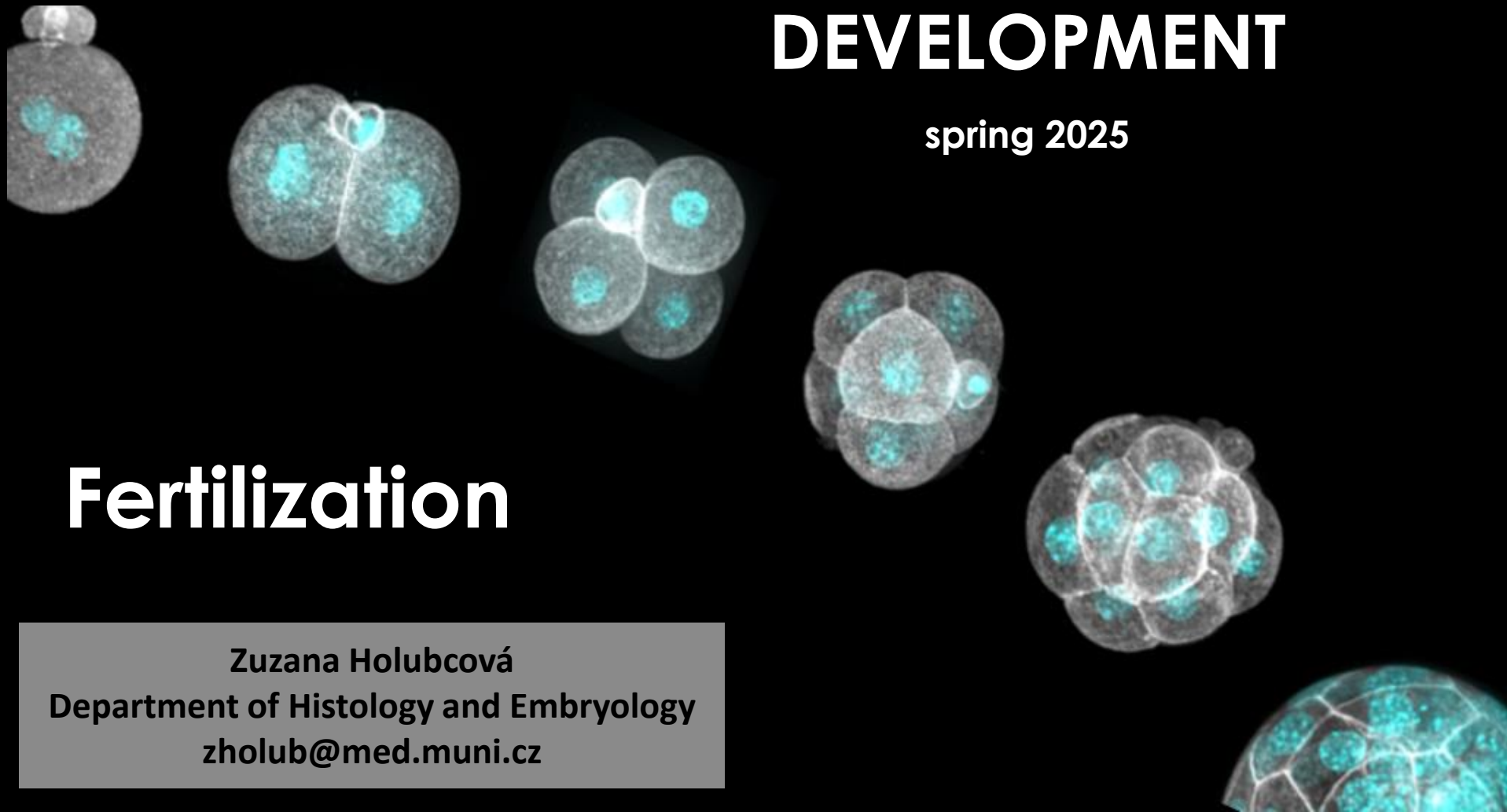
MUNI MED

Embryology II

PREIMPLANTATION DEVELOPMENT

spring 2025



Fertilization

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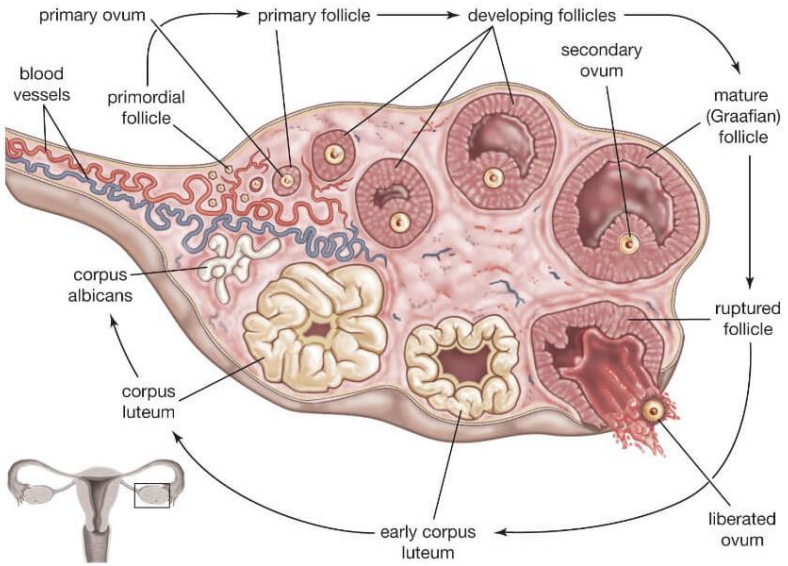
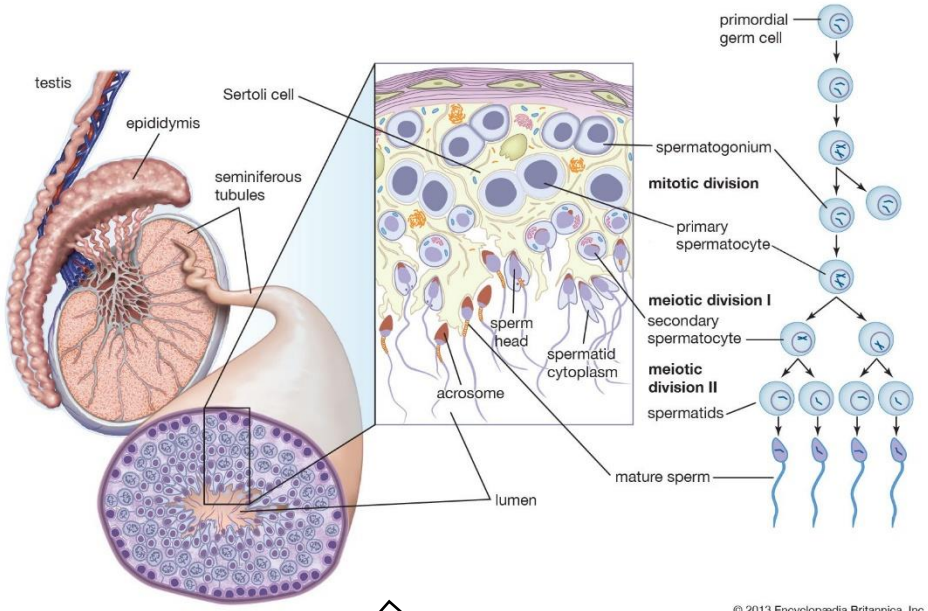
Preparation for fertilization

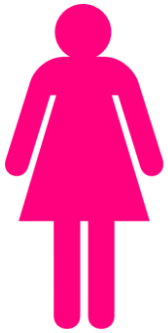
OOGENESIS

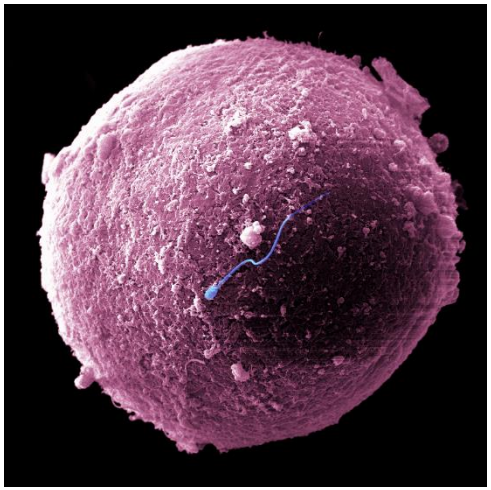


SPERMATOGENESIS



- entry to meiosis
- follicle formation
- oocyte and follicle growth
- dominant follicle selection
- oocyte maturation
- ovulation





INTERNAL FERTILIZATION

- completion of meiosis
- morphological transformation
- sperm maturation

(forming transient sperm reservoirs)
- capacitation
- hyperactivation
- acrosomal reaction
- cumulus and zona penetration

Preparation for fertilization

❖ Capacitation

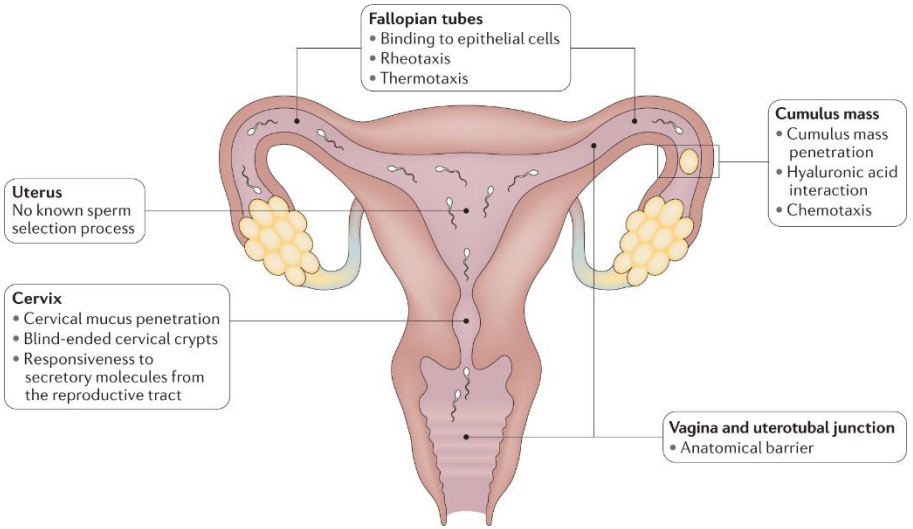
- series of biochemical and physiological changes in the sperm cell

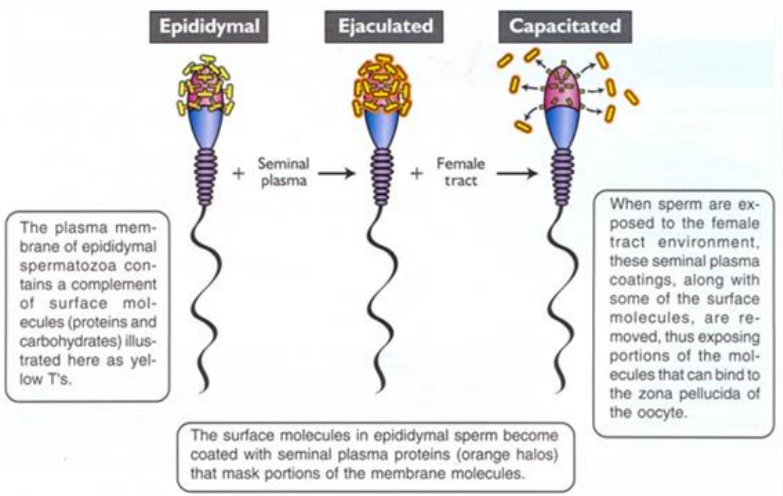
- takes place inside the female reproductive tract

- can be achieve in vitro in media containing appropriate compound and with high pH

- critical for sperm ability to reach fertilization site and interact with the oocyte

- changes in
  - **(1) membrane properties (fluidization)**
  - **(2) intracellular ion concentration**
  - **(3) activities of enzymes**





