

**11th Postgraduate Course for Training in
Reproductive Medicine and Reproductive Biology**

Fertilization

Françoise Urner

2001

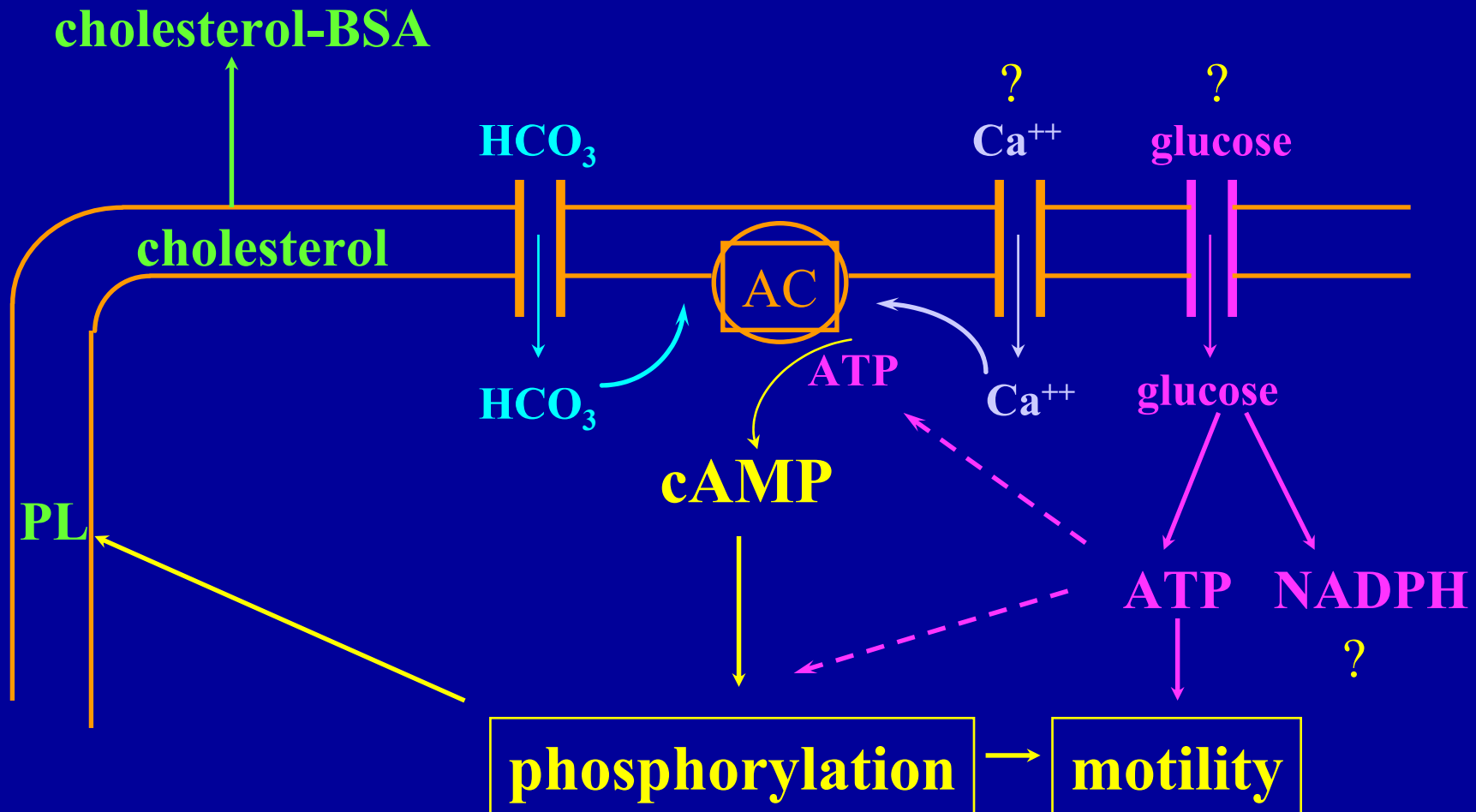
1

GAMETE MATURATION

Sperm capacitation

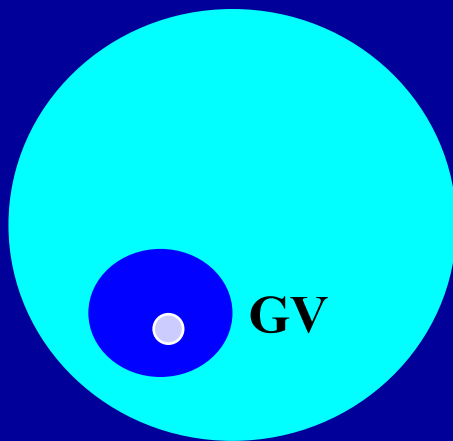
- **Modifications of the sperm plasma membrane**
- **Metabolic changes**
- **Post-translational modifications of proteins**
- **Activation of motility**

