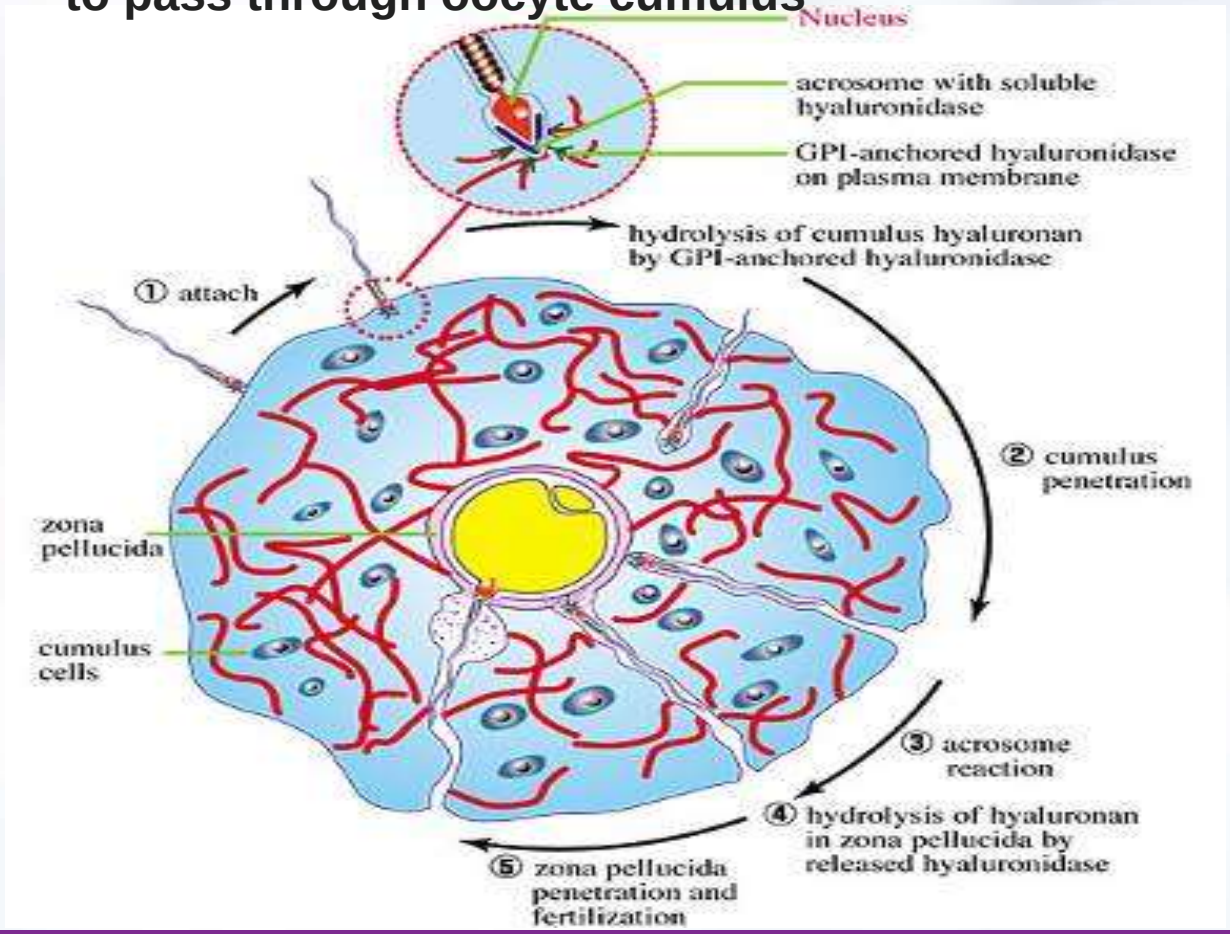
Denudation of Oocytes

Natural in vivo function:

Hyaluronidase enzyme breaks the HA bonds connecting cumulus cells

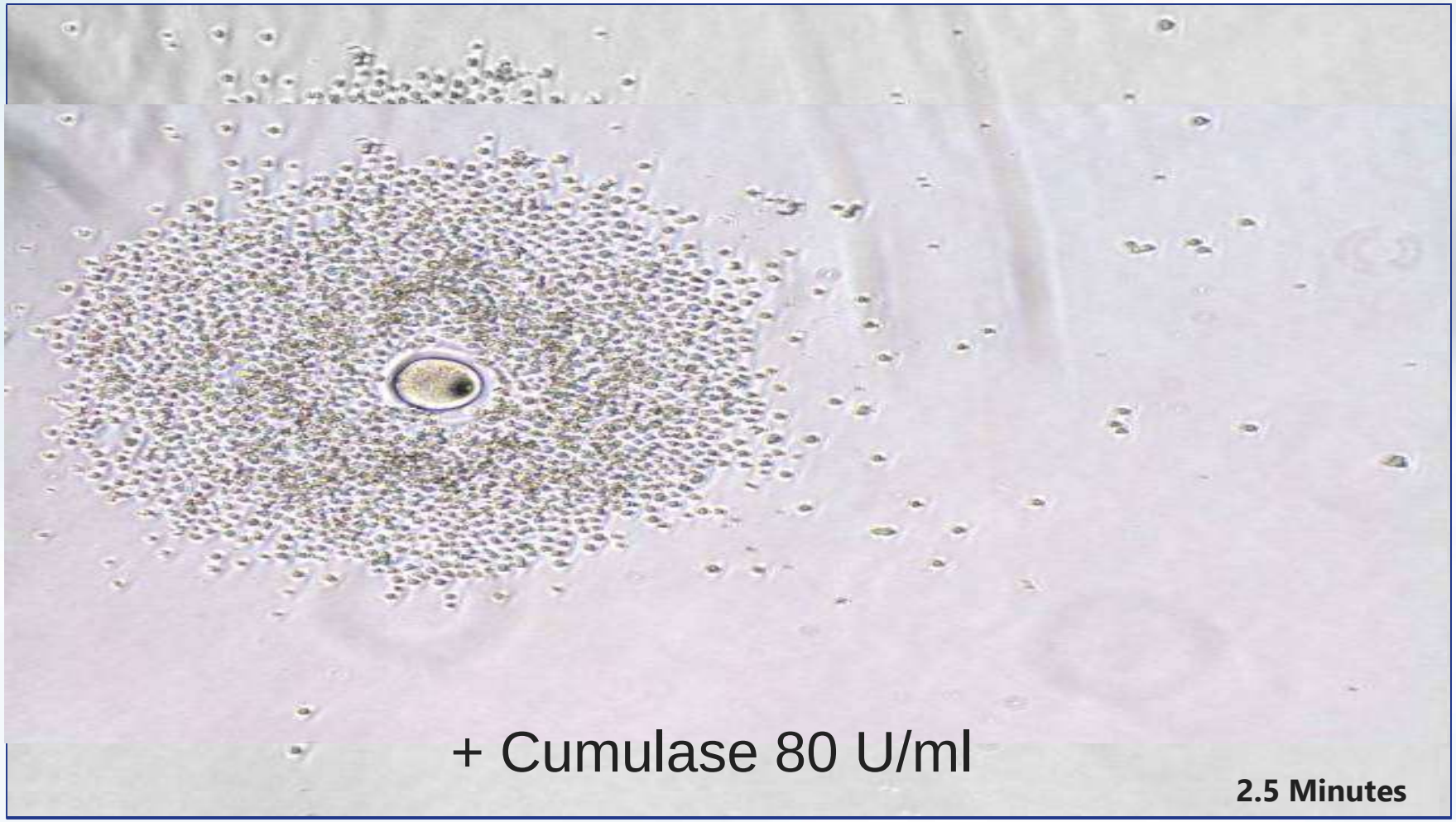


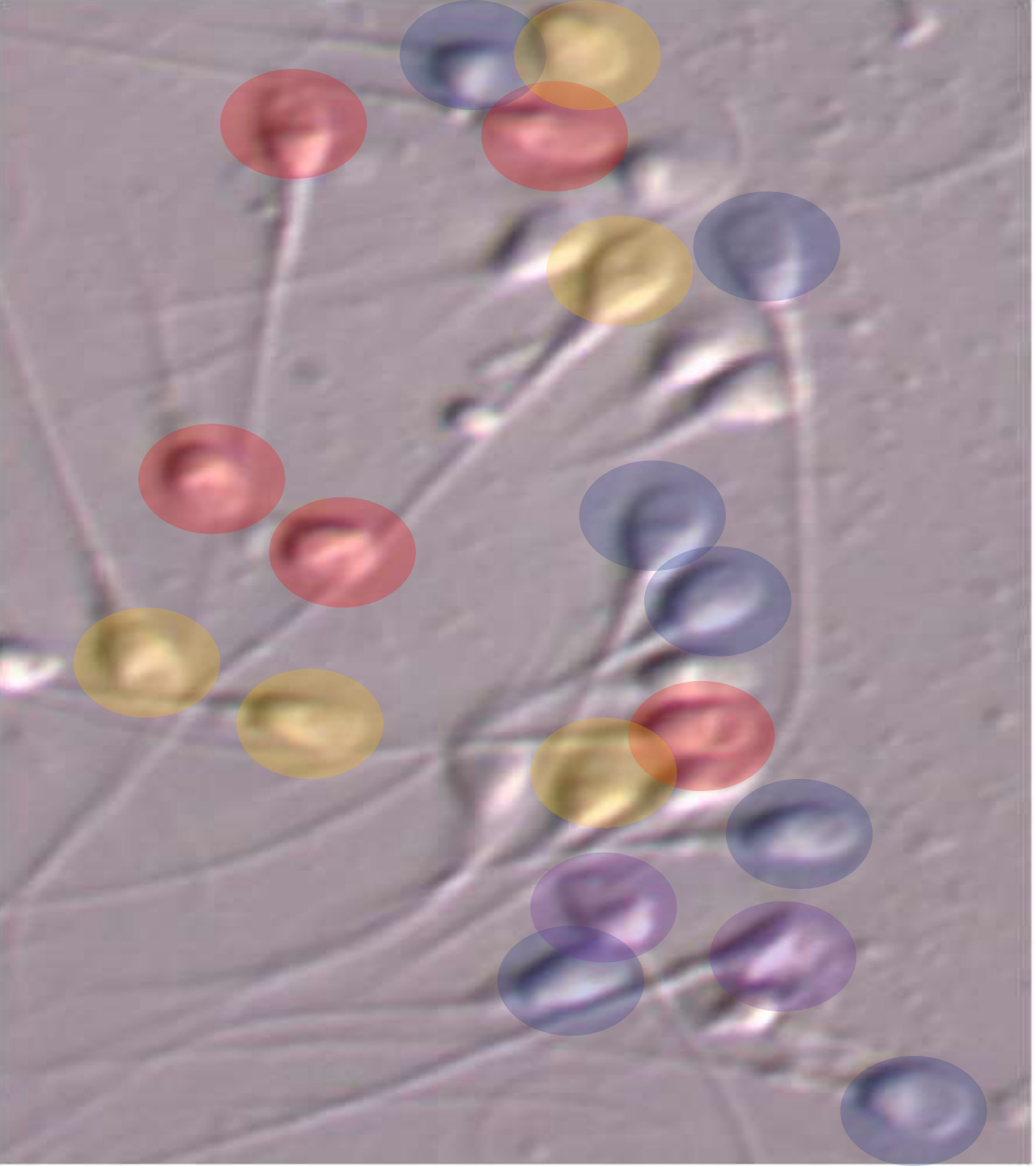
Sperm uses hyaluronidase enzyme to pass through oocyte cumulus