Preparation for fertilization

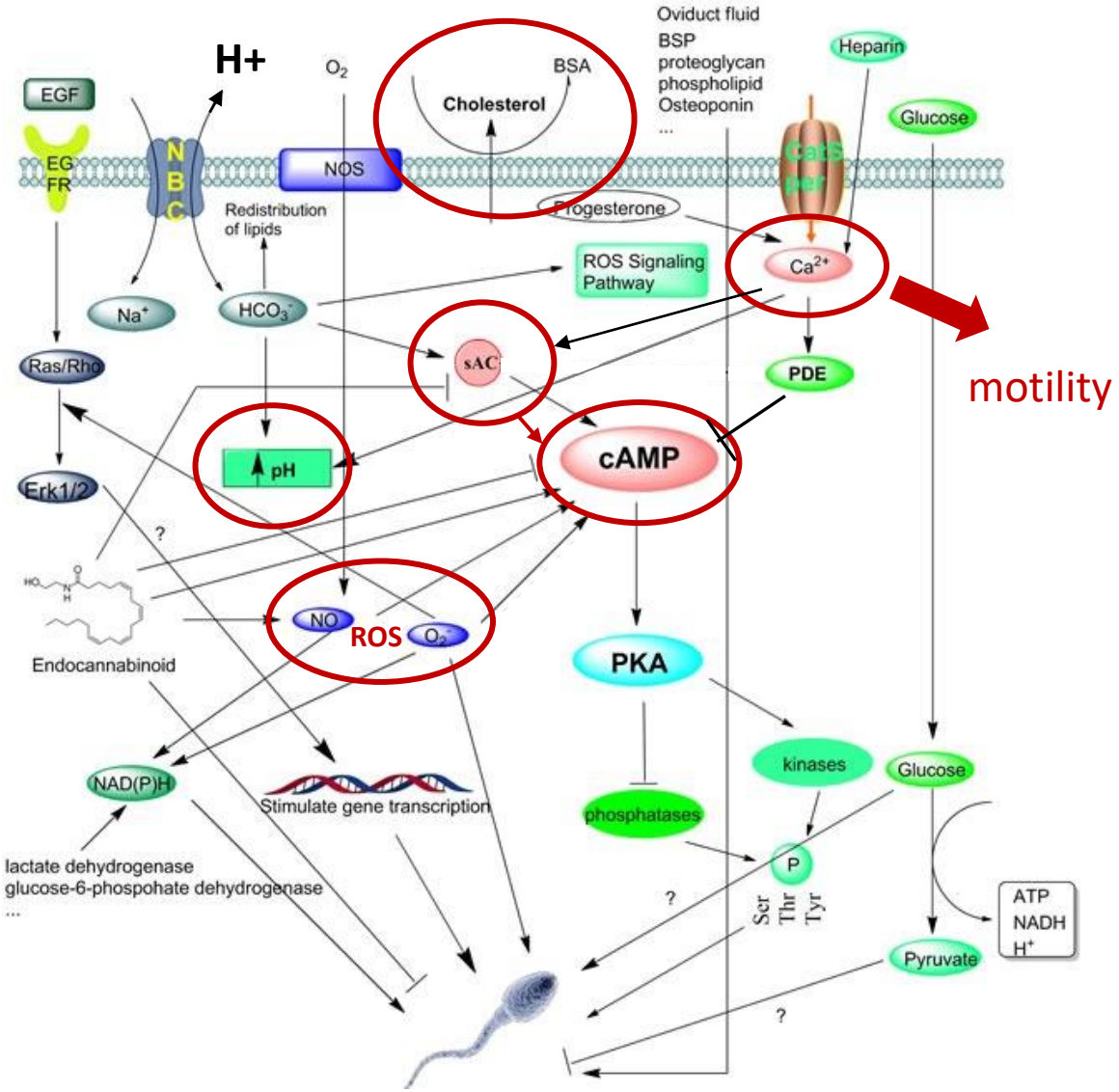
❖ Capacitation

- removal of cholesterol increases sperm membrane permeability

- leak of H+ → rise of pHi (alkalization)

- Ca2+ influx → activation of soluble adenylate cyclase (sAC) → Tyr-kinase phosphorylation

- physiological ROS generation

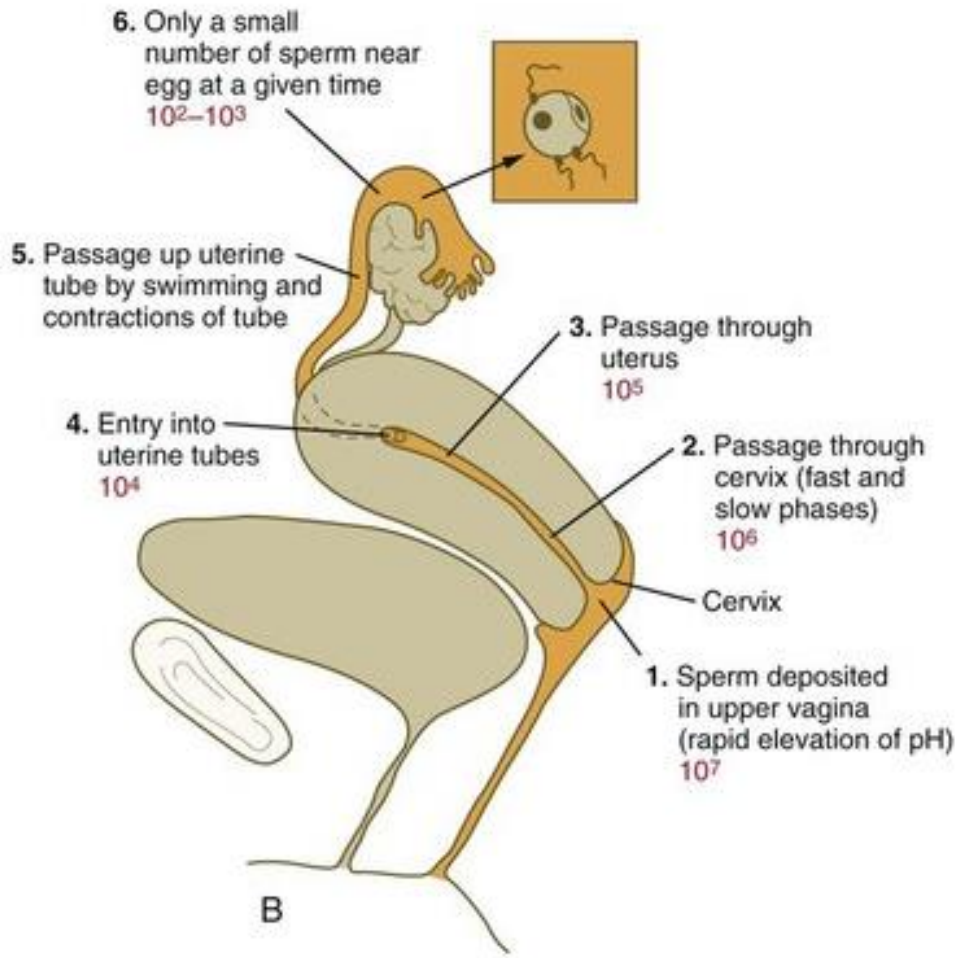


*NBC = Na+/HCO3 co-transporter

Jin and Yang, 2017

Preparation for fertilization

❖ Sperm transport

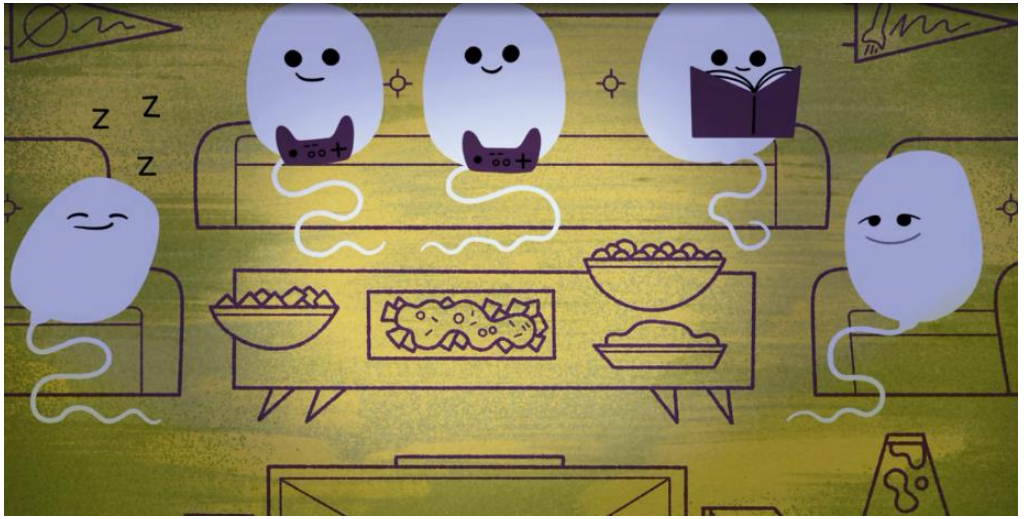


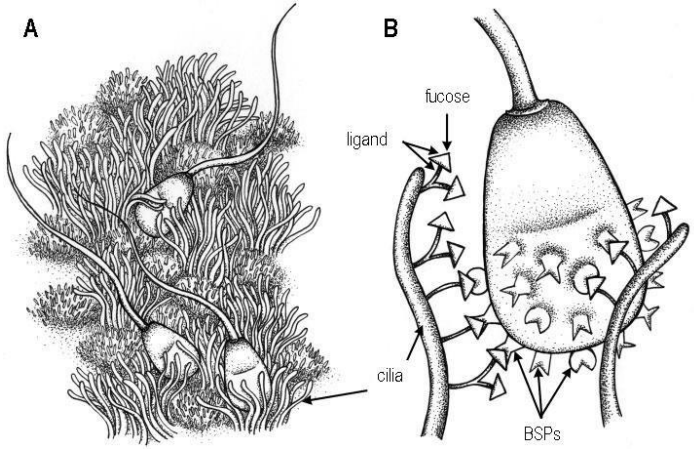
https://clinicalgate.com/transport-of-gametes-and-fertilization/

Preparation for fertilization

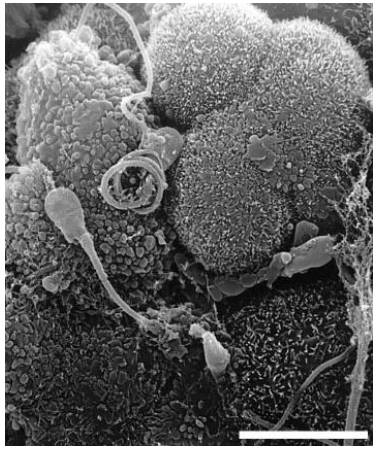
❖ Sperm reservoirs

- sperm deposition sites in lower oviduct (isthmus)
- adhesion to oviductal epithelium
- sperm membrane proteins bind to carbohydrate moities of oviduct epithelial cells
- storage of functional sperm before ovulation, delay of capacitation, maintenance of sperm cell viability

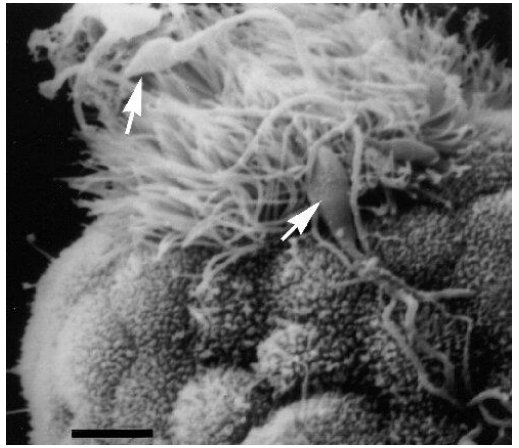




Thys et al 2009



Ellington 1998



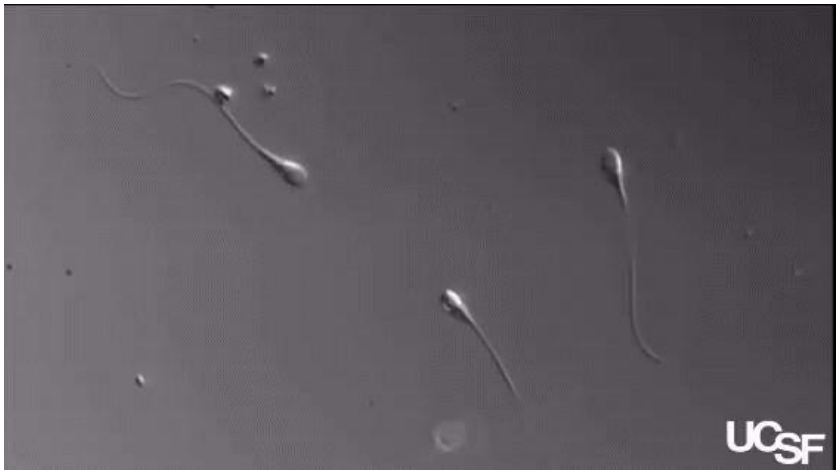
Suarez et al 2005

Preparation for fertilization

❖ Hyperactivation

- change from basal to hyperactivated motility
- symmetric flagellar bending with low frequency → asymmetring beating
- critical for sperm release from reservoir, propulsive movement in viscous elastic fluid, penetration through extracellular matrix of COC, and for drilling zona pellucida

Basal movement



Hyperactivated movement

https://www.youtube.com/watch?v=PFPNv2LX3XY

Preparation for fertilization

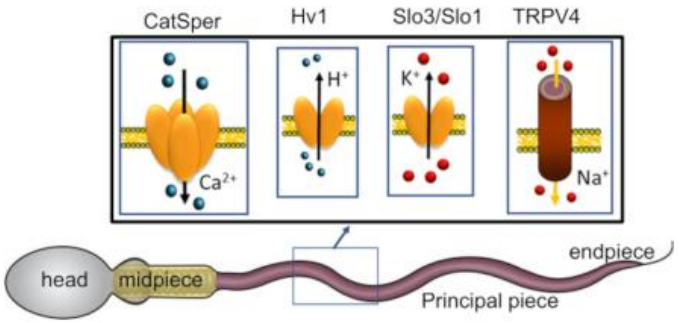
❖ Hyperactivation

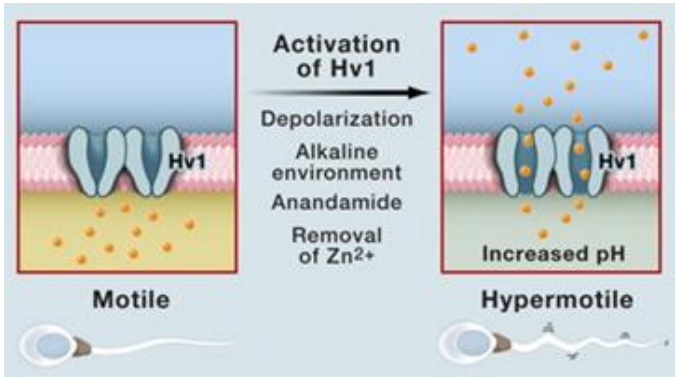
- caused by rapid **rise of intracellular Ca^{2+}** ([Ca2+]i)