Signaling pathway involved in capacitation

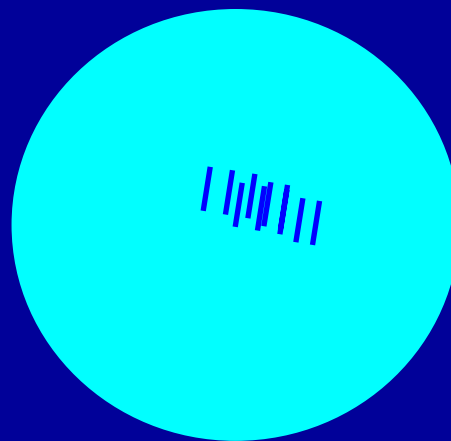


Oocyte maturation

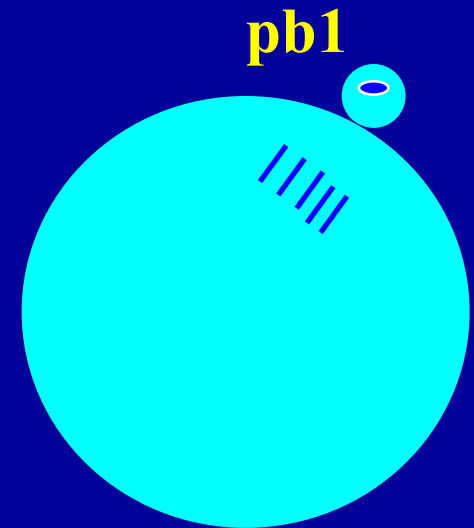
- Reinitiation of meiosis from prophase I to metaphase II
- Depends on the preovulatory surge of LH



Prophase I



Metaphase I



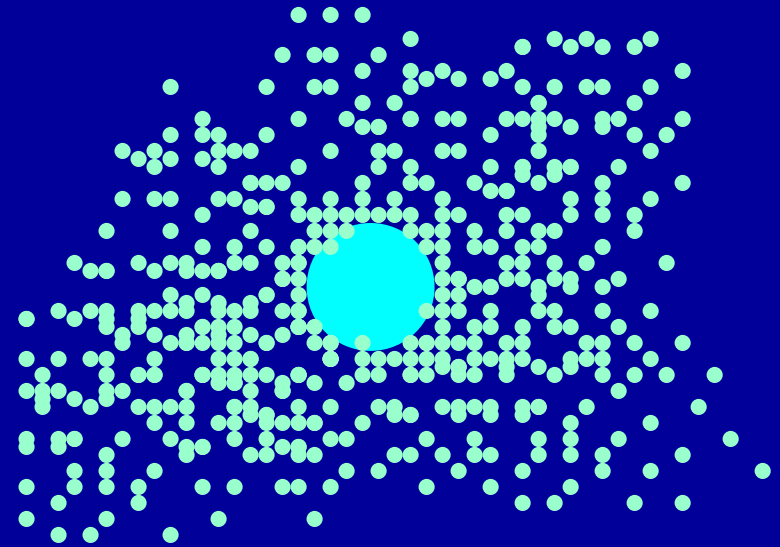
Metaphase II

2

**SPERM PENETRATION
OF CUMULUS CELL COMPLEX**

Cumulus cell complex

- **Cumulus cells : somatic cells surrounding the oocyte and embedded in a extracellular matrix containing hyaluronic acid**
- **Only capacitated and acrosome-intact sperm penetrate the cumulus complex to reach the oocyte**
- **Forward mobility is required for penetration**



- **Hyaluronidase activity of sperm surface molecules may participate in sperm progression**

3

**SPERM INTERACTION WITH
THE ZONA PELLUCIDA**

Zona pellucida

-
- **Glycoprotein coat surrounding the oocyte**
 - **Initial binding of sperm to the oocyte**
 - **Barrier to interspecies fertilization**
 - **Barrier to polyspermy**

Binding of sperm to the zona pellucida

Primary binding

- **Binding of capacitated and acrosome-intact sperm to ZP3 (oligosaccharide moiety)**
- **Specific receptors are present in the sperm plasma membrane, but many different molecules have been proposed as candidate receptors**