ICSI-Cumulase® (rHuPH20)

smoothly removes the cumulus matrix



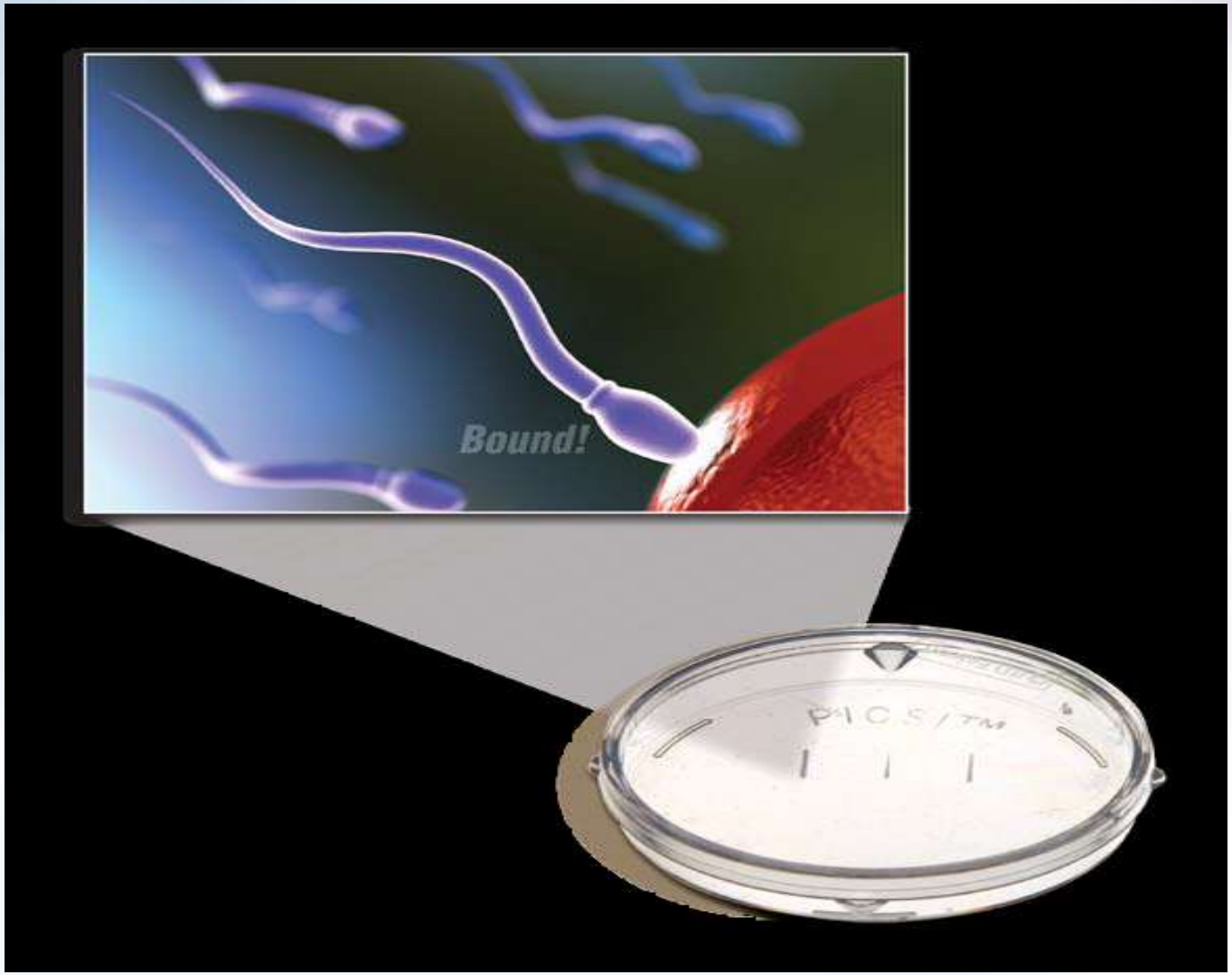


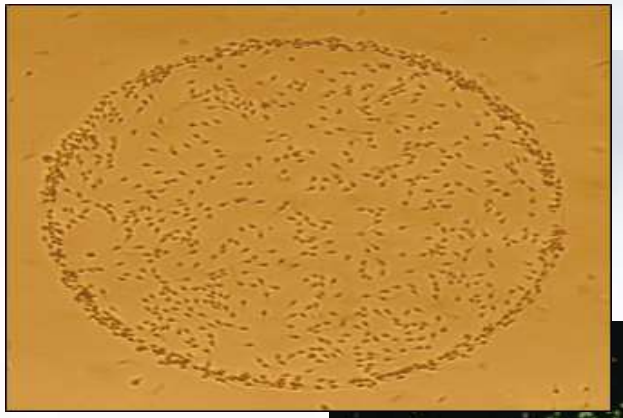
Grade	Description
I	No Vacuolation
II	<2 Small Vacuoles
III	>2 small or >=1 large Vaculoes
IV	Large Vacuole and other head abnormalities

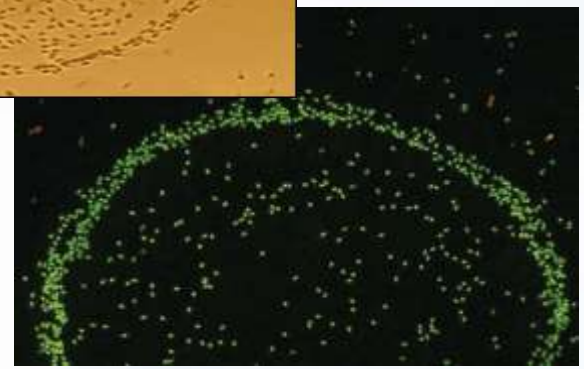
Reproductive BioMedicine Online

Vanderzwalmen et al, 17(5): 617-627, 2008

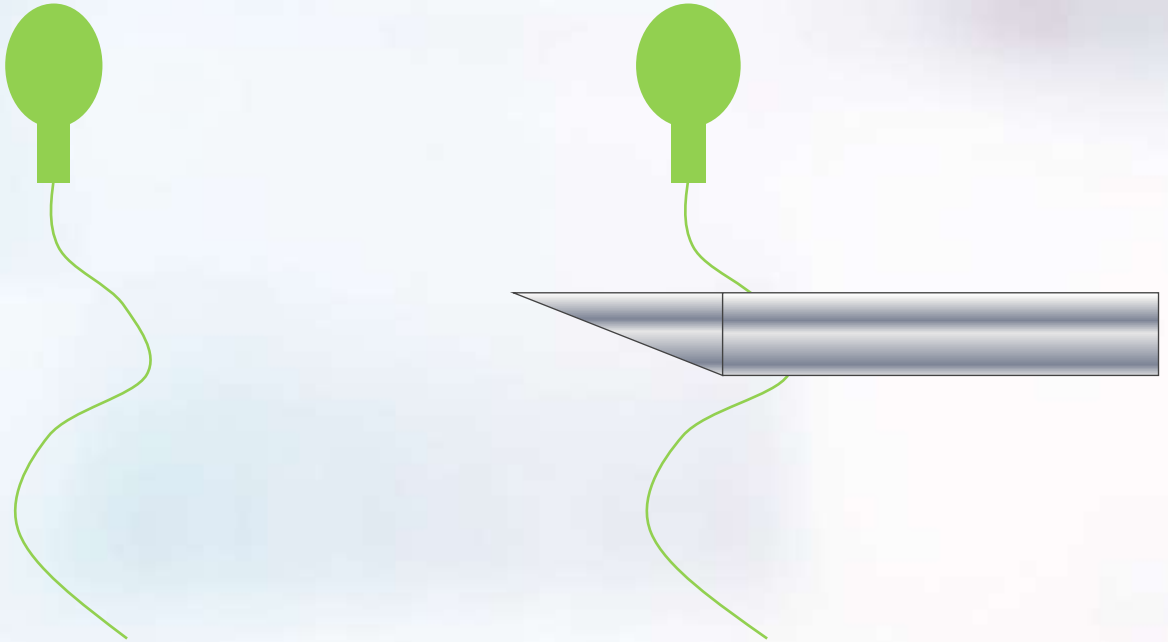
Sperm Selection – HA binding (PICSI)