- Ca2+ serves as a second messenger in cell signalling and directly enhaces dynamics of cytoskeletal components in sperm flagellum

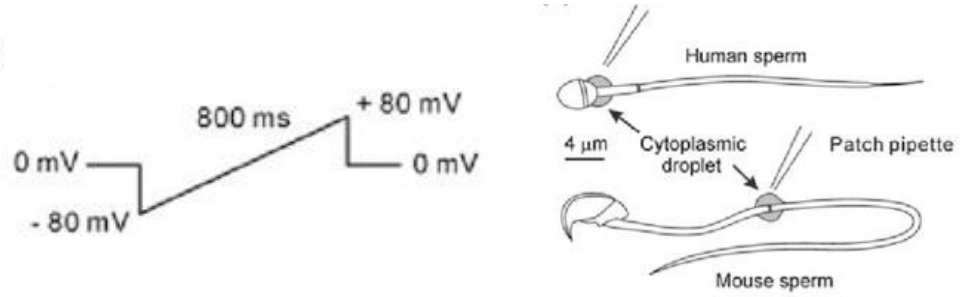
- primed by alkalic environment
  - multiple (voltage-dependent/pH-sensitive/ligand-activated) ion chanells in sperm midpiece and tail
  - H+ efflux increase rise of intracellular pH required for capacitation
  - oviduct lumen pH increases after ovulation providing favourable conditions for hyperactivation

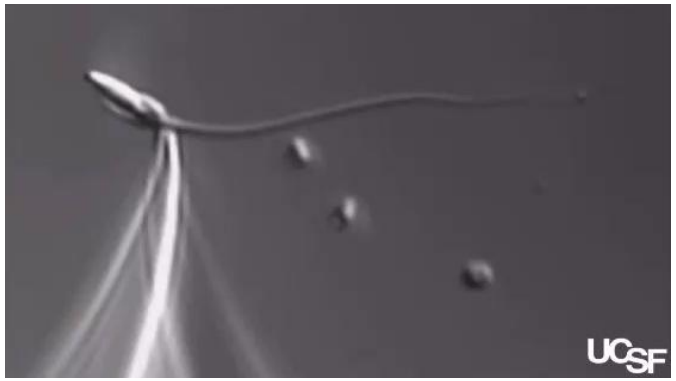
- ion movements accross membrane manifest as membrane depolarization





„patch-clamp" technique



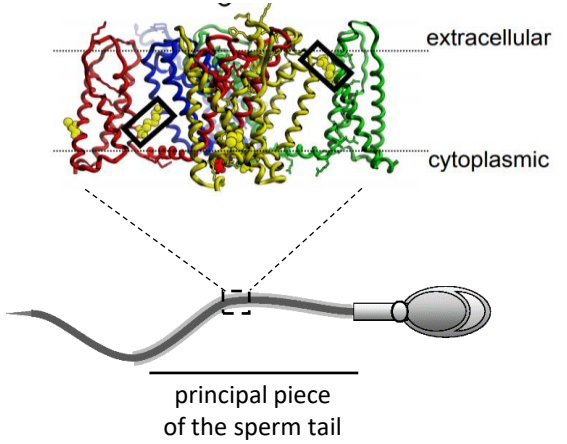


Preparation for fertilization

❖ Hyperactivation

CATSPER channel

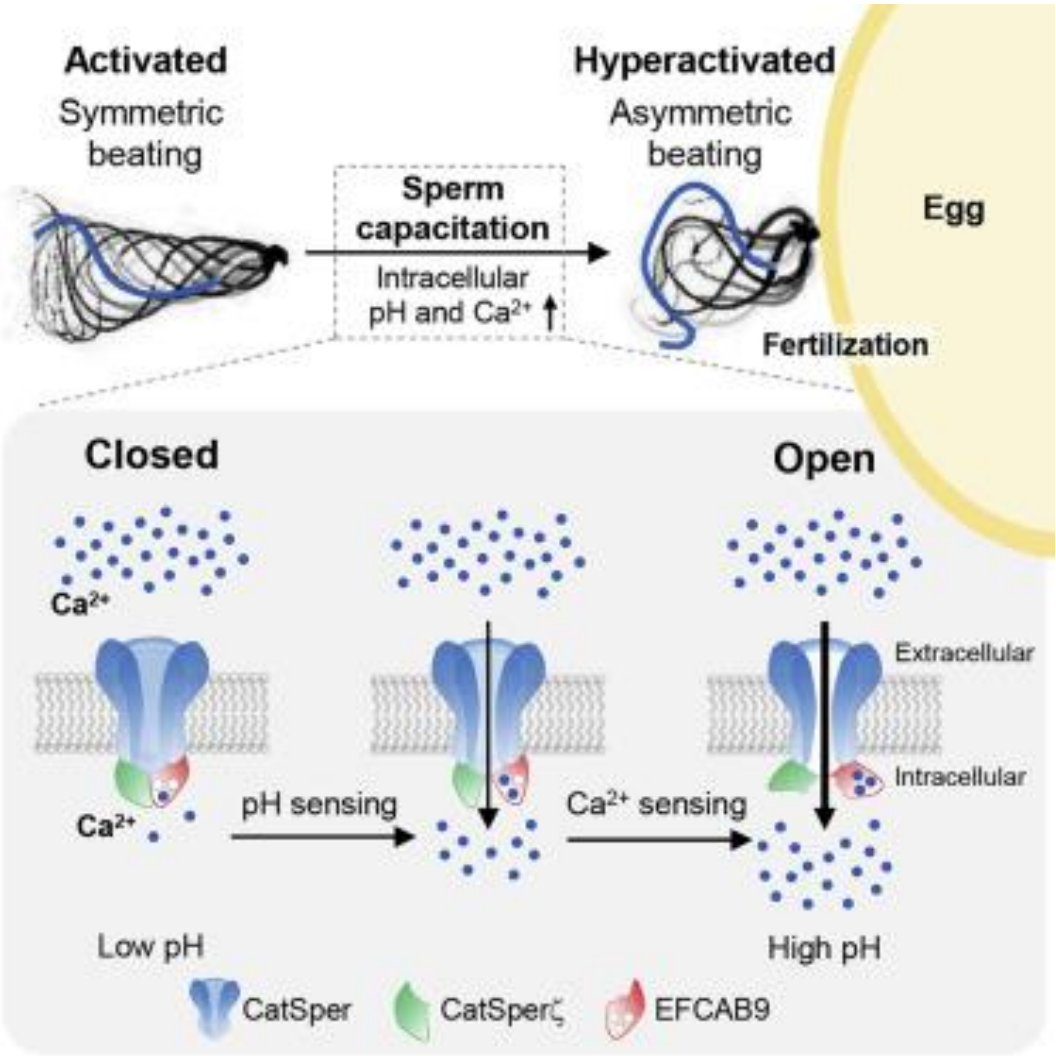
- **pH sensitive** ion channel
- testis-specific expression
- its opening causes **Ca2+ influx** resulting in rapid **rise of [Ca2+]i**



- mutation and altered experession linked to asthenospermia and male infertility

-/-

- normal mating, sperm count and morphology
- impaired motility, incapable to penetrate
- sterile



Ca2+ -

Hwang et al, Cell 2019

Preparation for fertilization

❖ Hyperactivation

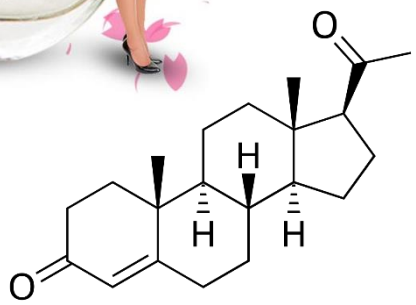
Nature 2011

LETTER

doi:10.1038/nature09769

The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm

Timo Strünker[1]*, Normann Goodwin[1]*, Christoph Brenker[1]*, Nachiket D. Kashikar[1]†, Ingo Weyand[2], Reinhard Seifert[1] & U. Benjamin Kaupp[1]

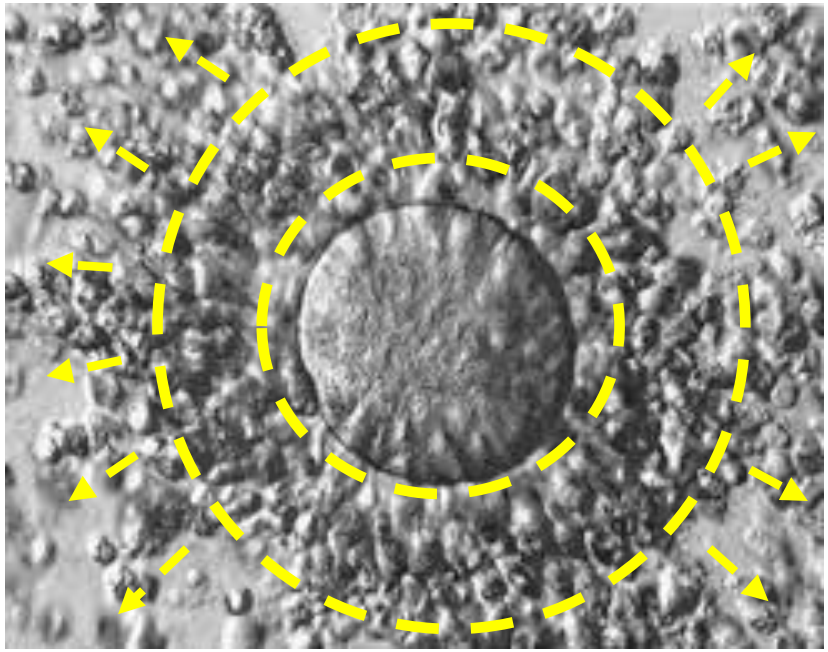


LETTER

doi:10.1038/nature09767

Progesterone activates the principal Ca^{2+} channel of human sperm

Polina V. Lishko[1], Inna L. Botchkina[1] & Yuriy Kirichok[1]



PROGESTERONE = chemoattractant and hyperactivation-inducing factor

Preparation for fertilization

❖ Hyperactivation

← *alkalization*

- sperm-specific Na/H+ exchanger SLC9C1 mediates i.c. alkalization
- ↑i.c. pH → conformational change of the blocker molecule EFCAB9 at CatSper pore and its opening

← *progesteron*

- binds to **ABHD2 - α/β hydrolase domain-containing protein 2 which** serves as a non-genomic progesterone receptor on the extracellular side of sperm membrane
- after progesterone binding lipid hydrolase ABHD2 degrades **endocannabinoid 2-arachidonoylglycerol (2AG)** enriched in the sperm membrane
- the replenishment of AG2 leads to the CatSper full opening and triggers sperm hyperactivation

- CatSper openning results in Ca2+ influx and triggers sperm hyperactivation

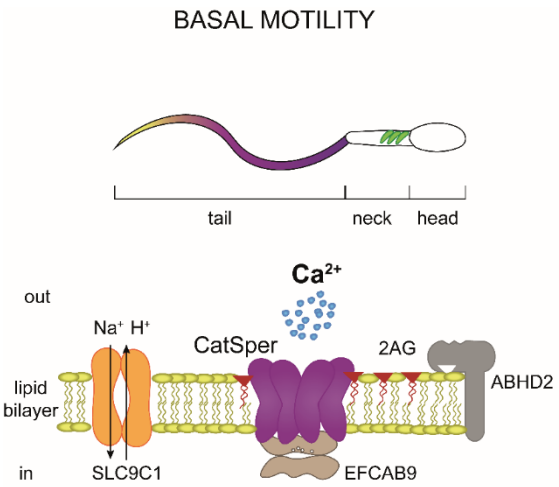
Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone

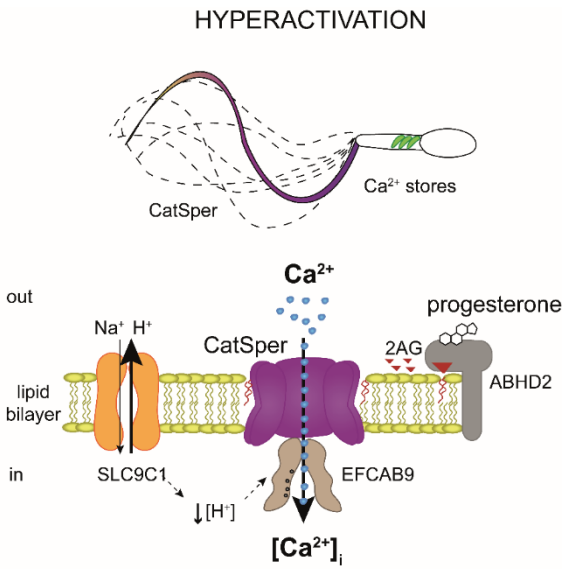
Melissa R. Miller,[1] Nadja Mannowetz,[1] Anthony T. Iavarone,[2] Rojin Safavi,[1] Elena O. Gracheva,[3] James F. Smith,[4] Rose Z. Hill,[1] Diana M. Bautista,[1] Yuriy Kirichok,[5] Polina V. Lishko[1]*

Miller et al Science 2016

Polina Lishko

Role of PGE1? Direct competition with 2AG?





