Fertilisation

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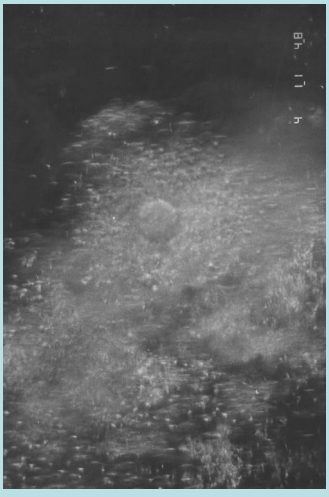
Objectives

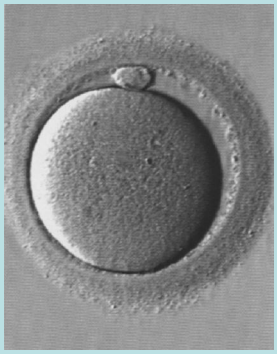
To understand the interactions between sperm and oocytes

To understand the initial post-fusion events

To understand the clinical attempts to overcome fertilisation impairment

Human oocytes collected for IVF with and stripped of cummulus cells





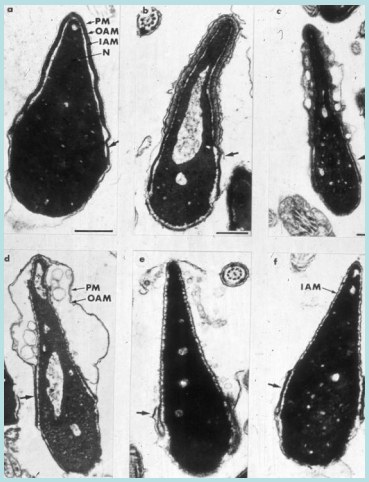
Fertilisation

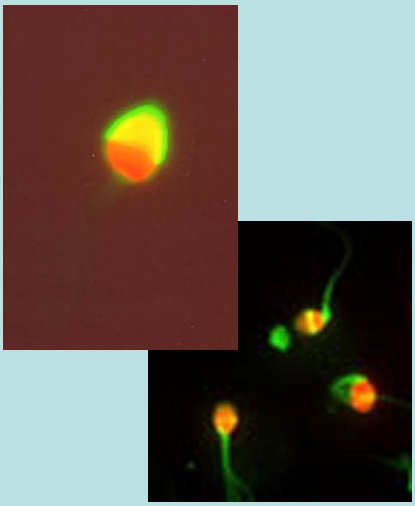
- A precise temporal sequence is required for fusion of mammalian spermatozoa and oocytes.
- Molecular interactions are species specific apart from notable exceptions.
- Freshly ejaculated sperm are unable to fertilise and require - capacitation which is associated with a vigorous motility characterised by large ALH & low linear velocities known as ‘hyperactivation’. and reversible changes in sperm head membranes.
- Irreversible changes in sperm head membranes are also a pre-requisite for normal fertilisation.

Sperm-egg binding

- Sperm attach loosely via plasma membrane and then bind tenaciously to specific sperm receptors on the zona pellucida.
- Following initial binding the sperm undergoes an irreversible form of lysosome exocytosis –
- The ***Acrosome reaction*** - characterised by fusion between plasma & outer acrosomal membranes. This fusion produces membrane vesicles and exposes the ZP to the enzymic contents.
- Solutions of human zonae induce AR in a dose and time dependent manner.