Secondary binding

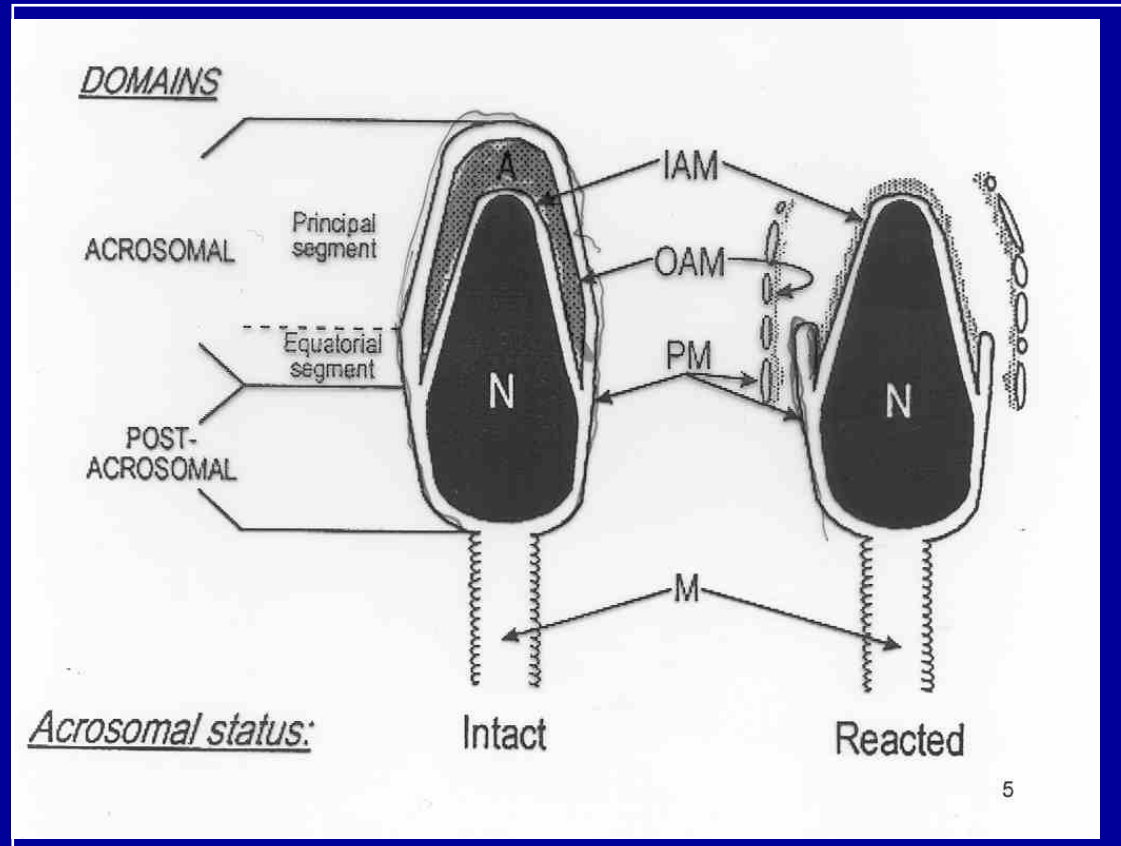
- **Binding of sperm to ZP2 following binding to ZP3 and acrosome reaction**
- **Specific receptors are probably present on the inner acrosomal membrane of the acrosome-reacted sperm**

Acrosome reaction

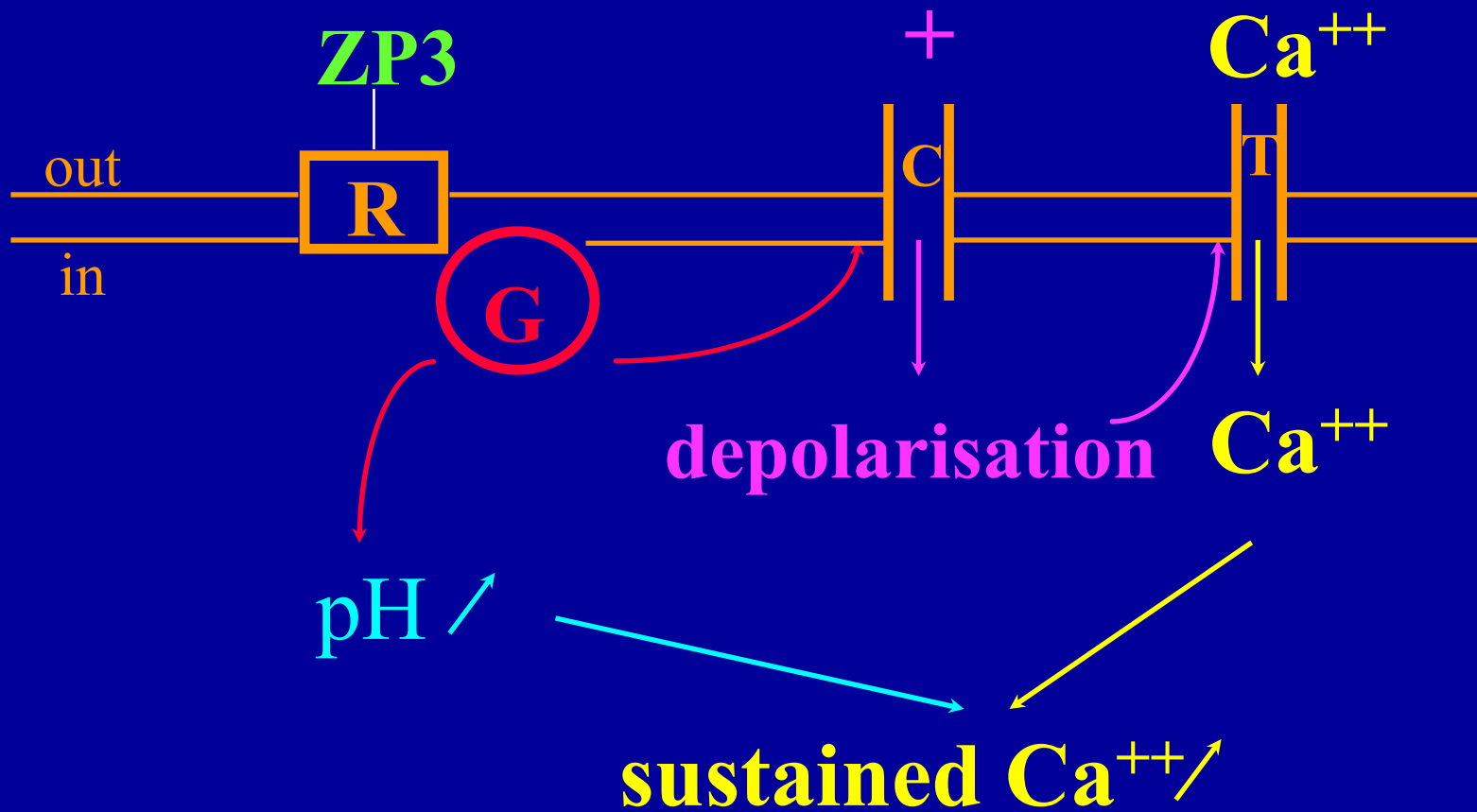
- **Triggered in sperm bound to ZP3**
- **Calcium-dependent event**
- **Essential for sperm penetration of the zona pellucida and sperm fusion with the oocyte plasma membrane**