Trebichalska and Holubcova, JARG 2020

Preparation for fertilization

❖ Hyperactivation

- ligand-sensitive nature of CatSper channel

„promiscuous“ channel

- opportunity for development of „male/unisex“ contraception based on prevention of hyperactivation

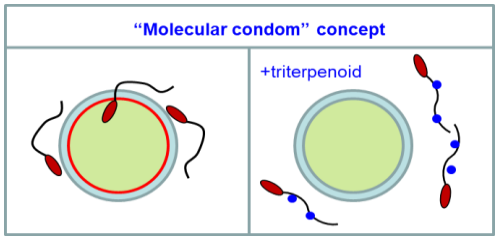


Table 1 Overview of the identified low molecular interactors of the CatSper channel

Type	Category	Name	CAS number	Concentration	Effect	Reference
endogenous	steroids	progesterone	57-83-0	nM	agonist	*Lishko et al., 2011* [24]; *Strunker et al., 2011* [25]
		testosterone	58-22-0	nM	partial agonist	*Mannowetz et al., 2017* [34]
		estrogen	50-28-2	nM	partial agonist	*Mannowetz et al., 2017* [34]
		hydrocortisone	50-23-7	nM	partial agonist	*Mannowetz et al., 2017* [34]
	endocannabidoids	2-arachidonoylglycerol	53847-30-6	µM	antagonist	*Miller et al., 2016* [27]
exogenous	analogues of cyclic nucleotide	8-bromoguanosine 3’,5’-cyclic monophosphate	31356-94-2	mM	agonist	*Brenker et al., 2012* [30]
	triterpenoids	pristimerin	1258-84-0	nM	partial agonist	*Mannowetz et al., 2017* [34]
		lupeol	545-47-1	nM	partial agonist	*Mannowetz et al., 2017* [34]
	nonsteroidal estrogens	α-zearalenol	36455-72-8	µM	agonist	*Brenker et al., 2018* [41]; *Schiffer et al., 2014* [36]
	endocrine disruptor	dichlorodiphenyldichloroethylene	72-55-9	pM - µM	agonist	*Tavares et al., 2013* [35]
		diethylstilbestrol	56-53-1	pM - µM	agonist	*Zou et al., 2017* [40]
	plasticizers	dibutyl phtalate	84-74-2	µM	agonist	*Schiffer et al., 2014* [36]
	anesthetic	ketamine	6740-88-1	mM	antagonist	*He et al., 2016* [38]
	odorants	bourgeonal	18127-01-0	µM	agonist	*Brenker et al., 2012* [30]
		undecanal	112-44-7	µM	agonist	*Brenker et al., 2012* [30]
		cyclamal	103-95-7	µM	agonist	*Brenker et al., 2012* [30]
		helional	1205-17-0	µM	agonist	*Brenker et al., 2012* [30]
	UV filters	benzylidene camphor sulfonic acid	56039-58-8	µM	agonist	*Brenker et al., 2018* [41]
		4-methylbenzylidene camphor	36861-47-9	µM	agonist	*Rehfeld et al., 2016* [37]
		methyl anthranilate	134-20-3	µM	agonist	*Rehfeld et al., 2016* [37]
		isoamyl p-methoxycinnamate	71617-10-2	µM	agonist	*Rehfeld et al., 2016* [37]

Trebichalska and Holubcova, JARG 2020

Preparation for fertilization

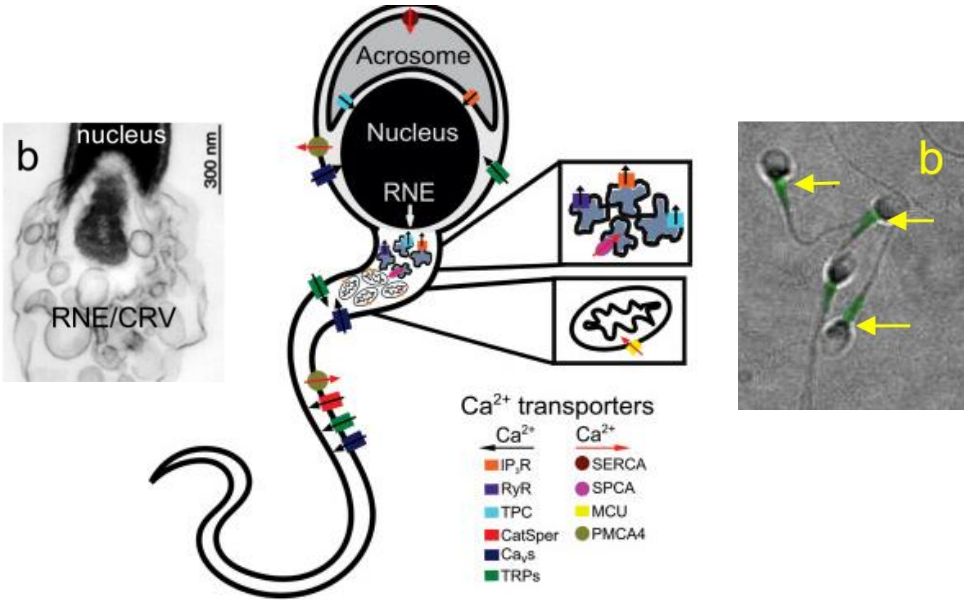
❖ Hyperactivation

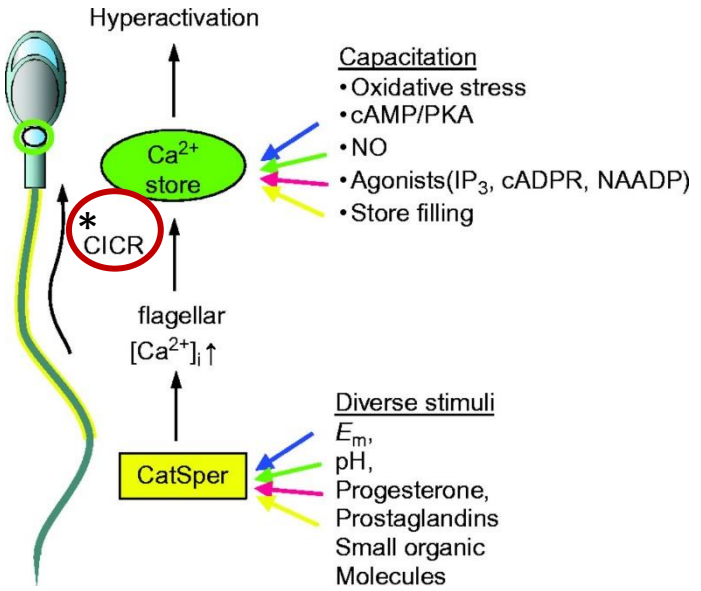
Ca2+ influx through CatSper

+

Internal Ca2+ stores mobilization

- Acrosome
- Mitochondria in the midpiece
- Redundant nuclear envelope (RNE) in the connecting piece



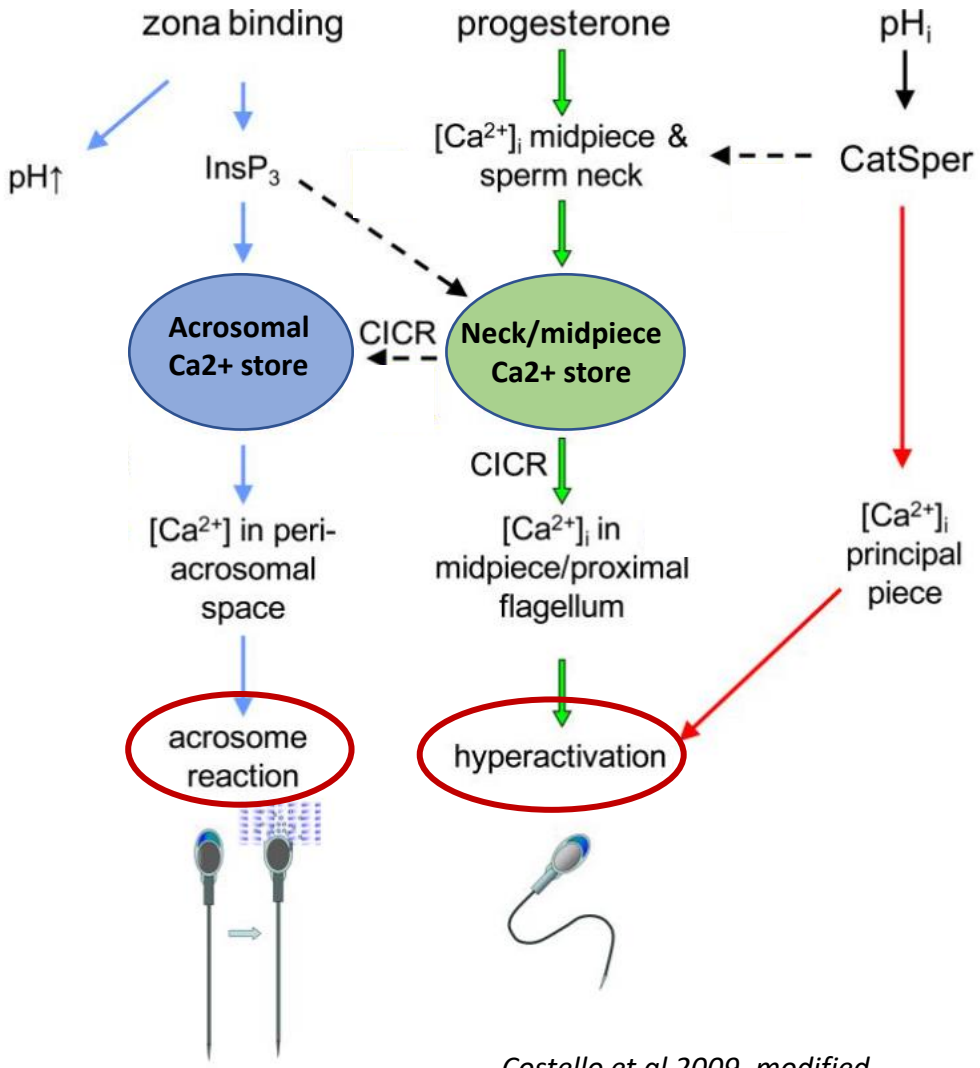


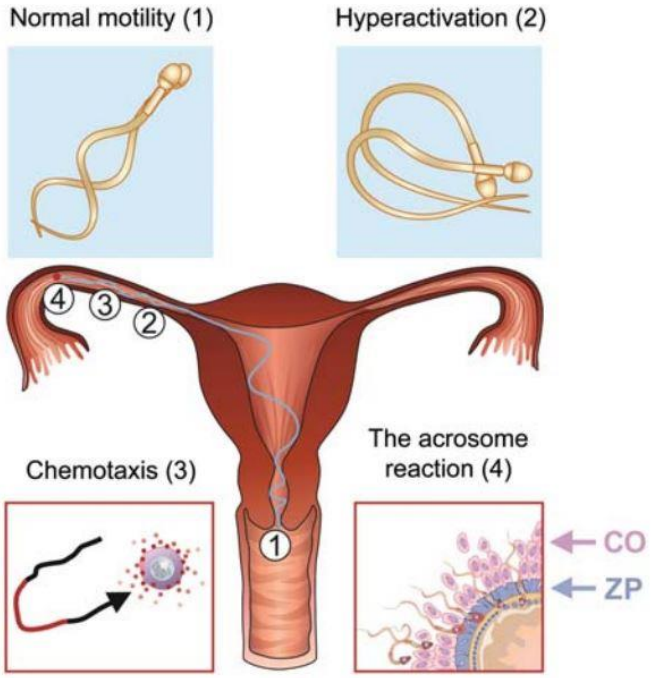
***calcium-induced calcium release**

- CatSperm mediated elevation of flagellar [Ca2+]i spreads forward and stimulates secondary Ca2+ release from intracellular stores in sperm midpiece and head region

