Università degli Studi di Bari Aldo Moro

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Scuola di Specializzazione in "Fisiopatologia della Riproduzione degli Animali Domestici"

Sperm capacitation and acrosome reaction

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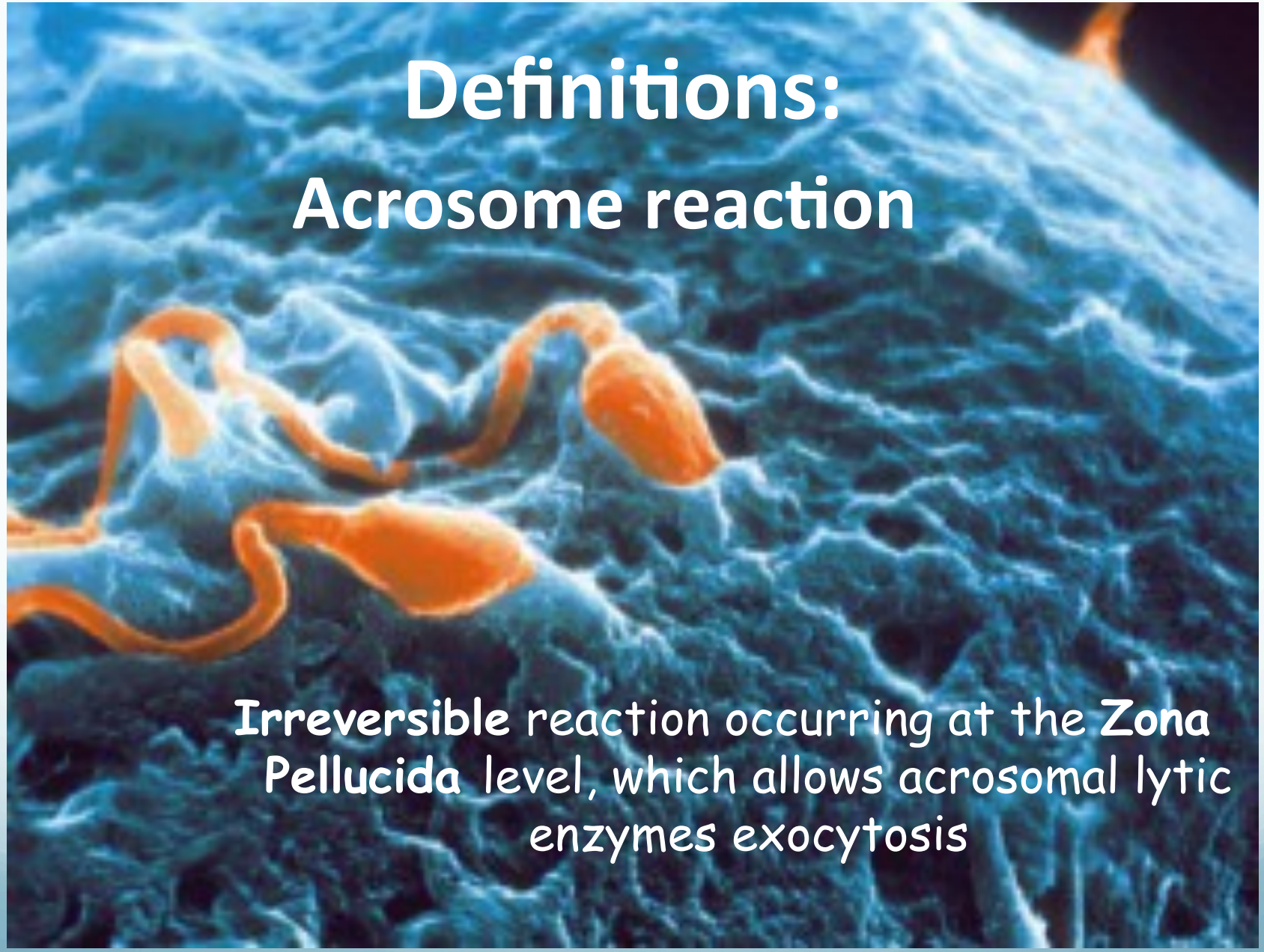
Summary

- Definitions
- Historical
- Physiology of capacitation/AR in vivo
- In vitro procedures
- Efficiency of in vitro procedures
- Use of fresh or frozen semen
- Sperm cap/AR assessment methods

Definitions: Capacitation

Process involving the spermatozoon in a complex series of physiological **reversible** reactions occurring in the **female reproductive tract**:

- Membrane phospholipids metabolism;
- Membrane cholesterol levels reduction;
- Membrane proteins reorganization;
- Hyperactivated motility



Definitions:

Acrosome reaction

Irreversible reaction occurring at the **Zona Pellucida** level, which allows acrosomal lytic enzymes exocytosis

Historical background

- Austin, 1951
- Chang, 1951
- Iritani and Niwa, 1971
- Iritani, 1980
- Yanagimachi, 1990

Spermatozoon morpho-functional maturation

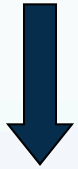
- **Male reproductive tract**

  Meiotic maturation (Testis)

  Morphological maturation (Testis)

  Motility acquirement (Epidydimis)

  Antigen acquirement for fertility (Epidydimis)



- **Female reproductive tract** Capacitation (uterus, oviduct)

  Acrosome Reaction (oocyte ZP)