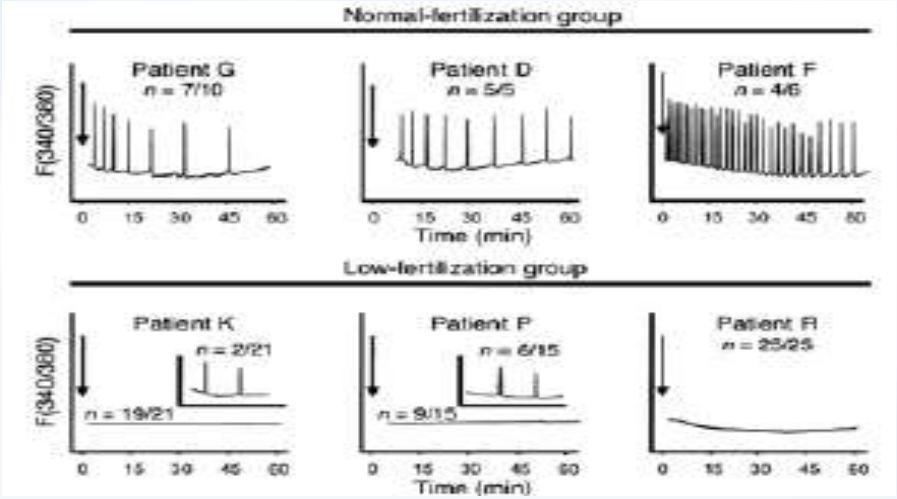


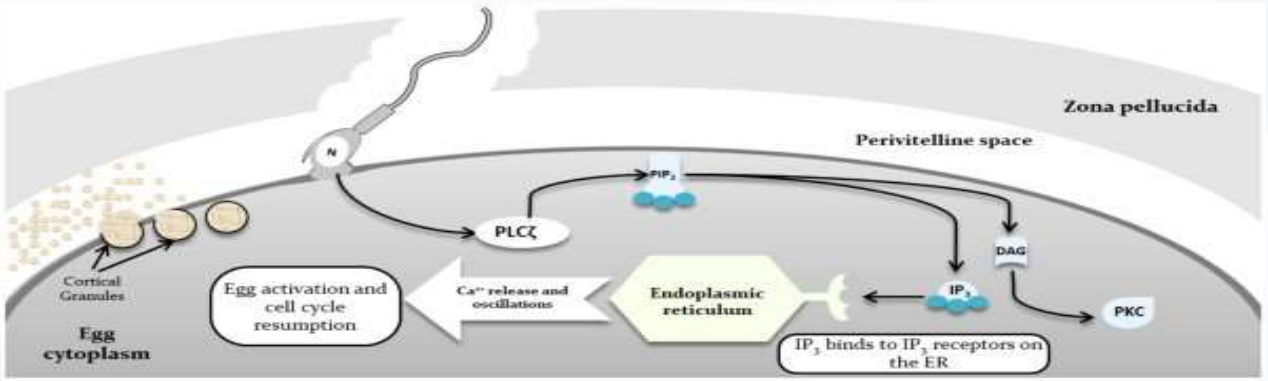




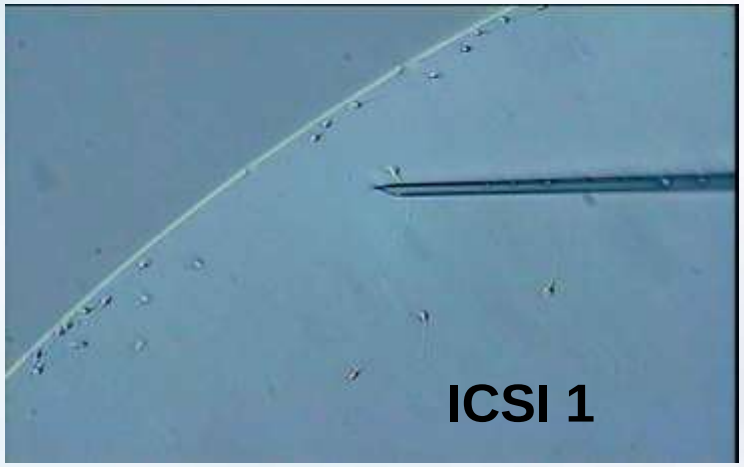
Sperm immobilisation for ICSI



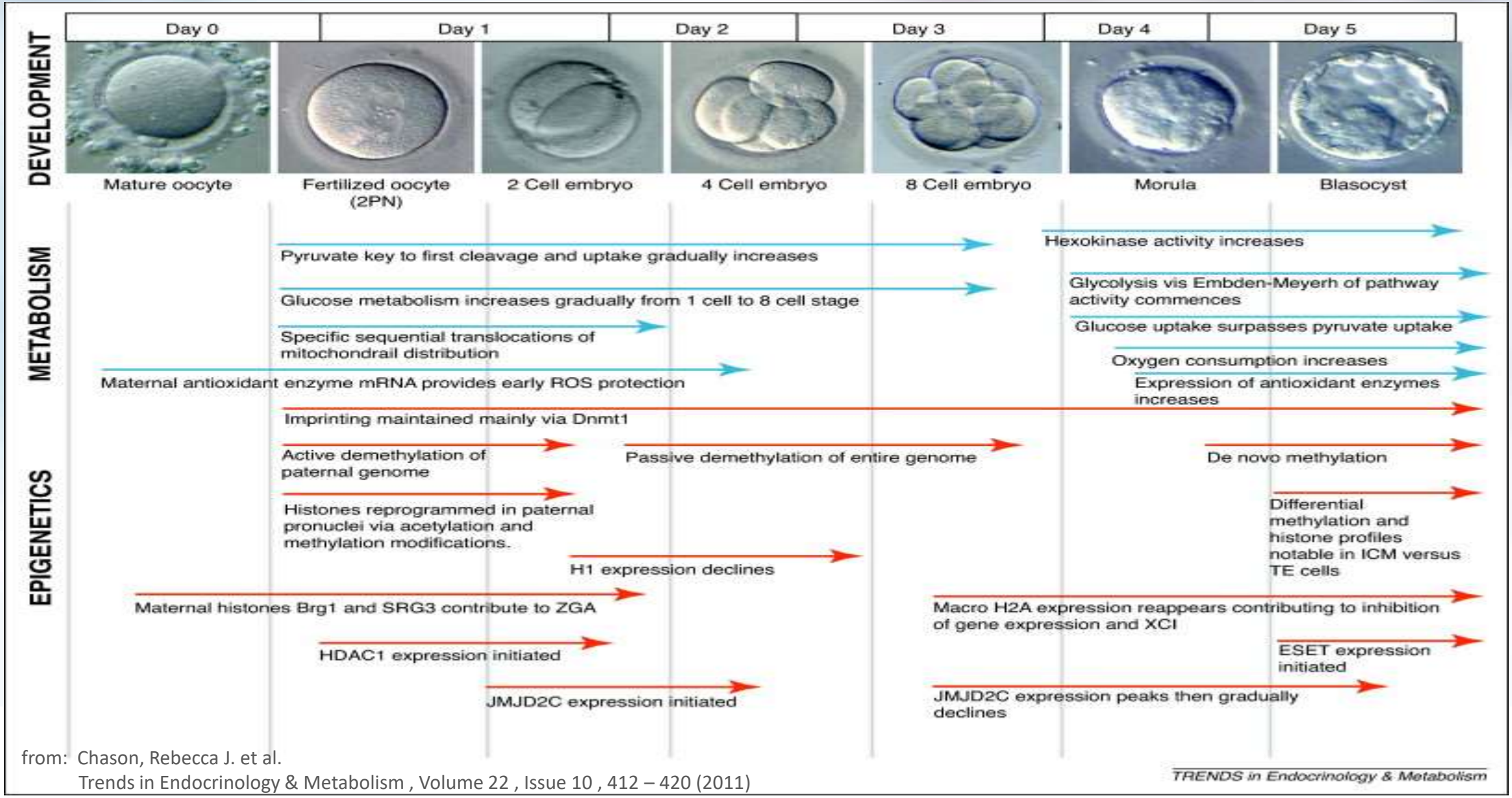




’Normal’ ICSI

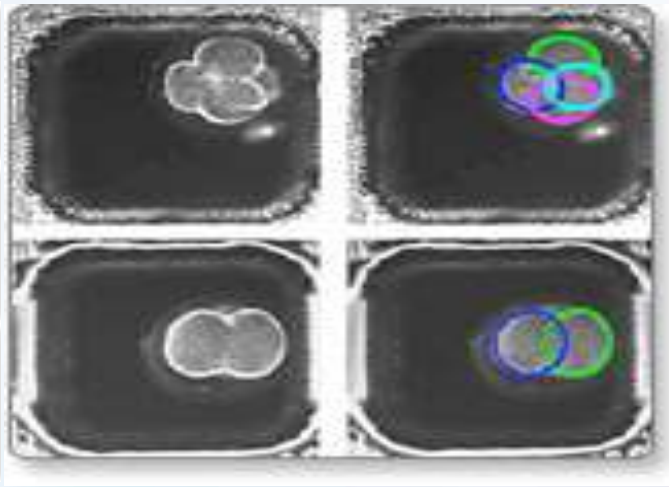


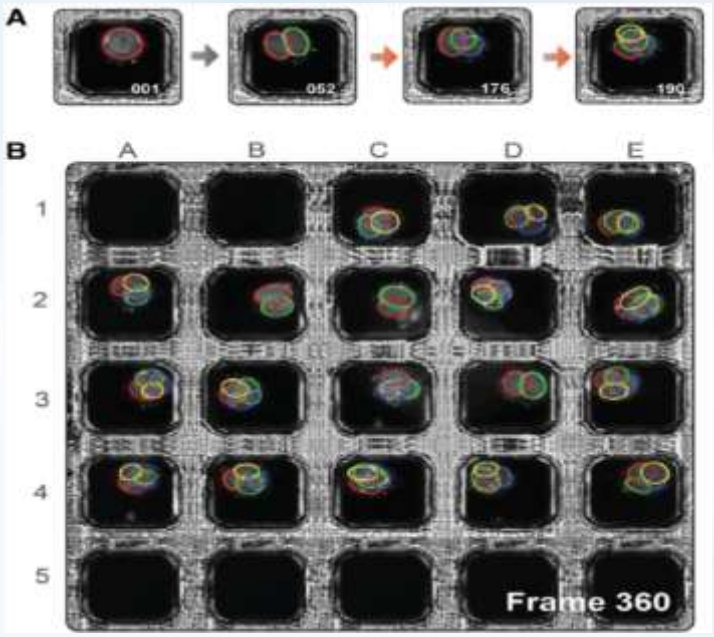
Embryo development

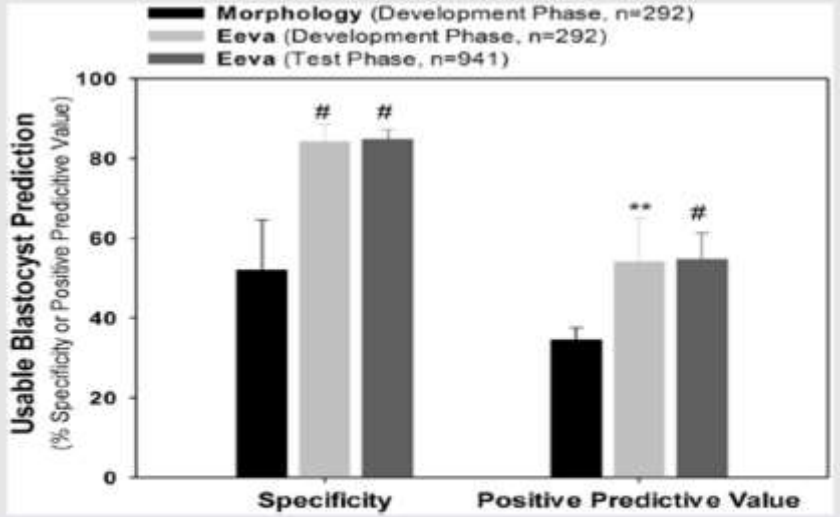


from: Chason, Rebecca J. et al.
Trends in Endocrinology & Metabolism , Volume 22 , Issue 10 , 412 – 420 (2011)

Incubators – with inbuilt optics



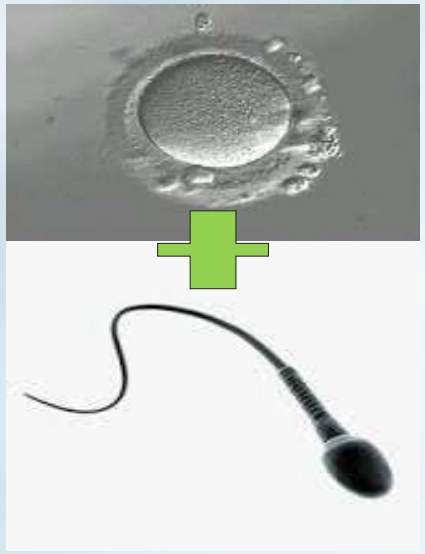




