Molecular aspects of fertilization

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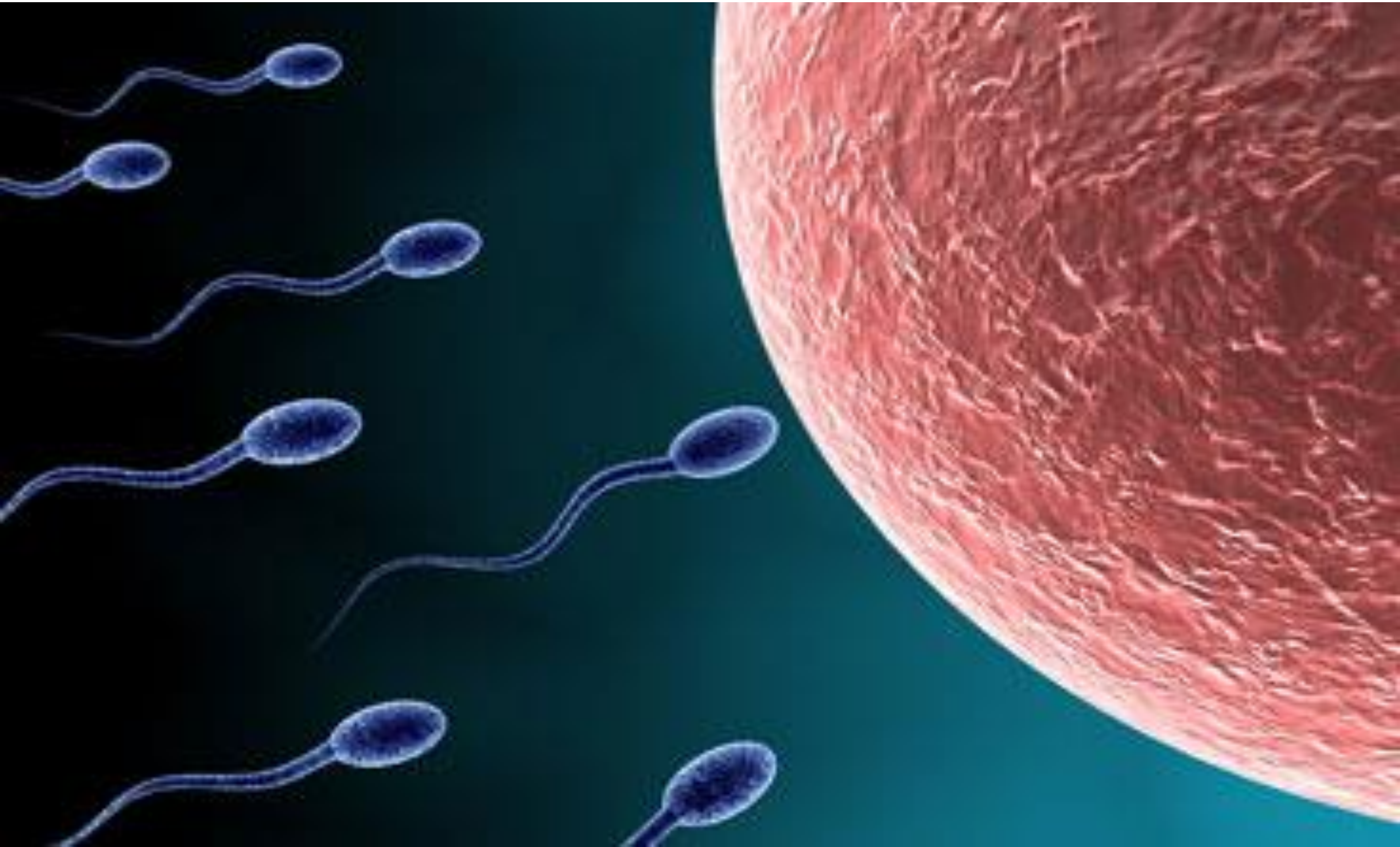
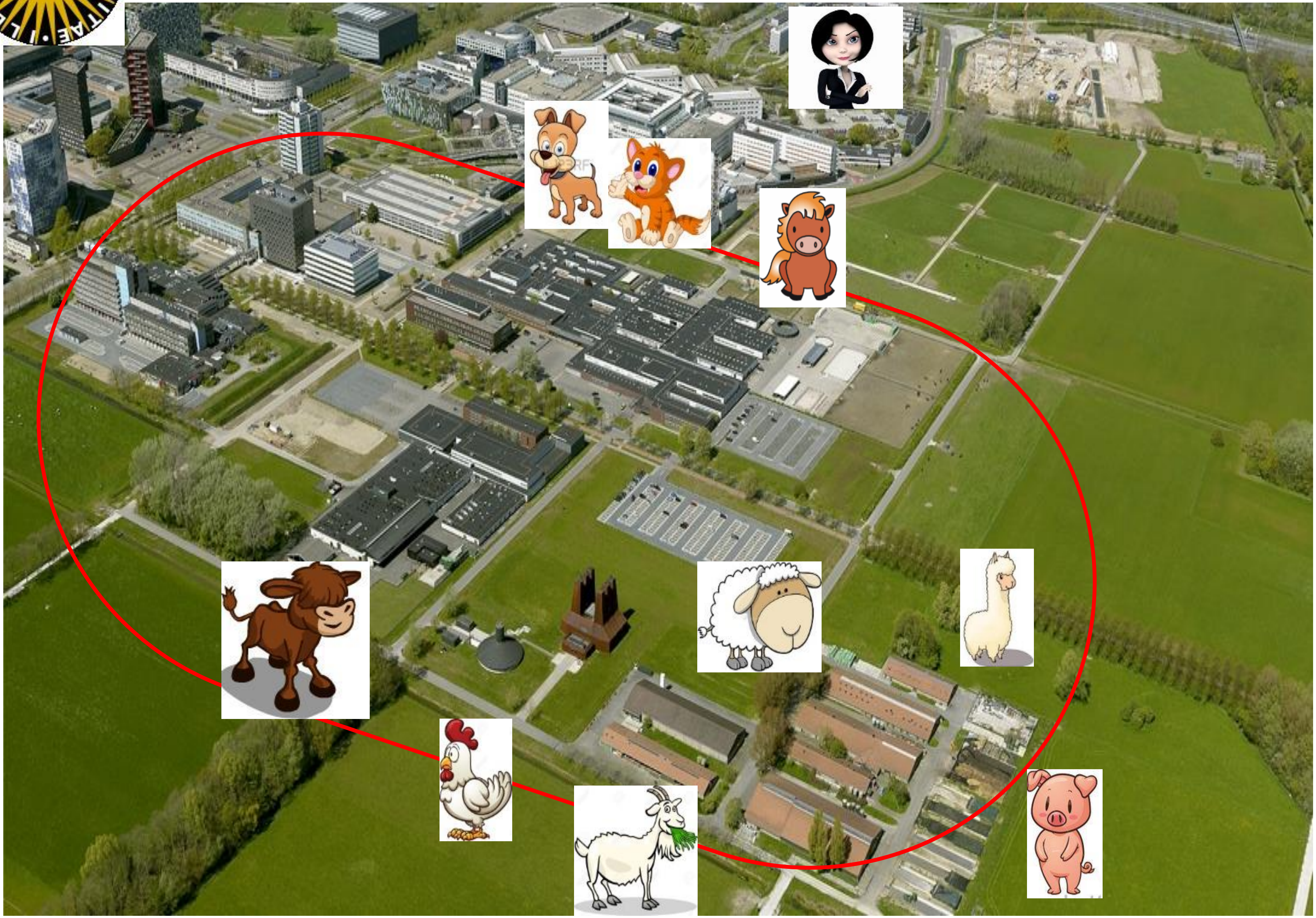
Diergeneeskunde

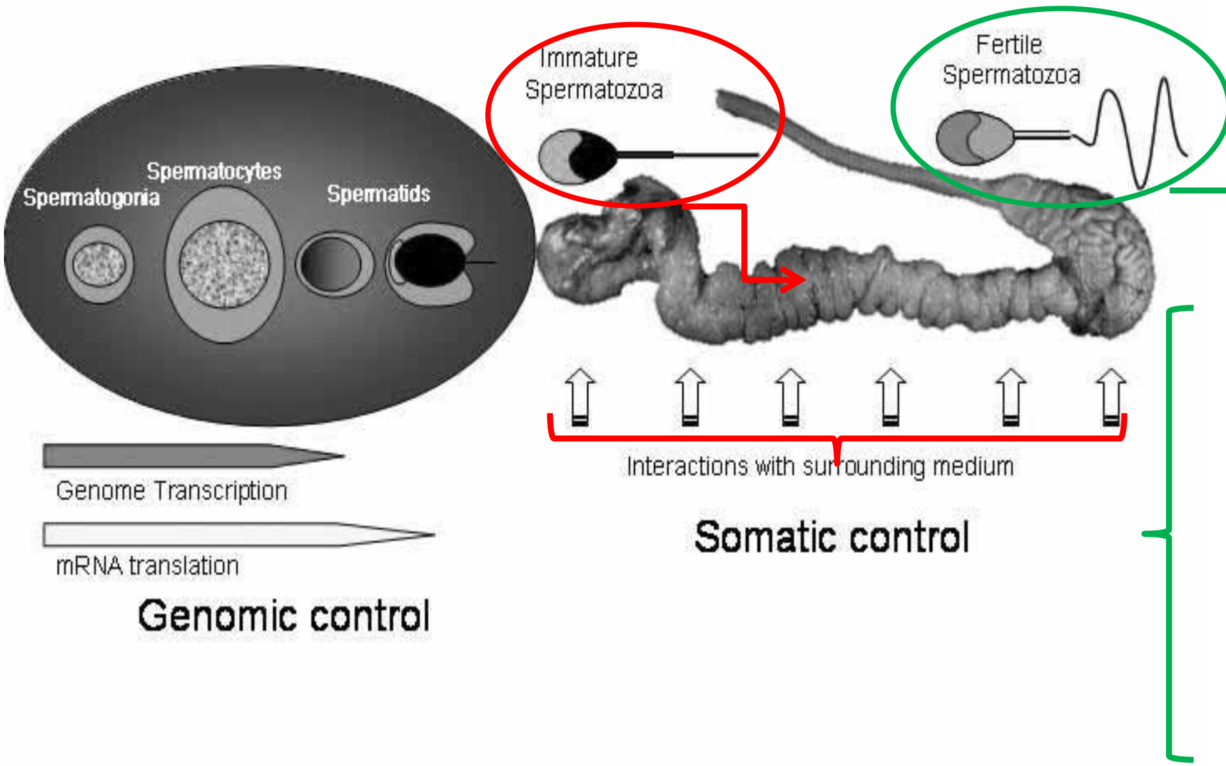
3rd CZU Prague May 3, 2024

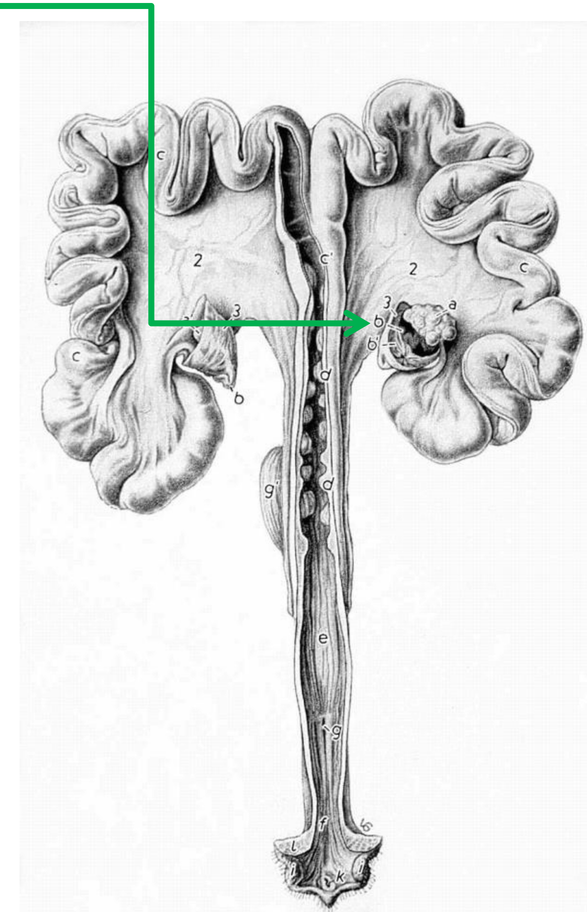


Utrecht University



The interacting structures from both gametes are after their release from the gonads subjected surface changes before they meet each other.



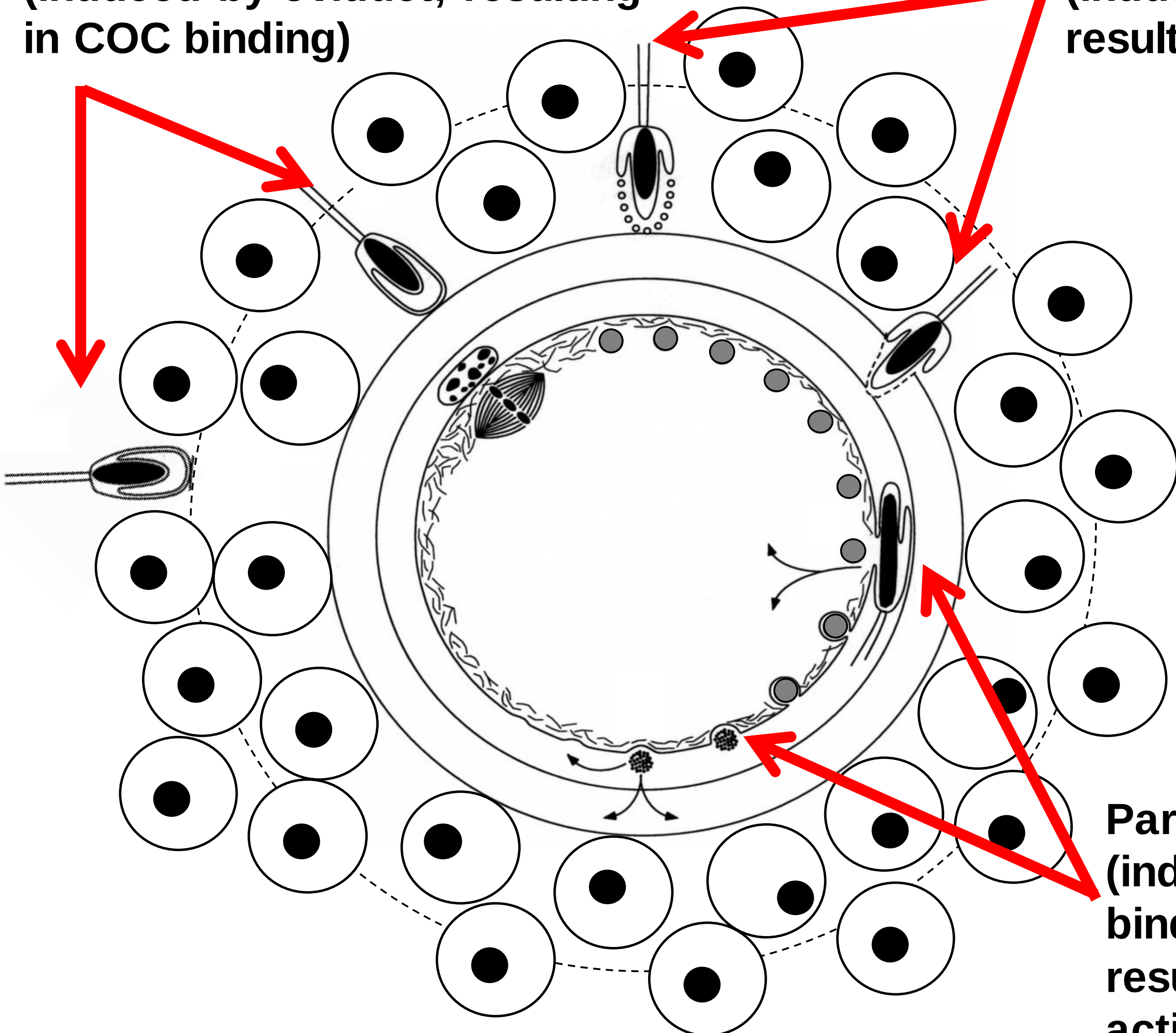


Oviduct conditions are mimicked in IVF media (SOF) or in in vtro capacitation media

Illustrations from Dacheux and from Senger

Part I: Sperm capacitation (induced by oviduct; resulting in COC binding)

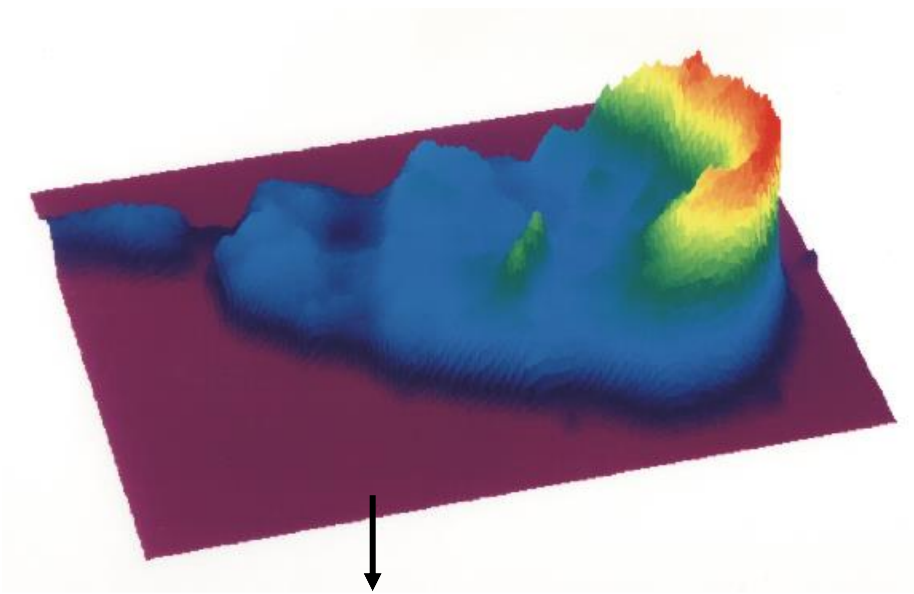
Part 2: Acrosome fusions (induced by zona binding; resulting in zona drilling)



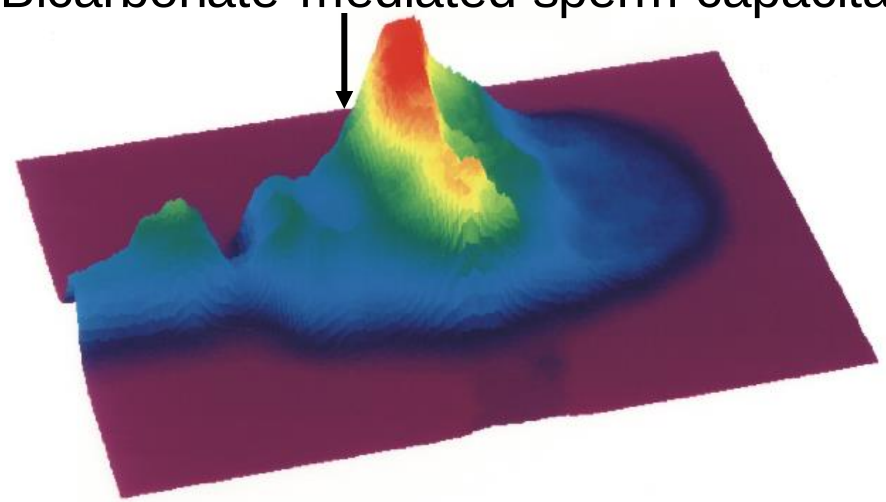
**Part 3: Fertilization fusion (induced by oolemma binding;
resulting in oocyte activation, polyspermy block and syngamy)**

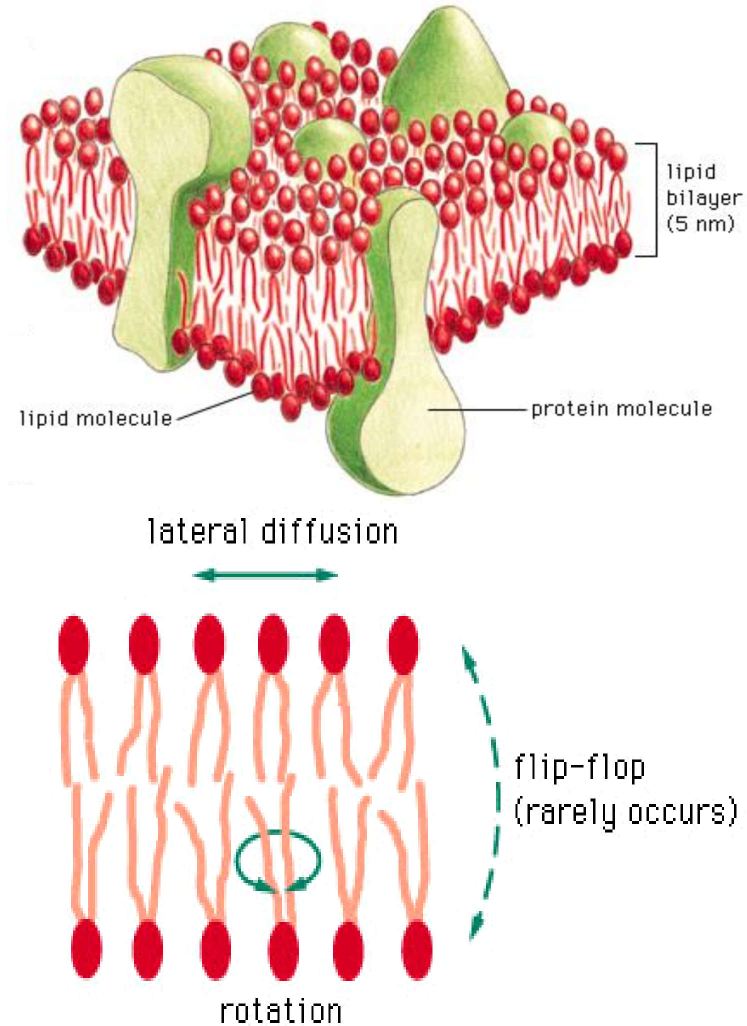
Gadella; Reprod. Fert. Dev. (2013)