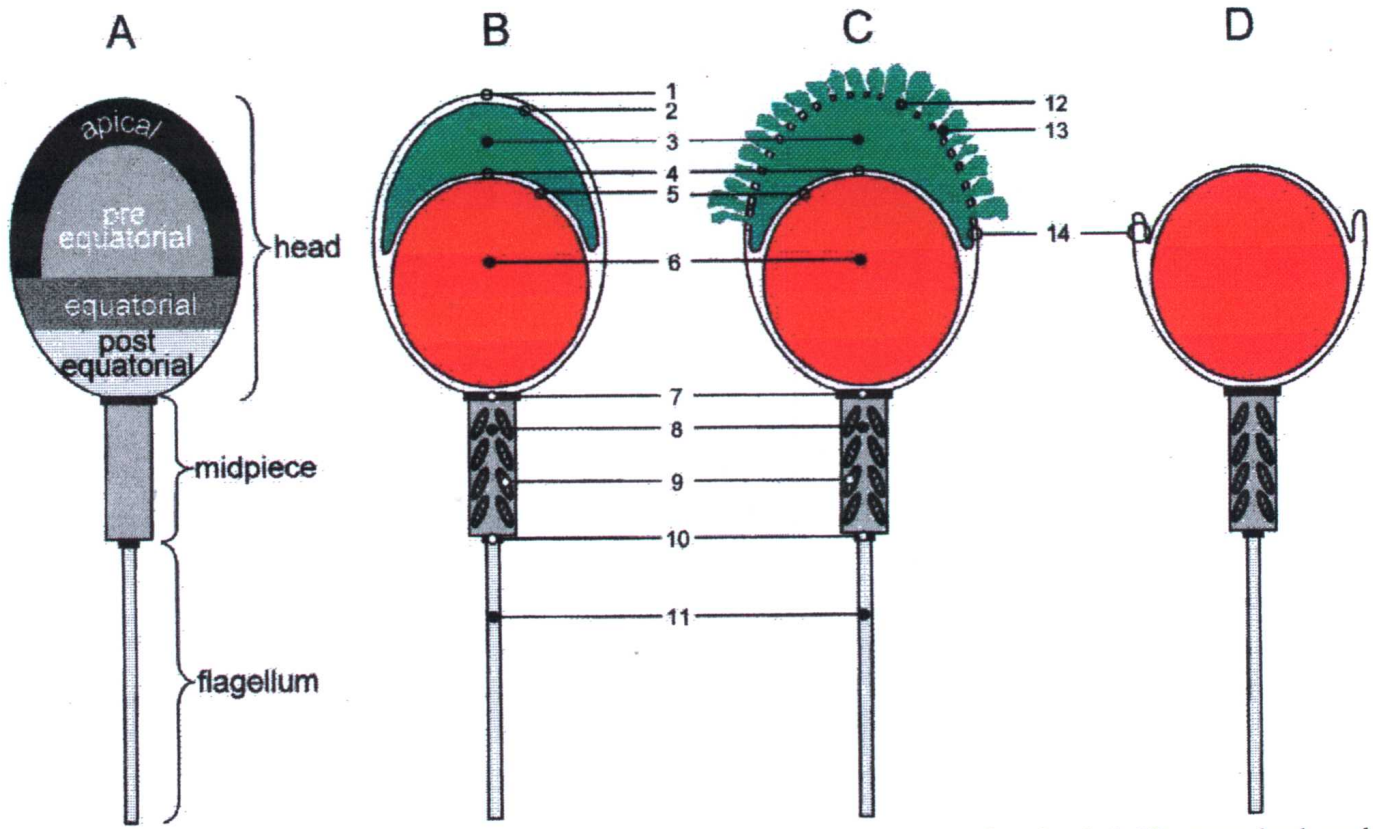


Fig. 1. Sperm cells are polarized cells with a head, flagellum and midpiece (A, schematic surface drawing). The sperm head can be subdivided in four regions: apical, pre-equatorial, equatorial and post-equatorial regions. The acrosome (large secretory vesicle, 3) is situated apical to the nucleus (B). After binding of the sperm cell to the oocyte with its apical plasma membrane, the plasma membrane fuses with the underlying outer acrosomal membrane at multiple sites (C). The acrosomal content (hydrolytic enzymes) will be secreted, which enables the sperm cell to digest the egg extracellular matrix (ZP). After the acrosome reaction has been completed, the inner acrosomal membrane forms a continuum with the remaining plasma membrane (D). This hairpin structure is involved in the primary binding of the sperm cell to the oolemma. Note that the representations (B), (C) and (D) are cross-sections through a flattened cell. 1: plasma membrane; 2: outer acrosomal membrane; 3: acrosomal content; 4: inner acrosomal membrane; 5: nuclear envelope; 6: nucleus containing highly condensed DNA; 7: posterior ring; 8: midpiece; 9: mitochondrion; 10: annular ring; 11: flagellum; 12: mixed vesicle (i.e. plasma membrane fused with outer acrosomal membrane); 13: acrosomal secretion; 14: hairpin structure.

Flesh & Gadella. **Biochim Biophys Acta 2000.**

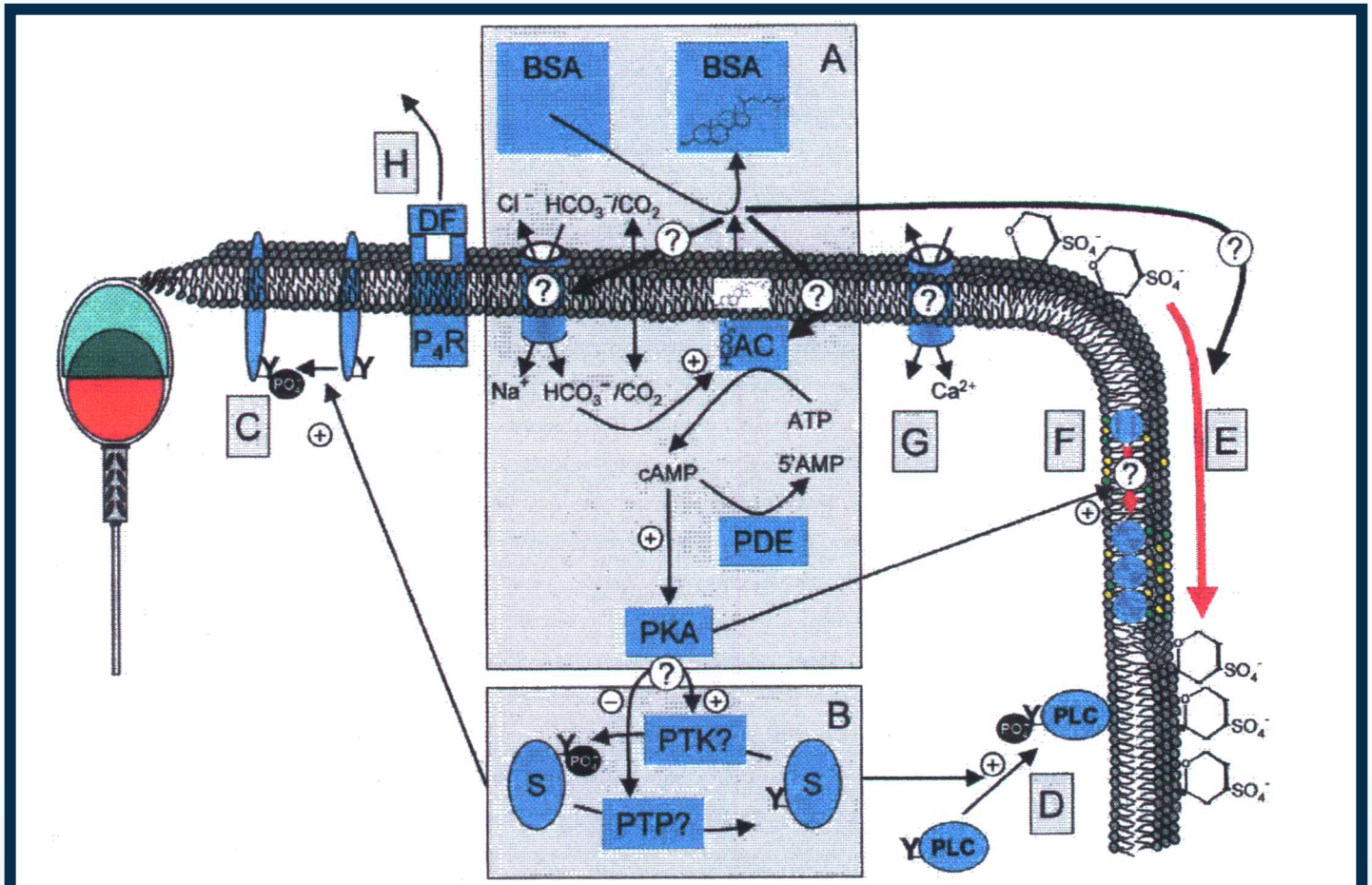
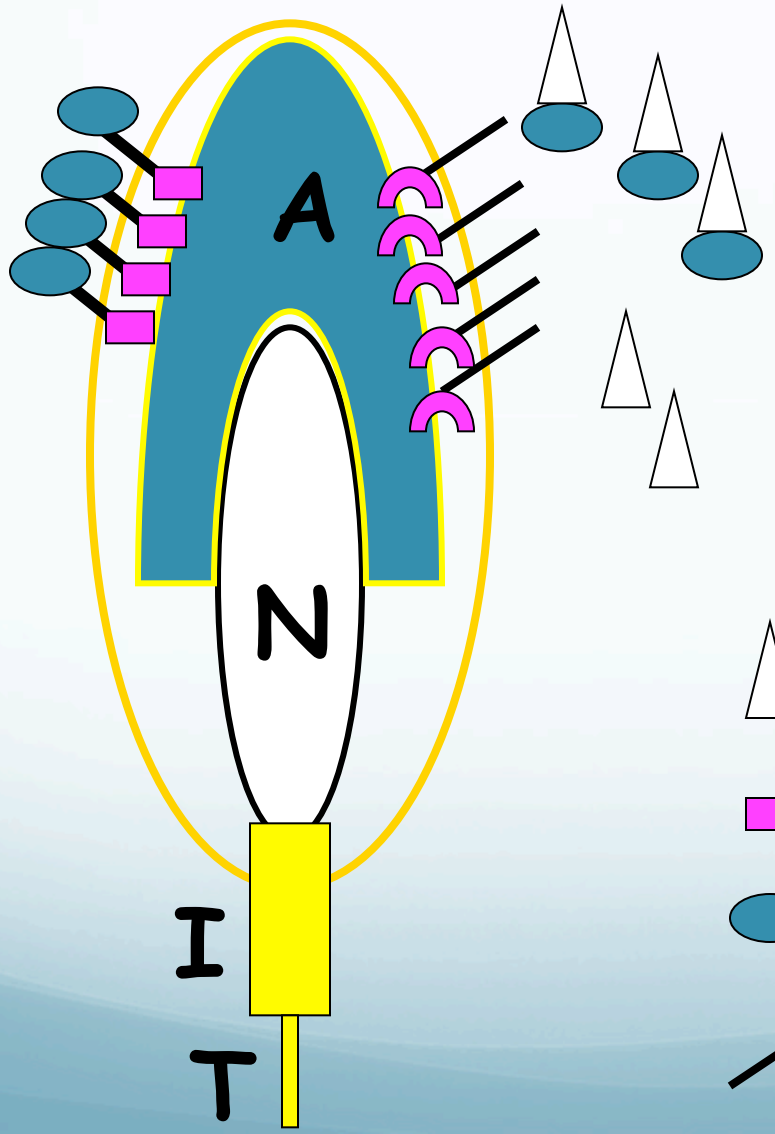