Preparation for fertilization

❖ Hyperactivation

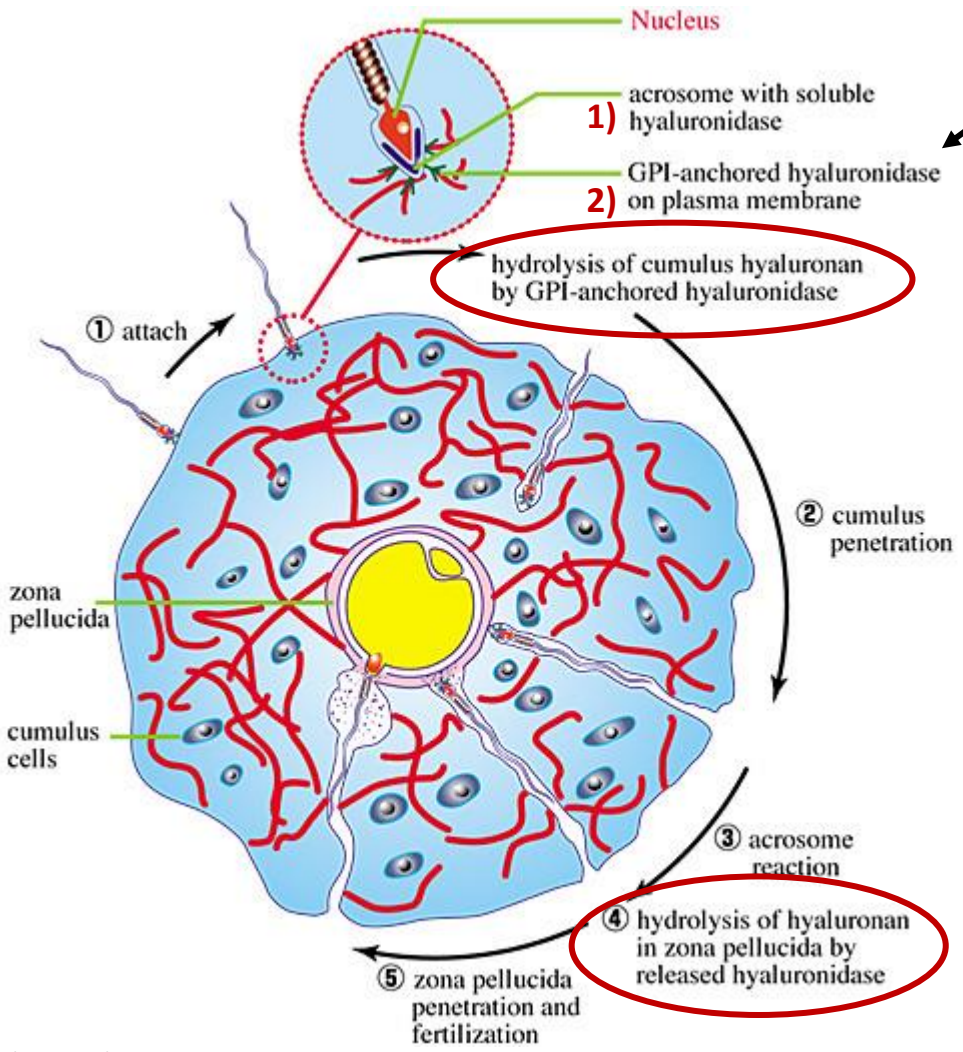




Costello et al 2009, modified

Preparation for fertilization

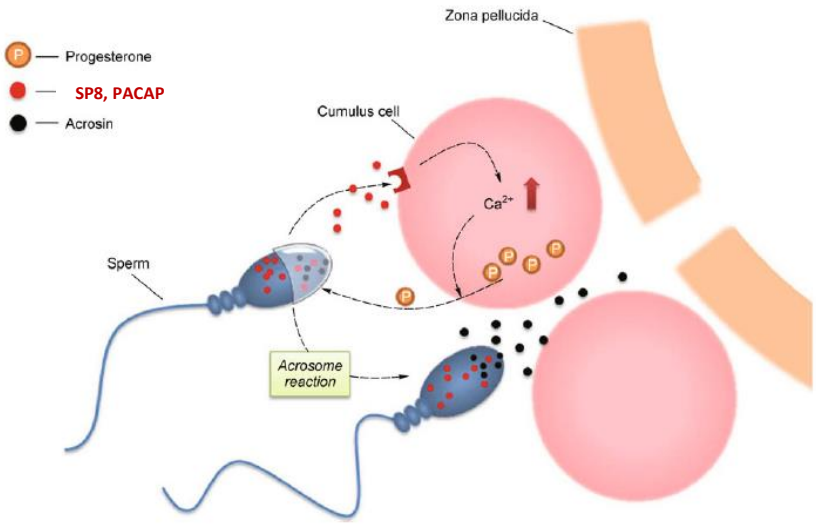
❖ Passage through cumulus



Salustri et al 1999

- **PH-20 and Hyal5**
  - glycosylphosphatidylinositol (GPI)-anchored proteins
  - localized on head of both human and mouse sperm
  - hyaluronidase activity
  - hydrolyses HA-rich cumulus matrix

- **NYD-SP8** and **PACAP**
  - sperm –derived surface factors
  - activate Ca2+ signalling in cumulus cells
    → ↑ progesterone release
    → acrosomal reaction

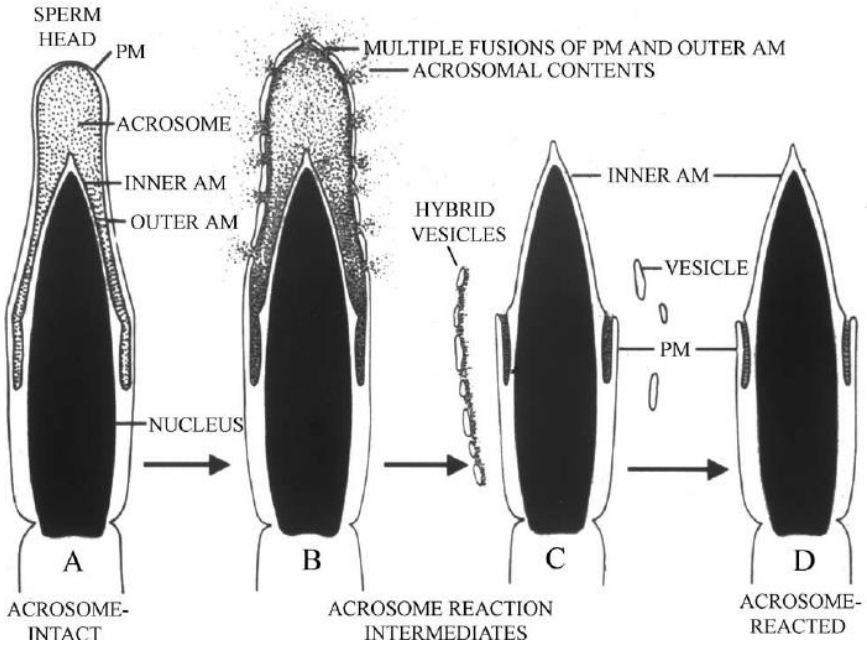


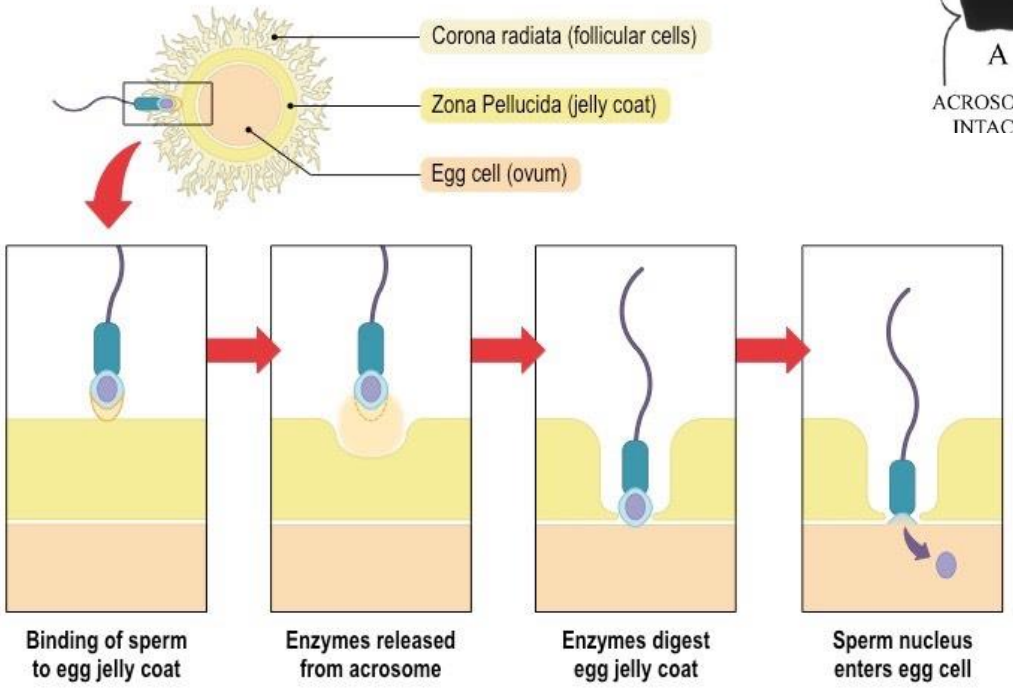
Sun et al 2011

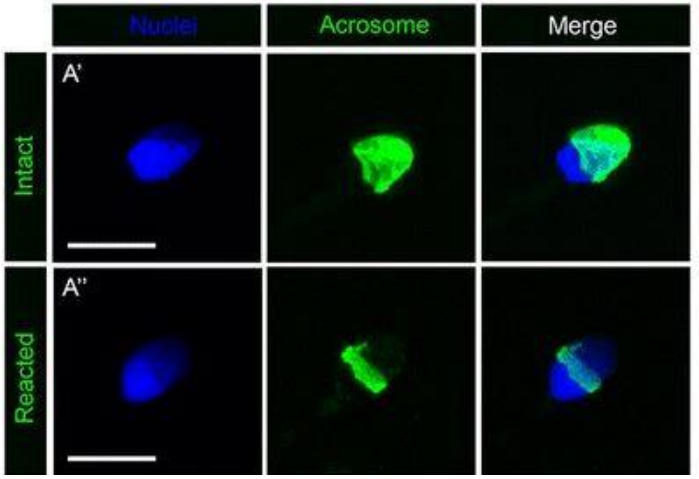
Preparation for fertilization

❖ Acrosome reaction

- membrane fusion and lytic enzyme release
- enables sperm to penetrate cumulus and zona pellucida surrounding the egg
- exposition of new set of surface antigens
- release of hyaluronidase and acrosin

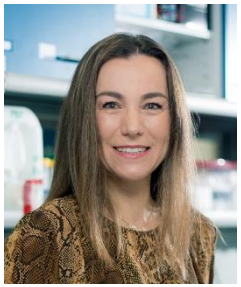






Preparation for fertilization

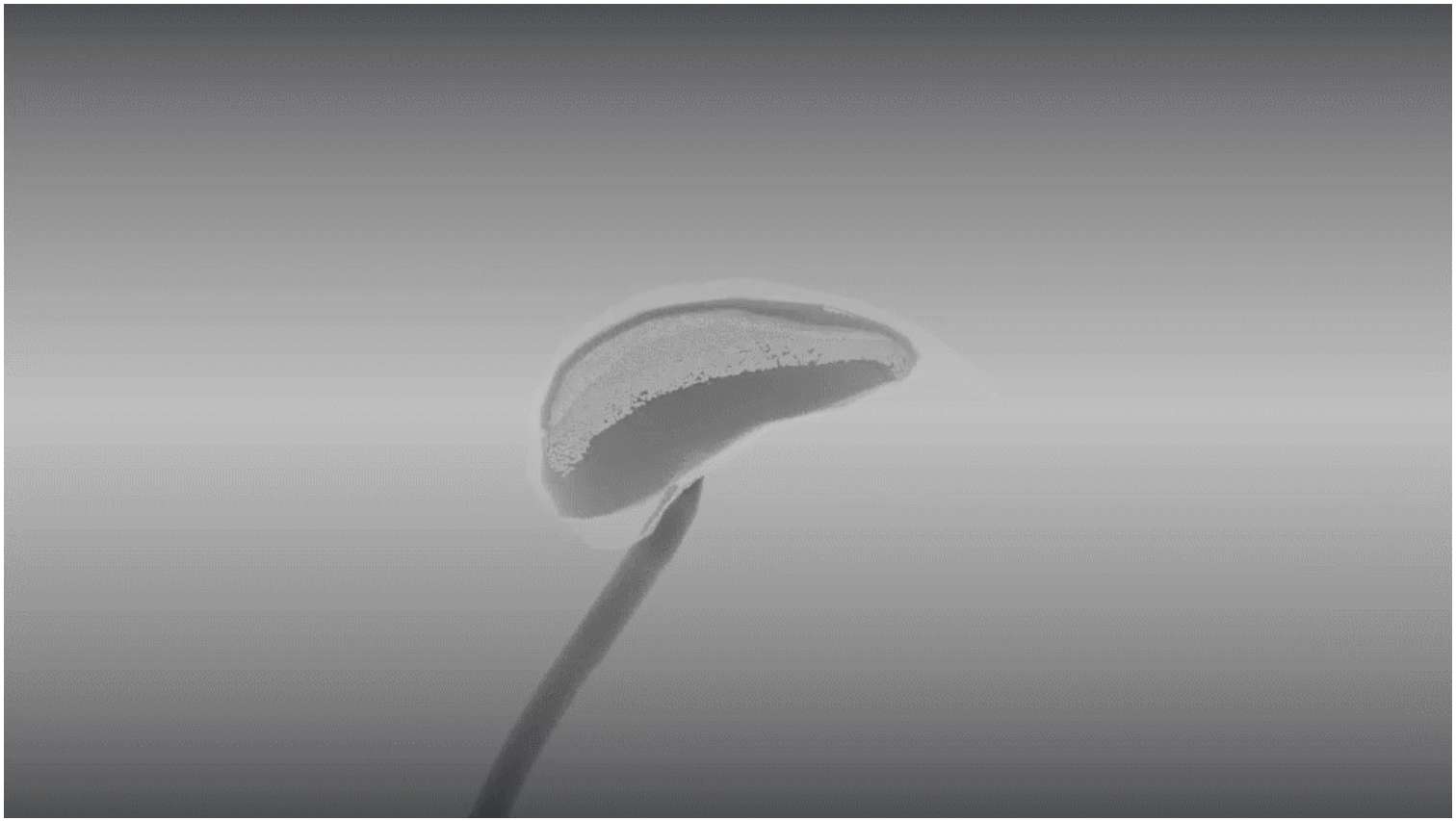
❖ Acrosome reaction



Kateřina Komrsková
(Dvořáková/Hortová)