Seminolipid migration from apical to equatorial surface area of the sperm head
→ Utrecht/Gottingen: *Gadella et al., J. Cell Sci. (1995)*



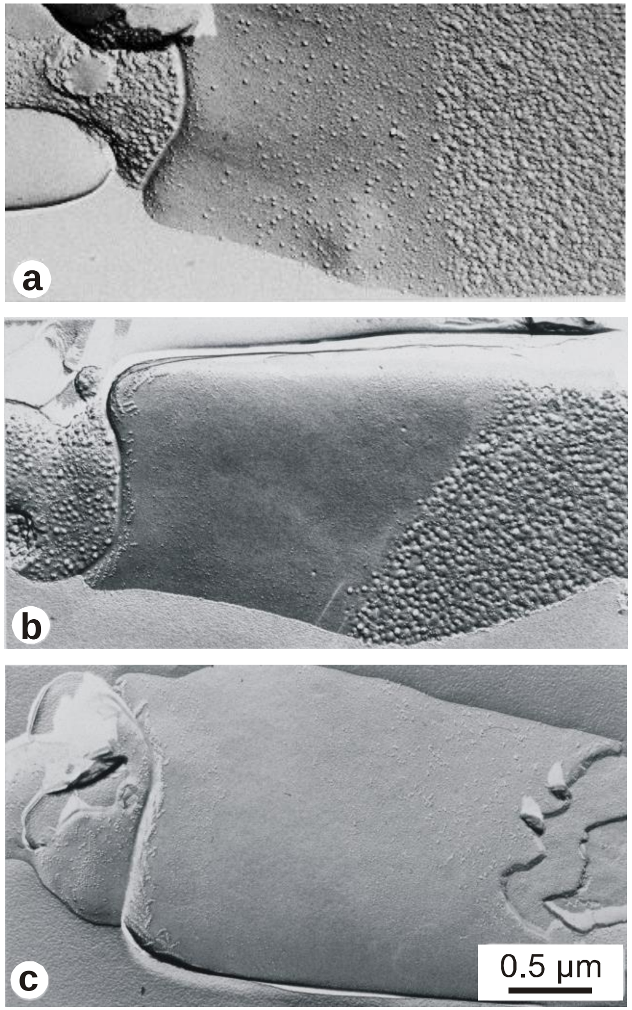
Bicarbonate mediated sperm capacitation



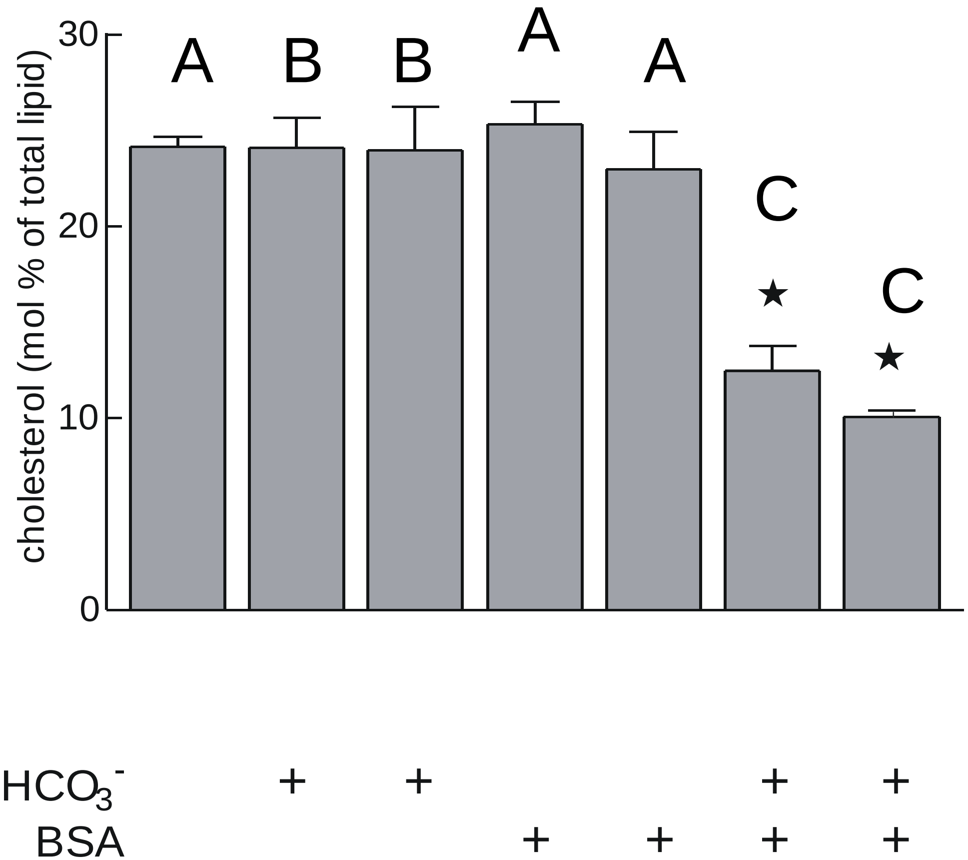


Cholesterol redistribution and depletion in boar sperm

Filipin staining type

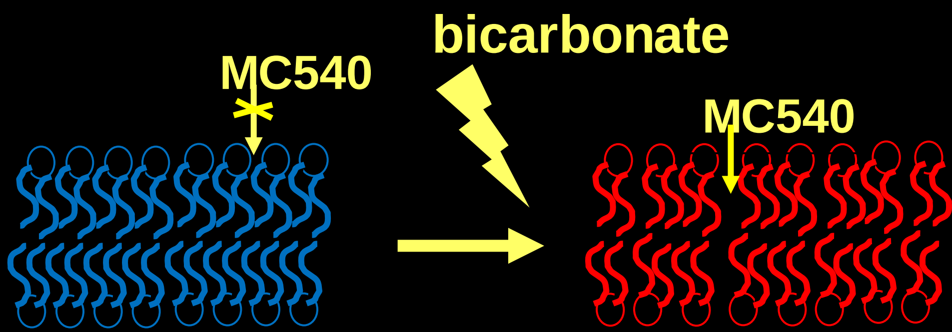


Flesch et al., J Cell Sci. 2001

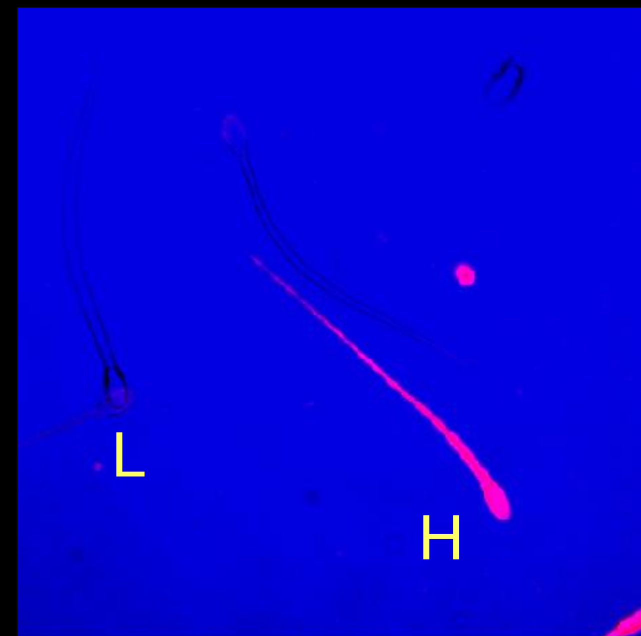


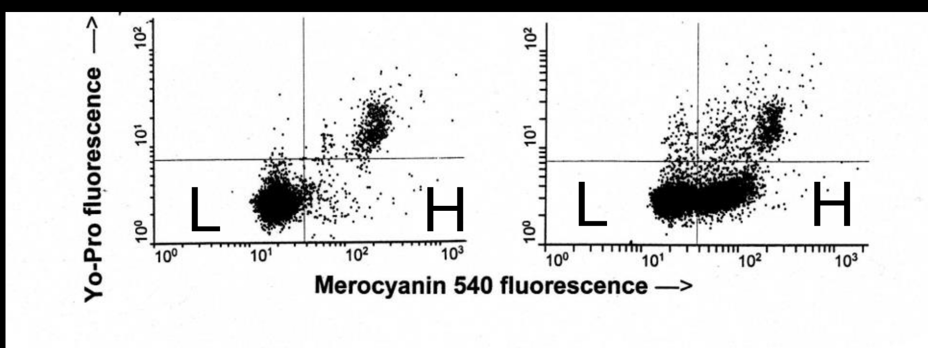
Cholesterol reordering is bicarbonate dependent.
Depletion of cholesterol only in presence of bicarbonate and BSA

Membrane fluidity changes: Cambridge *Gadella et al., Development (2000)*



Low fluorescence **High fluorescence**



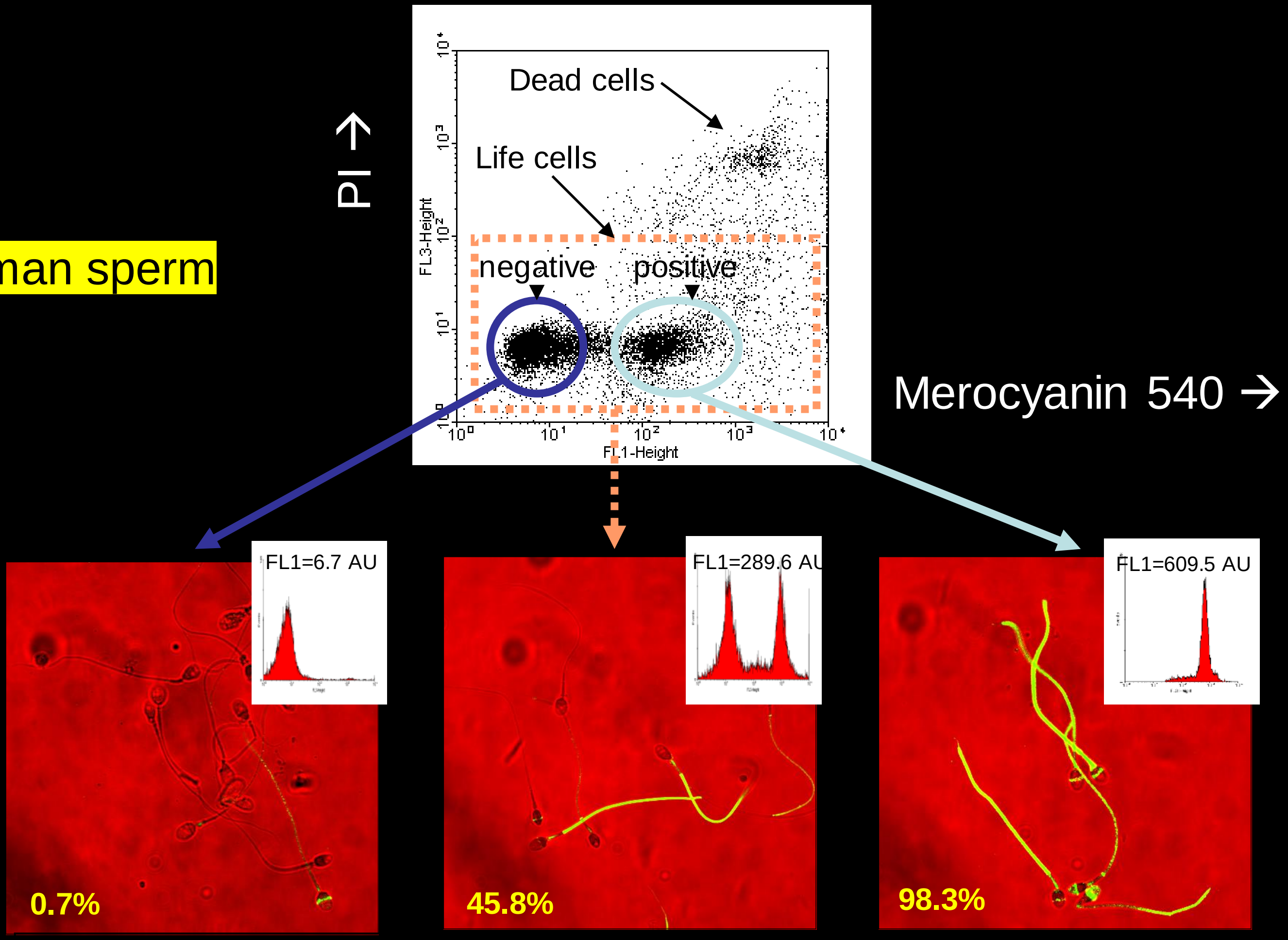


Non-capacitated **Capacitated (2 hrs)**

Sorting of responsive and non-responsive cells

Membrane responsive cells (head surface) are also hypermotile (tail PY)