Usable blastocyst prediction performance (% Specificity or % PPV) of Eeva compared with D3 morphology for two independent data sets in the development and test phases. Error bars represent upper 95% CI. $^{**}P<.01$, $^{\#}P<.0001$.

Conaghan. Validation of a time lapse screening tool. Fertil Steril 2013.

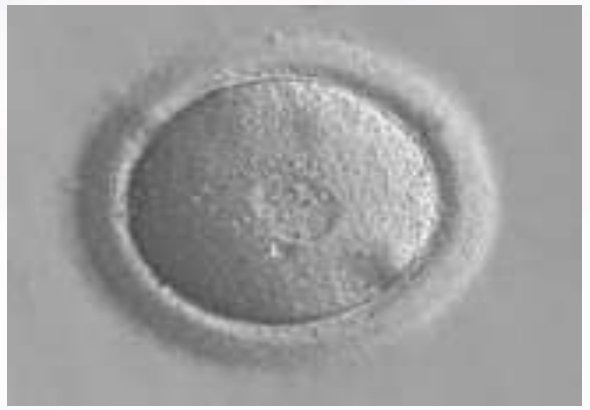
Assessing fertilisation



two polar bodies (PB) = excess genetic material discarded by oocyte

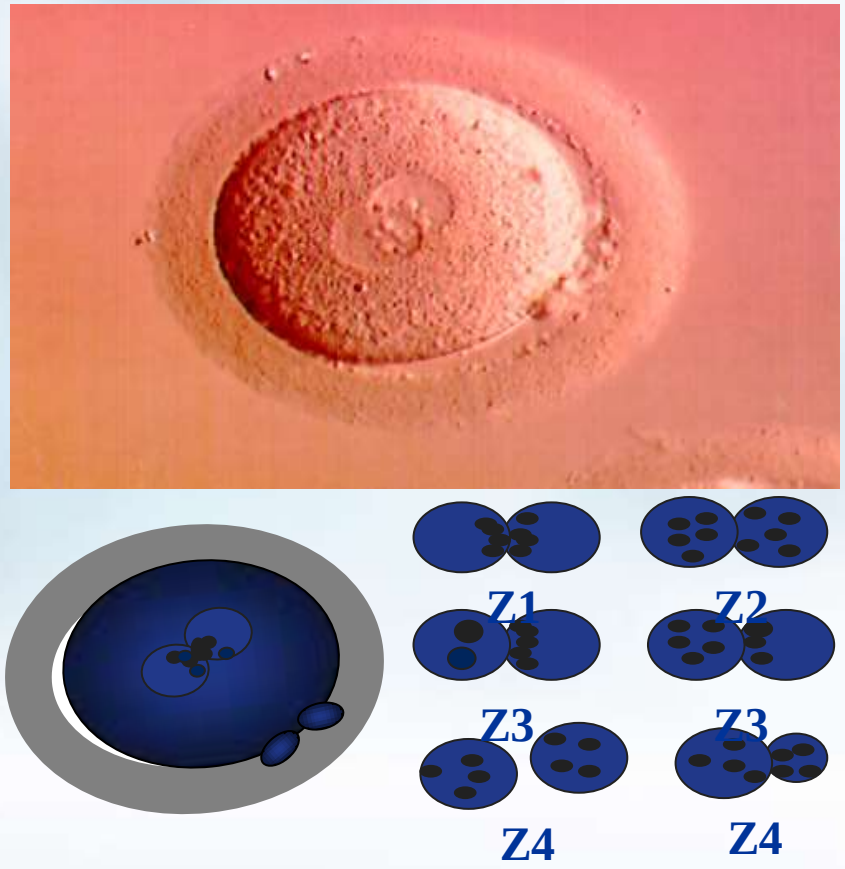
two pronuclei (**2PN**)
- one female (from oocyte)
- one male (from sperm)

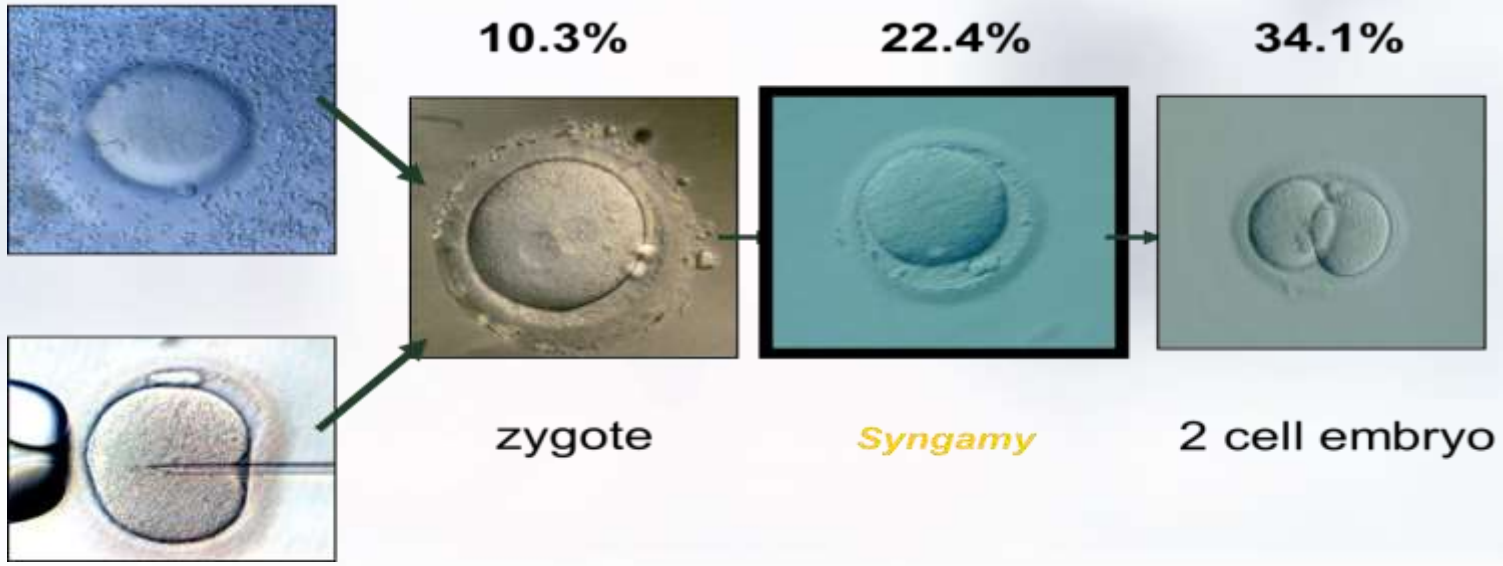
3PN
more than 1 sperm entered egg (polyspermy)
OR
retained PB (digyny)