Fig. 5. Proposed sequences of mammalian sperm capacitation. (A) Bicarbonate may enter sperm cells via ion channels or via diffusion as carbon dioxide. Intracellular bicarbonate switches on AC and concomitant production of cAMP activates PKA. The role of cholesterol efflux in the activation of PKA is unclear. Cholesterol efflux may induce increased bicarbonate entry or may affect AC. (B) PKA induces tyrosine (Y) phosphorylation of several substrates (S) most likely via activation of PTK or inhibition of protein tyrosine phosphatases (PTP). (C) Sperm–ZP binding proteins and other plasma membrane proteins become tyrosine phosphorylated via the bicarbonate induced activation of PKA. (D) Cytosolic PLC is tyrosine phosphorylated via the bicarbonate–PKA pathway. Tyrosine phosphorylated PLC is subsequently translocated to the plasma membrane. (E) PKA activation induces plasma membrane changes like lateral redistribution of seminolipid and translocation of aminophospholipids. (F) Aminophospholipids are translocated by the PKA dependent activation of a postulated scramblase. Most likely the efflux of cholesterol is involved in these plasma membrane transitions. (G) The entry of small amounts of calcium into sperm cells plays possibly an important role in capacitation. (H) Decapacitation factors (DF) are removed from the sperm cell surface, uncovering receptors like the postulated progesterone receptor (P_4R).