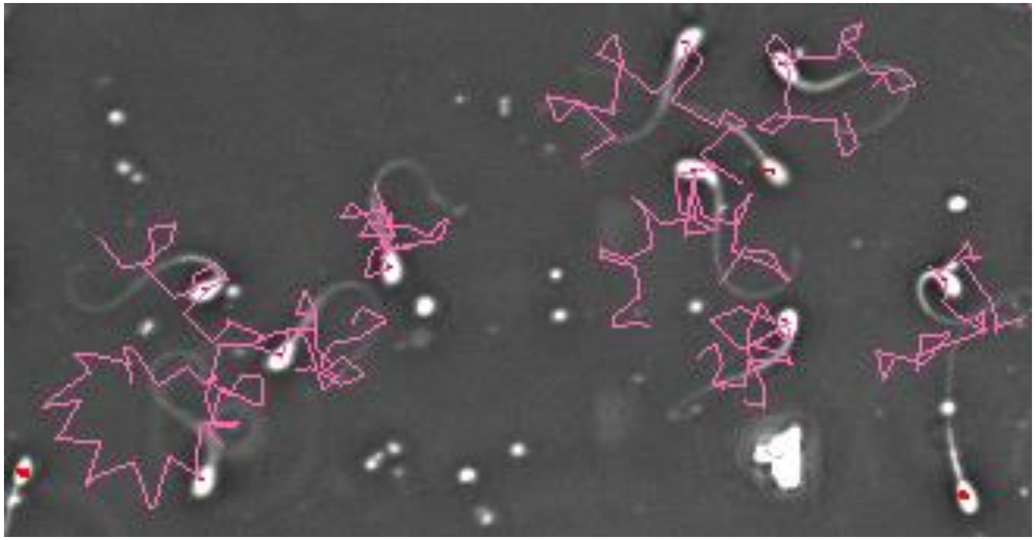
Human sperm



PY immunofluorescence

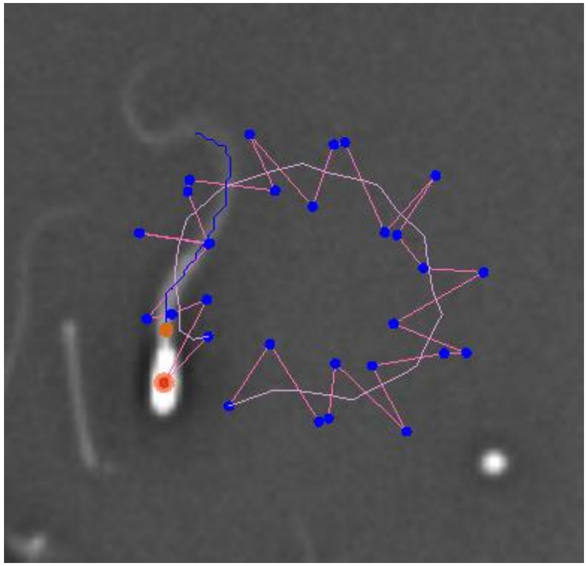
Hypermotility

Sydney *Bernecic & Gadella Biol Reprod (2021)*

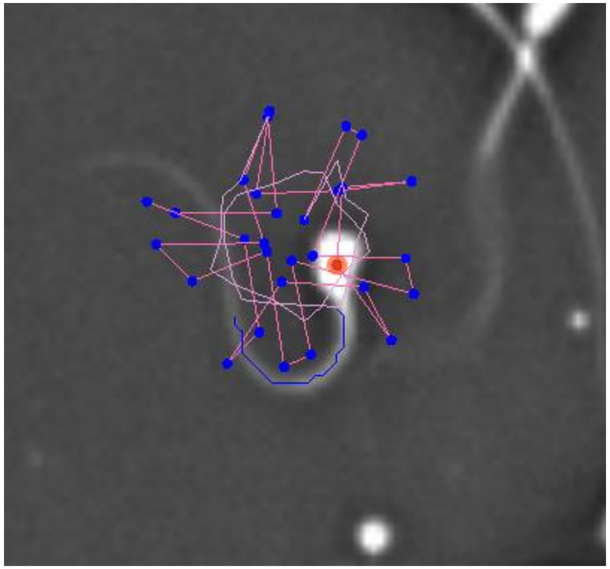


Non depleted

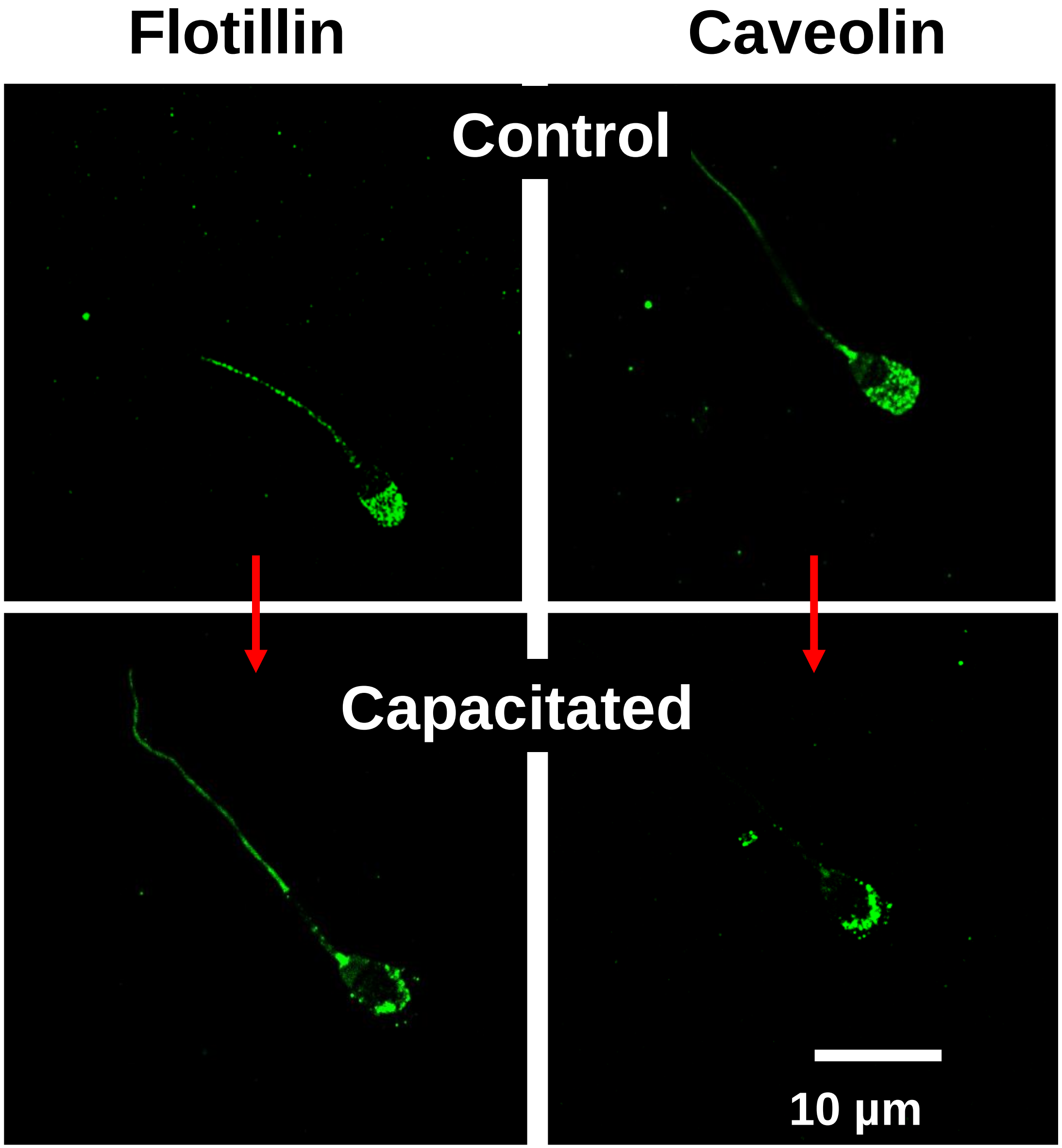
Cholesterol depleted



Ram sperm



Ca^{2+} + Bic + BSA induces aggregation of rafts proteins in the apical ridge

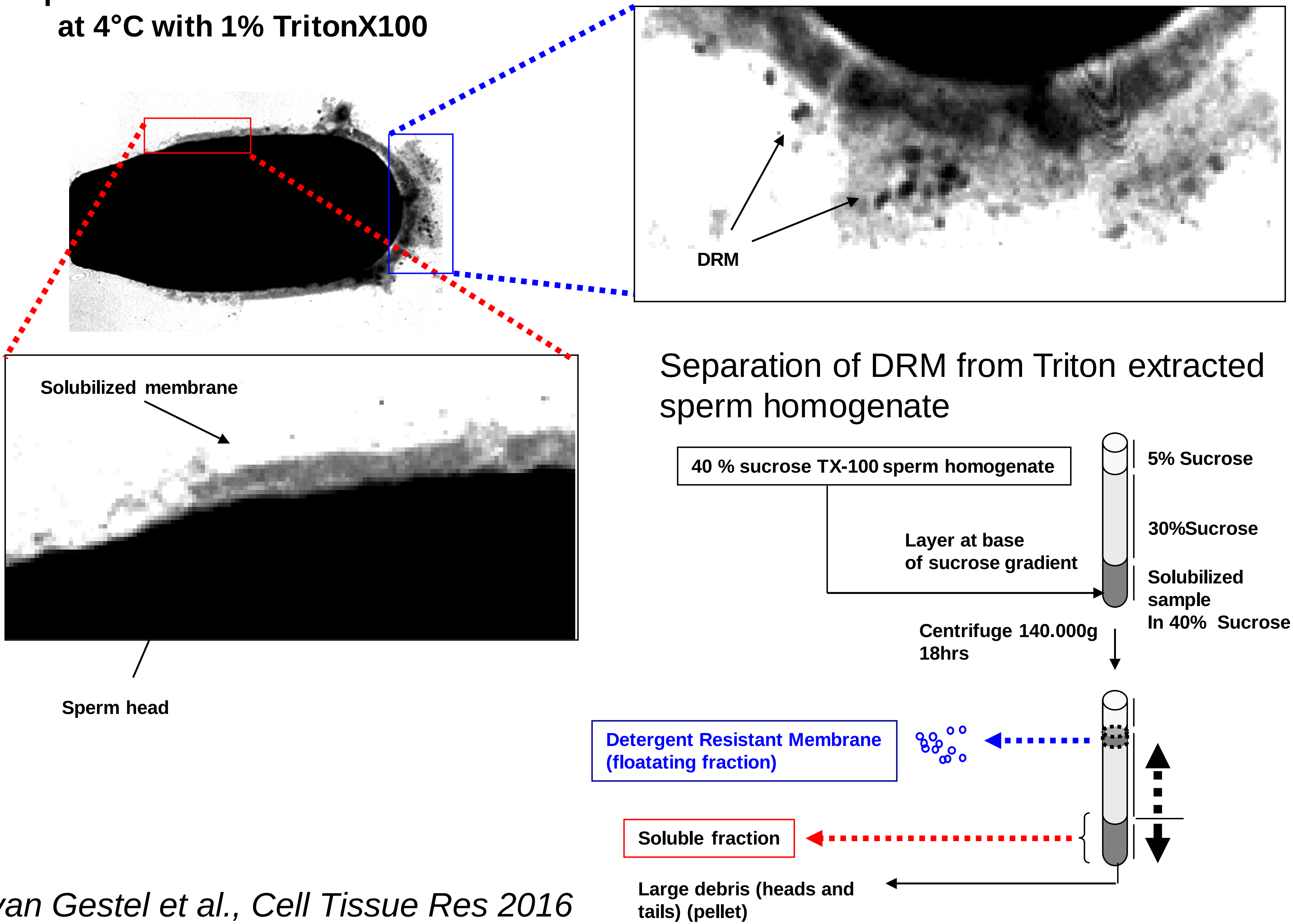


This redistribution to the apical ridge is matching the area where ZP binding takes place.

Tsai et al., Mol Membr Biol 2007

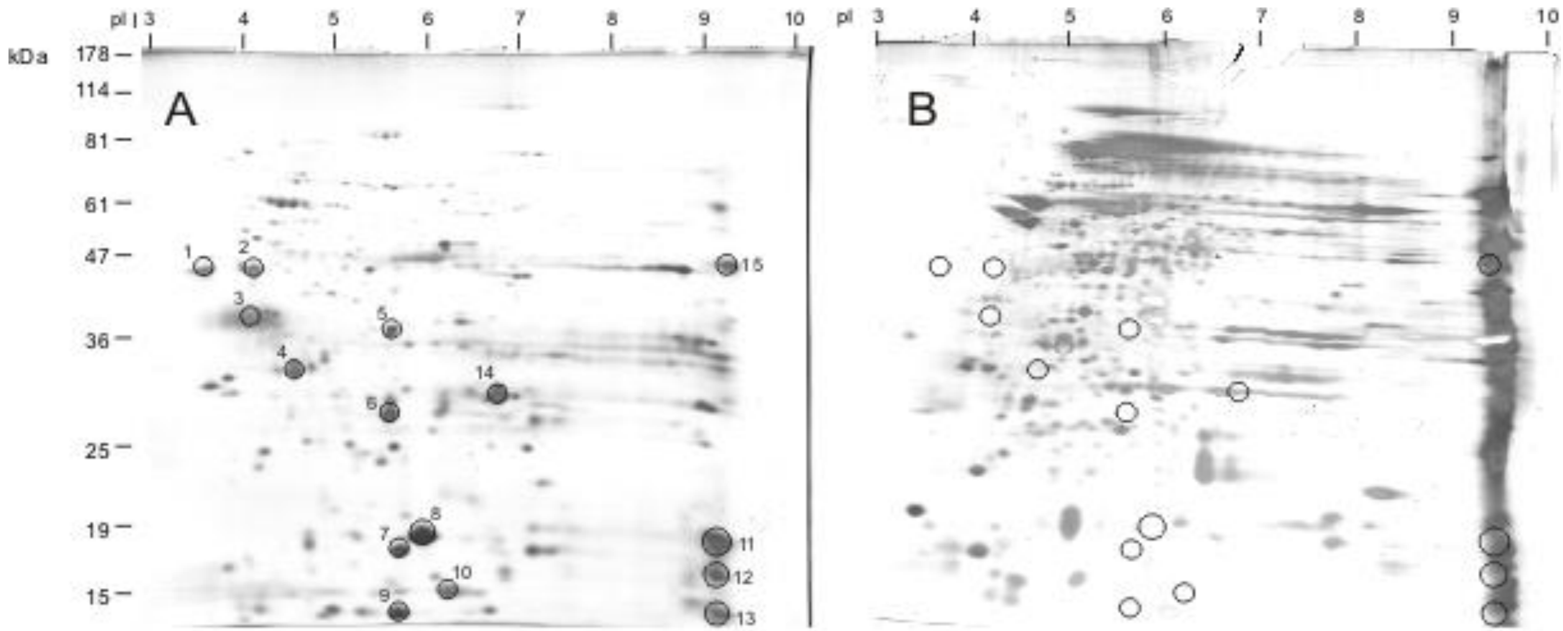
Sperm membranes solubilized at 4°C with 1% TritonX100

Separation of DRM from Triton extracted sperm homogenate



van Gestel et al., Cell Tissue Res 2016

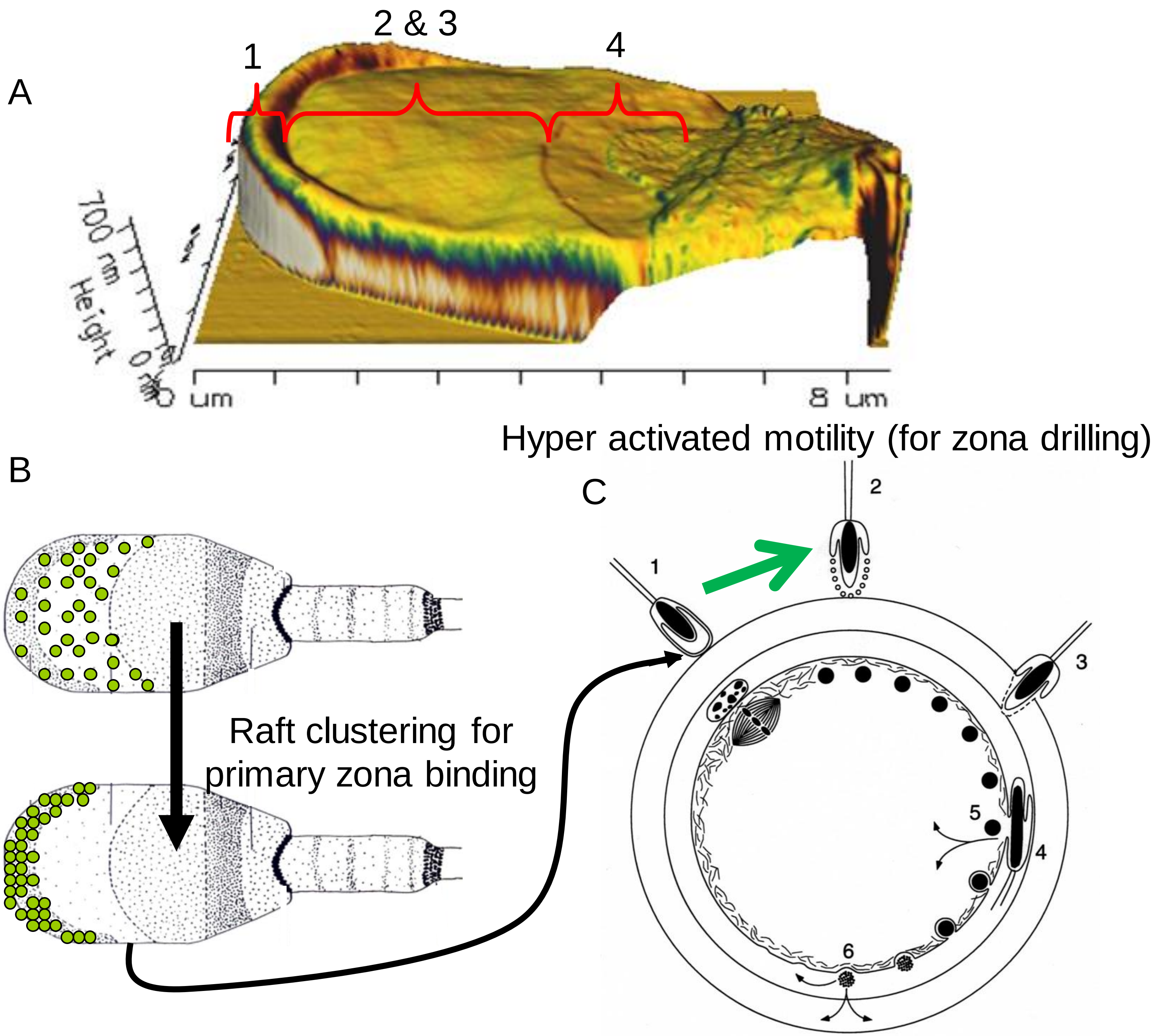
DRM fraction | **Whole cell membranes**



→ Sperm DRM's care highly enriched in zona binding proteins

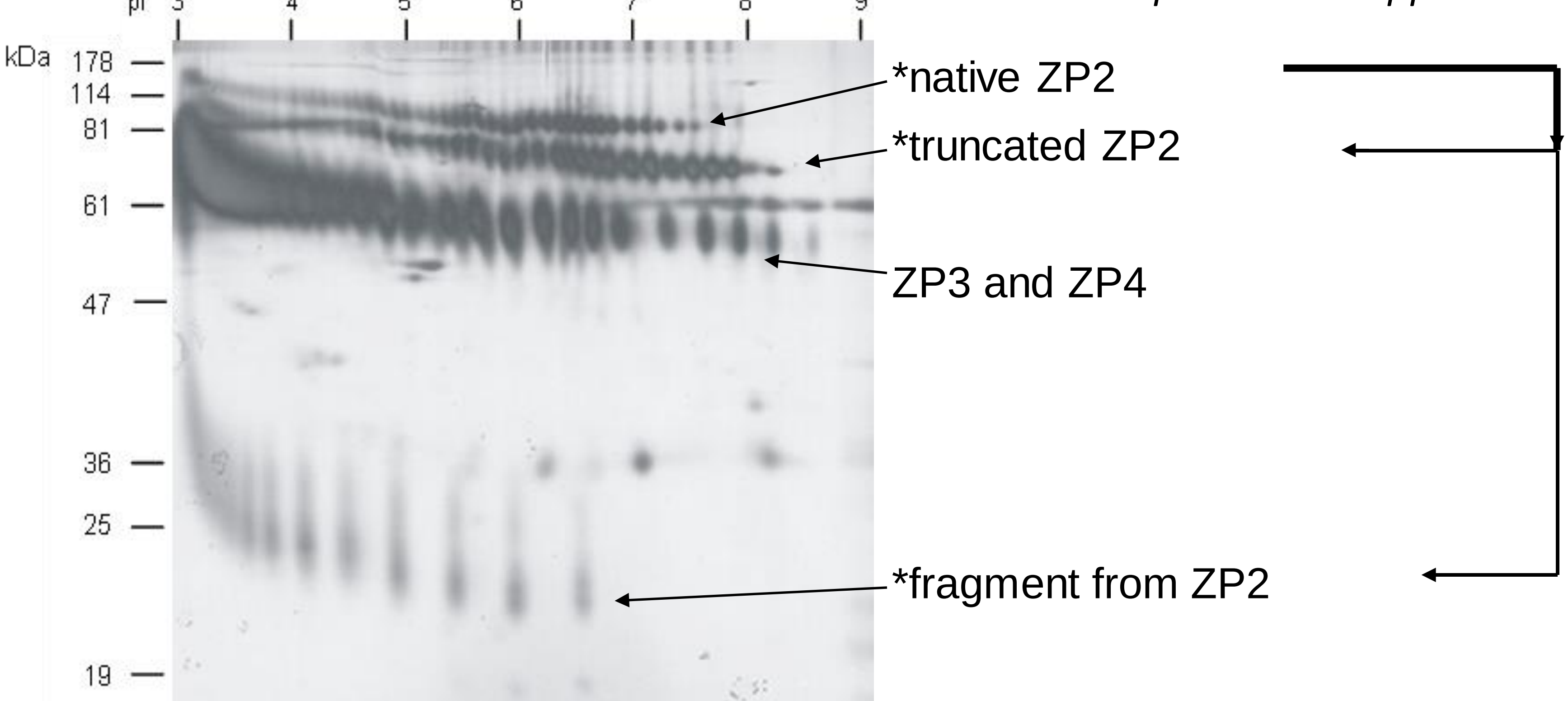
	spot	Protein assigned from database search of sequenced peptides	Accession no.	# peptides matched	comment
→	1	fertilin beta	28564477	6	B
	3	alpha-s1-casein precursor	1070620	3	B
→	4	sp32 precursor	1082952	15	B
	5	aldose (aldehyde) reductase	584742	10	A
	7	superoxide dismutase	66364	6	A
	8	peroxiredoxin 5	10305336	12	A
→	9	sp32 precursor	1082952	5	B
→	11	spermadhesin AQN-3	114083	3	A
→	12	spermadhesin AQN-3	114083	2	A
→	13	spermadhesin AQN-3	114083	2	A
→	14	sp32 precursor	1082952	9	B
	15	preproacrosin	1480413	2	A

Van Gestel et al., Mol Hum Reprod 2005

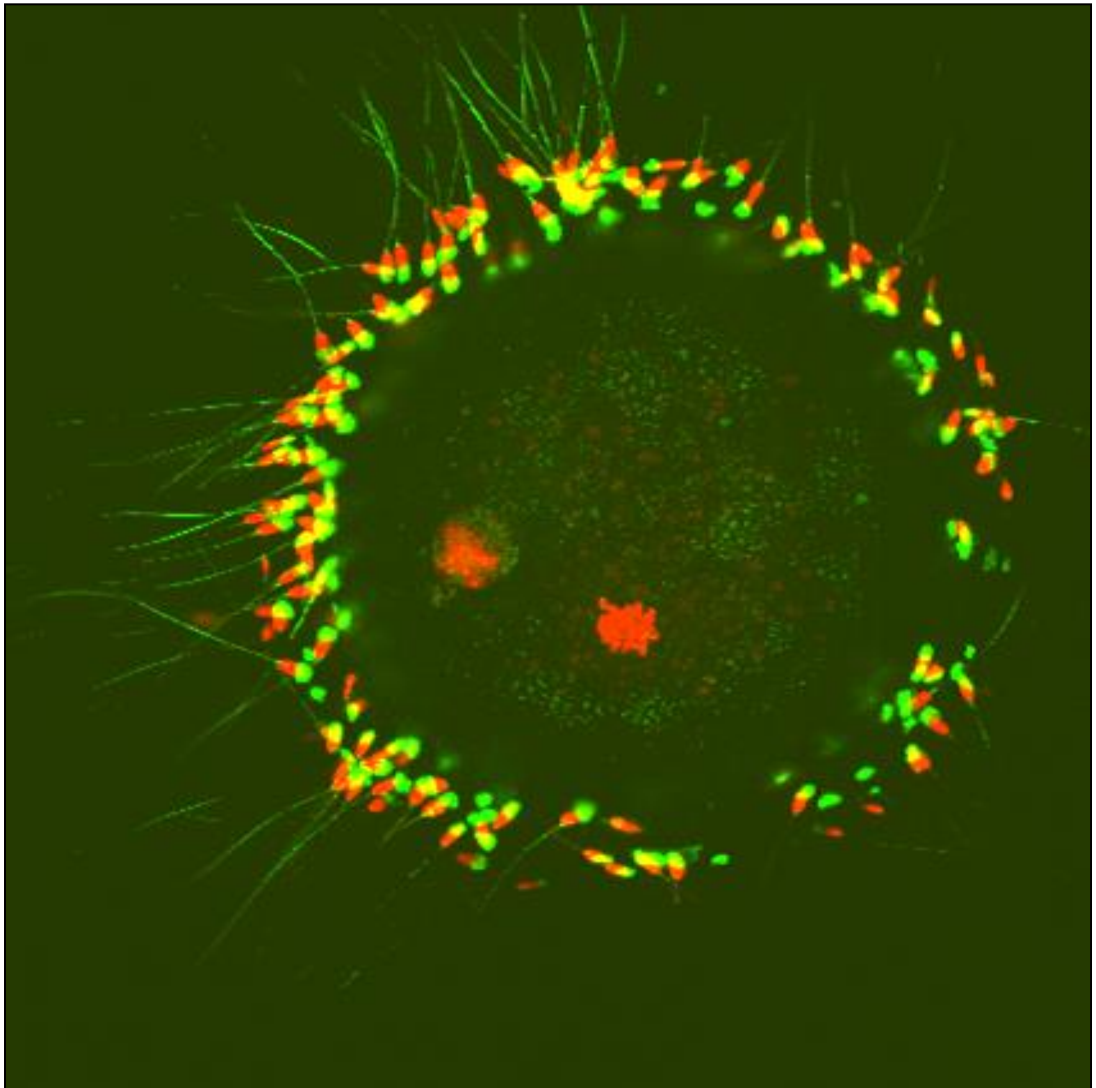

A
1
2 & 3
4
700 nm
Height
0 nm
0 um
8 um
B
Raft clustering for primary zona binding
Hyper activated motility (for zona drilling)
C
1
2
3
4
5
6

	ZP1	ZP2	ZP3	ZP4
Mouse	+	+	+	-(pseudogen)
Hamster	+	+	+	+
Human	+	+	+	+
Cattle & Pig	-	+*	+	+
Rabbit & Dog	-	+*	+	+