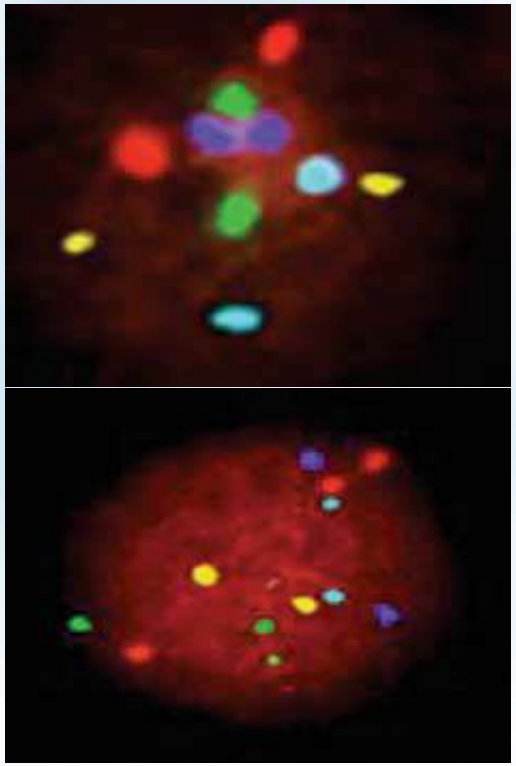
1PN
usually an activated egg – so female PN forms in absence of fertilisation or sperm nucleus decondensation