Acrosomal exocytosis

- Fusion between the plasma membrane (PM) and the outer acrosomal membrane (OAM)
- Exposure of the inner acrosomal membrane (IAM)
- Release of the acrosomal content (acrosin)
- Appearance of the equatorial segment (ES)



Calcium signaling pathway involved in acrosome reaction



Penetration of the zona pellucida

- **Strong motility is required (hyperactivated motility)**
- **Enzymatic digestion of the zona pellucida by acrosin facilitates penetration**

4

**SPERM PENETRATION
INTO THE OOCYTE**

Sperm binding to the oolemma and fusion

- Gamete binding occurs between the equatorial segment of the sperm and the microvilli of the oocyte surface**
- Specific surface molecules have been proposed to be involved in binding of the gametes**
- Following binding, sperm fusogenic molecules promote fusion between the plasma membranes of the equatorial segment and the microvilli**
- Subsequent incorporation of the whole sperm occurs as a phagocytosis-like process**

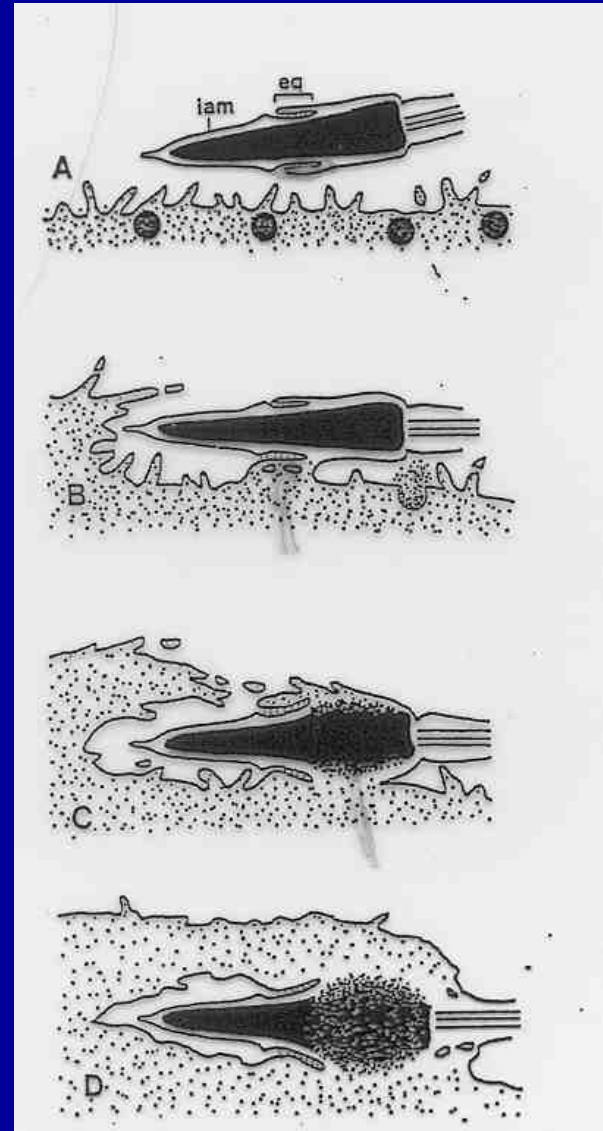
Gamete fusion

A: gamete binding

B: membrane fusion and pore formation

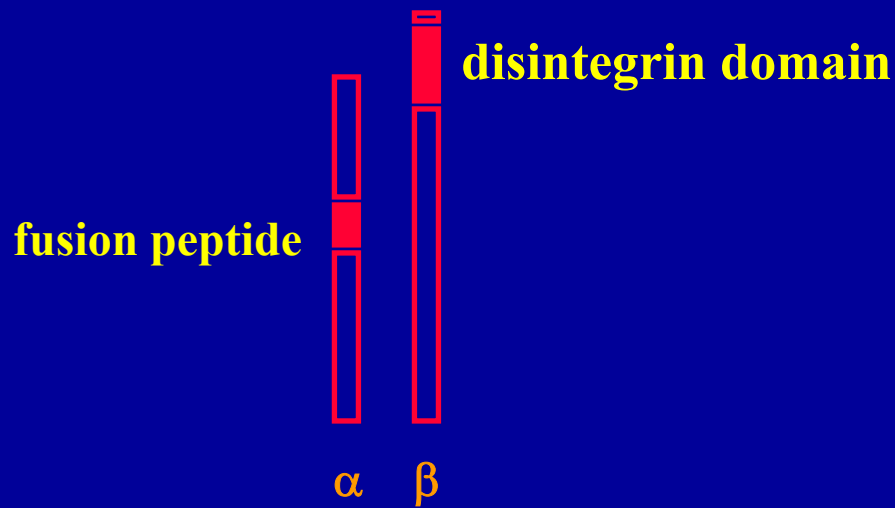
C: initiation of chromatin decondensation

D: incorporation of the head by phagocytosis

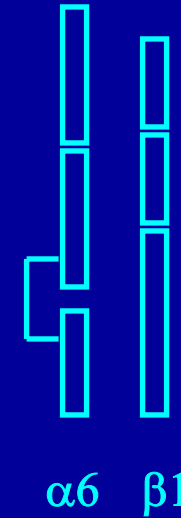


Fusion mediated by fertilin-integrin interaction

sperm: **fertilin**





oocyte: **integrin**



- **Binding of fertilin (disintegrin domain) to integrin**
- **Conformational change of the α and β subunits**
- **Exposure of the fusion peptide (α subunit)**
- **Insertion of the fusion peptide in the oocyte plasma membrane**
- **Fusion of the plasma membranes**

Sperm incorporation in the oocyte

- **Nucleus**  **paternal genome**
- **Centrosome**  **migration of the pronuclei + organisation of the mitotic spindle of the first cleavage**
- **Cytoplasm contains factors involved in oocyte activation**
(?)
- **Mitochondria are present during the first embryo cleavages**
- **Tail (axonemal microtubules, fibrous sheath) is present during the first embryo cleavages**

Sperm chromatin remodeling in the oocyte

- Removal of sperm nuclear envelope
- Reduction of disulfide bonds of protamines by glutathione
- Initiation of sperm chromatin decondensation
- Removal of protamines and replacement with maternal histones
- Chromatin recondensation
- Chromatin decondensation \Rightarrow male pronucleus formation