Gadella Soc Reprod Fertil Suppl. 2010



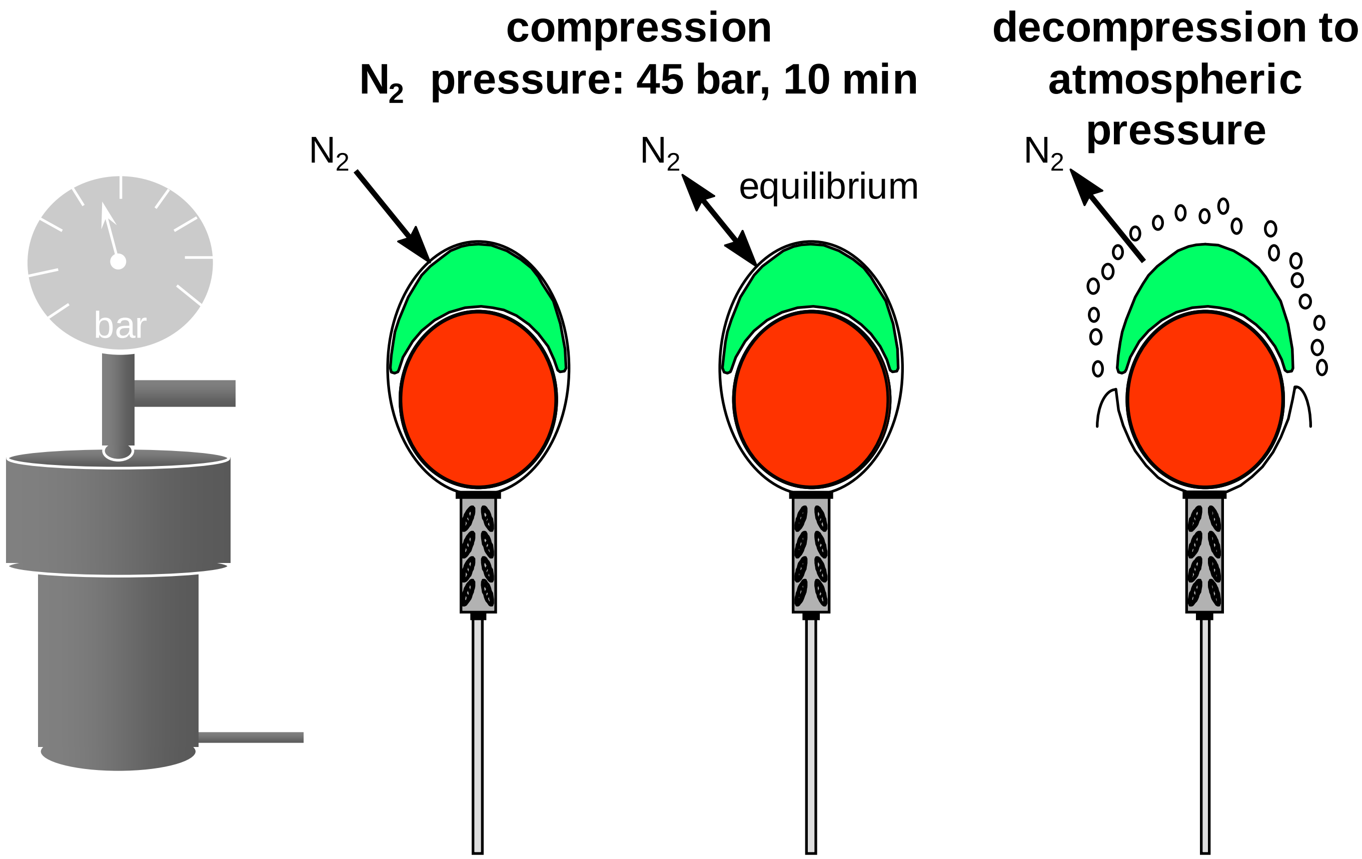
Capacitated sperm binds to the zona pellucida



horse

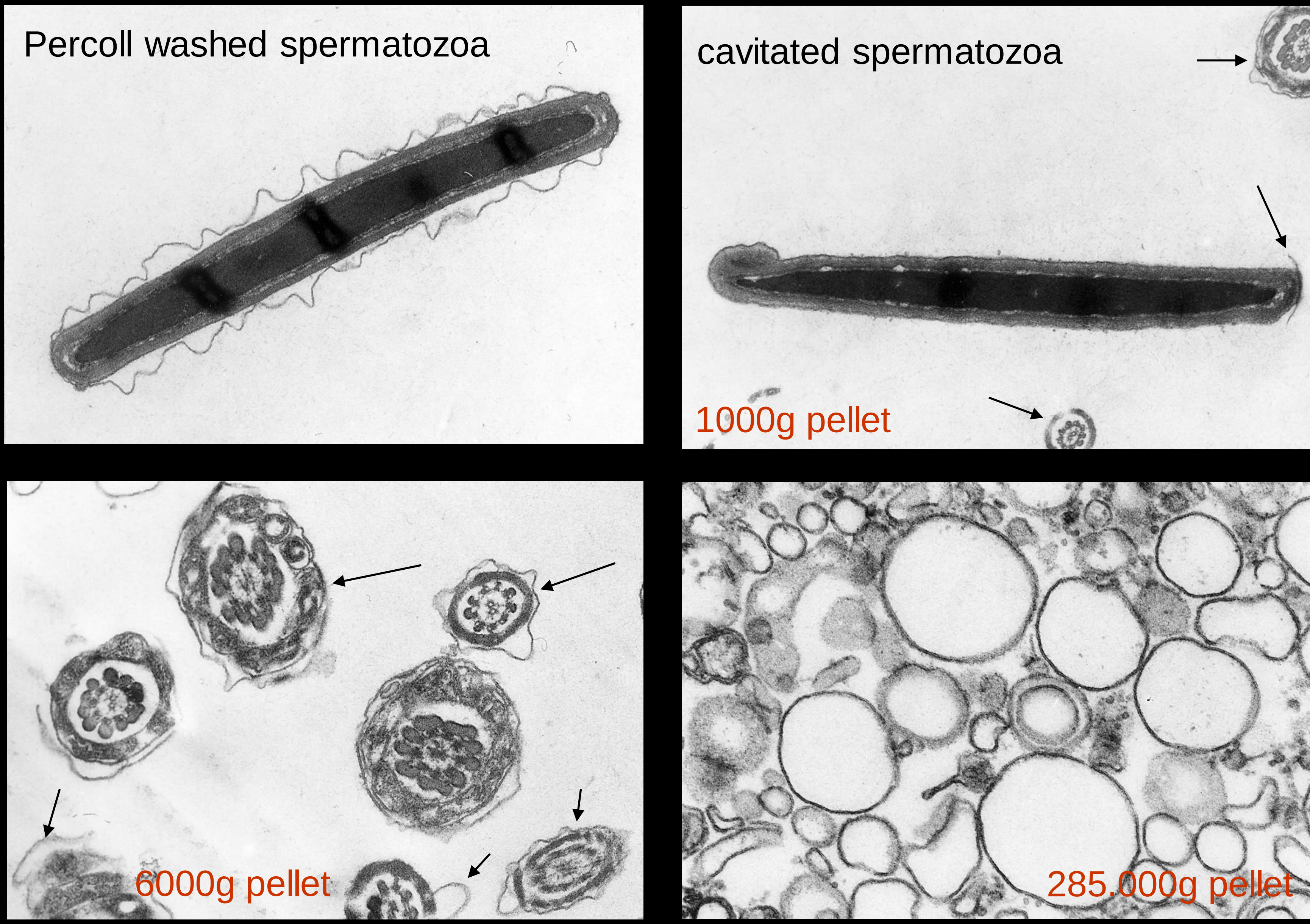
Note this picture is made after a IVF experiment. In vivo only a few sperm will bind to the zona.

Apical plasma membrane isolation for studying primary zona binding



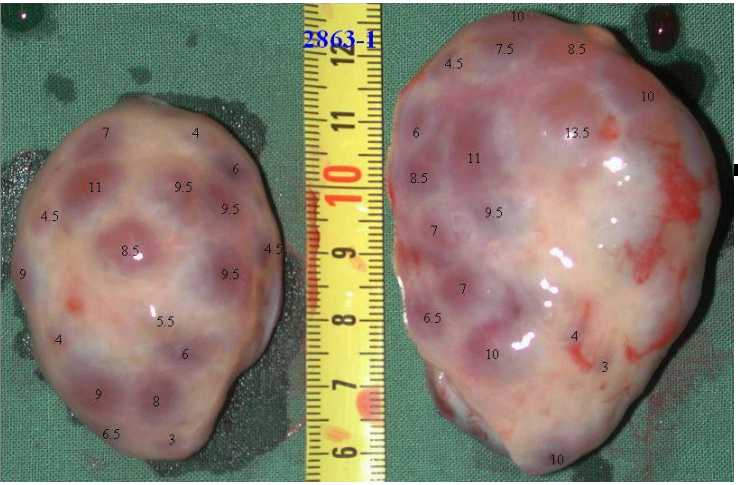
Flesch et al., Biol Reprod 1998

Apical sperm plasma membrane isolation



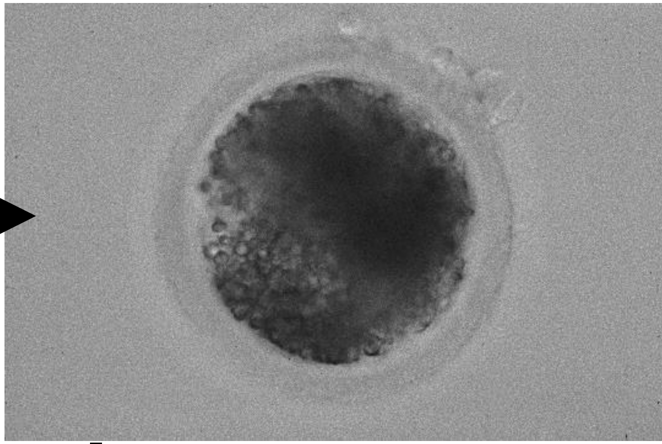
Isolation of zona ghosts

10.000 ovaries with mature follicles
From slaughterhouse

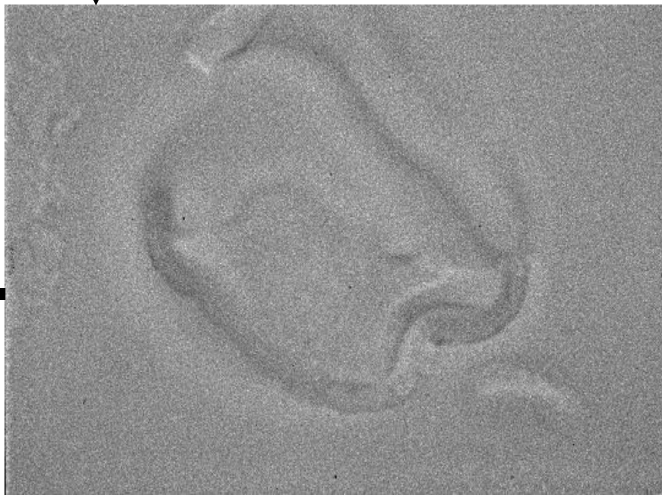


500.000 mature
oocyte cumulus
complexes

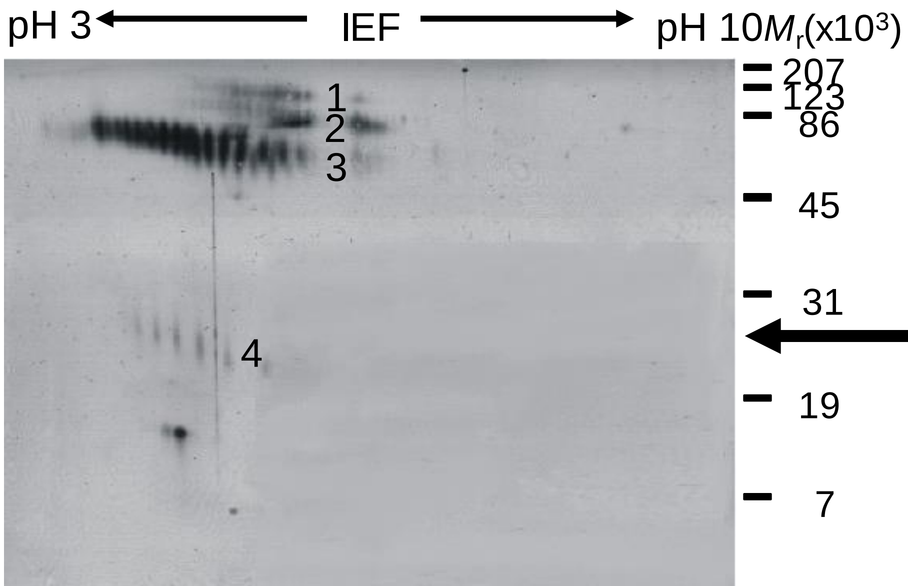
Removal of cumulus cells



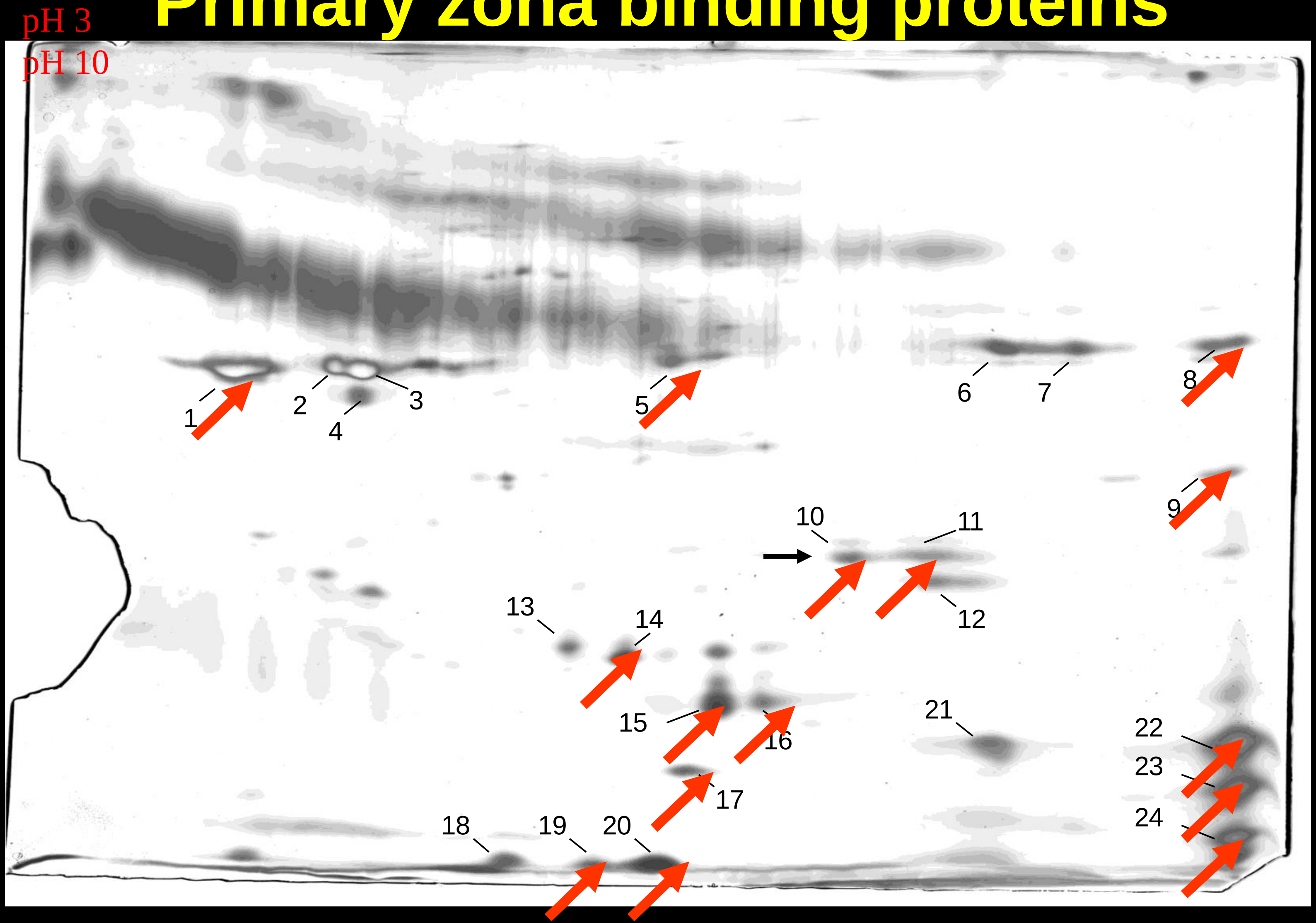
Homogenisation (Potter-
Elvehjem) and washing
of zona ghosts



2D Gel of
solubilised
zona proteins



Primary zona binding proteins



Zona binding proteins; more keys fit to the keyhole!

A zona binding complex is formed during capacitation

Zona adhering proteins from solubilized apical sperm plasma membrane

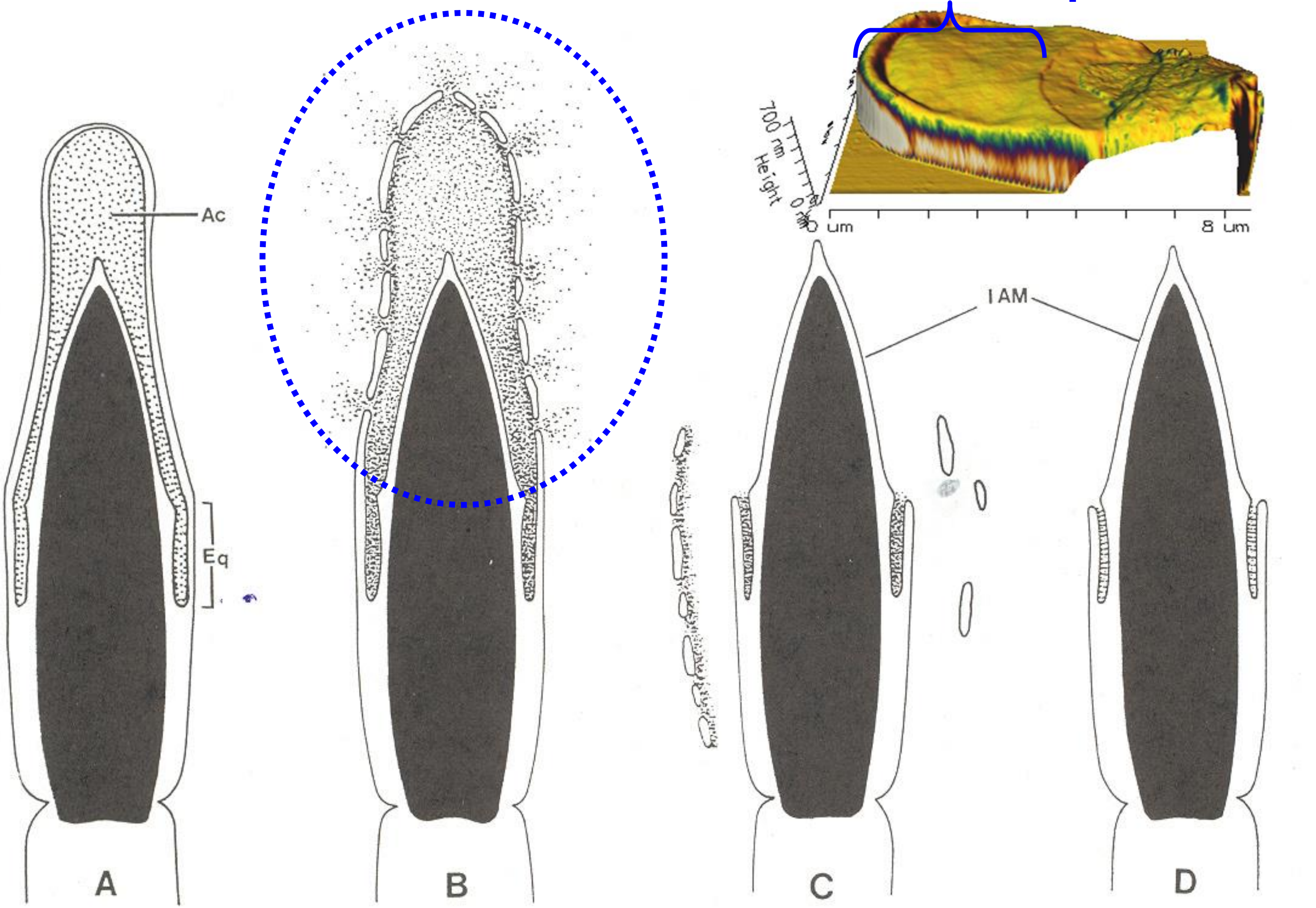
Spot No.	Protein assigned from database search of sequenced peptides	Number of peptides identified and used for searching	Accession No.	comment
→ 1	Fertilin beta ←	6	28564477	B
5	P47 ←	18	7459686	A
8	P47 ←	13	7459686	B
9	potassium channel	4	5542588; 28373976; 17942553	C
→ 10	SP32 precursor ←	2	1082952	B
→ 11	SP32 precursor ←	3	1082952	B
14	phosphatase	2	22218629, 13786807	C
16	carbonyl reductase ←	2	1827692, 27066006	C
→ 17	Peroxiredoxin 5	7	10305336	A
→ 19	SP32 precursor ←	2	1082952	B
→ 20	SP32 precursor ←	5	1082952	B
→ 22 and A	AQN-3 ←	2	543109	A
→ 23 and B	AQN-3 ←	3	543109	A
→ 24 and C	AQN-3 ←	2	543109	A