CD46

β1 integrin



Frolikova et al 2016

Preparation for fertilization

❖ Zona binding

zona pellucida

= meshwork of interconnected filament

- glycoproteins ZP1-4

- outer porous region (~25%)
- inner compact region (~75%)
- thickening from inside to outside
- -mutations of ZP genes linked to conventional fertilization failure and EFS

JCB: Report

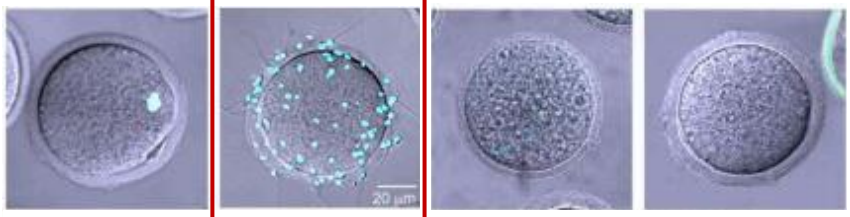
Human sperm bind to the N-terminal domain of ZP2 in humanized zonae pellucidae in transgenic mice

Boris Baibakov, Nathan A. Boggs, Belinda Yauger, Galina Baibakov, and Jurrien Dean

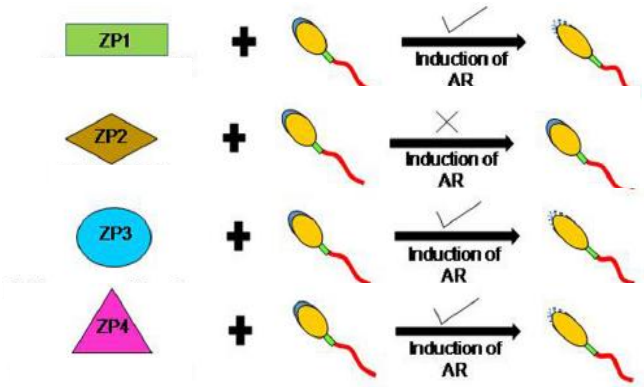
Jurrien Dean

- transgenic mice expressing human ZP proteins
- coincubation with human sperm

- human sperm binds to human ZP2



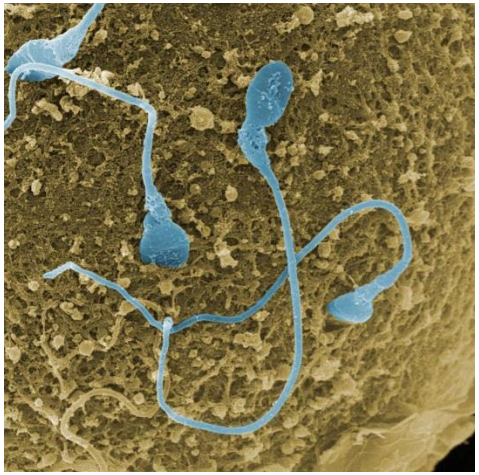
huZP1 rescue | huZP2 rescue | huZP3 rescue | huZP4 rescue



Baibakov et al 2012

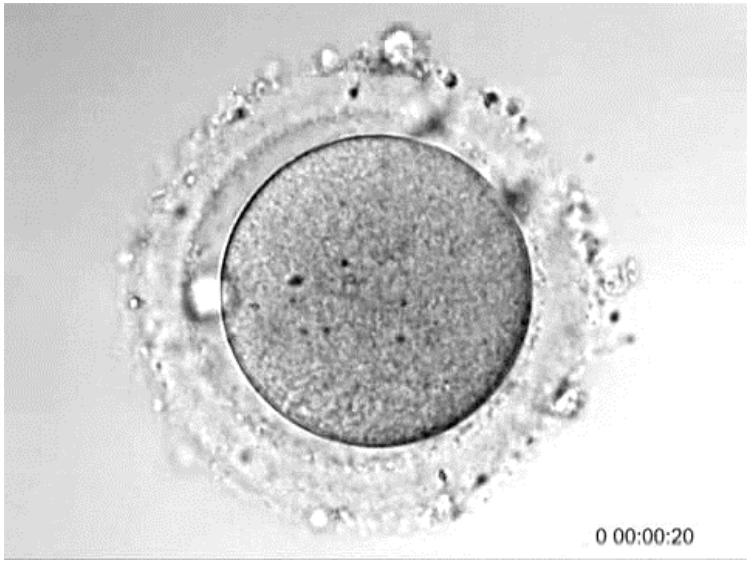
Preparation for fertilization

zona pellucida



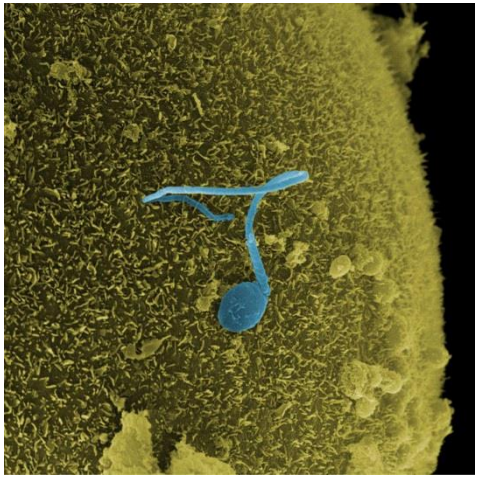
❖ Zona penetration

- hydrolysis of hyaluronan by acrosome-released proteases hyaluronidase and acrosin



Mio et al 2008

oolema



Sperm adhesion to microvilli

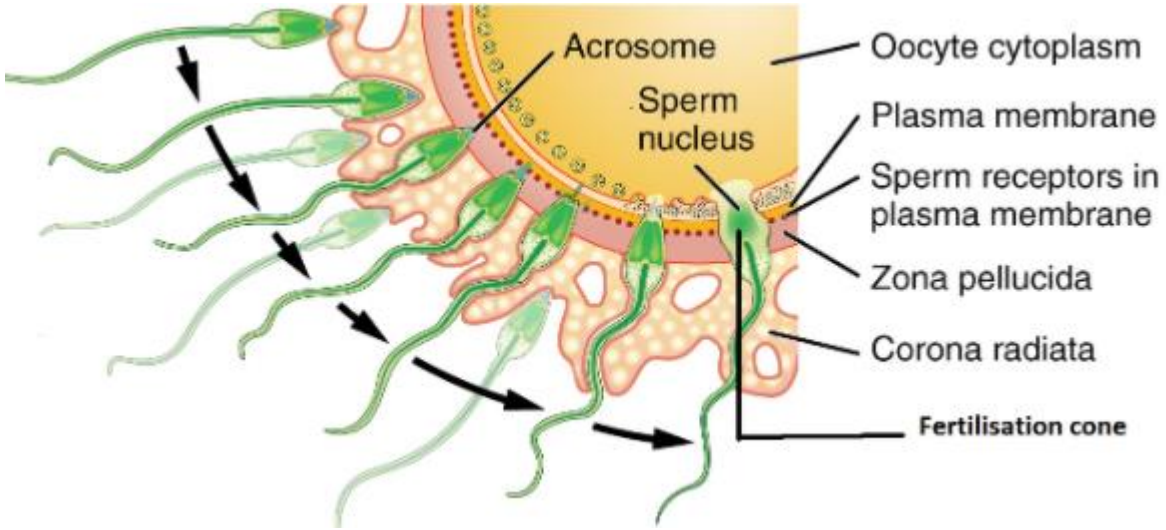
Gamete interaction

(1) **INITIAL APPROACH**

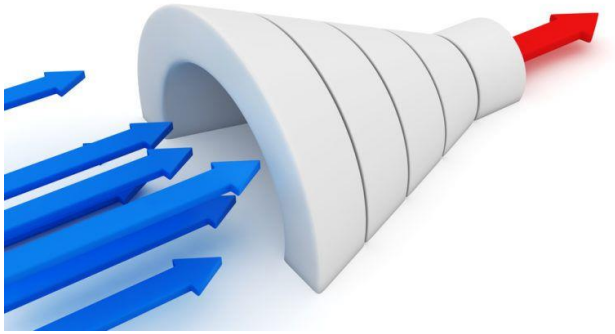
(2) **MOLECULAR RECOGNITION**

(3) **MEMBRANE APPOSITION**

(4) **MEMBRANE FUSION**

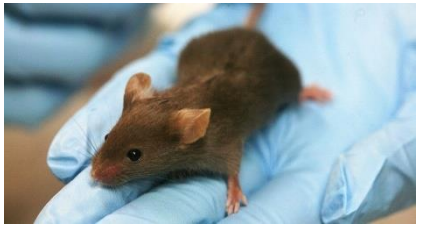


- from a number of genes shown to play a role in sperm-egg interaction only a few have been proven essential for fertilization in mouse model



-/-

- normal mating behaviour and gamete number and morphology
- BUT sex-specific reduction of fertility resulting from failed sperm-egg fusion

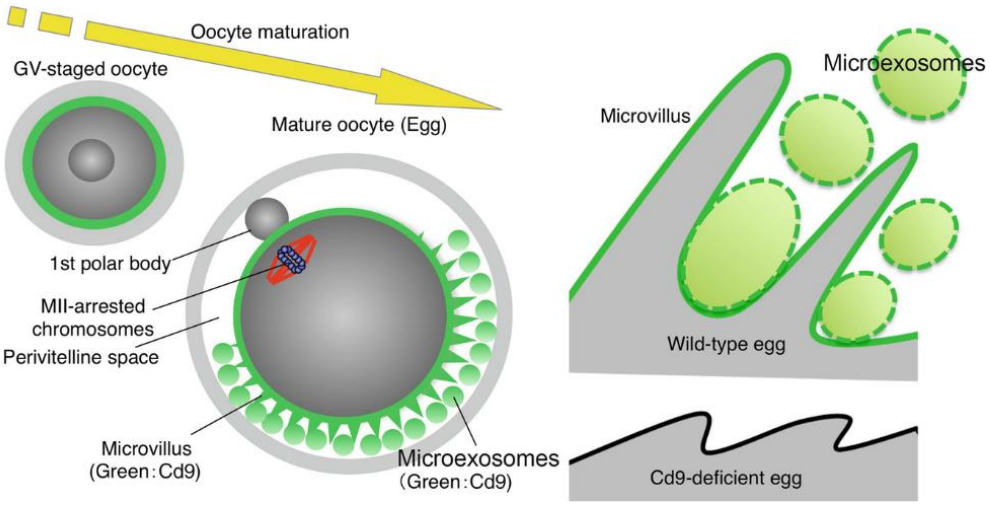


Key molecules of gamete recognition

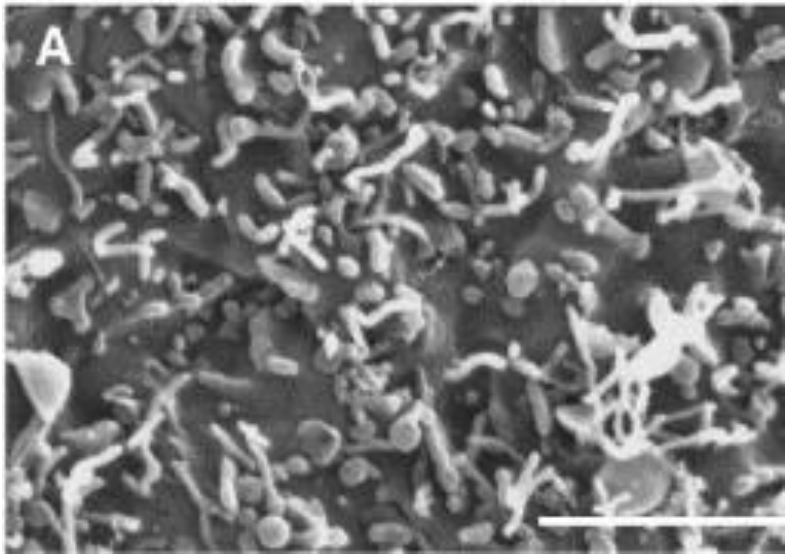
❖ *CD9*

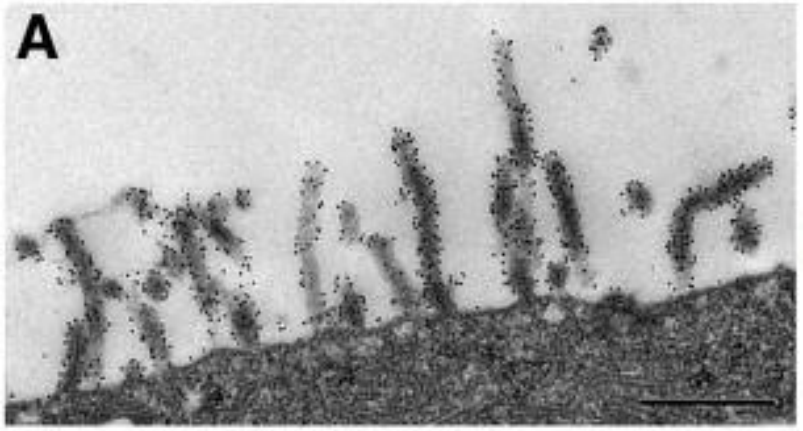
- localized on **oolema** and required for normal microvili morphology and distribution
- organizer of membrane architecture facilitating sperm-egg contact preceeding fusion