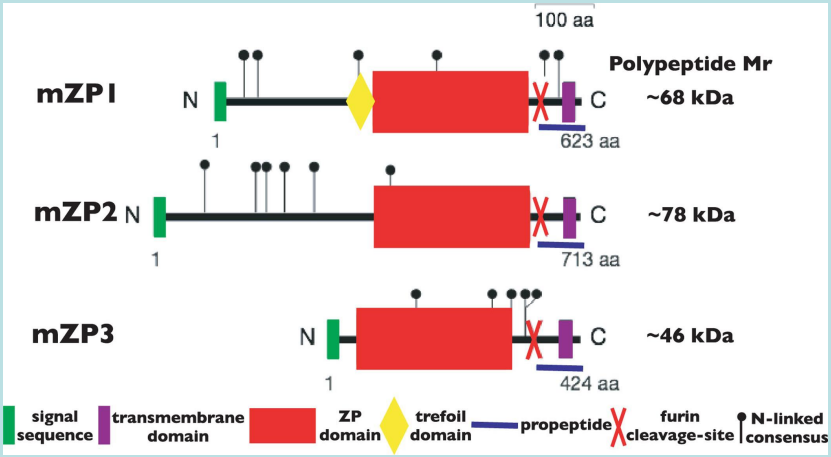
The acrosome reaction (AR)
Induced AR and seen by TEM & by Pisum sativum agglutinin and Propidium iodide staining (Univ.Utah)





Zona Pellucida Domain in Mouse (Wasserman)

Note absence of ZP4



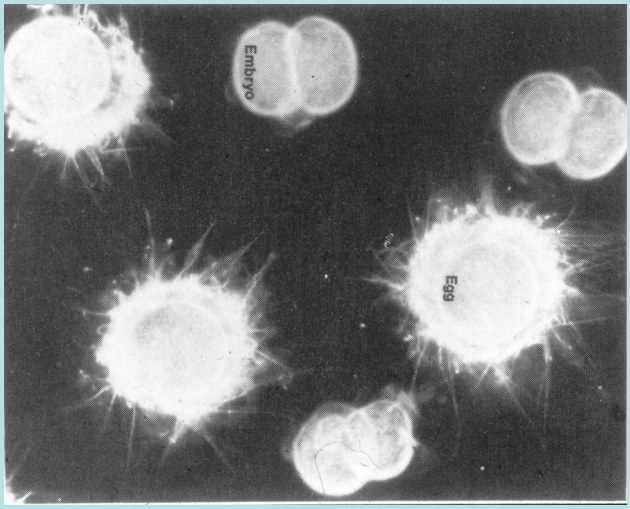
The sperm receptor (s)

- ZP3 is encoded by a single gene only expressed in the growing oocytes.
- ZP3 extracts inhibit sperm binding to oocytes.
- ZP3 only binds to acrosme intact sperm (mouse)
- Sperm bind to glass beads covalently linked to ZP3
- Sperm bind to embryonal carcinoma cells transfected with the ZP3 gene and secreting ZP3
- Purified ZP3 will induce the acrosome reaction and activate signal transduction system associated with cell exocytosis.
- A ZP4 is found in human ZP which can also induce AR

The sperm surface

- Each sperm has a large number of complimentary egg binding molecules.
- Gamete adhesion is carbohydrate mediated associated with O linked oligosaccharides.
- ZP3 binding has identified ß1,4-galactosyl transferase but it seems likely that other carbohydrate molecules are also involved.

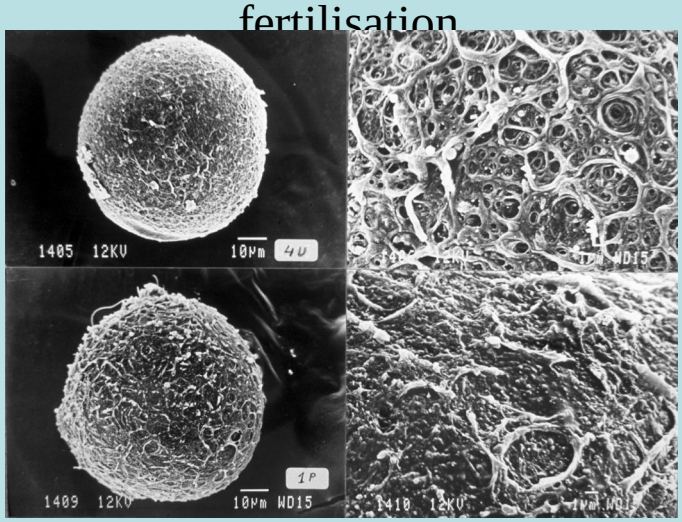
Sperm binding to mouse eggs and embryos after the block to polyspermy



The zona reaction & block to polyspermy

- Zona penetration is associated with vigorous sperm motility.
- A slit is made through the zona by a combination of physical movement and enzymic digestion allowing entry to the perivitelline space.
- Sperm-vitelline fusion induced the exocytosis of the many small lysosome like cortical granules at the egg periphery.
- The contents of cortical granules modify the properties of the ZP resulting in the block to polyspermy by the zona reaction.