→ **Predominant protein in DRM**

← **Known zona binding proteins**

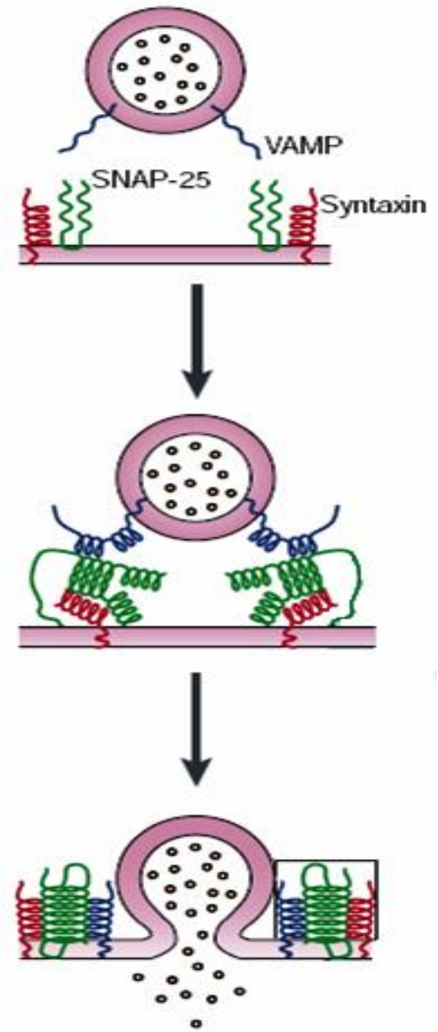
Van Gestel et al., Mol Hum Reprod 2007

Acrosome reaction & zona penetration

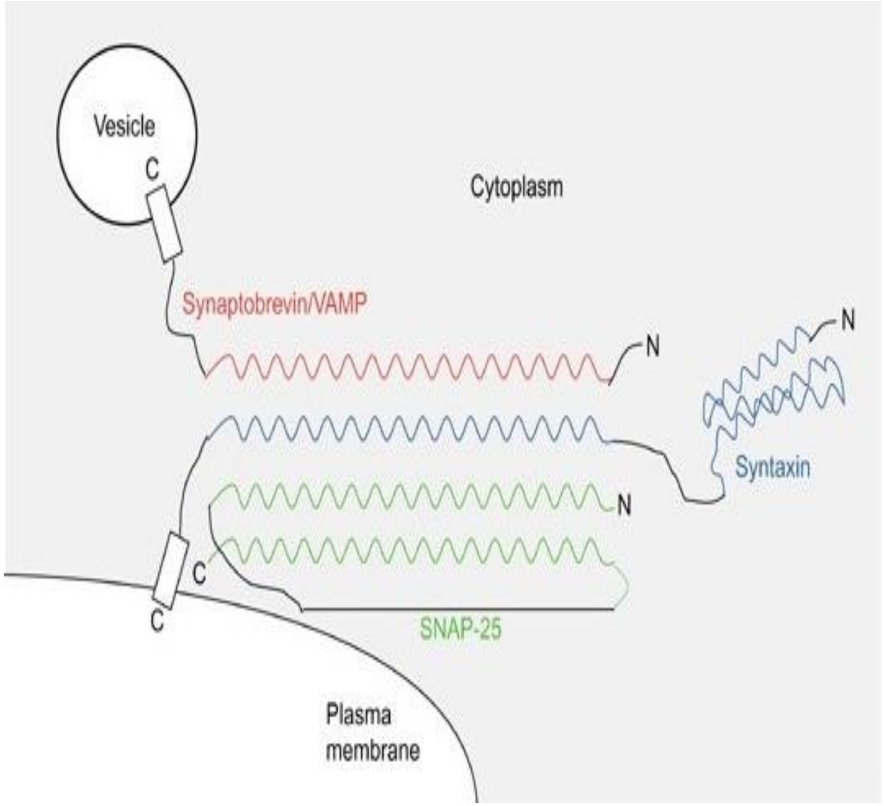


What are SNAREs?

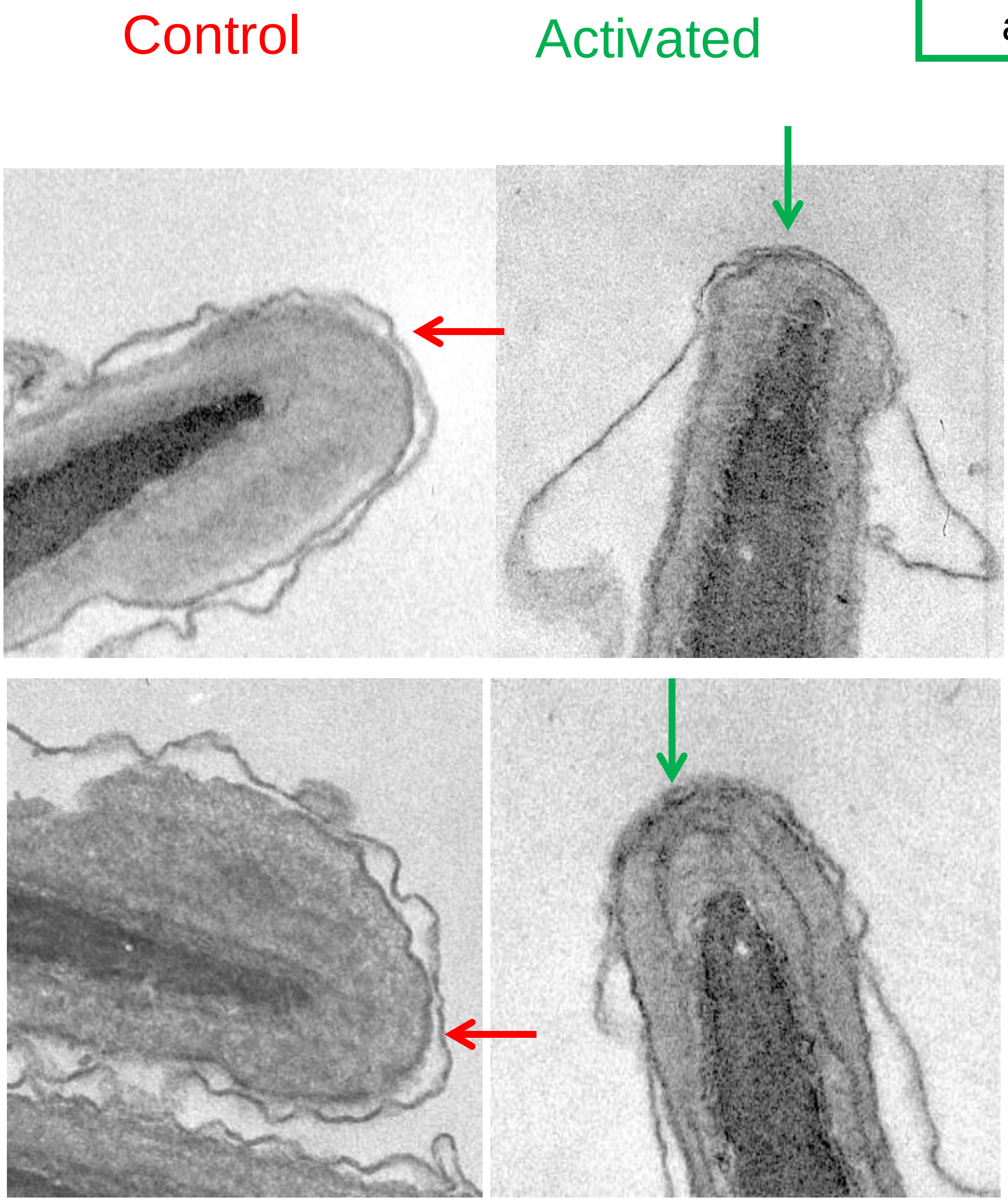
- **soluble NSF (*N*-ethylmaleimide sensitive factor) attachment protein receptors**



Chen Y.A. and Scheller R.H. (2001) Nat. Rev. Mol. Cel. Biol. 2(2) 98-106



Teng F.Y.H. et al (2001), Gen. Bio. 2(11) Review 30 12.1- 30 12.7

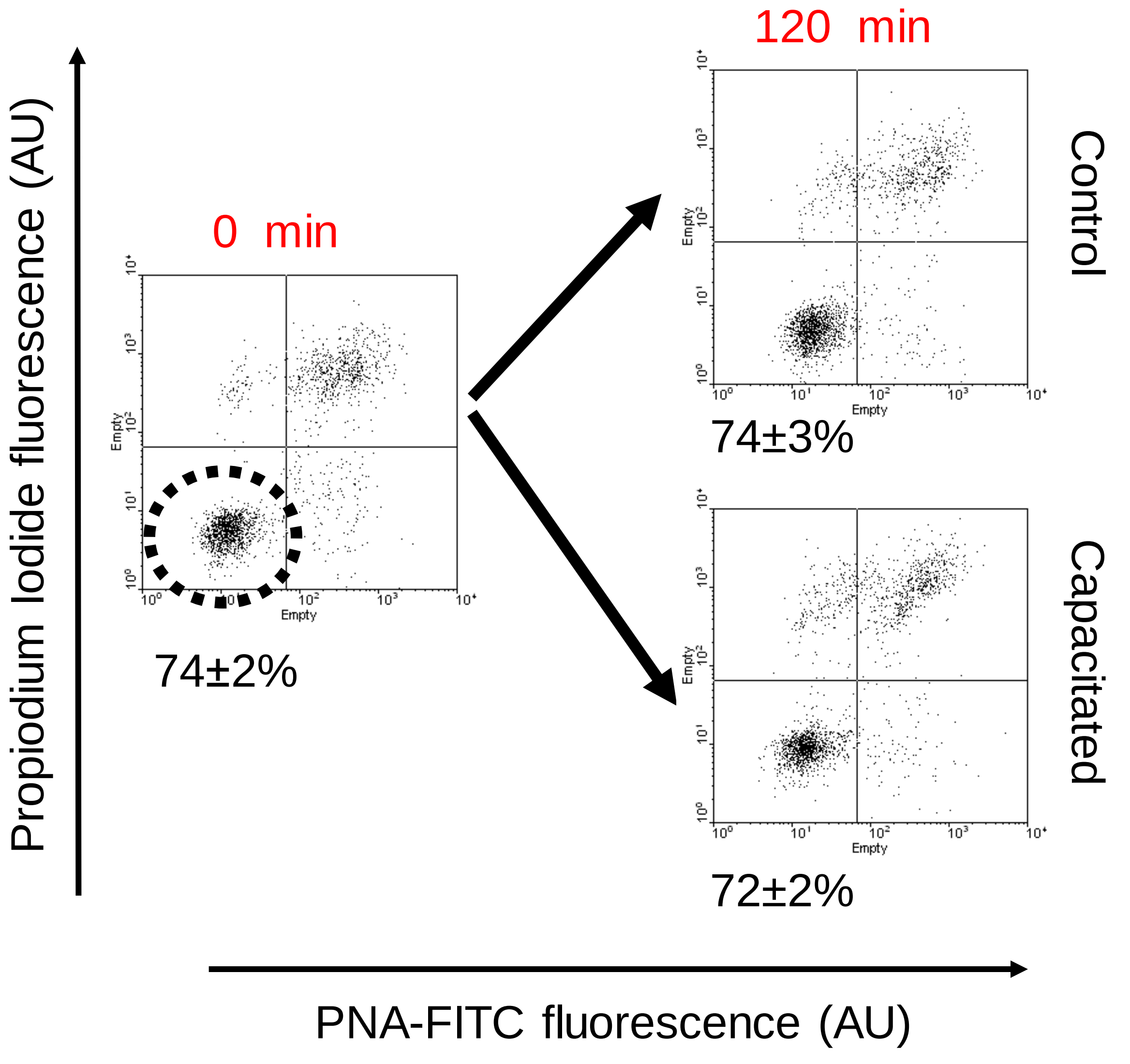


acrosome synapse formation

Closer contact of plasma membrane and acrosome membrane .

Tsai et al., Mol Membr Biol 2007
Tsai et al., PLOS One 2010; 2012

No induction of exocytosis



Cavitation now leads to isolation of bilamellar vesicles and removal of oam at pre-equatorial region

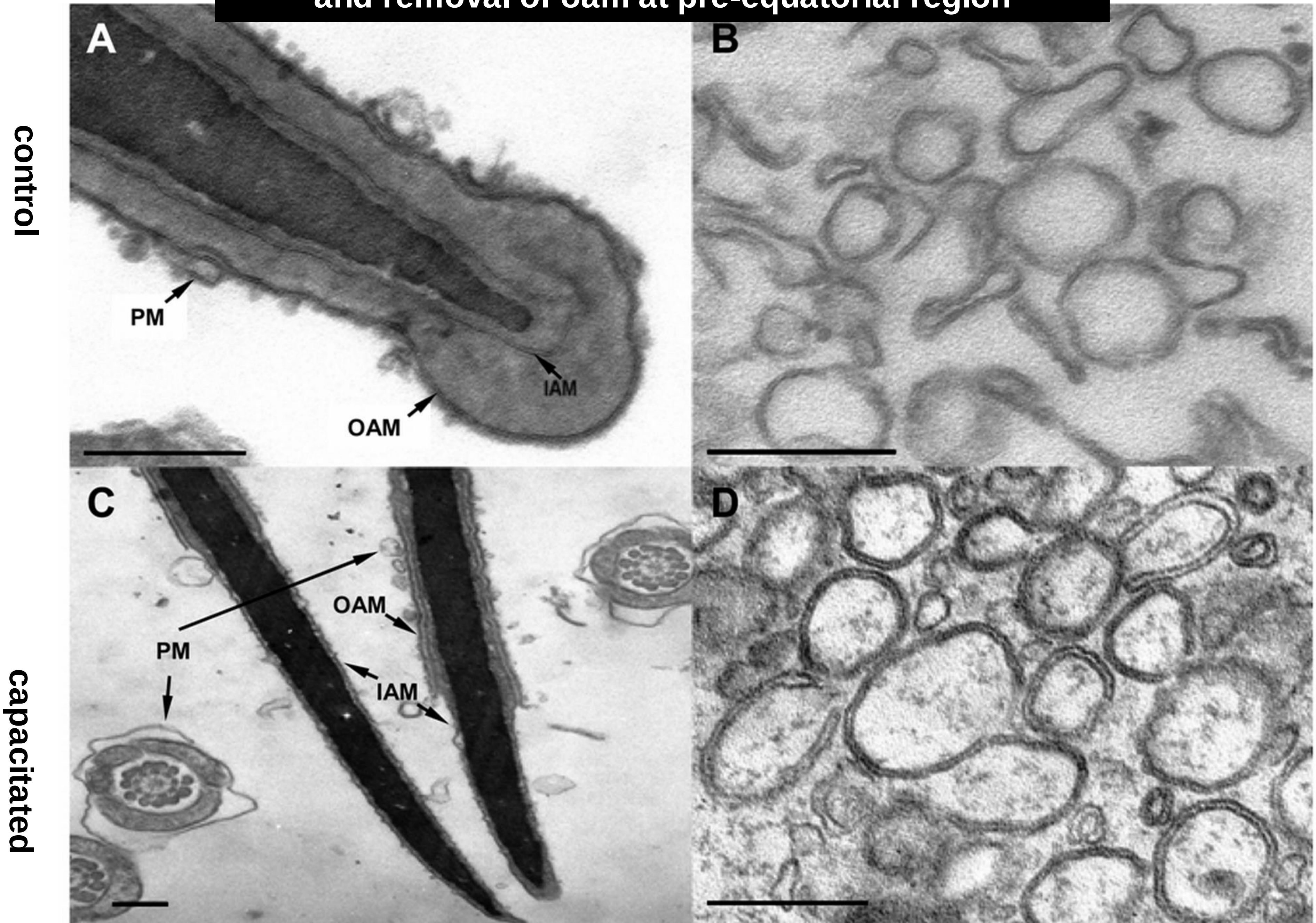
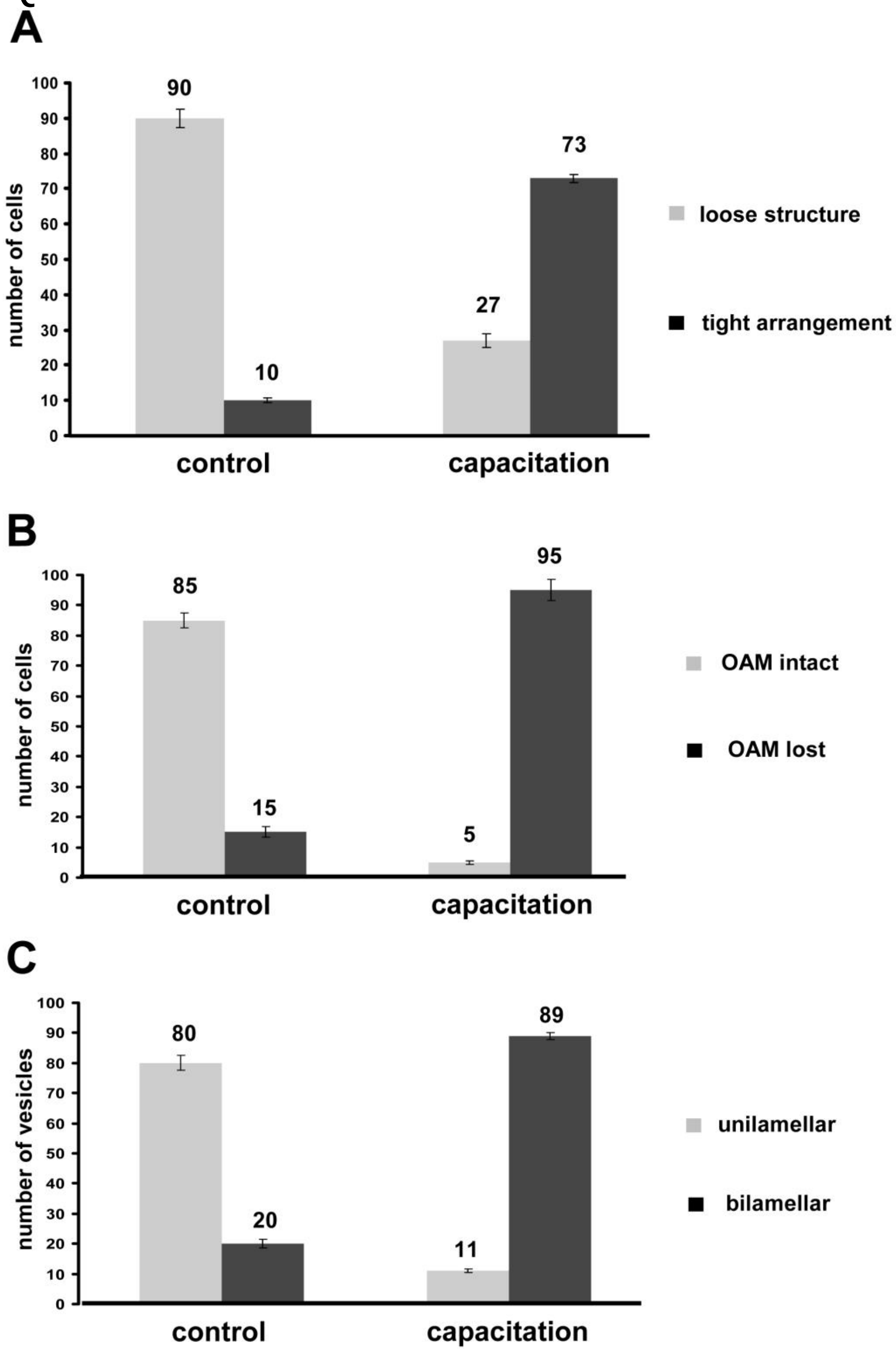


Figure 3

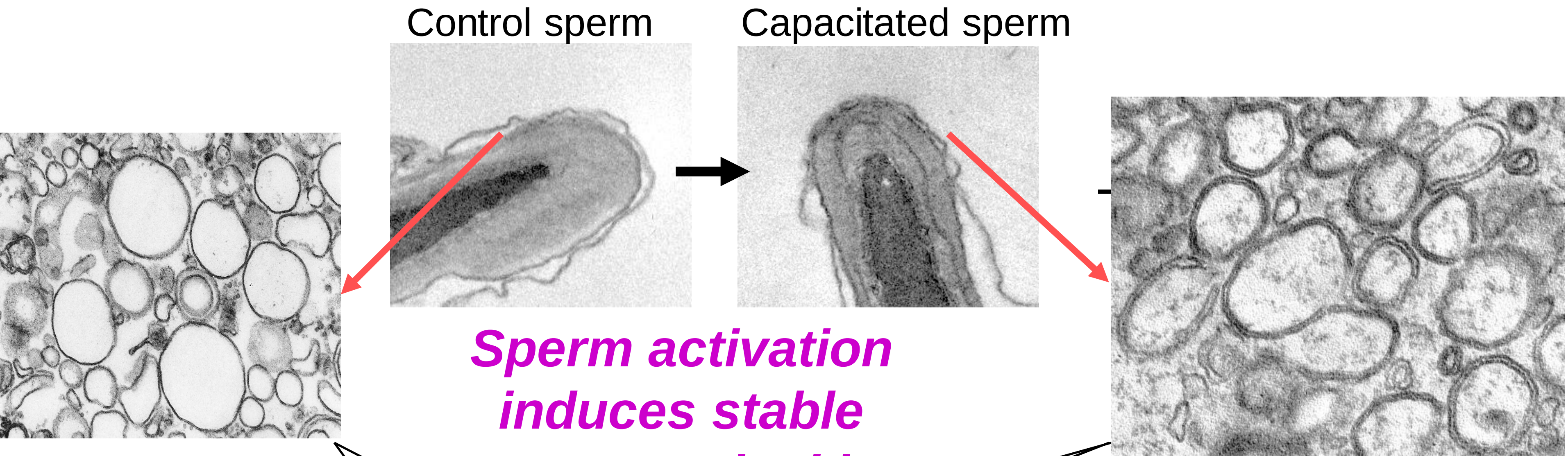
Quantification of altered ultrastructural features