- antiCD9-antibody inhibits sperm-egg binding in vitro
- microvesicles transport CD9 on sperm membrane before fertilization
- microvesicle-transported CD9 fragments can restore fertilisation capacity of CD9-/- oocytes



Miyado et al 2018



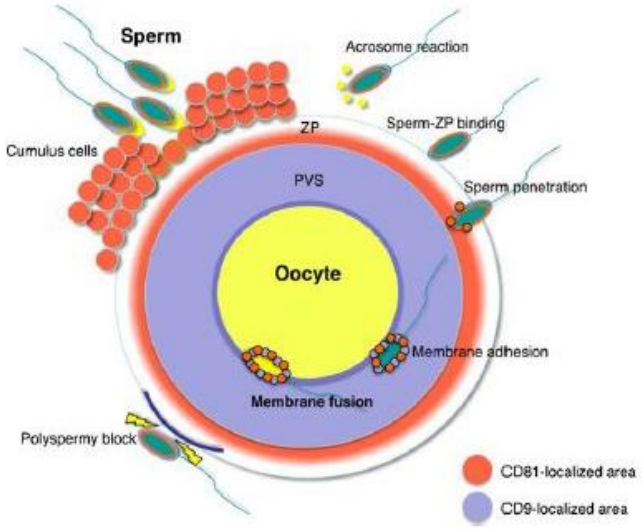


Runge et al 2007

Key molecules of gamete recognition

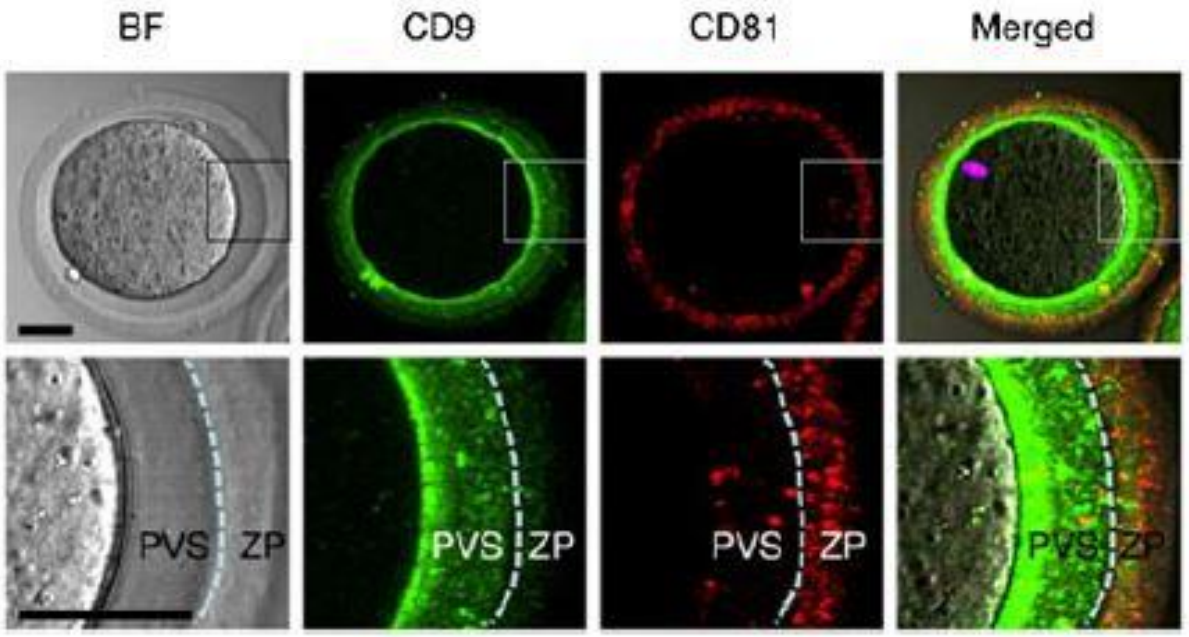
❖ *CD81*

- expressed on oocyte surface, associates with CD9
- incorporated to microvesicles, present in PVS
- depletion inhibits gamet fusion
- **CD81-/- subfertile,** CD81/CD9 -/- sterile
- CD9 microinjection reverse CD81-/- phenotype
- synergic affect with CD9?



- regulation of membrane architecture

- rearrangement of sperm membrane in preparation for adhesion/fusion?



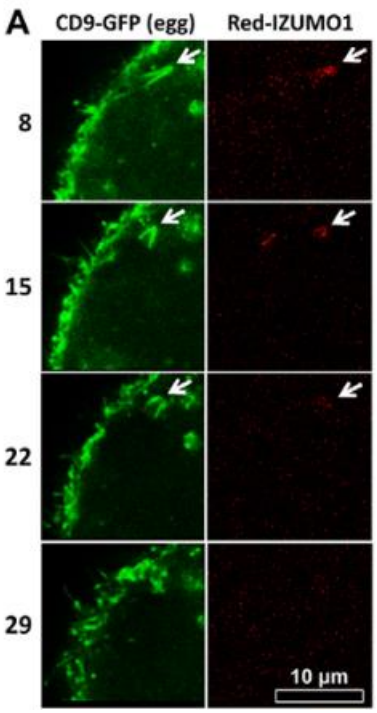
Ohnami et al 2012

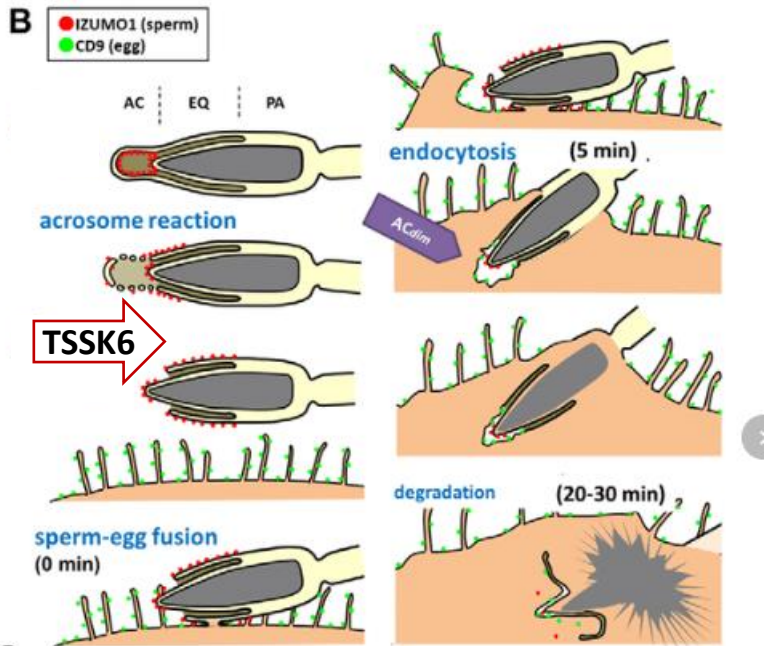
Key molecules of gamete recognition

❖ *Izumo1*

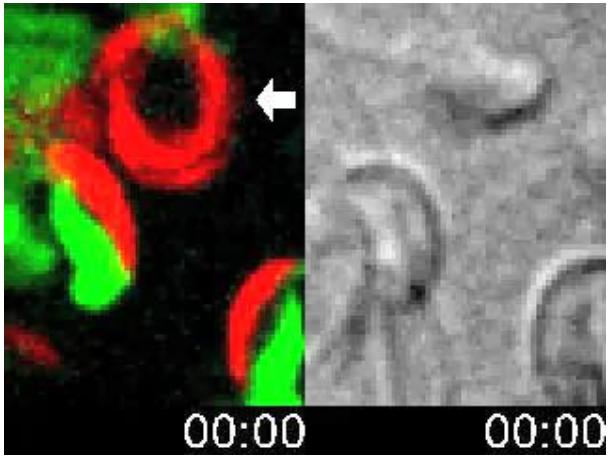
- expressed on the **sperm surface after acrosomal reaction**
- spermatozoa of -/- mise penetrate ZP but fail to fuse with the egg
- fertilization defect can be bypassed by ICSI
- antibody against Izumo has contraceptive affect
- **both Izumo and CD9 are enriched in adhesion area, but NO physical interaction between the two molecules**

Izumo= Japanese shrine of marriage





- localized to inner acriosomal membrane and redistributed after acrosomal reaction



Inoue et al 2005, Satouh et al 2012

Key molecules of gamete recognition

❖ ***Juno*** = Folate 4 receptor (Folr4)

- Izumo-complementary receptor expressed **on the egg´s surface**
- Juno-deficient eggs do not fuse with spermatozoa
- direct Juno-Izumo interaction is crucial for egg-sperm adhesion
- Juno mutations linked to clinical cases of idiopathic fertilization failure

Juno = Roman goddess of marriage and childbirth

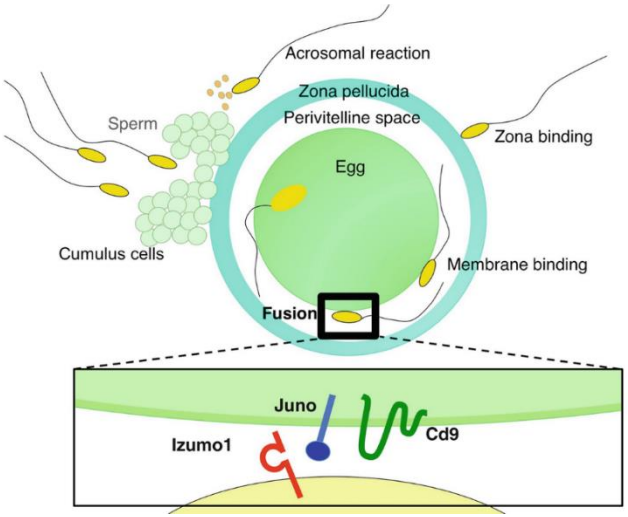
Bianchi et al 2014

ARTICLE

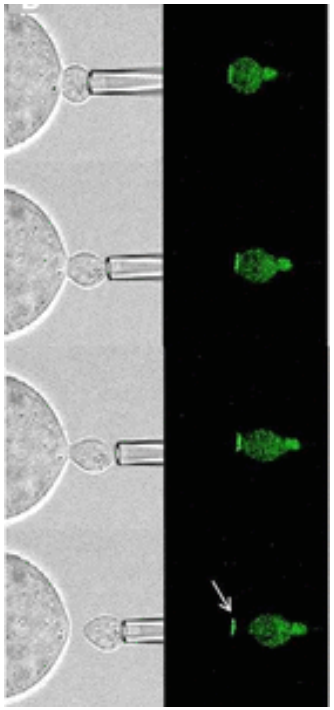
doi:10.1038/nature13203

Juno is the egg Izumo receptor and is essential for mammalian fertilization

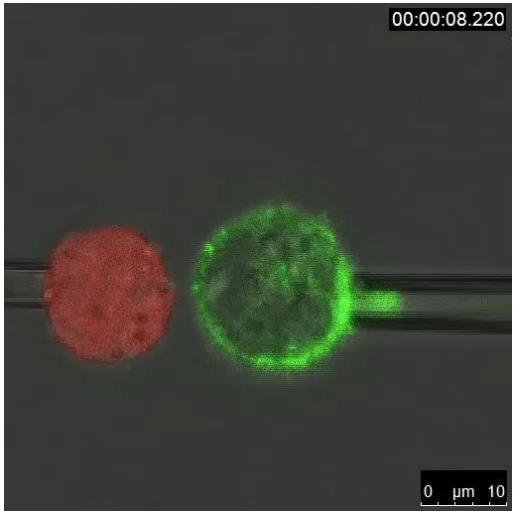
Enrica Bianchi[1], Brendan Doe[2], David Goulding[3] & Gavin J. Wright[1]



oocyte **Izumo-expressing cell**

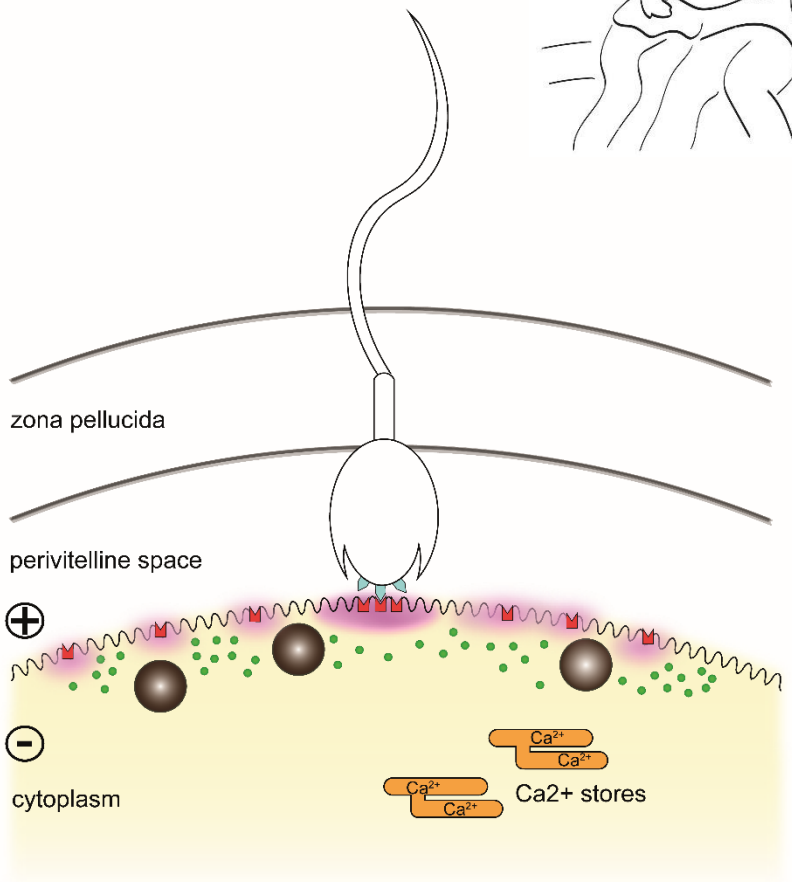


- ectopic expression of fluorescently tagged Juno/Izumo
- **firm adhesion but not fusion**