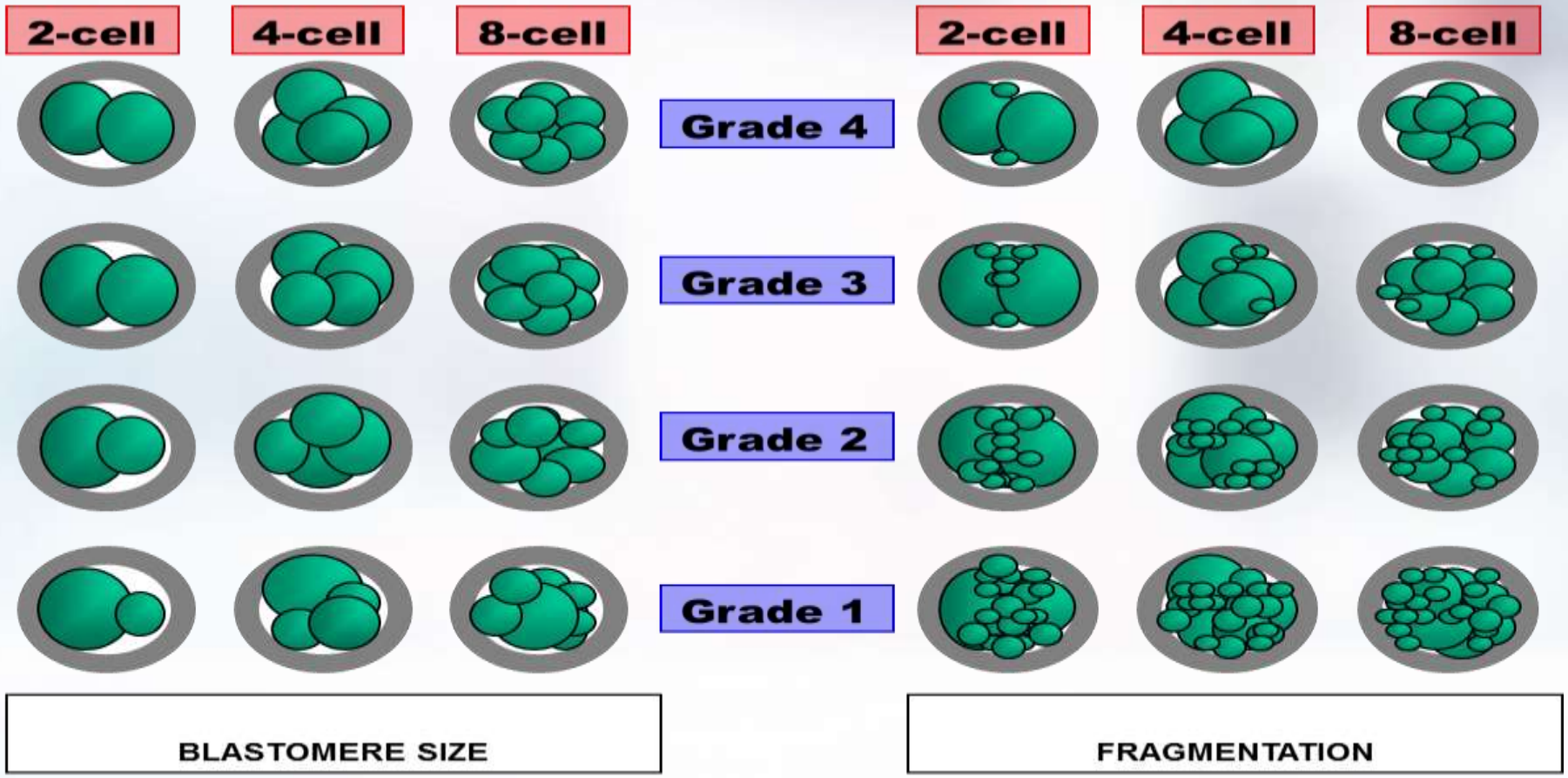
Embryo assessment – day 1



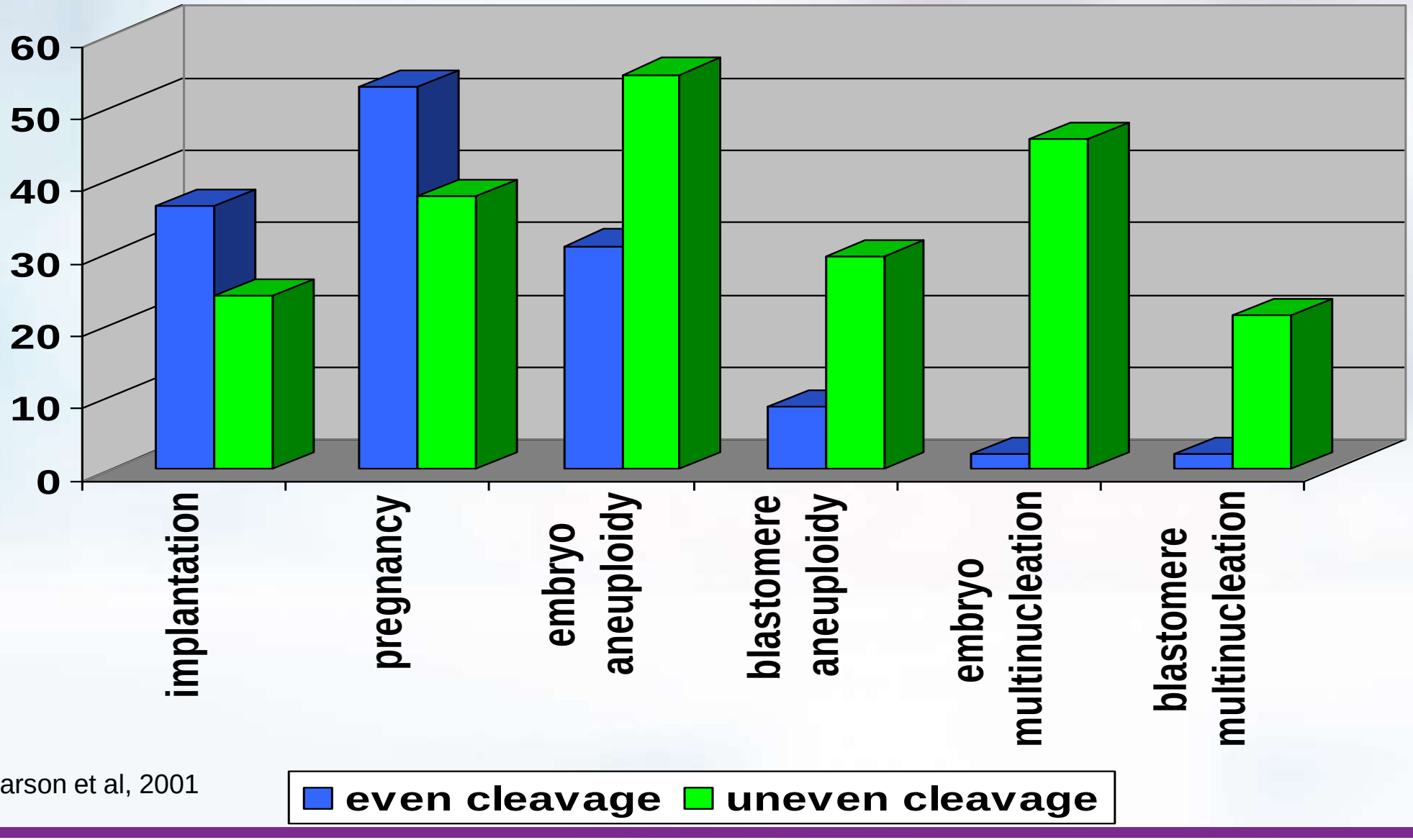


Embryo assessment – day 2/3



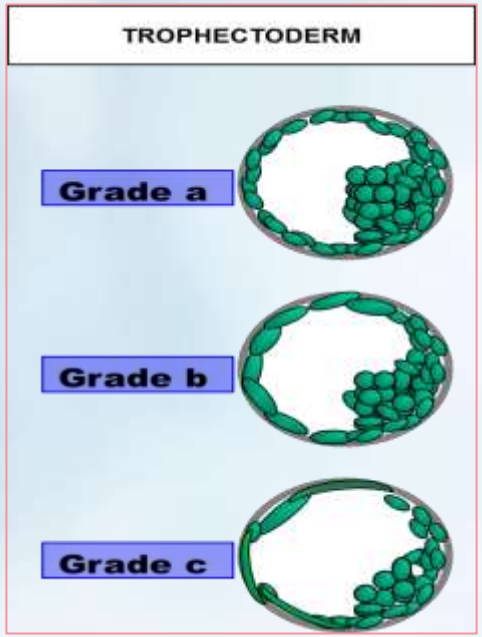


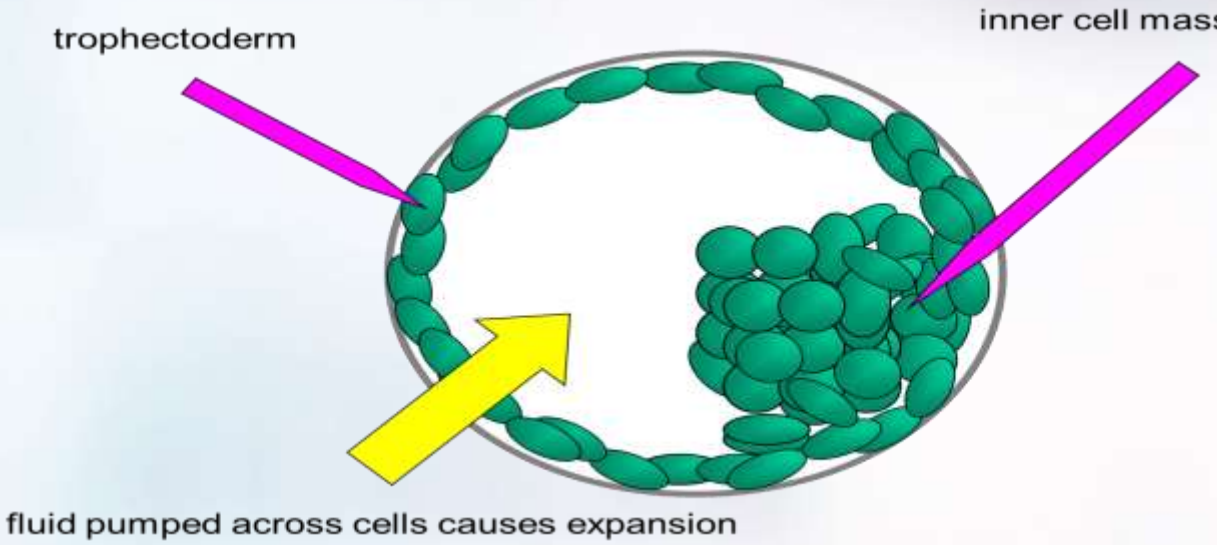
Cell division and genetic health

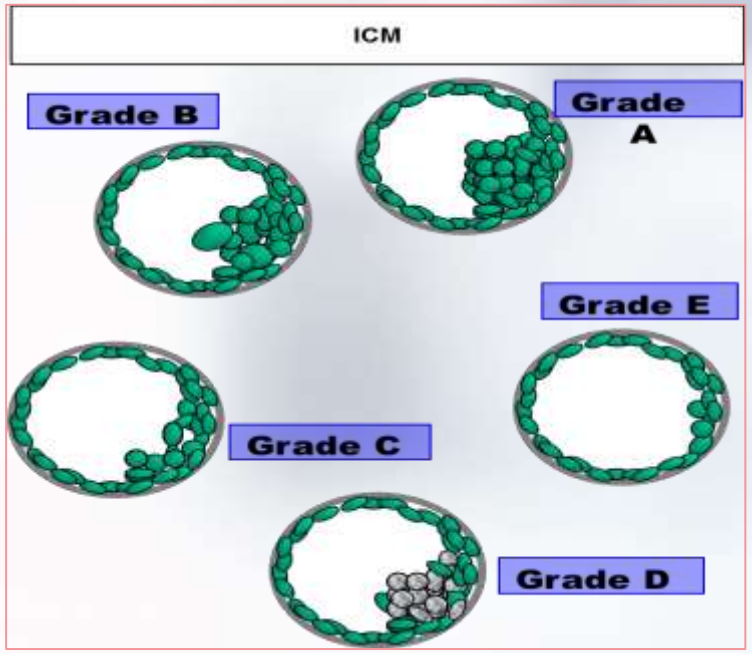


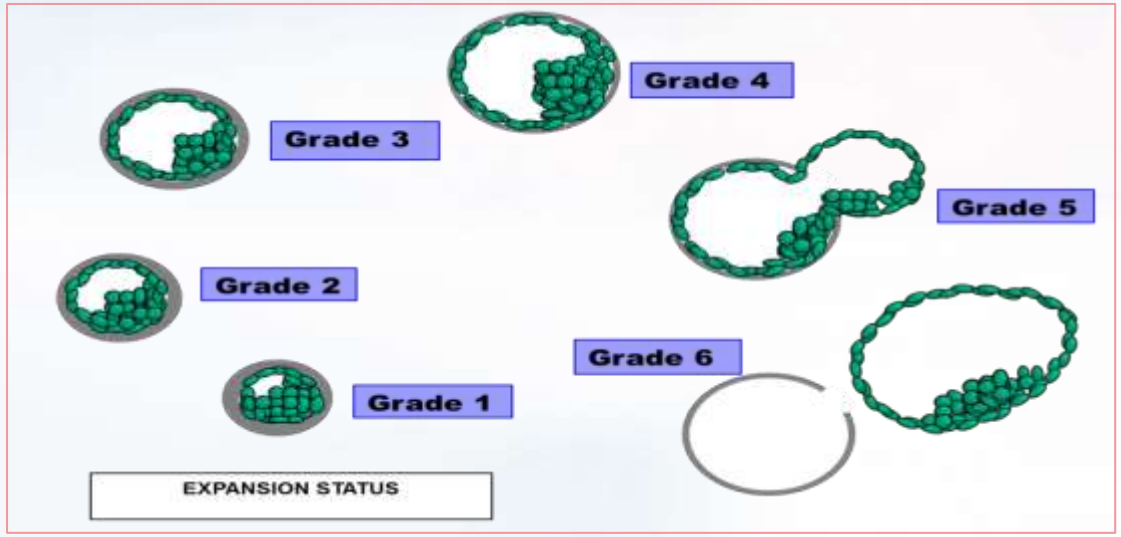
from Hardarson et al, 2001

Embryo assessment – day 5/6









Genetic testing

Preimplantation genetic diagnosis

PGD for detection of specific inherited genetic abnormalities

Preimplantation genetic screening

PGS for detection of spontaneously arising abnormalities

Polar body biopsy

video courtesy of
A Doshi

The Centre for Reproductive & Genetic Health

Blastomere biopsy

- laser
- simple aspiration – bevelled pipette

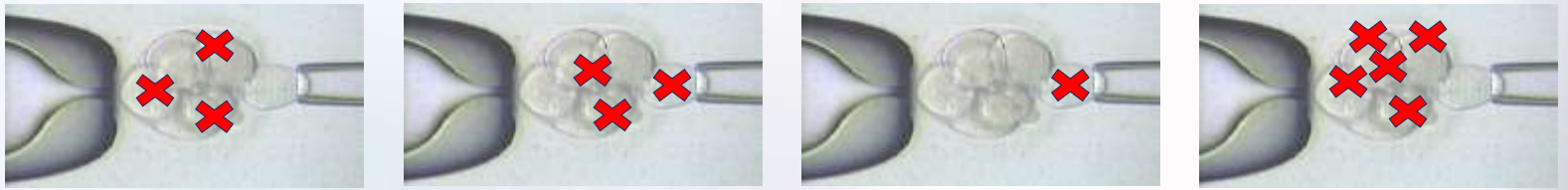
video courtesy of A Chatziparasidou & M Nijs