Flesh & Gadella **Biochim Biophys Acta 2000.**

Capacitation



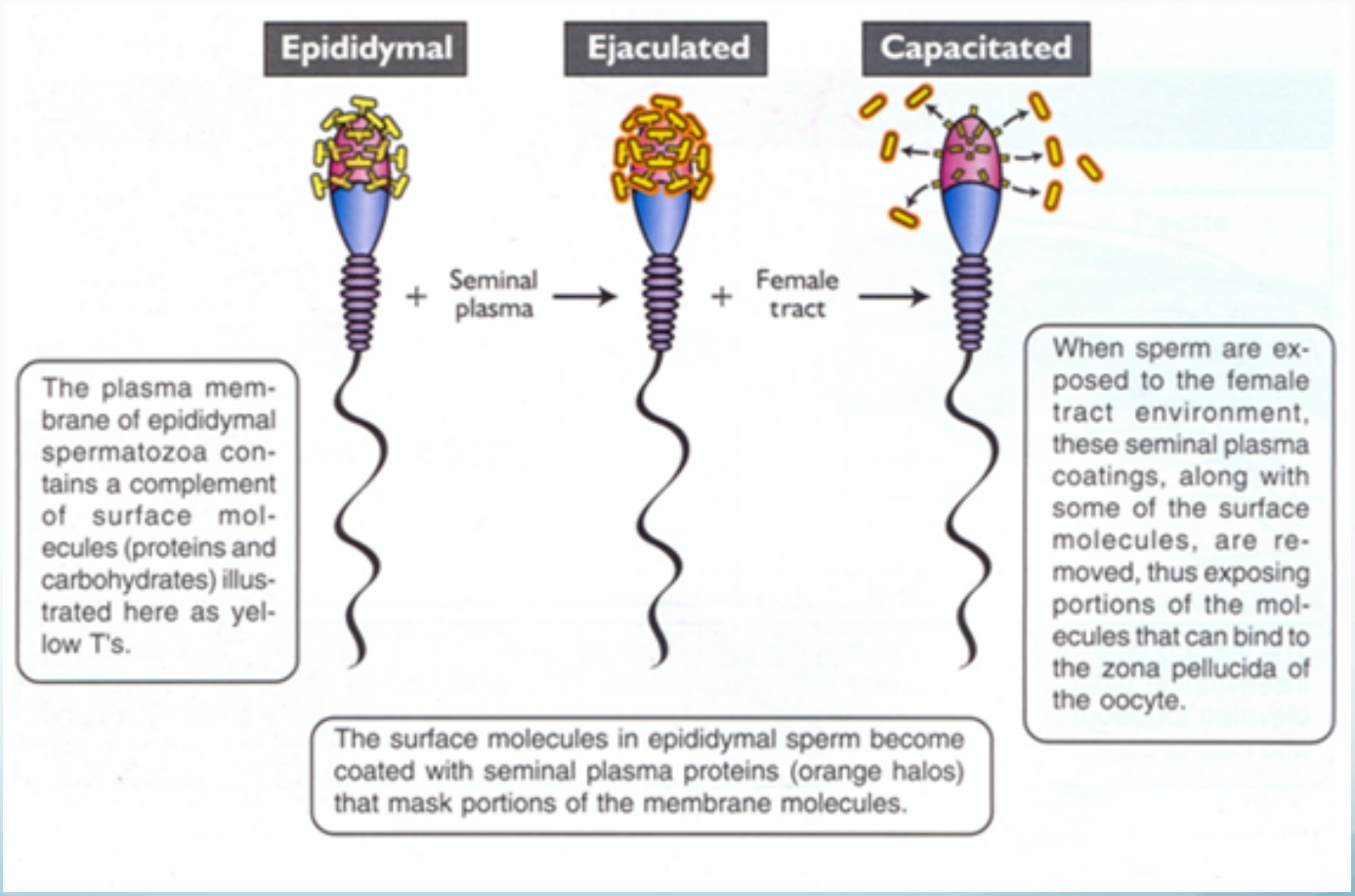
= Heparin

and = Calmodulin

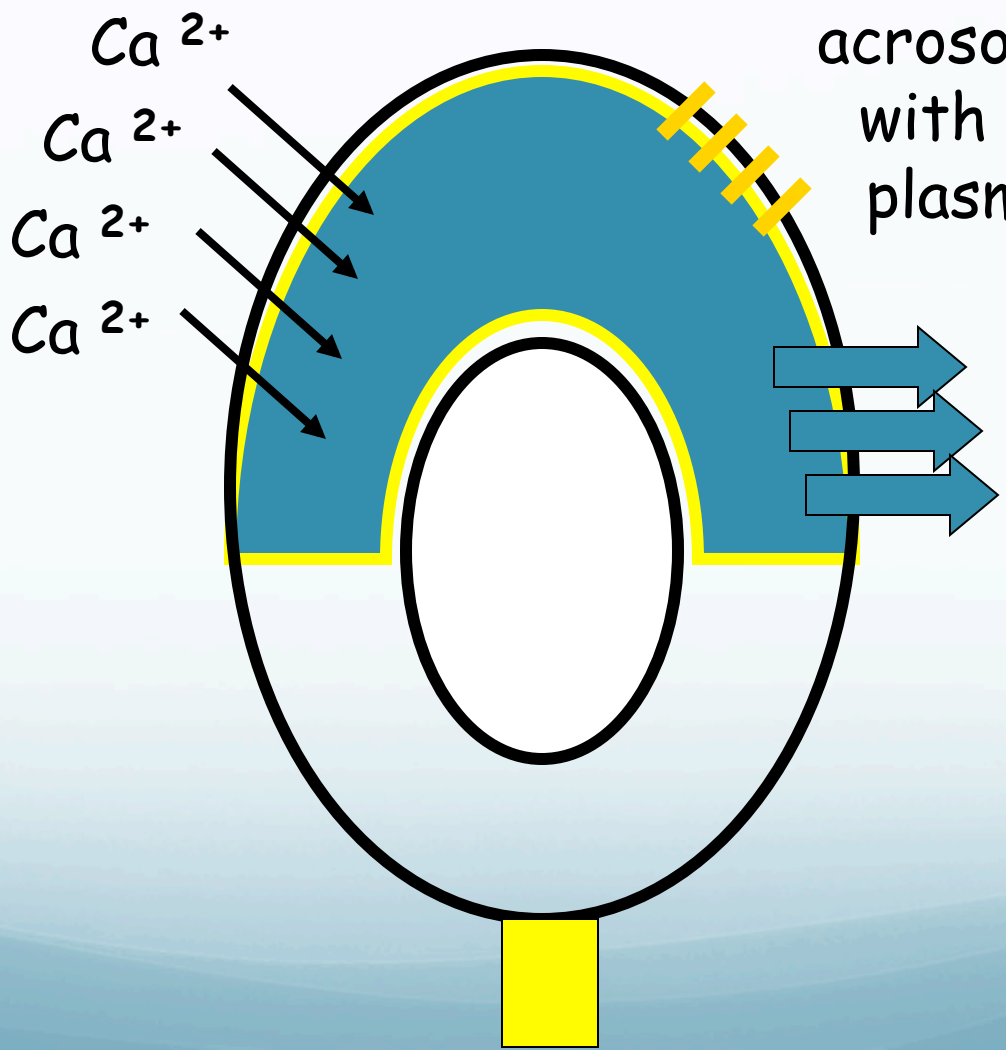
= Seminal plasma protein

= Cholinic phospholipid

Capacitation



Acrosome Reaction



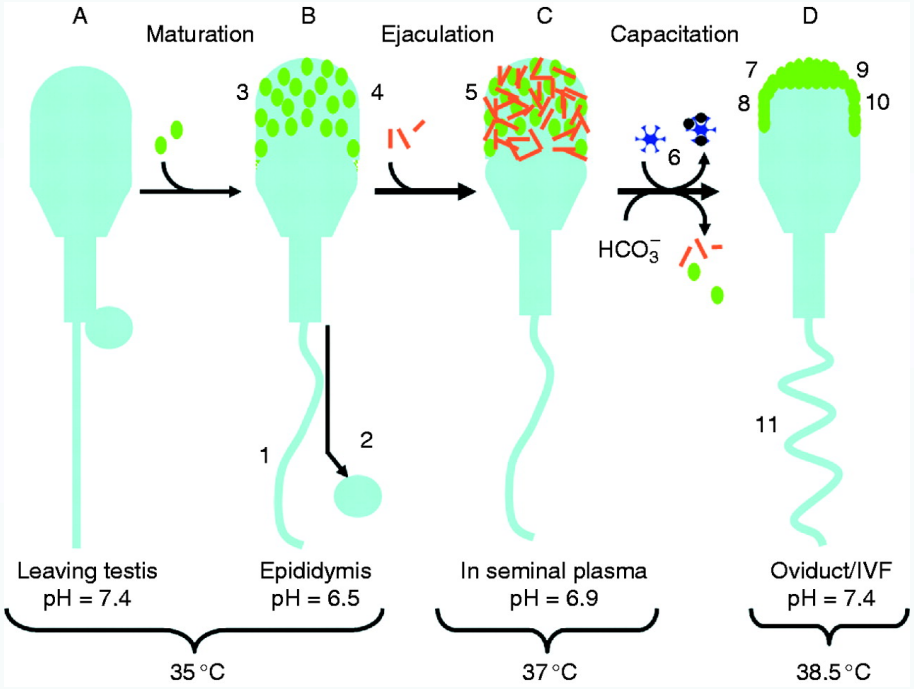
Fusion of the outer acrosomal membrane with the overlying plasma membrane

Acrosomal lytic enzymes exocytosis

Semen capacitation and Acrosome reaction

- Seminal plasma proteins reversible binding to spz membrane
- Glycosaminoglycans binding to seminal plasma proteins
- Cholinic phospholipid sites release
- Calmodulin steric configuration modification
- Ca^{2+} transport
- Phospholipase A activation
- Lysophosphatidylcholine synthesis
- Fusion of the outer acrosomal membrane with the overlying plasma membrane

Da Leahy & Gadella, 2011



Physiological sequence of surface changes rendering spermatozoa fit to fertilise the oocyte.
(**A**) When spermatozoa leave the testis, they are morphologically complete but lack the capacity to fertilise the oocyte as they are immotile and contain remnants of the cytosolic bridges that synchronise spermatogenic cells until spermiation (the cytoplasmic droplet).