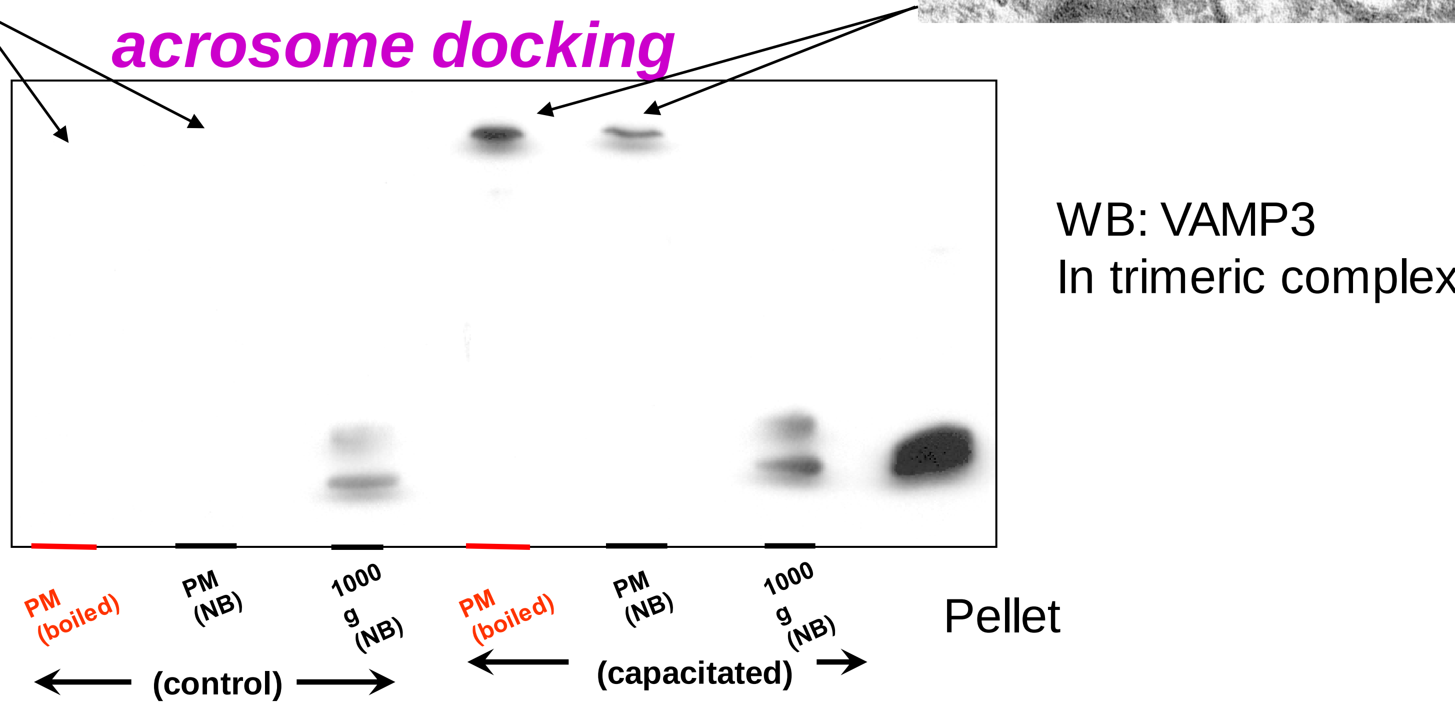
Intact sperm
(position of OAM to APM)

Cavitated heads

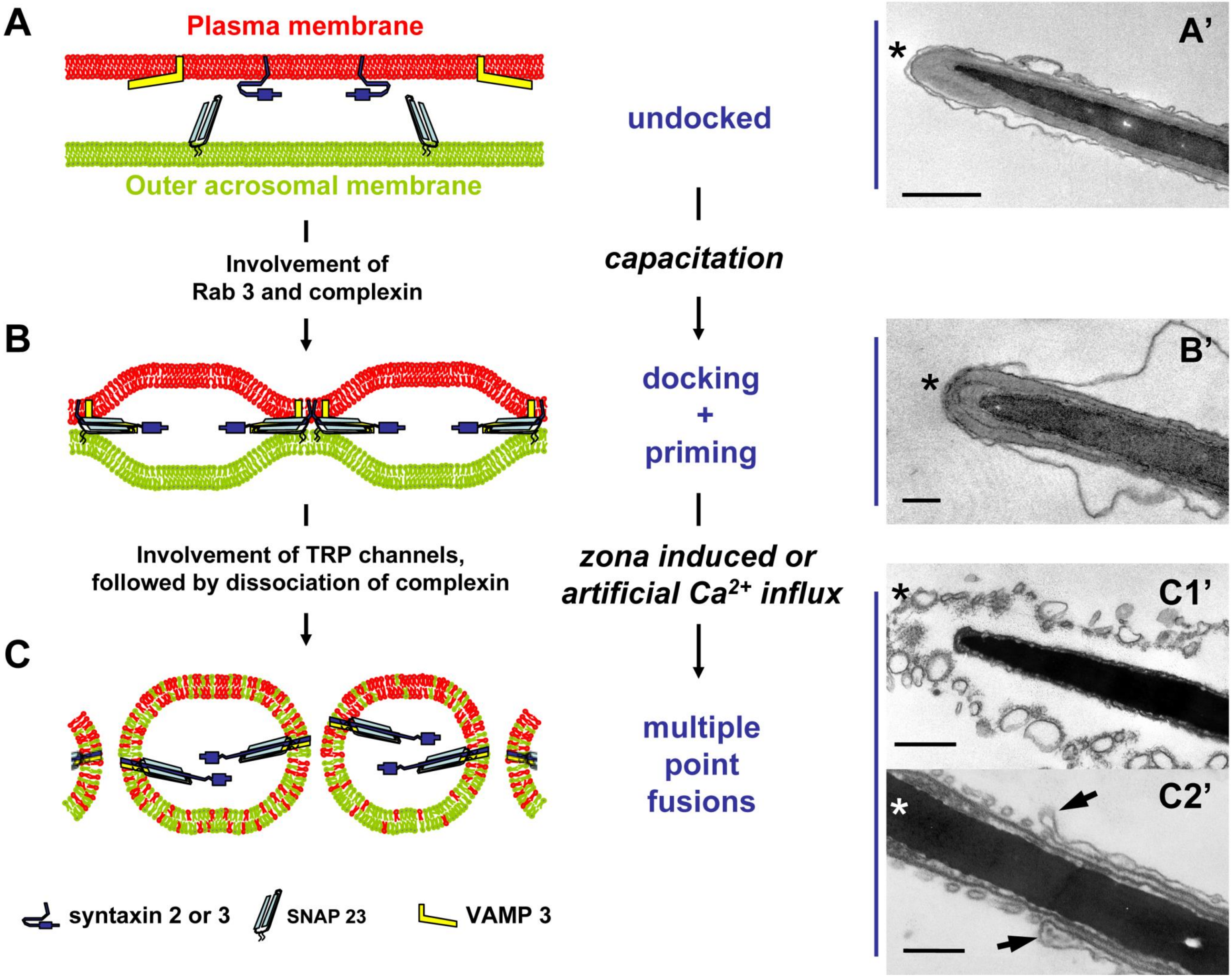
Cavitated UCF pellet

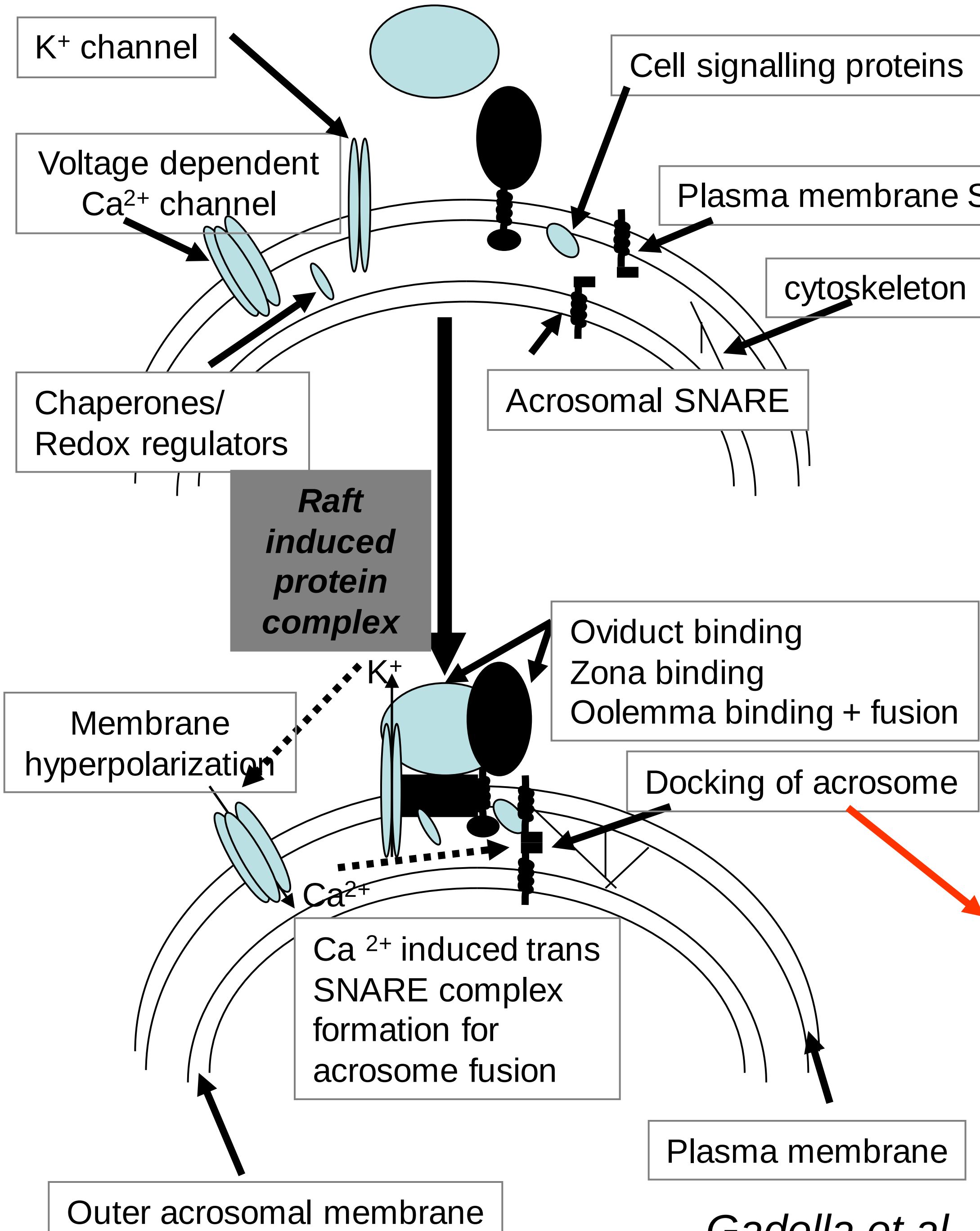


Sperm activation induces stable acrosome docking

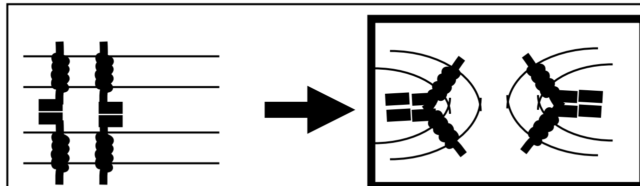


NB: complex also specific for DRM


A
Plasma membrane
Outer acrosomal membrane
Involvement of
Rab 3 and complexin
B
Involvement of TRP channels,
followed by dissociation of complexin
C
syntaxin 2 or 3
SNAP 23
VAMP 3
undocked
capacitation
docking
+
priming
zona induced or
artificial Ca2+ influx
multiple
point
fusions
A'
B'
C1'
C2'



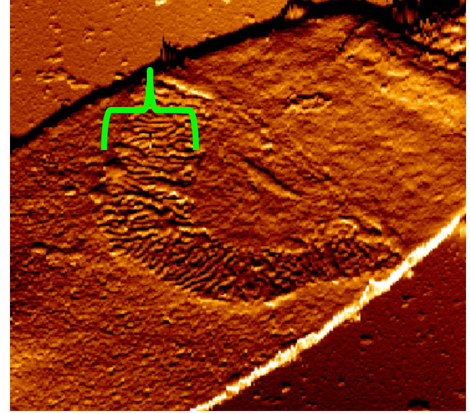
Putative formation of a multiple zona binding and acrosome docking protein complex in capacitating porcine sperm.

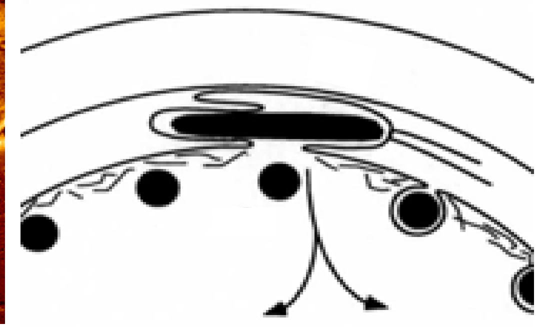


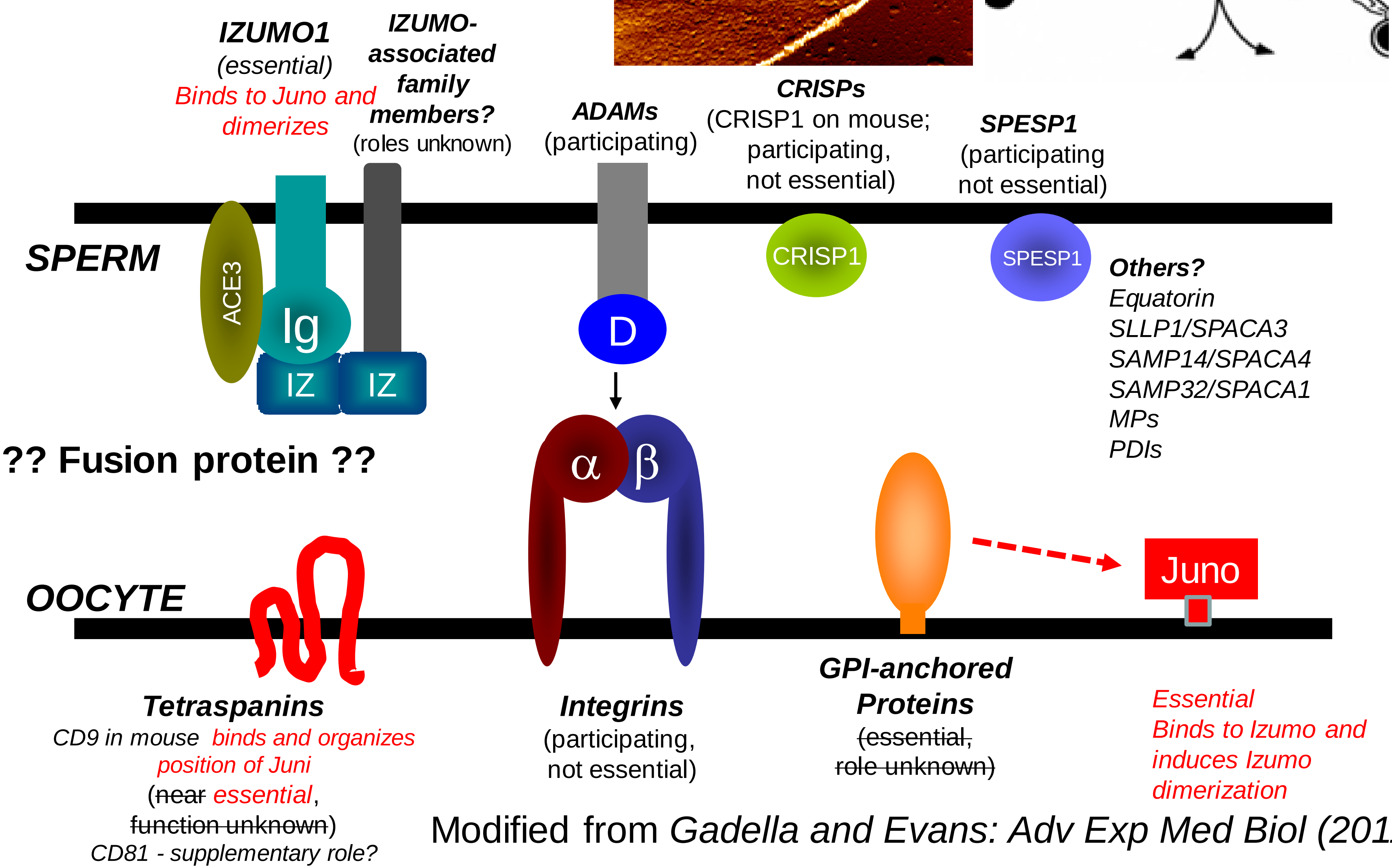
SNARE complex conformation change causes membrane fusion (induced by ZP)