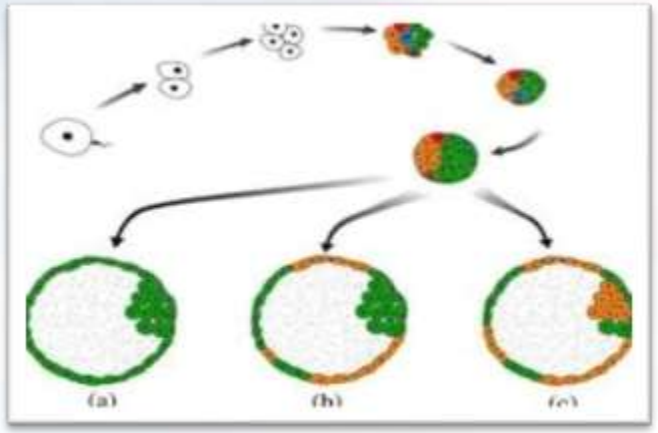
EMBRYOLAB

EMBRYOLAB ACADEMY

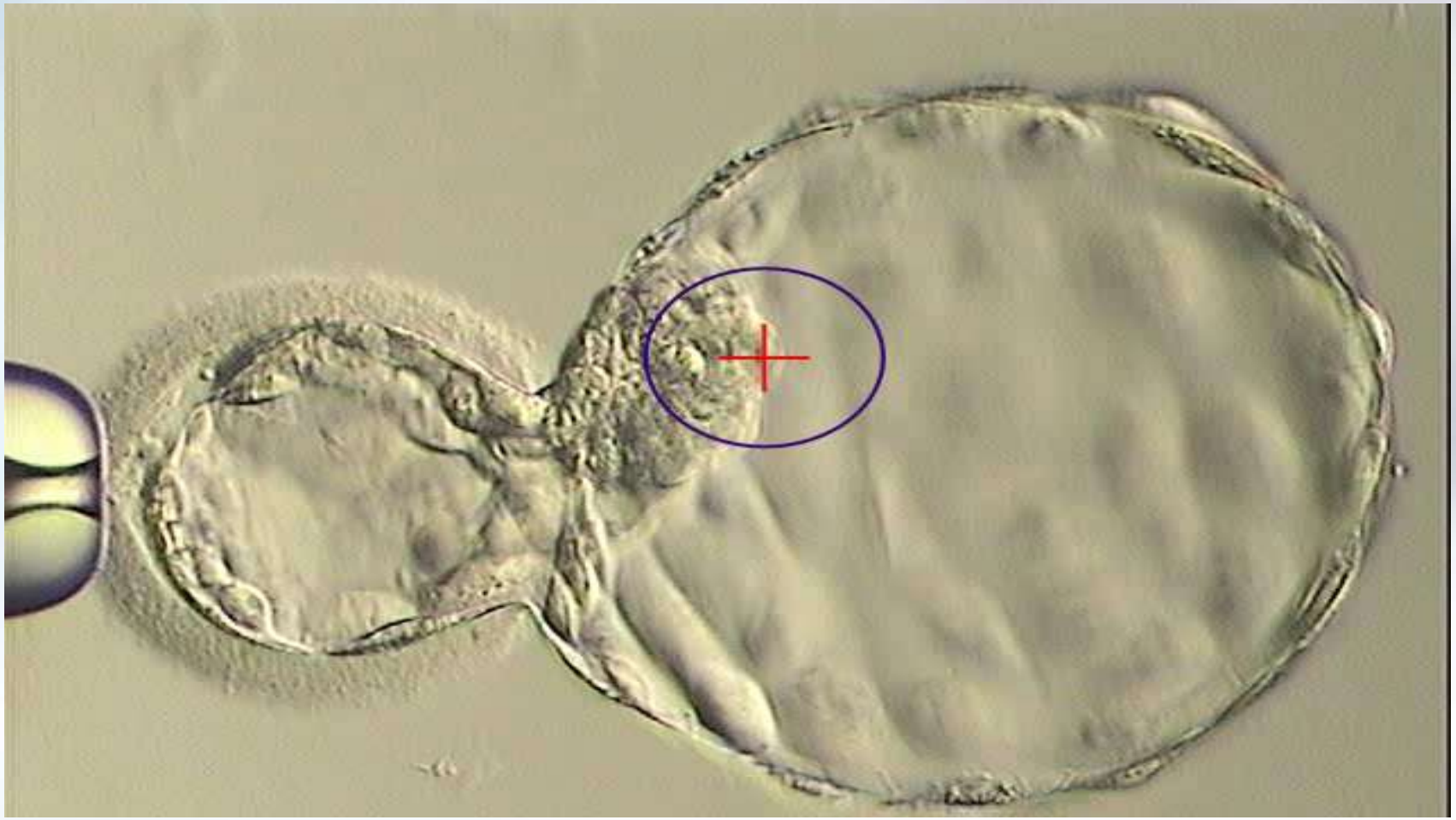
Preimplantation genetic screening

- Chromosomal mosaicism in early screening embryos,
- where embryo biopsy is not representative of the other blastomeres.





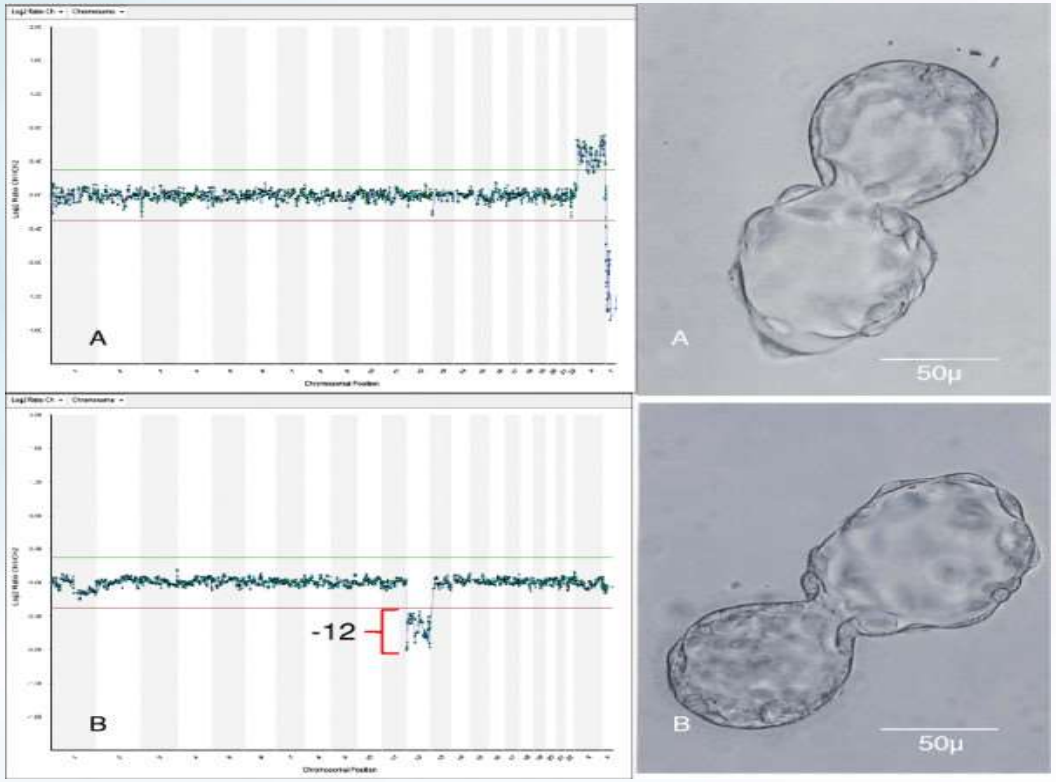
Trophectoderm Biopsy



Slide: Pacific Fertility, USA

Trophectoderm biopsy – clinical data

Yang et al. Molecular Cytogenetics 2012



First-time IVF patients with a good prognosis (age <35, no prior miscarriage) and normal karyotype.

Pilot study, prospective randomised
Transfer on day 5

44,9% aneuploidy rate in Blastocysts

Morphology + aCGH: 69,1% OPR
Morphology: 41,7% OPR p<009

Future applications

MtDNA

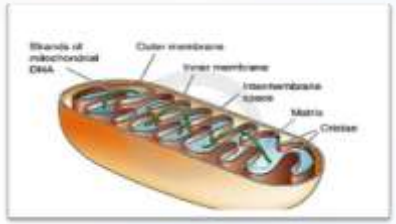
Information on chromosomal status, amount of mtDNA, and presence of mutations in the mitochondrial genome

379 embryos analysed with aCGH, quantitative PCR and NGS

123 were determined to be aneuploid

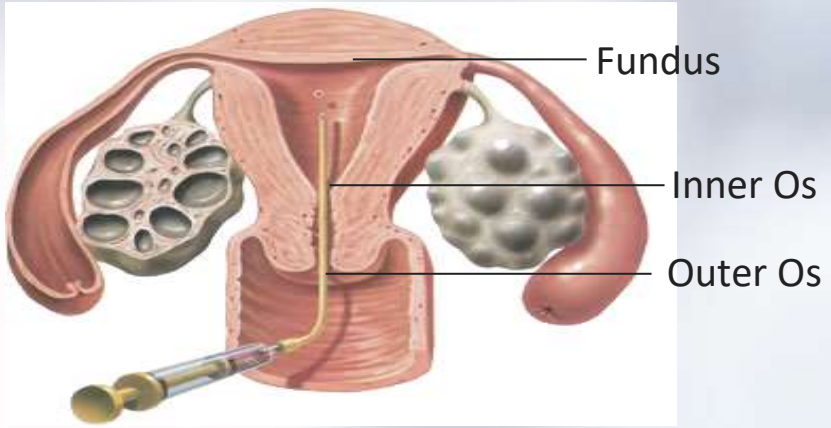
- Abnormal mtDNA levels are present in 30% of non-implanting euploid embryos, but are not seen in embryos forming a viable pregnancy
- The quantity of mtDNA was significantly higher in embryos from older women (P=0.003).
- MtDNA levels were elevated in aneuploid embryos, independent of age (P=0.025).
- Blastocysts that successfully implanted tended to contain lower mtDNA quantities than those failing to implant (P=0.007).

A novel biomarker?



Embryo transfer

- precise placement of the embryo inside the uterus is important
  - touching the fundus may cause the uterus to contract and harm chances of implantation
  - if the catheter is not inserted far enough the embryo may be unintentionally placed in the cervix
- difficult transfers:
  - trial transfer (normal catheter but no opening)
  - stylet (mandril; inner that helps form shape of catheter)
  - pre-formed (shaped before use)



Slow freezing v vitrification

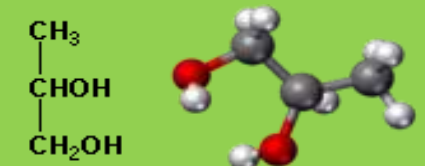
A critical appraisal of cryopreservation (slow cooling versus vitrification) of human oocytes and embryos

Hum. Reprod. Update (2012) 18(5): 536-554

Edgar DH and Gook DA

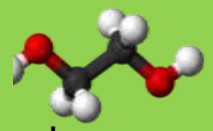
CONCLUSIONS: Available evidence suggests that vitrification is the current method of choice when cryopreserving MII oocytes. Early cleavage stage embryos can be cryopreserved with equal success using slow cooling and vitrification. Successful blastocyst cryopreservation may be more consistently achieved with vitrification but optimal slow cooling can produce similar results.