5

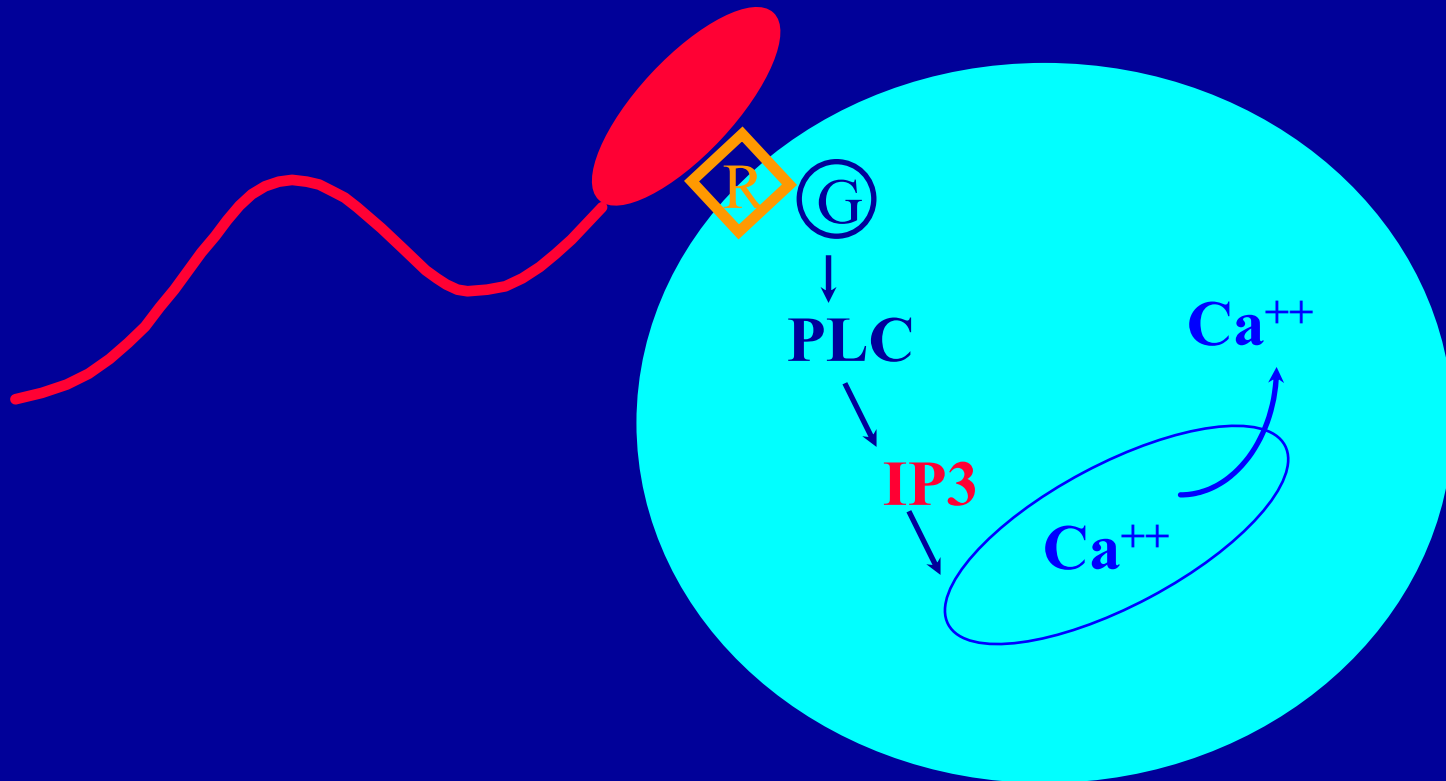
OOCYTE ACTIVATION

Oocyte activation

- **Sequence of events triggered by the fertilizing sperm in the oocyte and that are required to initiate embryonic development**
- **Repetitive calcium rises in the oocyte plays a central role in triggering activation**