(**B**) In the epididymis, spermatozoa (**1**) acquire motility characteristics, (**2**) lose the cytoplasmic droplet and (**3**) undergo some final chromatin condensation. The sperm surface undergoes various molecular alterations. The most important being the adsorption of proteins (green) that are involved in sperm–zona binding (**4**) and surface stabilising factors.

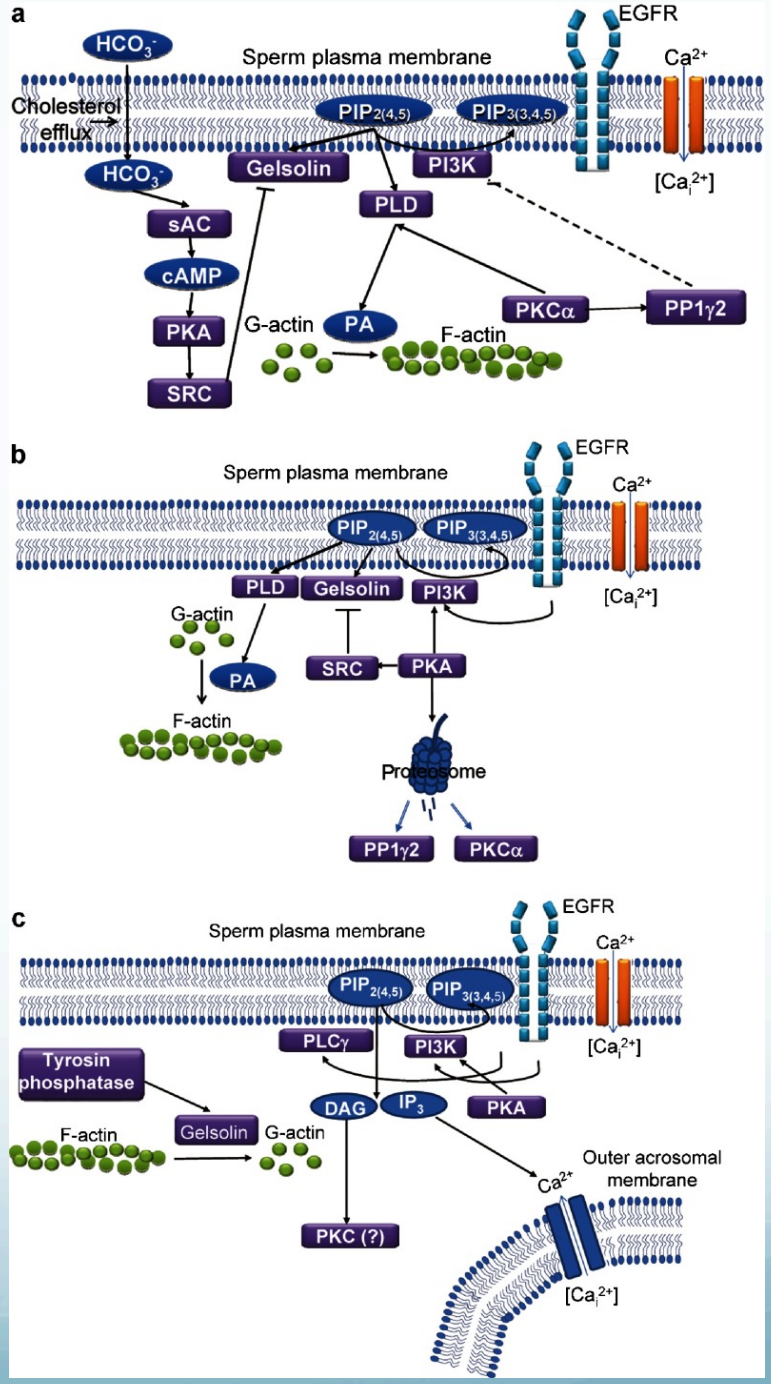
(**C**) At ejaculation, spermatozoa are mixed with seminal plasma from the accessory sex glands. Seminal plasma glycoproteins (orange) adhere tightly to the sperm surface and efficiently stabilise the cell during transport through the lower portion of the female genital tract (**5**).

(**D**) When spermatozoa reach the upper portion of the female genital tract (the isthmus of the oviduct), they are triggered to capacitate. The mechanisms are unknown but it is thought that migration of spermatozoa through the uterus and the uterotubal junction causes the removal of decapacitation factors (orange) and that spermatozoa respond to female-derived stimulatory signals (Holt & Fazeli 2010). Cholesterol (black) is oxidised and removed (**6**) from the sperm surface by albumin (blue), (**7**) epididymal proteins (green) aggregate into membrane rafts at the apical ridge of the sperm head, (**8**) a partial lipid scrambling takes place in the same area (**9**) and seminolipids migrate towards the equatorial area of the sperm head. At the cytosolic site, the apical ridge of the sperm head is now efficiently and stably docked to the outer acrosomal membrane (**10**). Functionally, the apical head area is now prepared for fertilisation as it contains protein complexes capable of interacting with the zona pellucida or the extracellular matrix of the cumulus mass. At the sperm tail, an increase in the pH of the fluid environment evokes proton extrusion and signal transduction cascades provoke tyrosine phosphorylation events, which generate hyperactivated motility, probably by enhancement of anaerobic ATP generation (**11**).

from Ickowicz:

Mechanisms of sperm CAP/AR: role of PK

2012 Asian J Androl



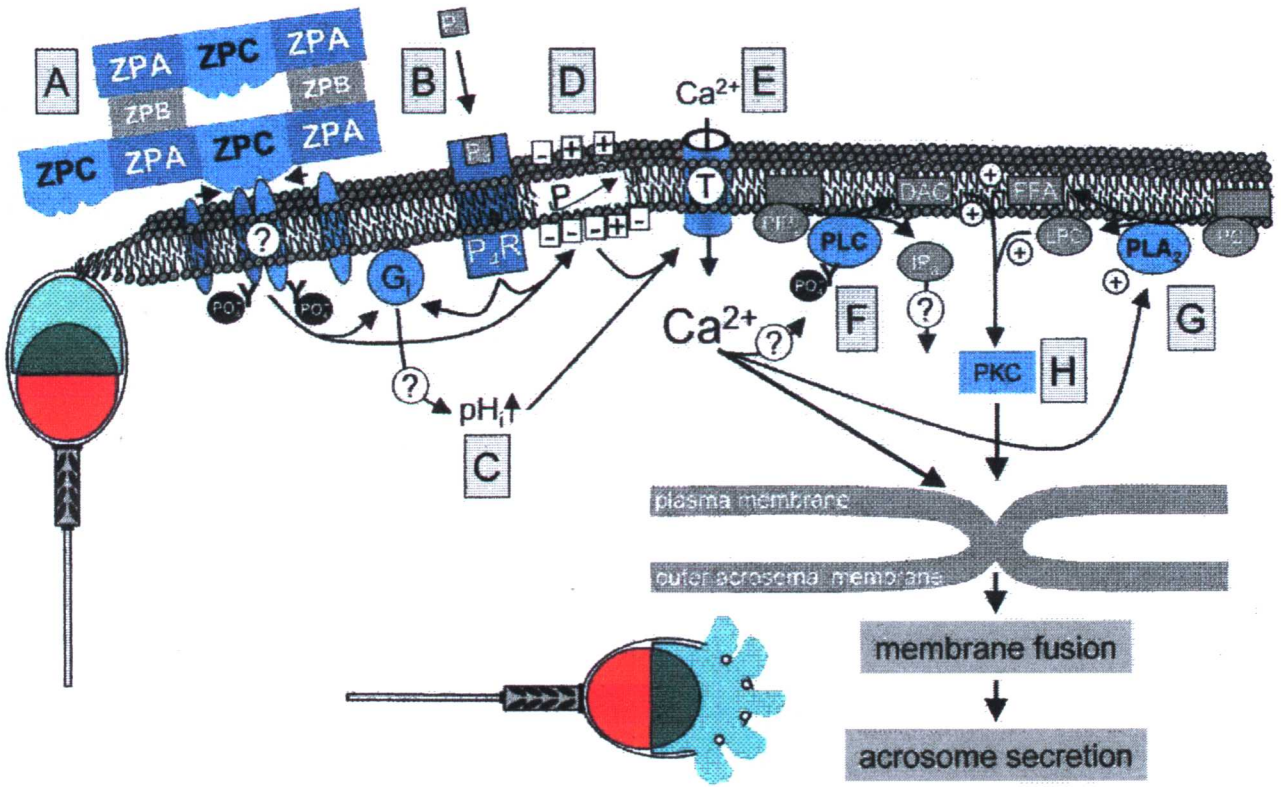


Fig. 6. Proposed sequence of the ZP and progesterone induced acrosome reaction. (A) ZP proteins (most likely ZPC) bind to sperm ZP receptors, leading to aggregation and tyrosine (Y) phosphorylation. (B) The direct environment of the ZP contains high levels of progesterone that can bind to its non-genomic receptor (P_4R) on the sperm surface. Both ZP and progesterone have a dual effect on sperm cells. (C) The intracellular pH (pH_i) is increased via G-proteins (G_i) and (D) the plasma membrane potential depolarizes. (E) Both the increased pH_i and depolarization induce the entry of calcium via a T-type voltage dependent Ca^{2+} channel. (F) The higher intracellular Ca^{2+} levels activate PLC that has been translocated to the plasma membrane during capacitation. PLC converts PIP_2 to DAG and IP_3. (G) Increased Ca^{2+} levels activate PLA_2, which degrades PC to LPC and free fatty acids (FFA). (H) The role of IP_3 is unclear, but DAG, FFA and LPC activate PKC. Both the increased intracellular Ca^{2+} levels and PKC activation are necessary for the fusion of the plasma membrane with the underlying acrosomal membrane, which leads to the subsequent secretion of acrosomal enzymes.

Flesh & Gadella. **Biochim Biophys Acta 2000.**

Table 1

The acrosome reaction can be induced in vitro by several agents

Inducer	Possible mechanism	Reference
Protein:		
Solubilized ZP	receptor activation (physiological)	[289]
Progesterone	receptor activation? (physiological?)	[327]
Glycoconjugate	mimic ZP activation?	[384]
Glycosamineglycan sulfate	mimic ZP activation?	[385]
Angiotensin II	receptor mediated?	[386]
Atrial natriuretic peptide	receptor mediated?	[387]
Trypsin inhibitor	?	[388]
Sialic acid binding protein	?	[389]
Lipid:		
Arachidonic acid	membrane perturbation? receptor mediated?	[390]
Platelet activating factor	membrane perturbation? receptor mediated?	[391]
Lysophospholipids	membrane perturbation? receptor mediated?	[150]
Fluid:		
Follicular fluid	multiple? activators including progesterone	[392]
Chemical agents:		
Calcium ionophore	Introduction of Ca^{2+} into the cell	[393]
cAMP analogue	capacitation effect?	[394]
cGMP analogue	capacitation effect?	[318]
Other:		
Ethanol	membrane perturbation	[393]
Low temperature	'cold shock' membrane perturbation	[394]
Electropermeabilization	introduction of Ca^{2+} into the cell	[395]

The mechanism of action of these agents is very diverse, ranging from the physiological relevant activation of sperm ZP receptors by solubilized ZP proteins, to perturbation of membranes by ethanol.

Flesh & Gadella. **Biochim Biophys Acta 2000.**

Efficiency of in vitro capacitation procedures

- Staining techniques (NO colorimetriche)

- Fluorescence microscopy: CTC-Hoechst 33258;

- Flow Cytometry: Merocianina 540-PI

- Oocyte penetration tests

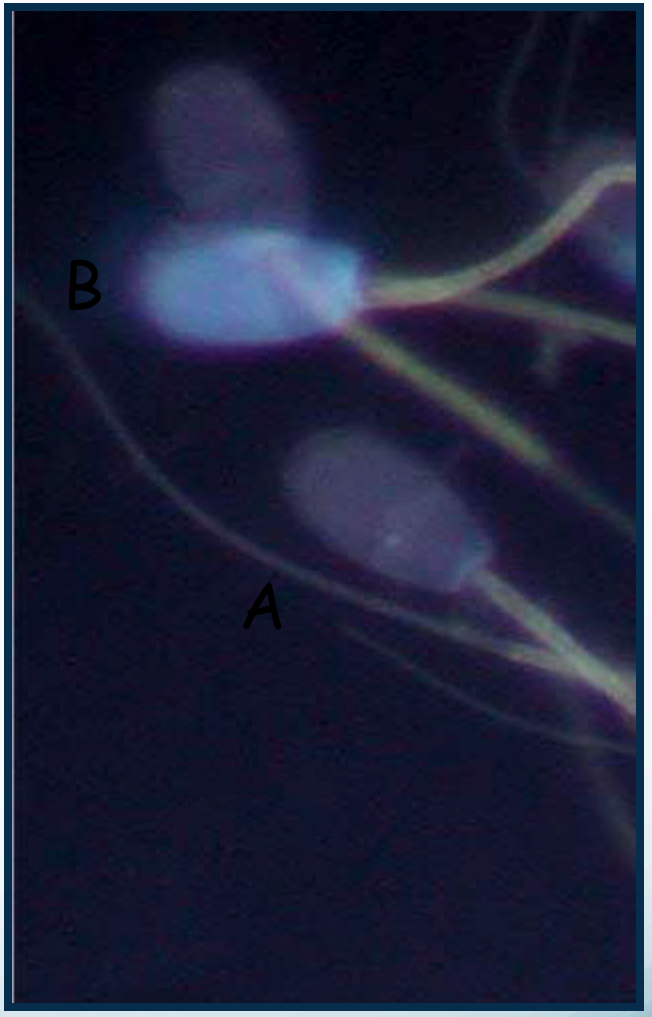
Electronic microscopy: frozen ZP penetration

- Sperm-zona binding

Fluorescence microscopy

HOECHST 33258

- FLUORESCENT NON-INTERCALATING SUPRAVITAL DYE SPECIFIC FOR ADENINE AND THYMINE .
- IT EMITS BLUE LIGHT AT 420 nm WHEN EXPOSED TO A LIGHT WITH A WAVELENGTH OF 330-380 nm
- IT ALLOWS TO DISTINGUISH BETWEEN VIABLE (A) AND DIED (B) SPERMATOZOA, ACCORDING TO THEIR MEMBRANE INTEGRITY AND PENETRATION CAPABILITY