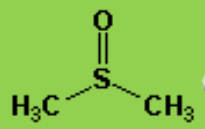
Choice of cryoprotectant



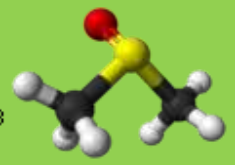
PROH

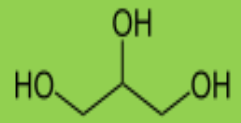
Ethylene glycol



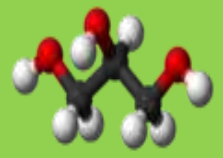


DMSO

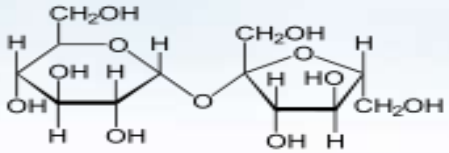




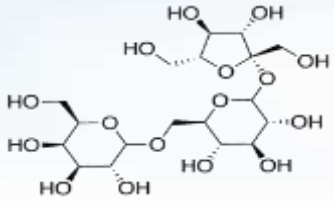
Glycerol



permeating - intracellular



Sucrose

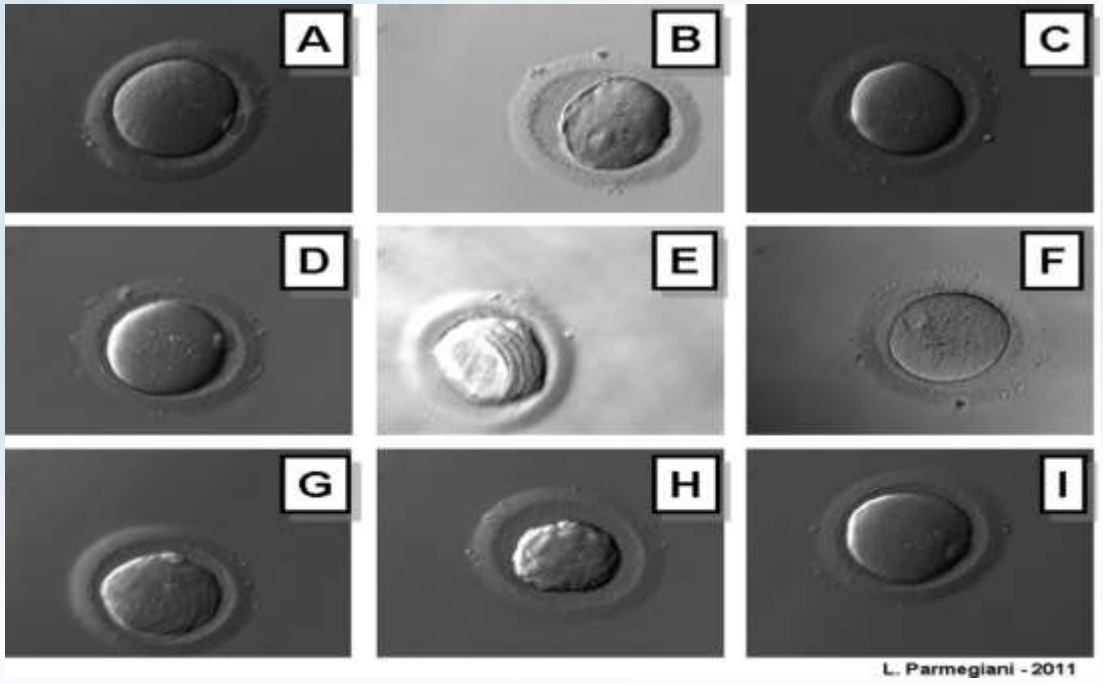


Raffinose

non-permeating - extracellular

- no clear evidence to favour any one system
- optimise system within each laboratory
- ORIGIO: PROH/EG; Sage: DMSO/EG
- anecdotally, DMSO favoured for oocytes?

Time required for equilibration



- stage-dependent
- 5 – 15 minutes
- options

  - observe and wait to see full (90%) re-expansion

  - establish median (fixed) time for your laboratory

- blastocysts – effect of collapse

  - collapsed: use 5 minutes

  - non-collapsed: check for re-expansion of cells NOT blastocoel

Collapsing blastocysts

- some clinics doing well without collapsing
- preferred options
  - puncture with ICSI needle
  - laser
  - small tip (micro-pipetting)
- generally advised
- wait for 50% shrinkage and then move straight to VM

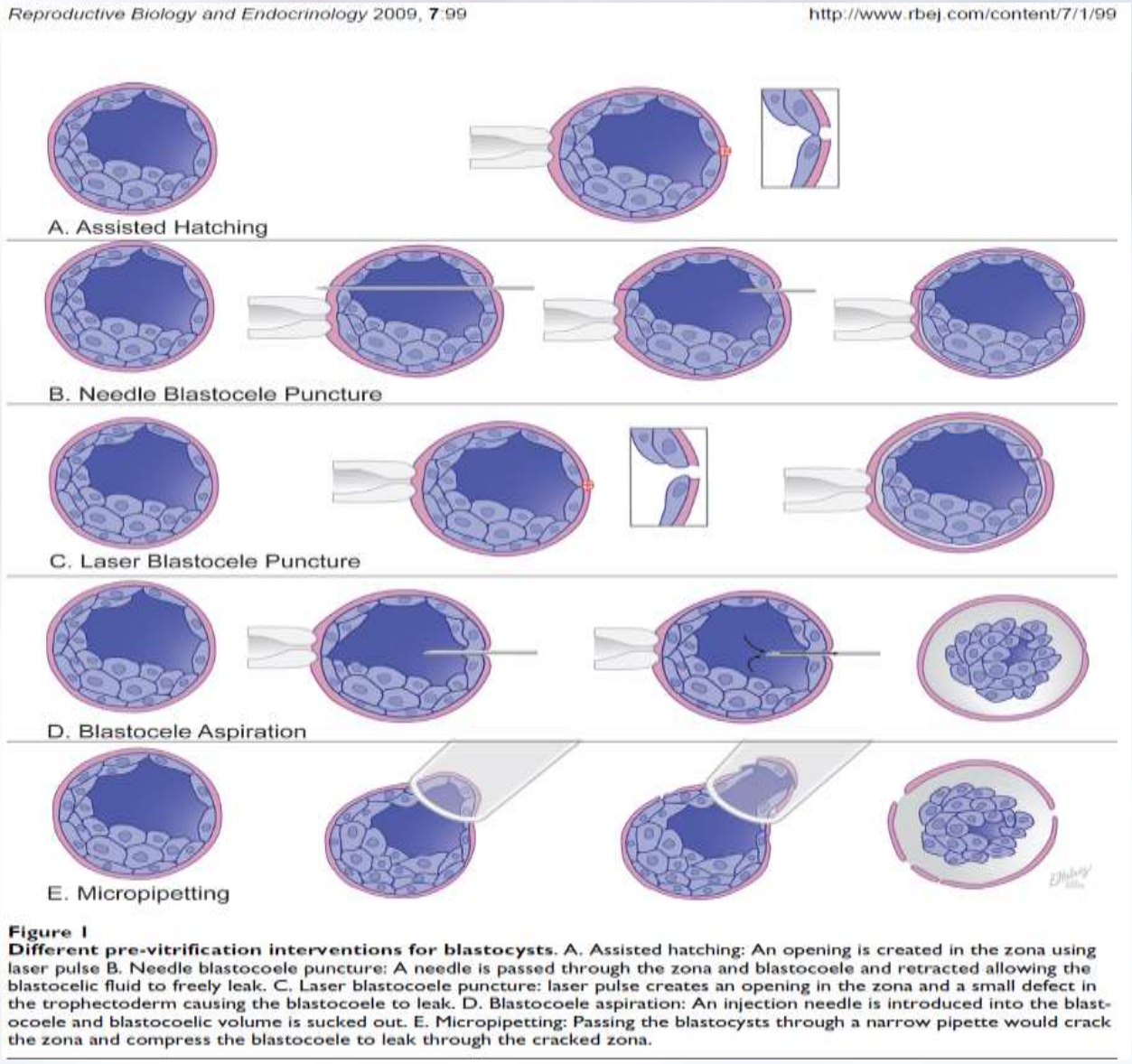
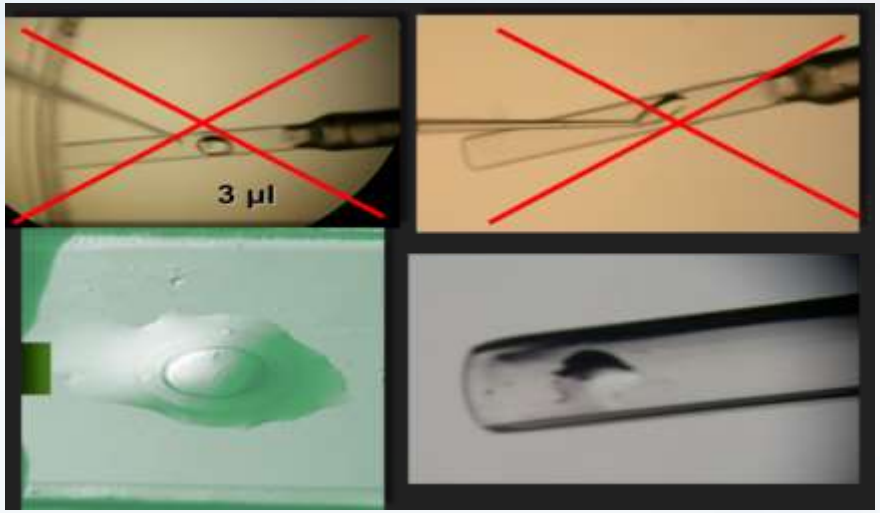


Figure 1
Different pre-vitrification interventions for blastocysts. A. Assisted hatching: An opening is created in the zona using laser pulse B. Needle blastocoele puncture: A needle is passed through the zona and blastocoele and retracted allowing the blastocelic fluid to freely leak. C. Laser blastocoele puncture: laser pulse creates an opening in the zona and a small defect in the trophectoderm causing the blastocoele to leak. D. Blastocoele aspiration: An injection needle is introduced into the blastocoele and blastocoelic volume is sucked out. E. Micropipetting: Passing the blastocysts through a narrow pipette would crack the zona and compress the blastocoele to leak through the cracked zona.

Choice of carrier: open v closed

- simple device
- loading

- minimal volume
- numbers
- maximal cooling
- timing (not too soon as evaporation significant)



Summary

To optimise any lab, one needs…

- the right people with…
- the right skills, provided with
- the right equipment in
- the right facilities.

To get the best outcomes, one must also …

- optimise egg quality with good stimulation regimens
- provide good culture conditions
- be able to choose the right gametes and/or embryo(s) for treatment and transfer
- be skilled at replacing embryos