Gadella et al., Int J Dev Biol 2018

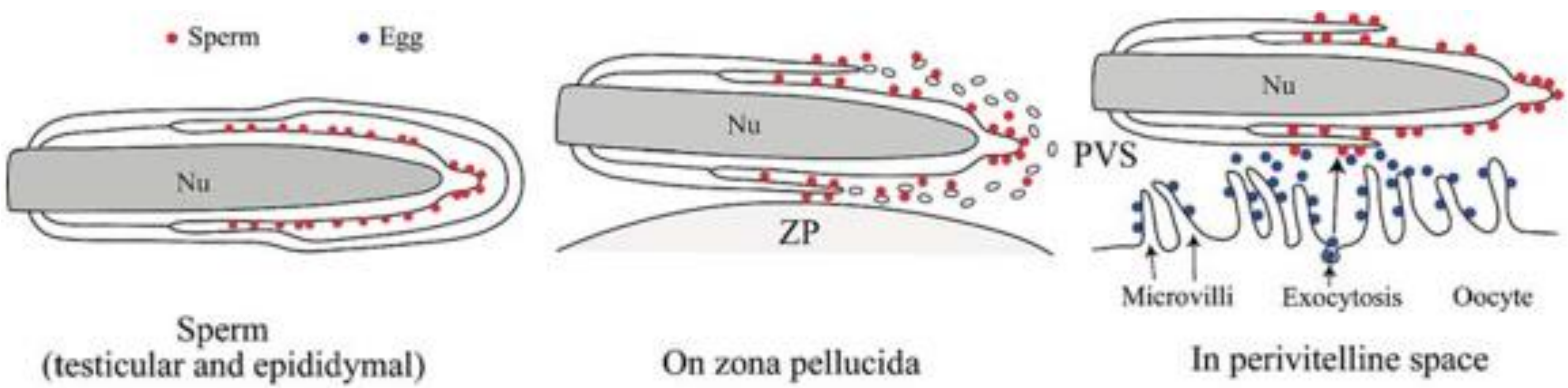
Fertilization fusion



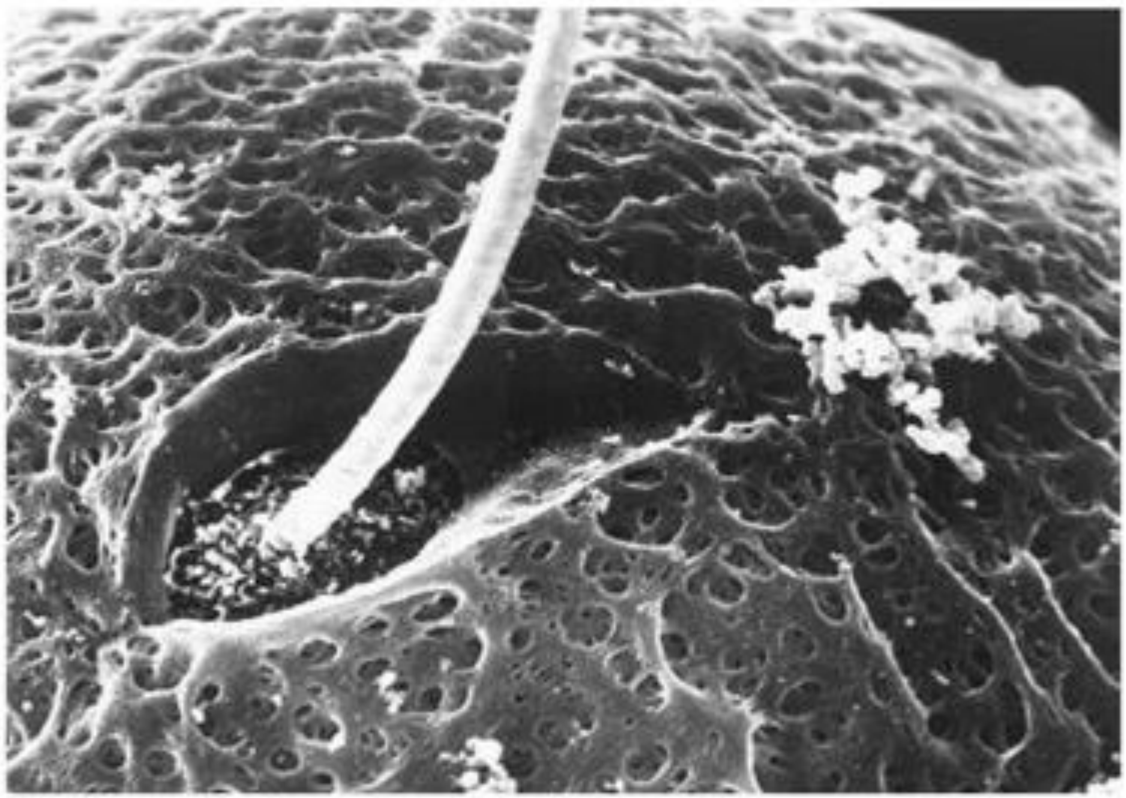


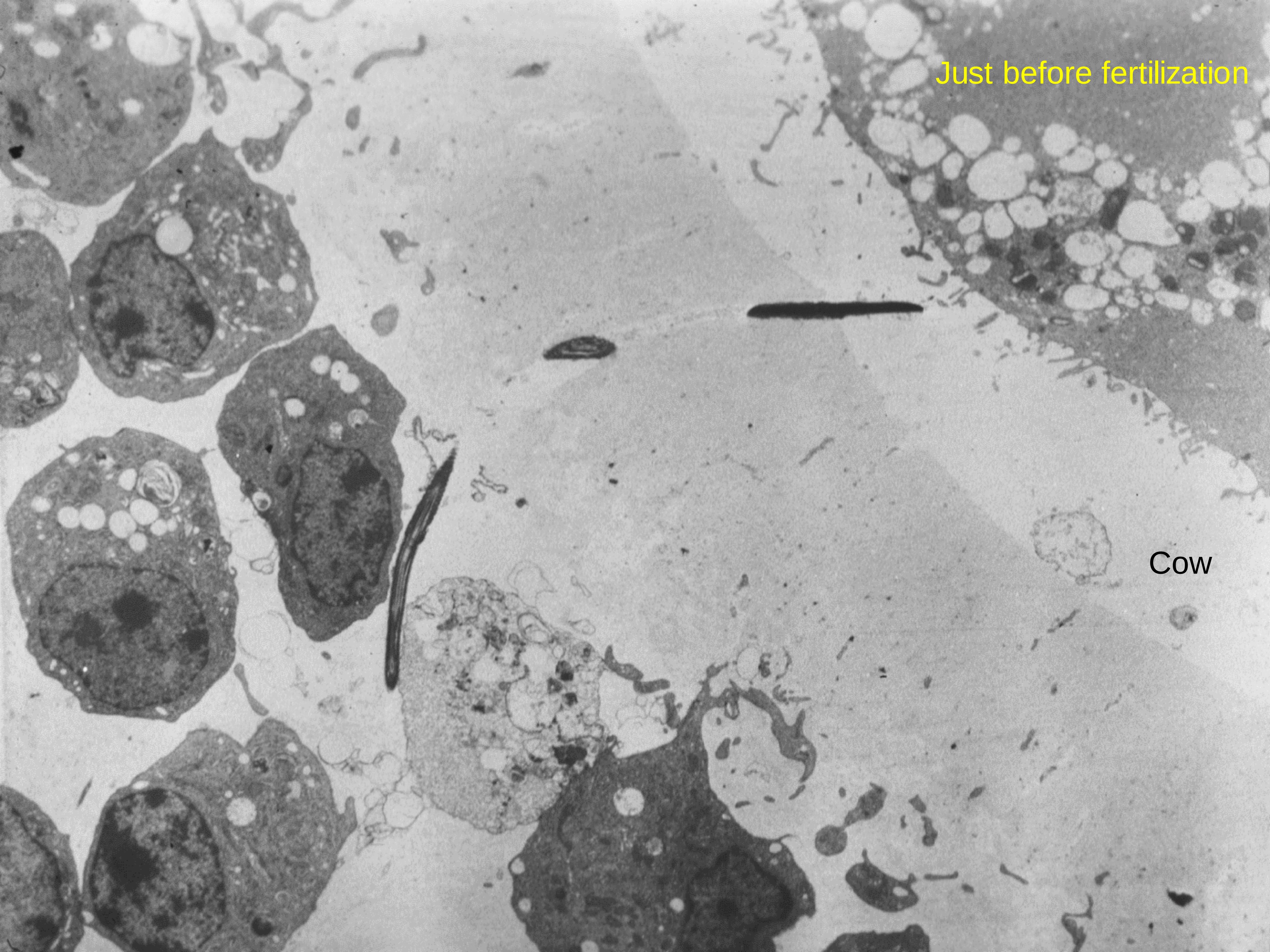


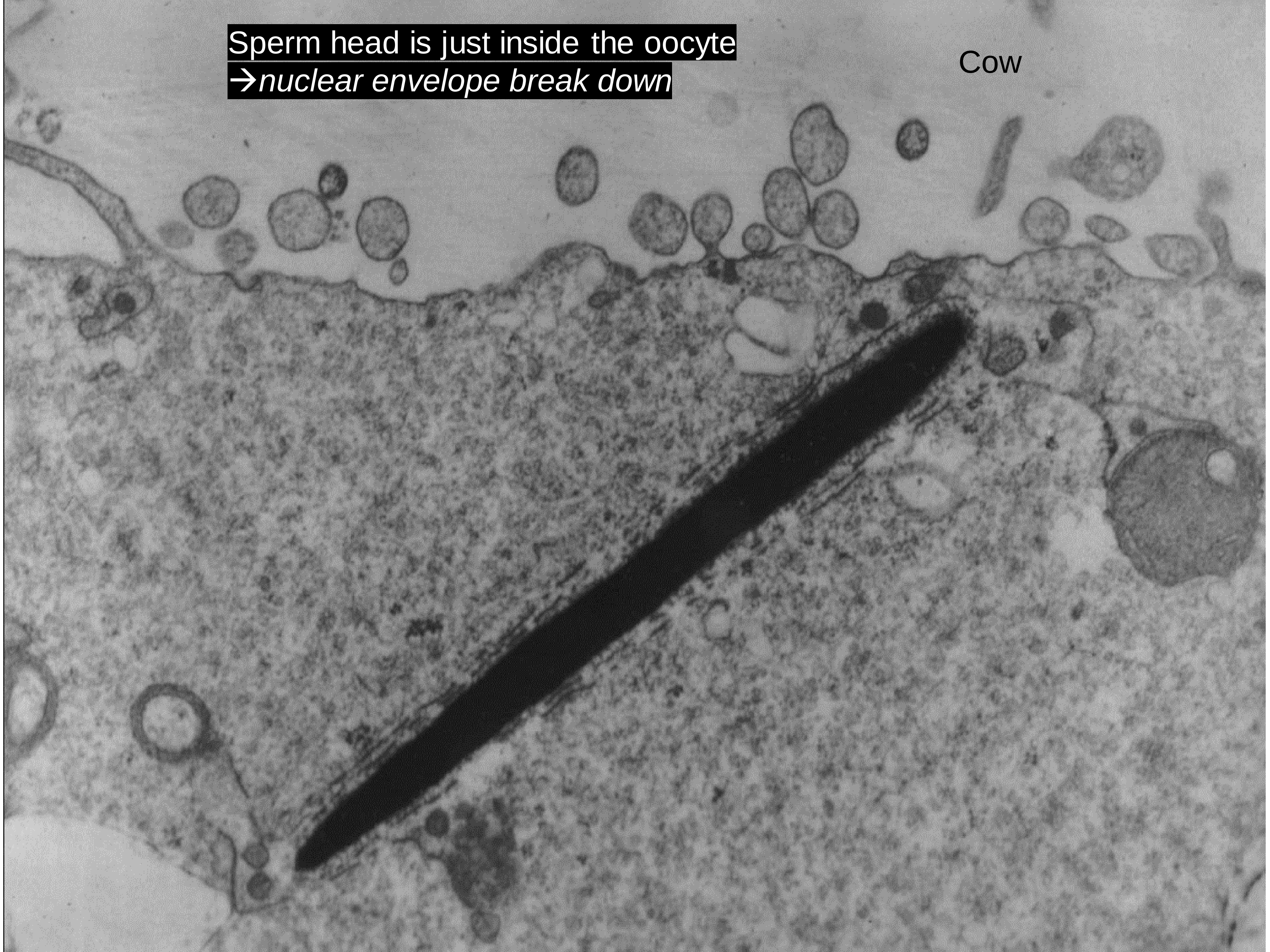
Modified from *Gadella and Evans: Adv Exp Med Biol (2011)*



Gadella and Evans: Adv Exp Med Biol (2011)

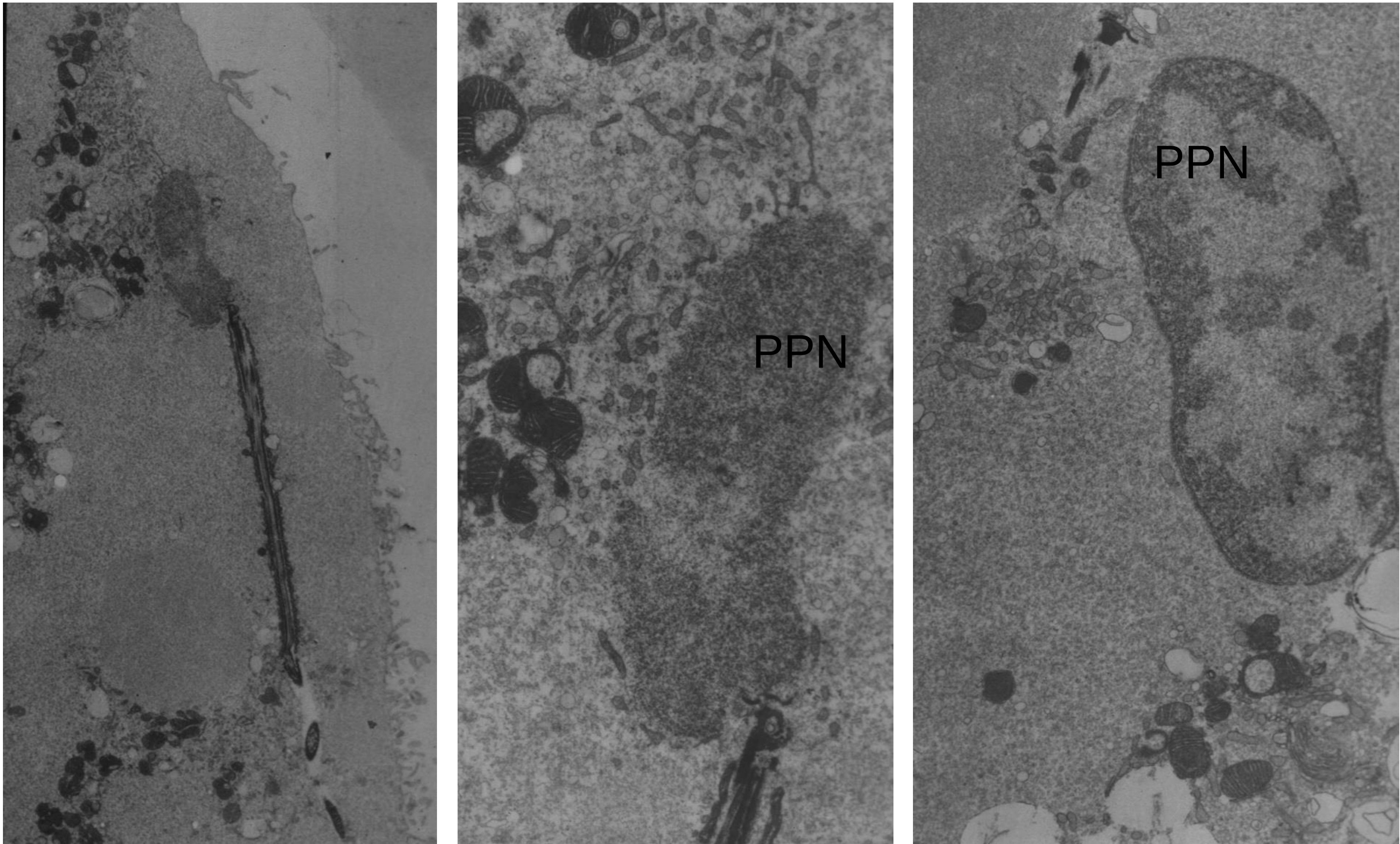


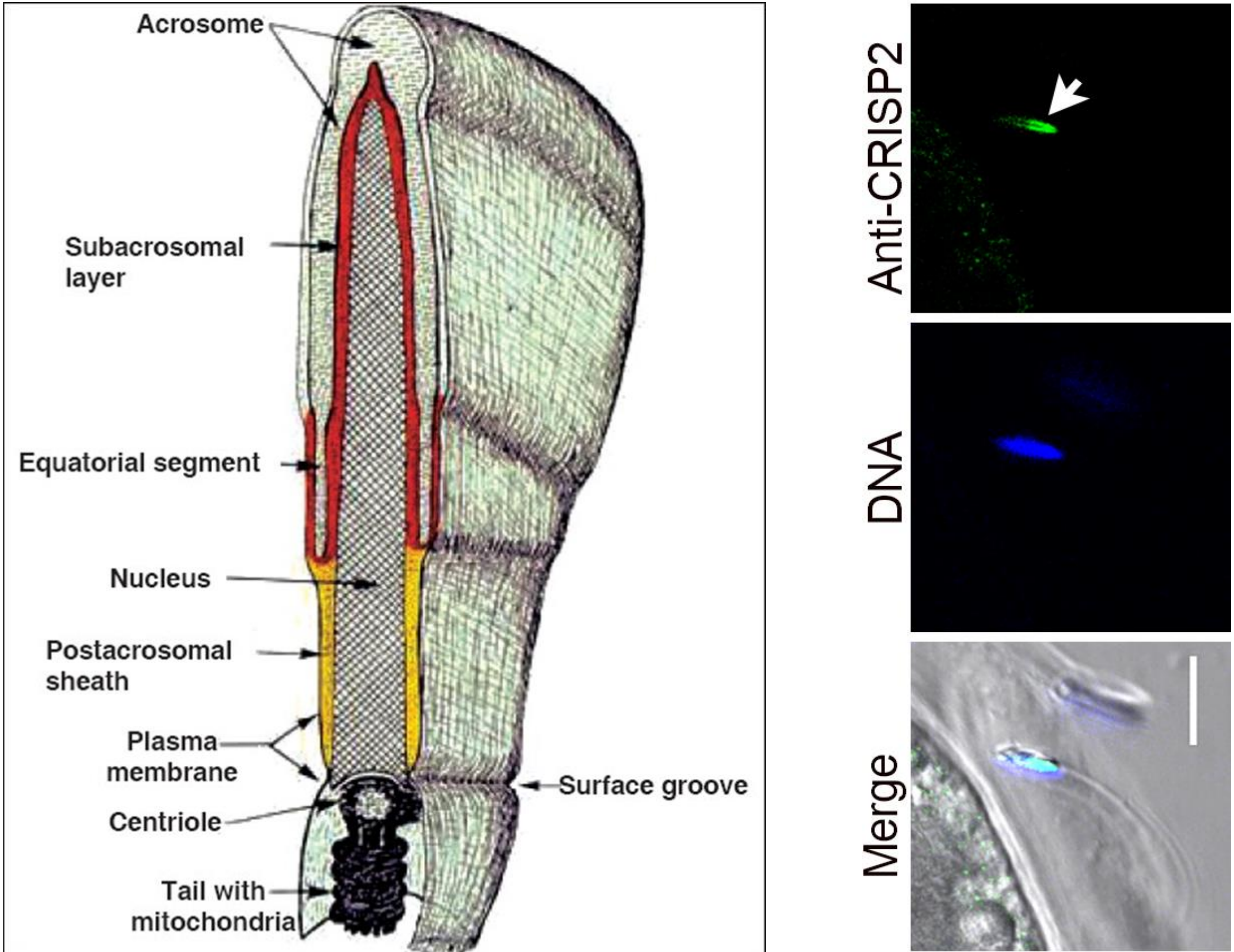

Just before fertilization
Cow


Sperm head is just inside the oocyte
→*nuclear envelope break down*
Cow

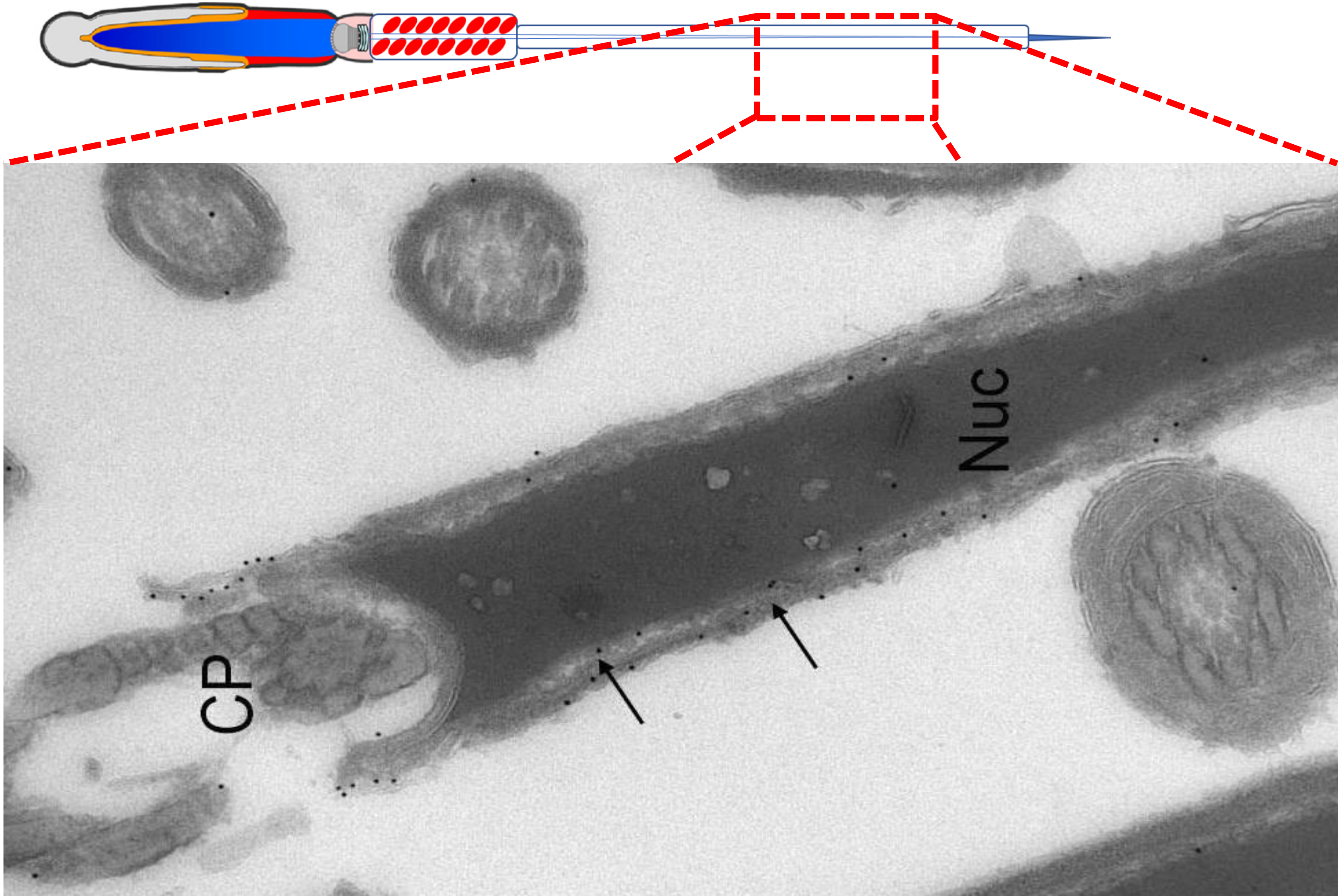
Decondensation and pronucleus formation - paternal