Albrizio et al. Reproduction 2004

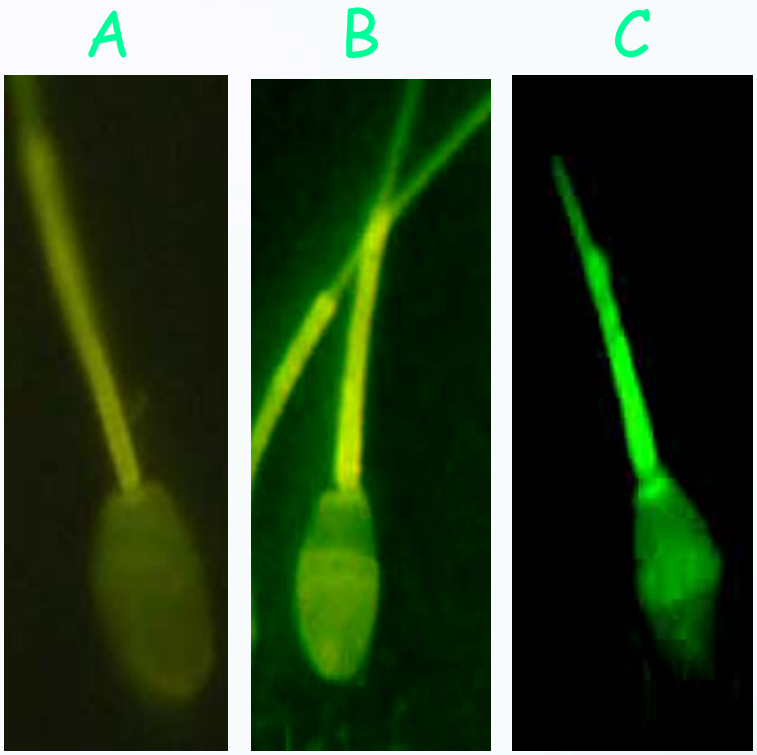
EXPERIMENTAL PROTOCOL:

- add 0.5 μl of Hoechst 33258 solution (10 μg/ml) to 50 μl of semen treated with a testing substance
- incubate for 2', add 500 μl of 2% PVP and centrifuge at 900xg for 5'
- remove the supernatant and re-suspend the pellet with 45 μl of TALP, thus proceed with CTC treatment

CHLORTETRACYCLINE (CTC)

- ANTIBIOTIC ABLE TO BIND HYDROPHOBIC MEMBRANE REGIONS IN A CALCIUM-DEPENDING MANNER.
IT EMITS GREEN LIGHT AT 470 nm WHEN EXPOSED TO A LIGHT WITH A WAVELENGTH OF 400-440 nm

- IT ALLOWS TO DISTINGUISH NORMAL (A), CAPACITATED (B) AND ACROSOME REACTED (C) SPERMATOZOA, ACCORDING TO THEIR DIFFERENT MEMBRANE CHANGES

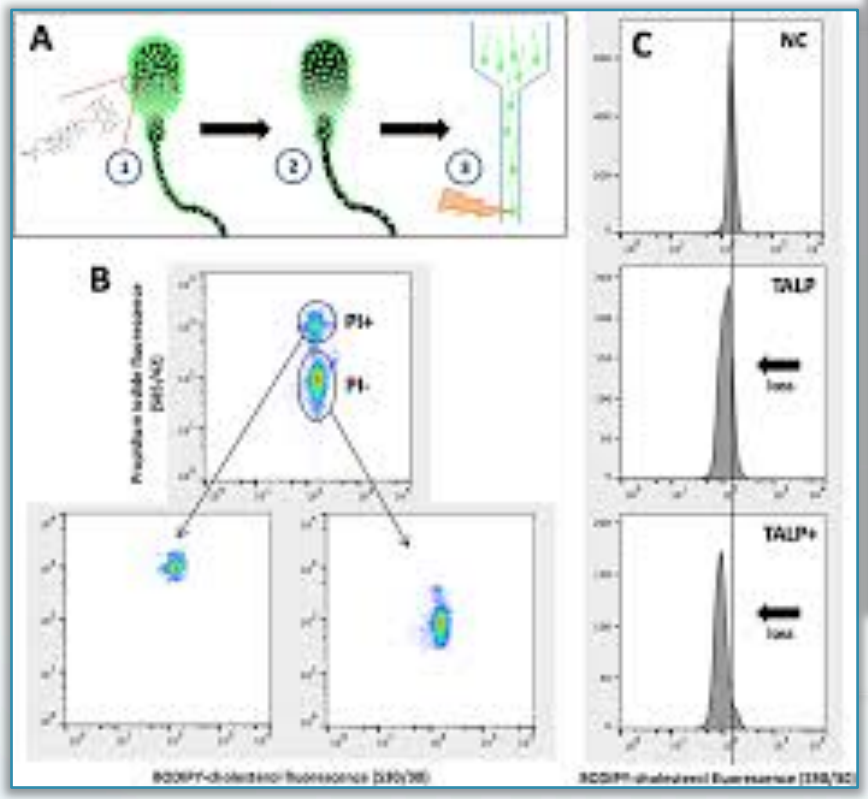


Albrizio et al. Reproduction 2004

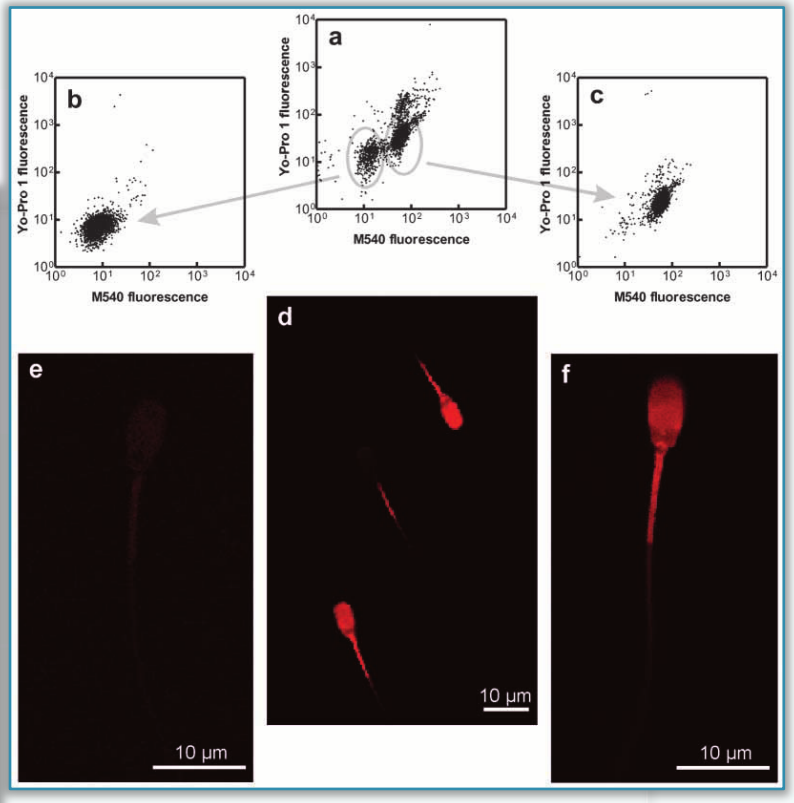
EXPERIMENTAL PROTOCOL:

- dissolve 0.0019 g of CTC and 0.0044 g of L-cysteine in a solution containing Tris 20mM, NaCl 130mM at pH=7.8
- mix 50 μl of spermatozoa, previuosly treated with Hoechst 33258, with 50μl of CTC solution
- add 15 μl of 12.5% glutaraldehyde in Tris 1M pH=7.0 in order to fix cells
- assemble the slides by putting 5 μl of trated sperm and observe in 1 hour

Flow cytometry



Da Bernecic et al., 2019



Da Flesh et al., 2001

Merocyanine 540/Yo-Pro-1 staining

Flow cytometry for capacitation evaluation

- Sperm cells in Tyrodes Medium/bicarbonate containing:
  - 2.7 μM of Merocyanine 540 (sensible to membrane phospholipidic variations)
  - 25nM of Yo-Pro-1 (specific for nucleic acids; relatively membrane impermeable)
  - 0.5mg/ml PVA
  - 0.5mg/ml PVP
- Incubate at 37°C for 30 min
- Flow cytometry for capacitation evaluation is carried out by FACS Vantage SE: Merocyanine 540/Yo-Pro-1 are excited by Argon ions laser at λ=488 nm.
- Fluorescence is measured at λ emmisions rispectively of 520 and 575 nm.
- 8.000-10.000 spermatozoa/second are analyzed.

FITC-PNA/PI staining

Flow cytometry for acrosome reaction evaluation

- The outer acrosomal membrane express specific markers (glycoproteins)
- These glycoproteins can be detected by lectins, such as PNA (Peanut Agglutinin)
- These lectins have to be linked to fluorescent probes, such as FITC (Fluorescein isothiocianate)
- Incubate sperm cells with 5μg/ml of FITC-PNA (acrosome reaction marker) and 1μM of PI (died cells marker).
- Analyze with FACS scan

SYBR 14/PE-PNA/PI staining

Flow cytometry for simultaneous viability and acrosome integrity evaluation

Add to 1 ml of diluted semen:

- 100 nM SYBR-14 (*component A, 10 times diluted in DMSO)
- 2.5 mg/ml PE-PNA (in buffer as Nagy et al., 2003)
- 12 mM PI (*non-diluted component B)

* LIVE/DEAD Sperm Viability kit, Molecular Probes, OR, USA

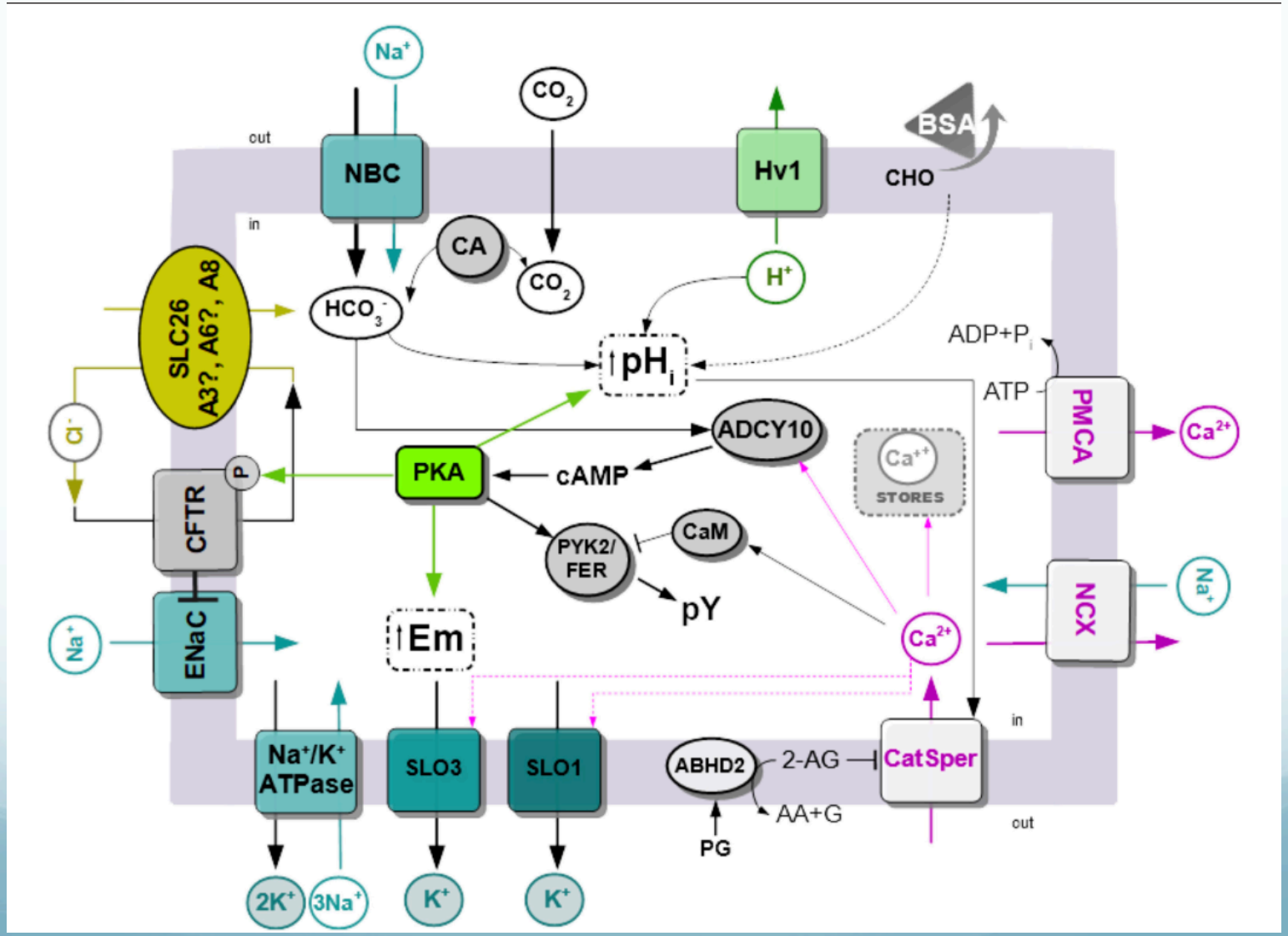
Nagy et al., 2003 Biol Reprod 68, 1828-35.

Fresh and frozen semen

- Freezing procedures affect sperm viability, capacitation and acrosome reaction

Signaling pathways and ion fluxes involved in sperm capacitation

(da Molina et al., 2018)



Recommended readings

- **Gordon I.**"Capacitating bovine sperm" In: "Laboratory production of cattle embryos" CABI Publishing Dublin 2003 II ed., Chapter 5.
- **Flesh FM & Gadella BM.** Dynamics of the mammalian plasma membrane in the process of fertilization. Biochim Biophys Acta 2000; 1469: 197-235.
- **Rathi et al.,**2001. Evaluation of in vitro capacitation of stallion spermatozoa. Biol Reprod 65: 462-470.
- **Nagy et al.,** 2003. A triple stain flow cytometric method to assess plasma- and acrosome-membrane integrity of cryopreserved bovine sperm … Biol Reprod 68:1828-1835.
- **Molina et al.,** 2018 Molecular basis of human sperm capacitation. Front. Cell and Dev Biol *6:72. doi: 10.3389/fcell.2018.00072*