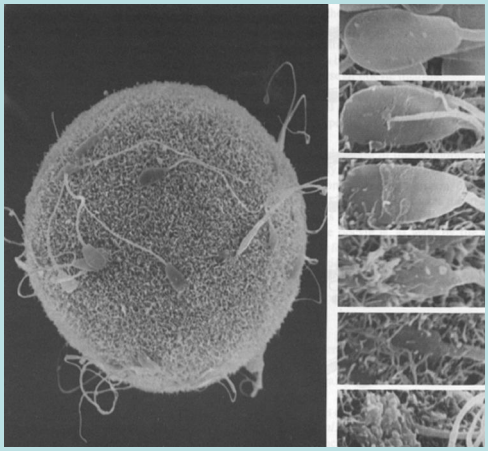
The impact of cortical granule exocytosis and impact on the zona pellucida before and after fertilisation

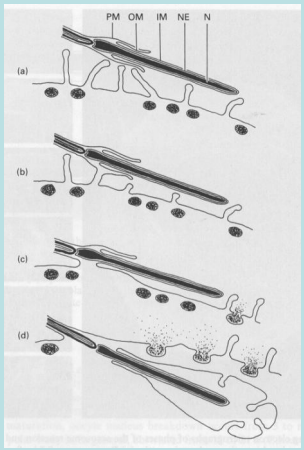


Before

After

Sperm egg fusion - (a zona free cow oocyte by SEM)