- **Activation events include: cortical granules exocytosis, resumption of meiosis, pronucleus formation, DNA synthesis, first cleavage**

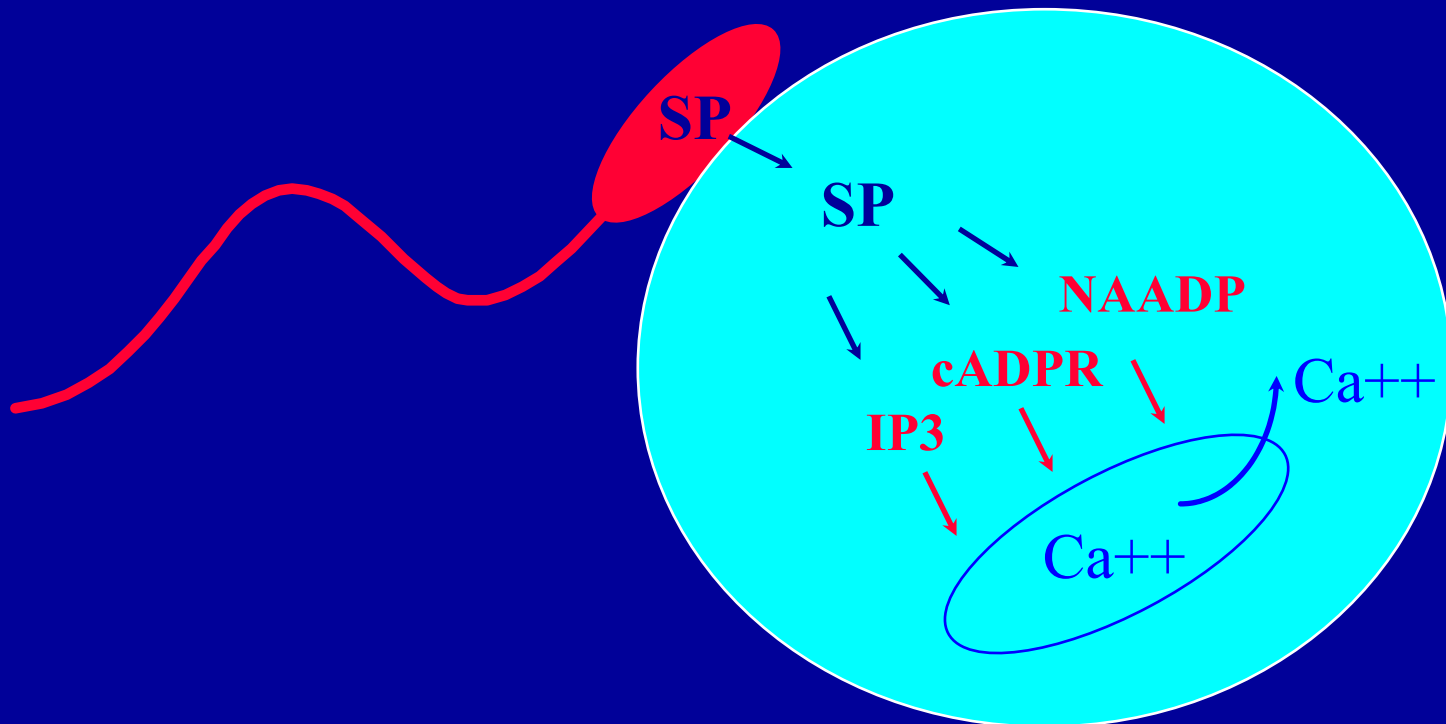
Calcium signaling pathway: receptor hypothesis

- The sperm bound to the oolemma activates a signal transduction pathway leading to a rise in calcium
- Activation is triggered prior to gamete fusion