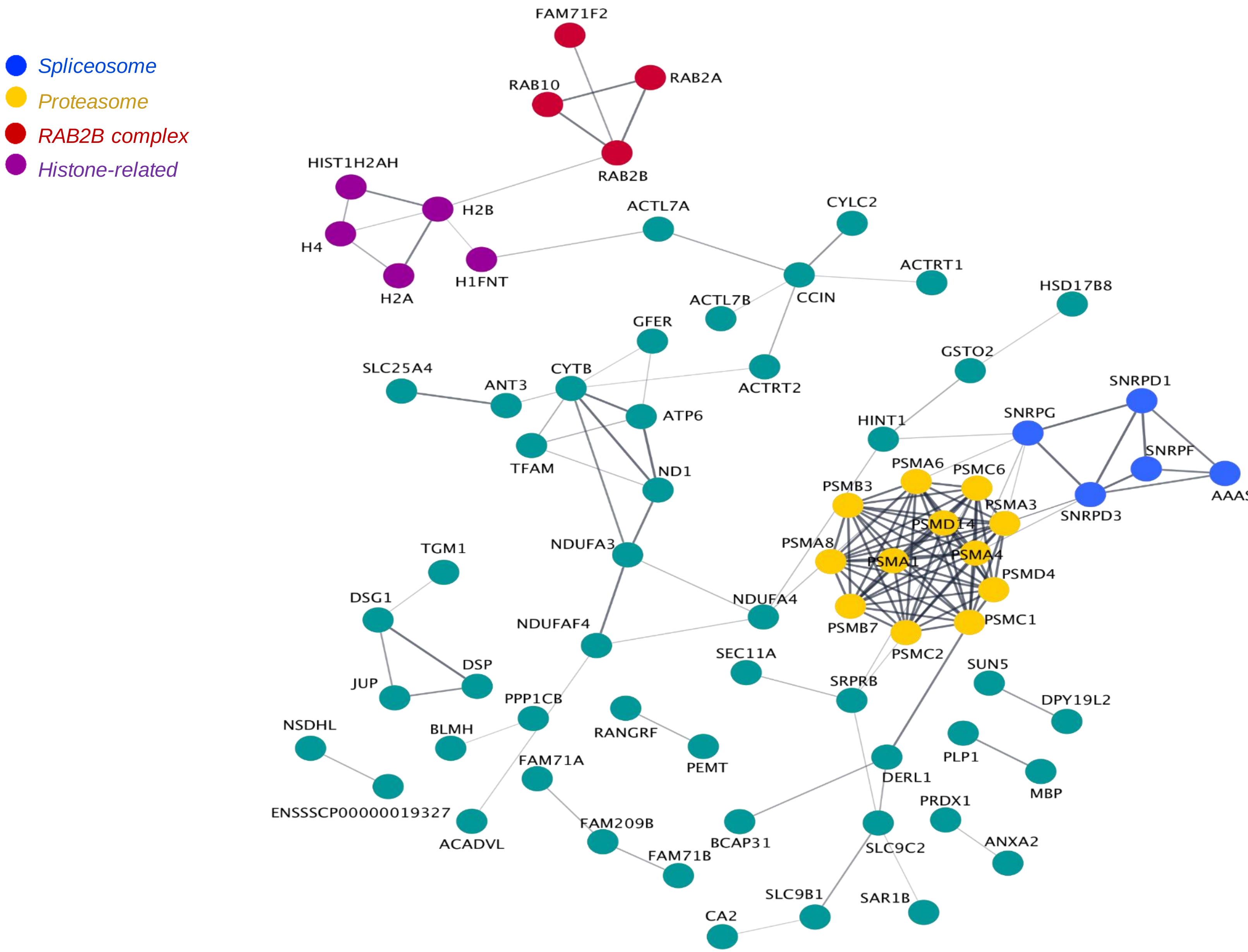
Cow

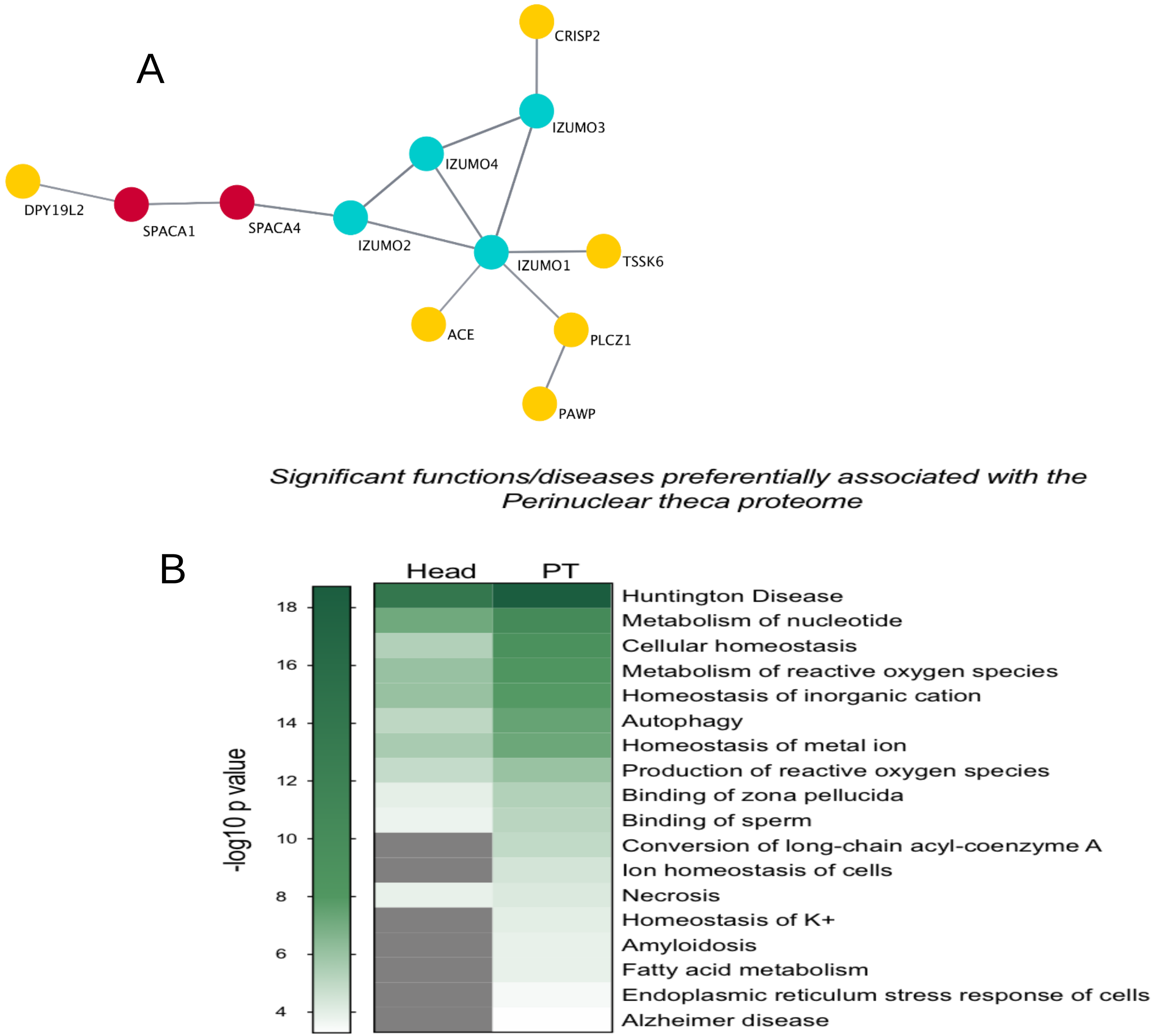



Acrosome
Subacrosomal layer
Equatorial segment
Nucleus
Postacrosomal sheath
Plasma membrane
Centriole
Tail with mitochondria
Surface groove
Anti-CRISP2
DNA
Merge

The perinuclear theca protects the sperm nucleus (CRISP2 immunolabelling, *Zhang et al.*, *Biol Reprod. 2021, 2022, Front Cell Dev Biol 2022, Andrology 2023*)



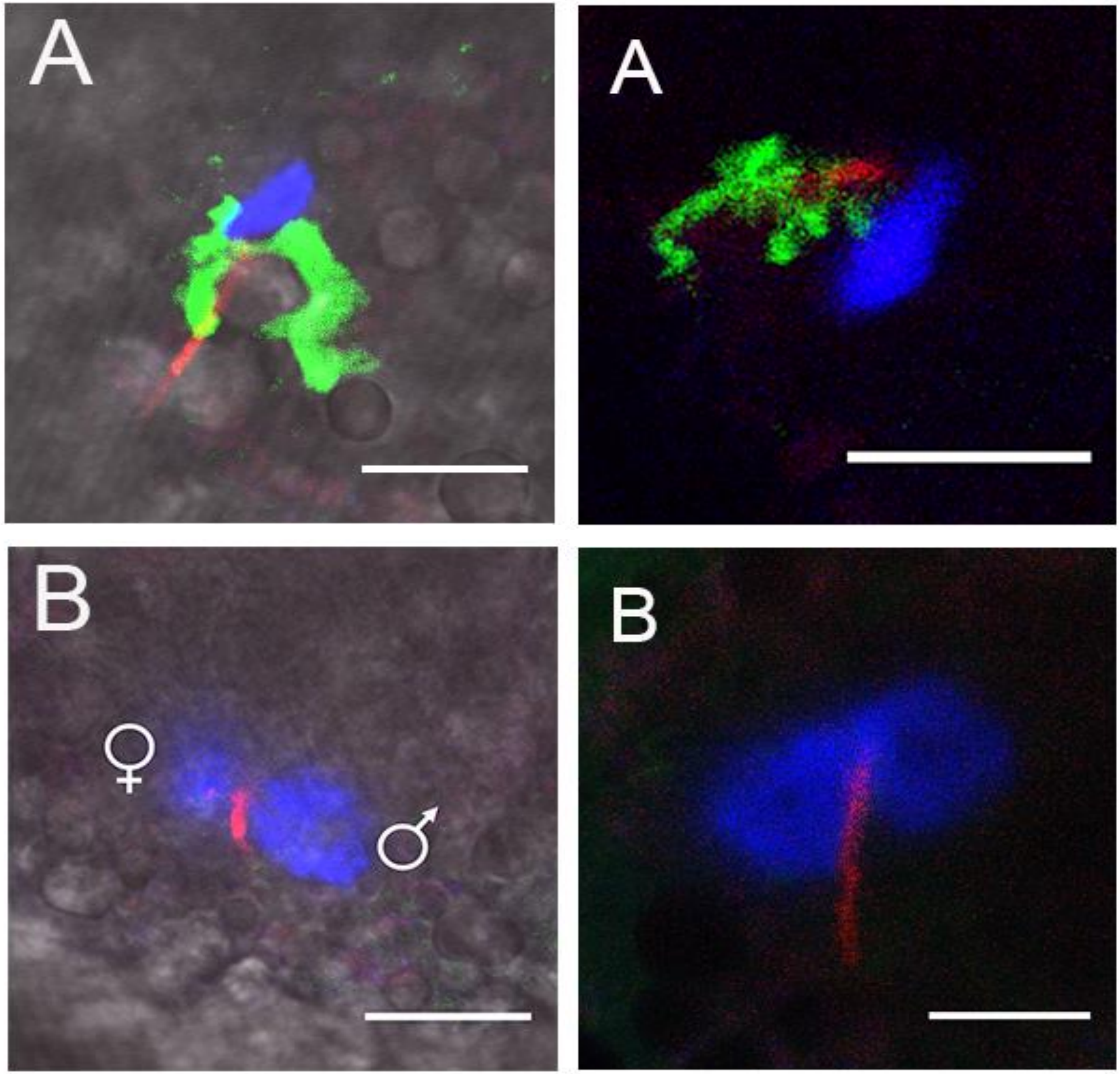

Spliceosome
Proteasome
RAB2B complex
Histone-related
FAM71F2
RAB2A
RAB10
RAB2B
HIST1H2AH
H2B
H4
H1FNT
H2A
ACTL7A
CYLC2
ACTRT1
CCIN
ACTL7B
HSD17B8
GFER
SLC25A4
CYTB
ANT3
ATP6
ACTRT2
GSTO2
SNRPD1
SNRPG
HINT1
SNRPF
AAAS
SNRPD3
TFAM
ND1
PSMA6
PSMC6
PSMB3
PSMA3
PSMD14
PSMA8
PSMA1
PSMA4
PSMD4
NDUFA3
TGM1
NDUFA4
DSG1
NDUFAF4
PSMB7
PSMC1
PSMC2
SEC11A
DSP
JUP
SUN5
SRPRB
PPP1CB
DPY19L2
NSDHL
BLMH
RANGRF
PLP1
FAM71A
PEMT
DERL1
MBP
ENSSSCP00000019327
PRDX1
ACADVL
FAM209B
BCAP31
ANXA2
FAM71B
SLC9C2
SLC9B1
SAR1B
CA2

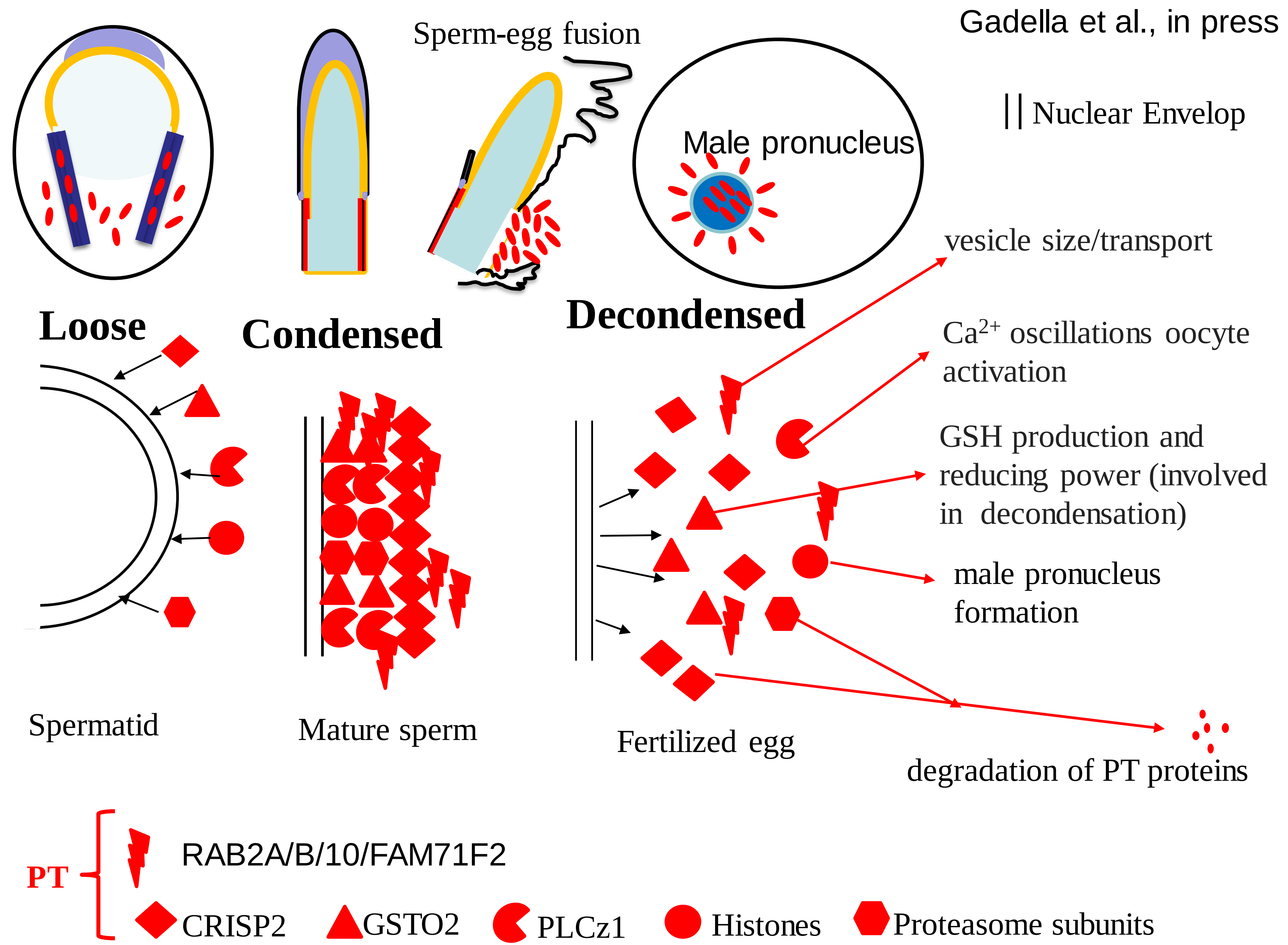

A
CRISP2
IZUMO3
IZUMO4
DPY19L2
SPACA1
SPACA4
IZUMO2
IZUMO1
TSSK6
ACE
PLCZ1
PAWP
Significant functions/diseases preferentially associated with the Perinuclear theca proteome
B
Head
PT
-log10 p value
18
16
14
12
10
8
6
4
Huntington Disease
Metabolism of nucleotide
Cellular homeostasis
Metabolism of reactive oxygen species
Homeostasis of inorganic cation
Autophagy
Homeostasis of metal ion
Production of reactive oxygen species
Binding of zona pellucida
Binding of sperm
Conversion of long-chain acyl-coenzyme A
Ion homeostasis of cells
Necrosis
Homeostasis of K+
Amyloidosis
Fatty acid metabolism
Endoplasmic reticulum stress response of cells
Alzheimer disease

CRISP2 (green) decondensation and degradation in just fertilized oocytes <u>within 5-8 h after mixing with sperm</u>

**both before male pronucleus formation (A 5 h)*

**both before mitochondrial degradation (B 8 h)*




Gadella et al., in press
Sperm-egg fusion
Male pronucleus
|| Nuclear Envelop
Loose
Condensed
Decondensed
vesicle size/transport
Ca2+ oscillations oocyte activation
GSH production and reducing power (involved in decondensation)
male pronucleus formation
degradation of PT proteins
Spermatid
Mature sperm
Fertilized egg
PT
RAB2A/B/10/FAM71F2
CRISP2
GSTO2
PLCz1
Histones
Proteasome subunits

CONCLUSIONS

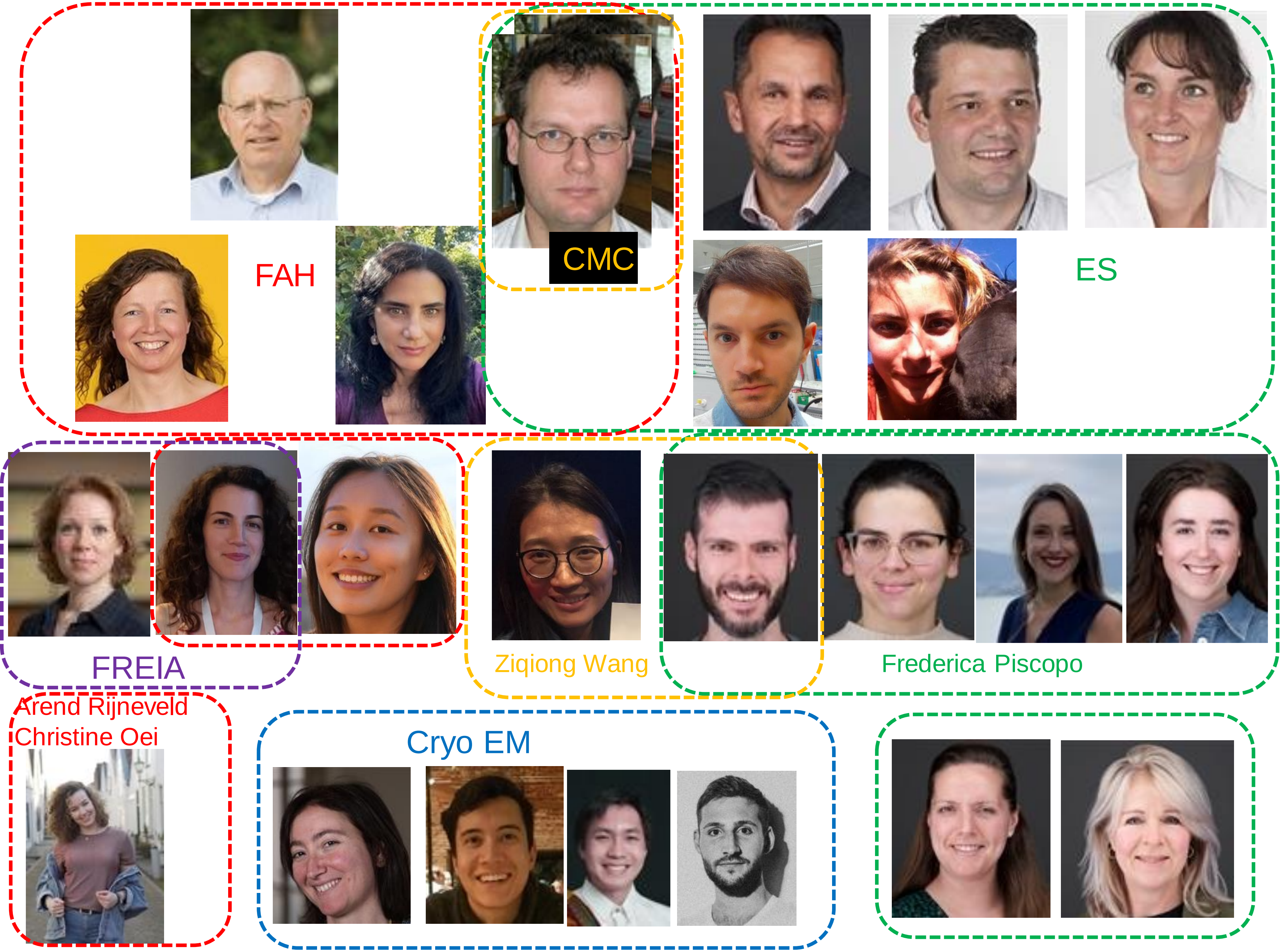
1. Sperm capacitation leads to hyperactivated motility and to surface alterations in the sperm head and to the priming of the acrosome to the sperm surface.

2. This does not result in exocytosis as the primed SNARE complexes are stabilized, complexin 2 and other proteins play a role in this process.

3. These changes are important preparative steps for zona binding and for sperm to become responsive for the zona-induced acrosome reaction.

4. Relevance to hexagonal sperm-egg fusion structure (and appearance of filaments) needs to be further investigated.

5. The role of the perinuclear theca components post-fertilization should be further investigated.


FAH
CMC
ES
FREIA
Ziqiong Wang
Frederica Piscopo
Arend Rijneveld
Christine Oei
Cryo EM