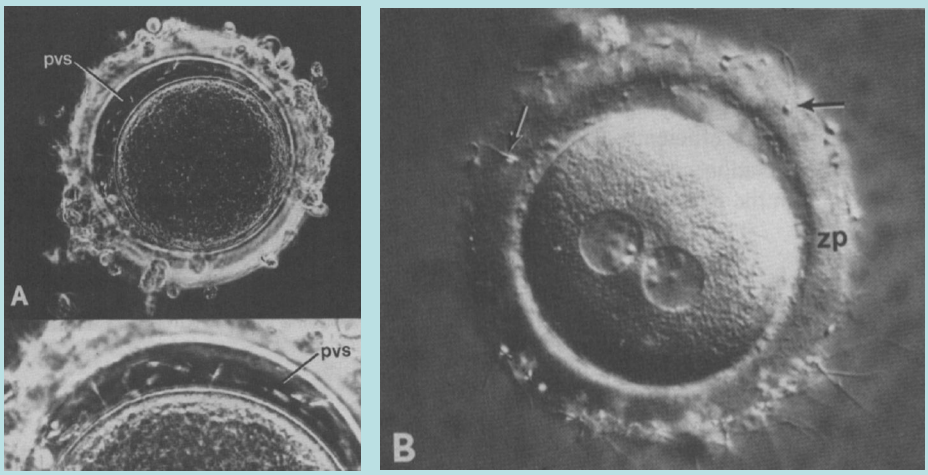




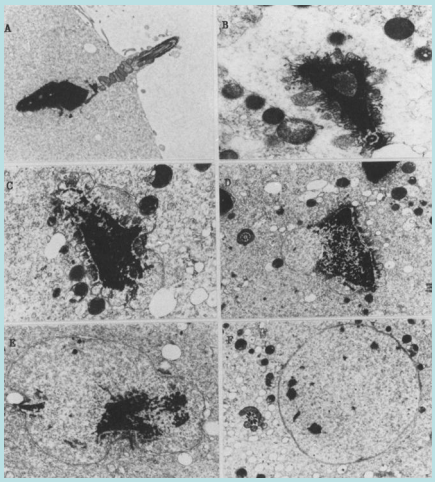
The block to polyspermy

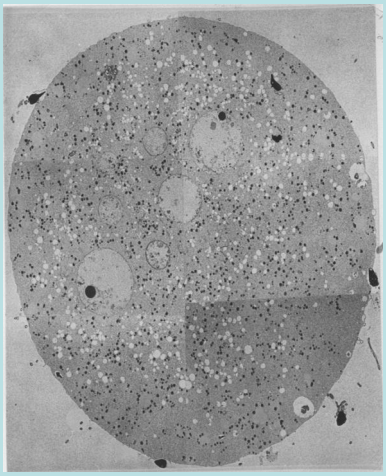
- Within seconds of sperm attachment the oolemma is depolarised (-70mV to +10mV in the sea urchin) - rapid block
- Cortical Granule discharge release is associated with a plasma membrane block in the mouse - slow block
- Calcium spikes occur within the oocyte.

The block to polyspermy in the rabbit (left) and human (right) showing different mechanisms

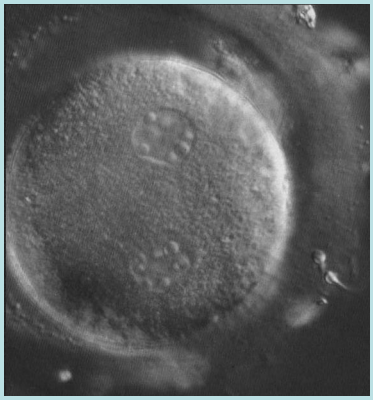


Sperm head penetration, de-condensation and formation of sperm pronuclei





The migration of pronuclei



Determination of fertilisation by light microscopy

- Presence of sperm in zona or within periviteline space.
- Presence of a sperm tail in ooplasm
- Swelling sperm head in ooplasm
- Male and female pronuclei.
- Presence of first and second polar bodies.
- Changes in appearance of ooplasm *eg. Loss of cortical granules.*
- Rotation of meiotic spindle.

Formation of nucleoli

- Nucleolar precursor bodies (NPB)are thought to associated with rRNA.
- Patterns may be associated with subsequent embryo development.
- As the pronuclei move to the center NPB may align along apposing membranes.

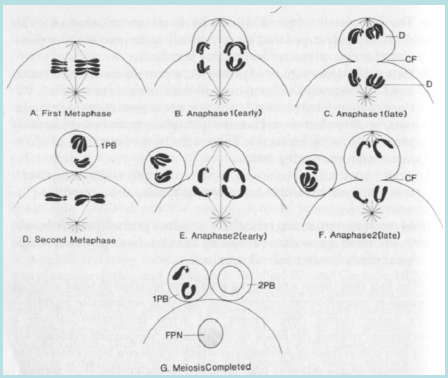
Sperm-oocyte activation factor(s)

- Perinuclear theca (PT) structures esp. around equatorial segment have been associated with cytosolic sperm factors that induce calcium oscillation.
- Oscillin identified with glucosamine 6 phosphate in mouse but failed to move beyond a candidate molecule.
- Stat 4 - possible two component candidate.
- Sperm cytosol phospholipase C-*zeta*

Post fusion events

- The quiescent egg is activated by penetration by the sperm.
- Activation is manifest by the resumption of meiosis in the egg.
- The sperm nuclear envelope disappears and the condensed nucleus de-condenses due to the reduction of sulphur-sulphur bonds
- Concurrently the female pronucleus forms with both pronuclei regulated by growth factors.
- Sperm centriole replicates and envelopes breakdown
- The oocyte cytoskeleton is responsible for migration of the two pronuclei towards the centre of the oocyte.
- When in close proximity the pronuclear membranes inter-digitate, breakdown and the chromosomes align on a single metaphase spindle.
- Diploid nuclei develop and mitotic division is initiated.