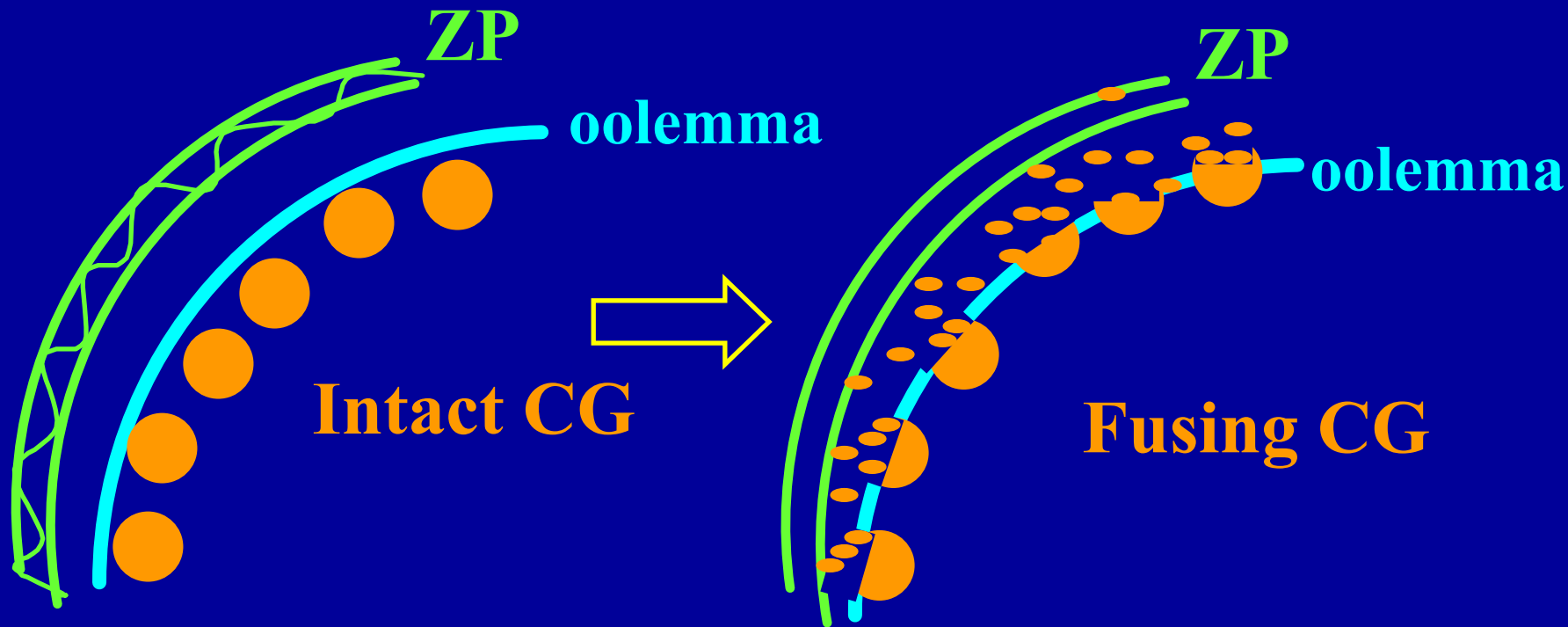
Calcium signaling pathway: sperm factor hypothesis

- Activation is initiated by a sperm factor introduced into the ooplasm following gamete fusion and responsible for a rise in calcium
- Sperm factor: oscillin, Phospholipase C, SOAF ??