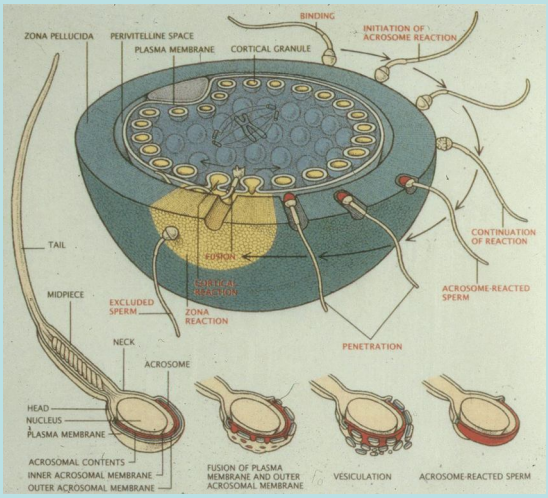
Extrusion of the second polar body.



Cytoplasmic changes

- A cytoplasmic wave of organelles esp. mitochondria can be visualised as the formation of a clear halo in the zygote.
- Possible clustering around the pronuclei may indicate localised energy requirements.
- Higher ATP levels are associated with higher implantation of embryos and

Summary of Fertilisation



What happens to sperm structures?

- The sperm proximal centriole is acquired by the zygote (in most species - but not mouse) & provides centre for MTOC development with peri-centriolar material from the oocyte
- Sperm mitochondria appear to be selectively eliminated after acquiring ubiquitin.
- The presence of paternally derived mitochondrial disease indicates the possibility of ptmitDNA survival & population studies indicate fusion of mtDNA
- The sperm tail elements degenerate 2-3 days after fertilisation.

Mitochondrial Eve ?

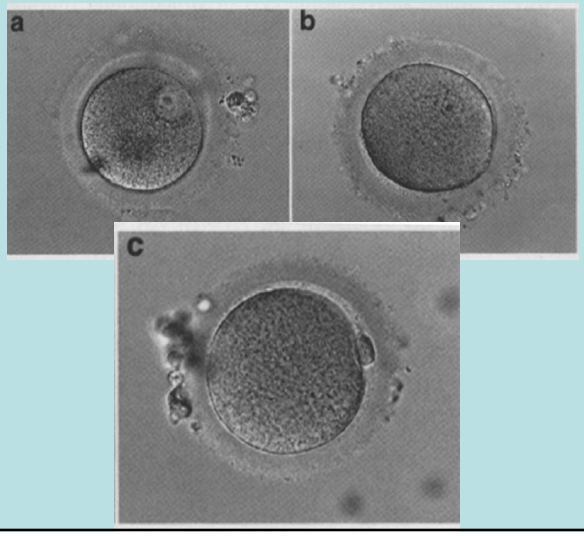
Science 2004, 308 p981

A mitochondrial disease was shown to be paternally inherited.

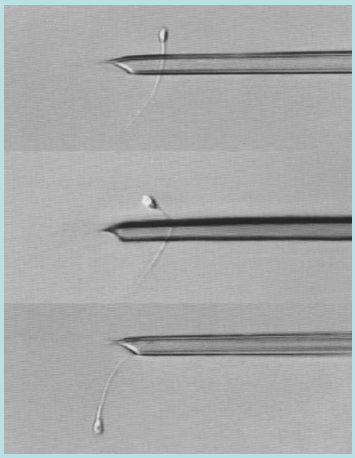
0.7%muscle mitochondria were maternal/paternal hybrids - mtDNA swap.

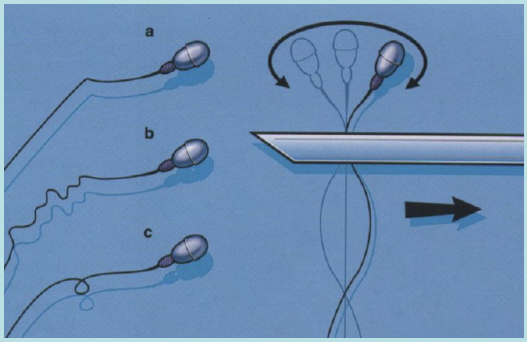
Sperm would provide only 0.01% of the mitochondria in the zygote if they survived.

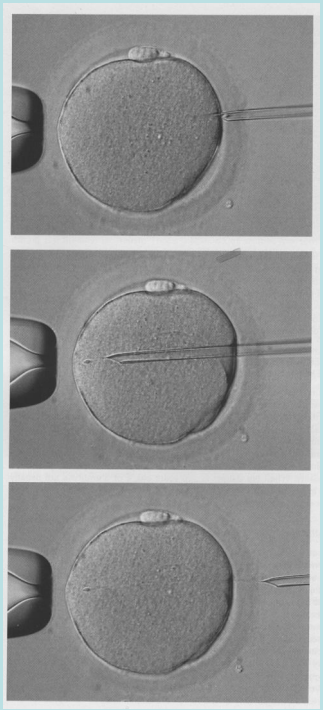
Human oocytes - selection for ICSI

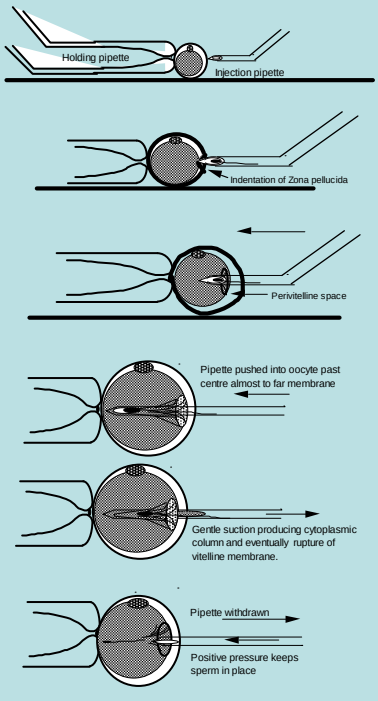


Immobilisation of sperm for ICSI









Prevalence of birth defects after ICSI(Belgium) with W.Australian Registry (BMJ 1997)- *different classifications!*

Birth defects	ICSI N-420	Registry n-100454
Major	14 (3.33%)	31 (0.03%)
Minor	84 (20.00%)	3 (0.003%)
Cardiovascular	14 (3.33)	672 (0.67%)
Urinogenital	6 (1.43%)	1080 (1.08%)
Gastrointestinal	4 (0.95%)	521 (0.52%)

Factors associated with poor outcome and ICSI

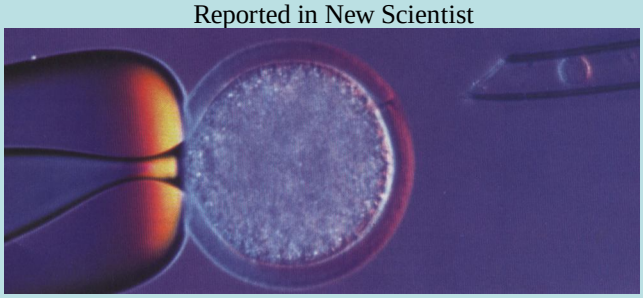
- Only one oocyte
- Abnormal oocytes
- Totally immotile sperm
- Round headed sperm
- Oocyte damage
- Sperm with damaged DNA ?

Developments in ICSI

- Nuclear transfer of somatic nuclei into oocytes for reprogramming to haploid.
- Maturation of gametes in vitro and vivo with transplantation.
- Addition of genes during in vitro maturation of sperm for addition of new genes at fertilisation – transgenics.
- Epigenetic impact remains unclear but is of major concern.

IVF with three parents

ASRM October 2003
Reported in New Scientist



Kaguya-Homoparental

Tomohiro Kono - Nature April 2004


Kaguya, the mouse with two mothers

- Modified H19 gene & promotion of IGF-2 appears to mimic male imprinting.
- Combined with immature eggs 'prior to oocyte imprints' appears to allow homoparental development.

The numbers game !

Dolly- 1st mammalian clone	Kaguya- 1st homoparental mammal
277 egg/ adult fusions	457 egg constructs
12% Blastocyst	91% Blastocyst
29 embryo / 13 ewes	371 embryo / 26 females
1 Clone	2 mice
	18 dead; 8 anomalies

Useful References

and acknowledgments

- Johnson & Everitt, (2007) Essential Reproduction
- Knobil & Neill eds (ed 2, 1994, ed 3,2005) The Physiology of Reproduction.
- Longo, F. Fertilisation