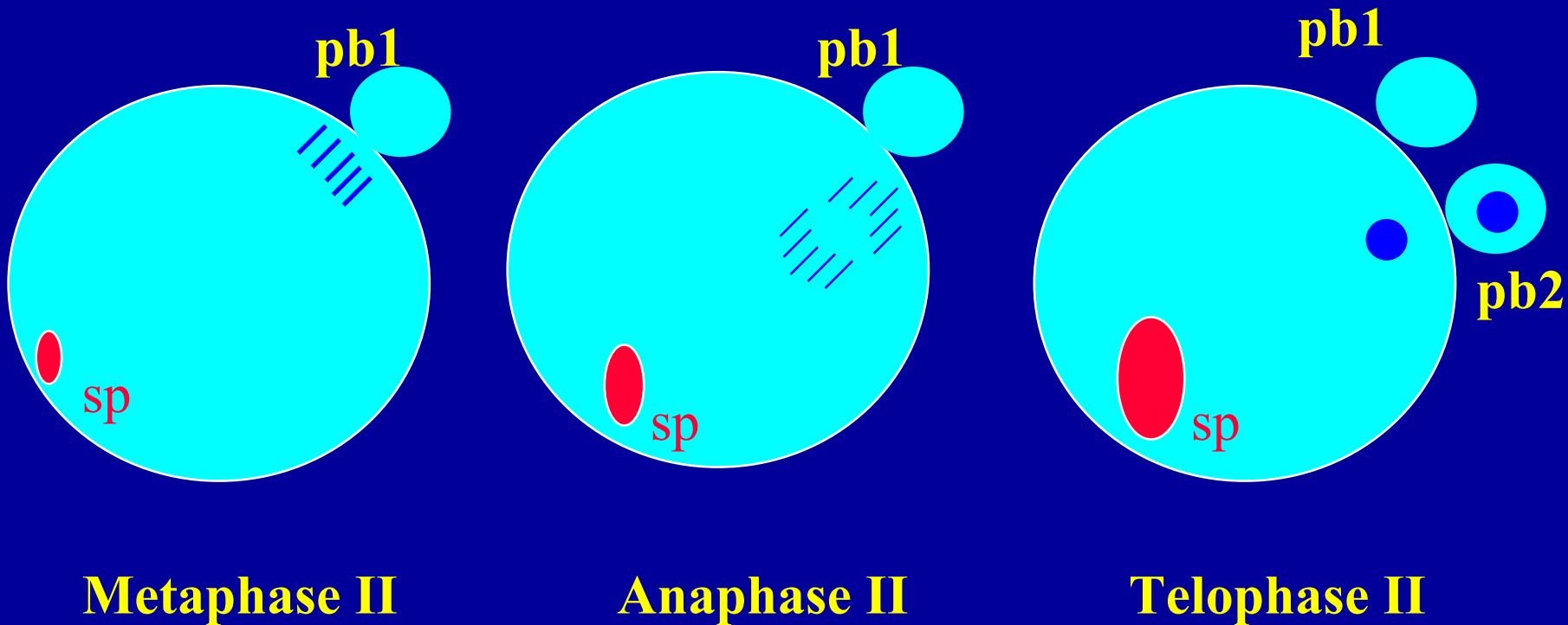
Exocytosis of cortical granules

- The cortical granules fuse with the oolemma and release their content into the perivitelline space to modify the ZP
- Modification of the zona pellucida prevents polyspermia



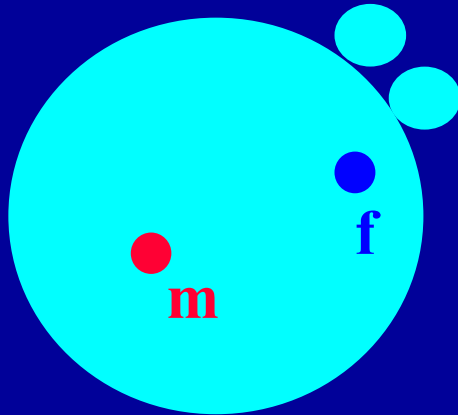
Resumption of meiosis

- Occurs during sperm chromatin decondensation
- Meiosis arrested at the metaphase II stage is reinitiated to the telophase II stage



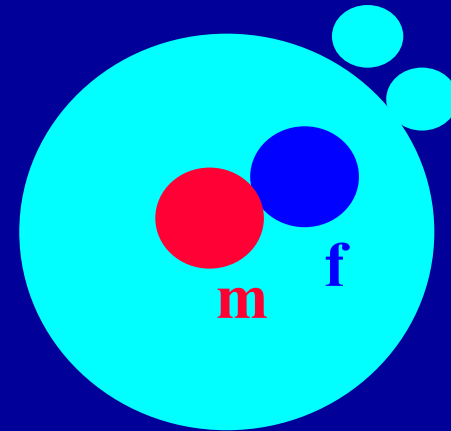
Pronuclei

1



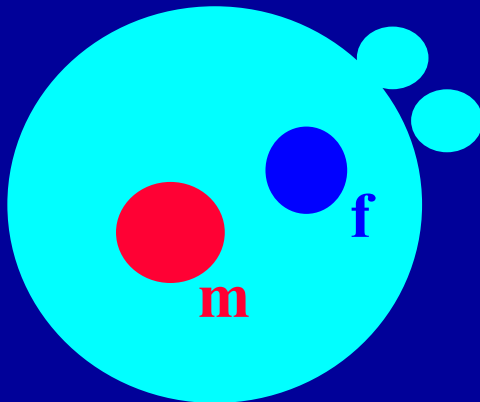
- Pronuclear envelope

3



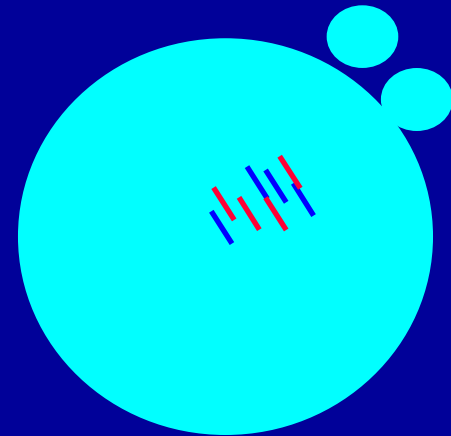
- Apposition of the pronuclei

2



- Enlargement + migration of the pronuclei
- DNA replication

4



- Chromosomes condensation

Summary

- **Maturation of the gametes (capacitation, meiotic maturation)**
- **Sperm penetration of the cumulus cell complex**
- **Binding of sperm to the zona pellucida**
- **Sperm acrosome reaction**
- **Zona pellucida penetration**
- **Binding of sperm to the oolemma (oocyte activation ?)**
- **Sperm fusion with the oolemma (oocyte activation?)**
- **Sperm chromatin decondensation**
- **Resumption of meiosis**
- **Pronuclei formation**
- **DNA synthesis in both pronuclei**
- **Association of paternal and maternal chromosomes**
- **First cleavage**