Chalbi et al 2014

Key molecules of gamete recognition



JUNO-IZUMO COMPLEX
is vital for membrane tethering
BUT
lacks **fusogenic** activity

further factors must be involved

PROPOSED MODEL:
Izumo-Juno binding induces accumulation of CD9 at adhesion site thus promoting CD9-mediated clustering of membrane proteins that participate in assembly of the cell fusion machinery

Chalbi et al 2014, Bianchi et al 2014

Izumo
Juno
CD9
CD81?
membrane potential
cortical granule
Zn vesicles

Key molecules of gamete recognition

❖ *MAIA*

- Fc receptor-like 3
- named after fertility goddess

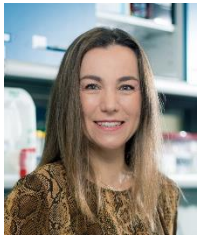
Vondrakova et al 2022

SCIENCE ADVANCES | RESEARCH ARTICLE

DEVELOPMENTAL BIOLOGY

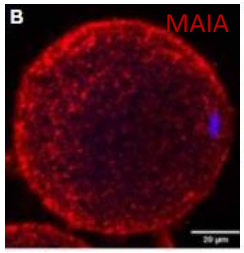
MAIA, Fc receptor–like 3, supersedes JUNO as IZUMO1 receptor during human fertilization

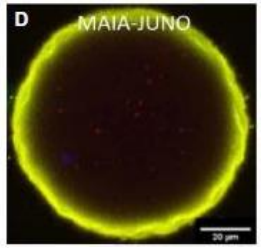
Jana Vondrakova[1]†, Michaela Frolikova[1]†, Lukas Ded[1], Jiri Cerny[2], Pavla Postlerova[1,3], Veronika Palenikova[1], Ondrej Simonik[1], Zuzana Nahacka[4], Krystof Basus[1], Eliska Valaskova[1], Radek Machan[5], Allan Pacey[6], Zuzana Holubcova[7,8], Pavel Koubek[9], Zuzana Ezrova[4], Soojin Park[10], Ruiwu Liu[11], Raghavendran Partha[12], Nathan Clark[13], Jiri Neuzil[4,14], Masahito Ikawa[10], Kent Erickson[15], Kit S. Lam[11], Harry Moore[16]*, Katerina Komrskova[1,17]*

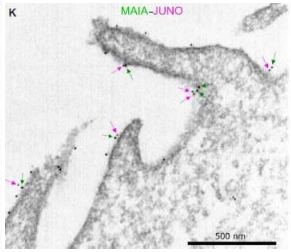


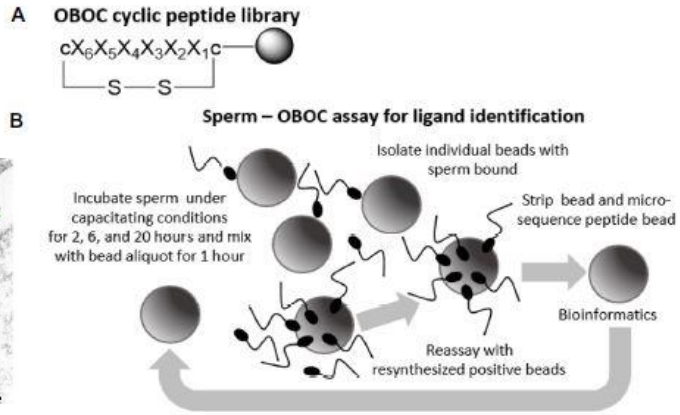
Kateřina Komrsková (Dvořáková/Hortová)

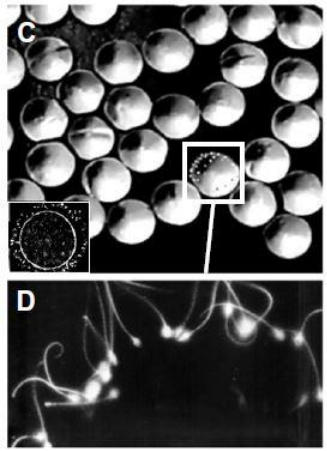
- localized on oolema of unfertilized human oocytes
- close association with Juno



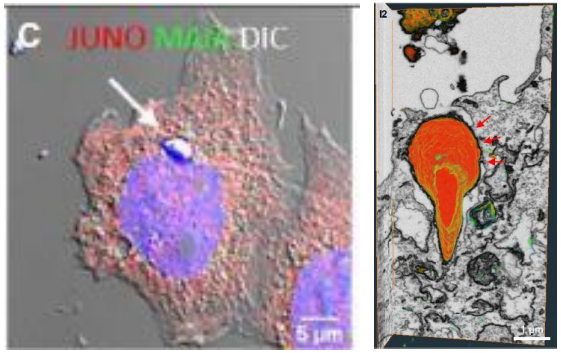








- transgene coexpression of MAIA+Juno led to sperm binding to primary cultured cells

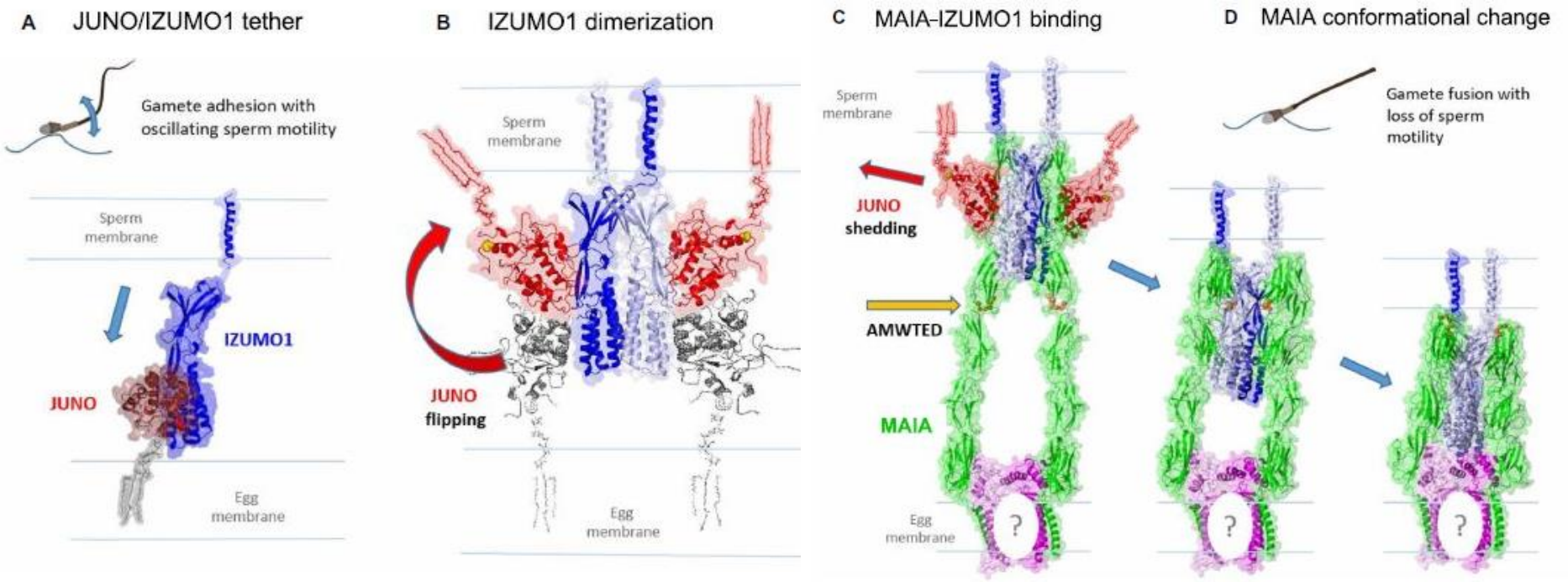


- MAIA facilitates sperm fusion in the presence of Juno

Key molecules of gamete recognition

❖ *MAIA*

PROPOSED MODEL OF MAIA/Juno-Izumo1 BINDING



? -unknown or CD9 protein

Juno/Izumo1 interaction ensures thethering of sperm and egg membranes. Fertilizing sperm display oscillatory motility.

Izumo1 dimerization triggers the conformational change of Juno and its shedding from the egg surface.

MAIA binds in the emptied Juno/Izomo1 binding pocket.

MAIA conformational change to extracellular Fc domains, leading to close membrane proximity enabling gamete fusion with the loss of sperm motility.

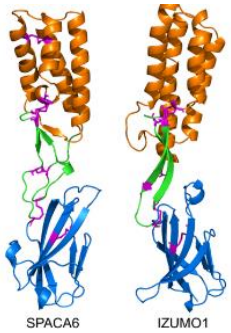
Key molecules of gamete recognition

❖ *SPACA6*

Barbaux et al 2020

SCIENTIFIC REPORTS
nature research

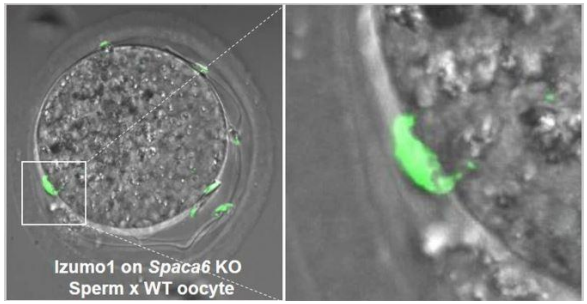
- mouse/human sperm expressed surface protein
- SPACA6-/- male mice infertile due to sperm inability to fuse with egg
- anti-SPACA6 Ab inhibits human IVF
- structural similarity with Izumo1



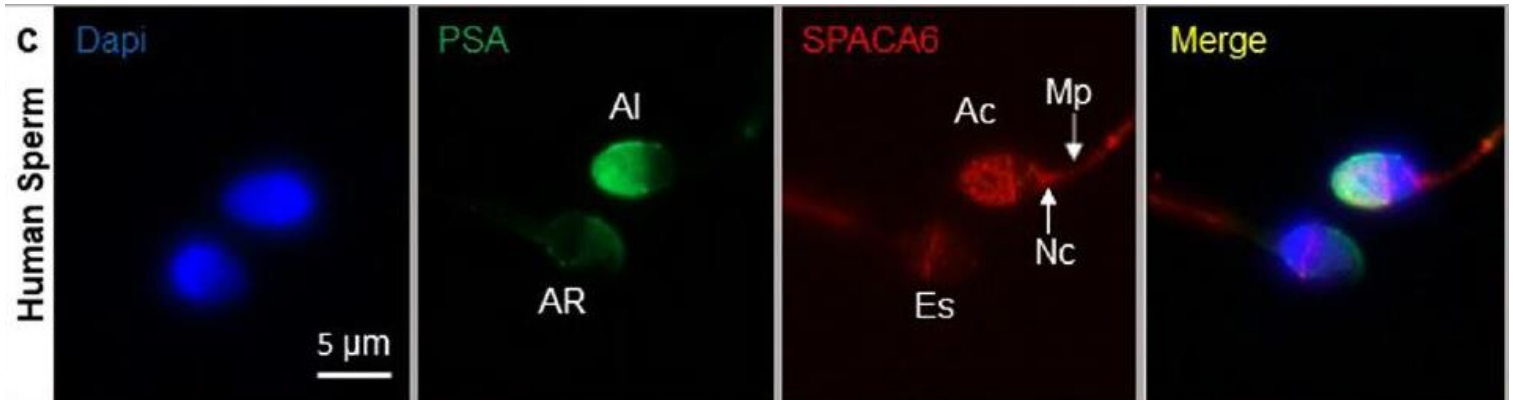
OPEN

Sperm SPACA6 protein is required for mammalian Sperm-Egg Adhesion/Fusion

Sandrine Barbaux[1], Côme Ialy-Radio[1], Myriam Chalbi[1], Elisa Dybal[1], Méline Homps-Legrand[1], Marcio Do Cruzeiro[1], Daniel Vaiman[1], Jean-Philippe Wolf[1,2] & Ahmed Ziyyat[1,2*]



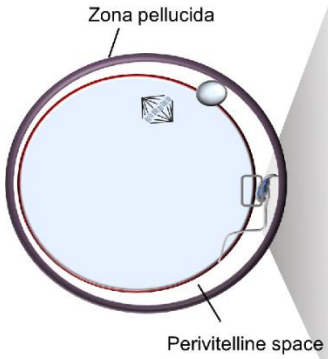
- absence of SPACA6 has no effect on Izumo localization
- SPACA6 alone does not induce adhesion!

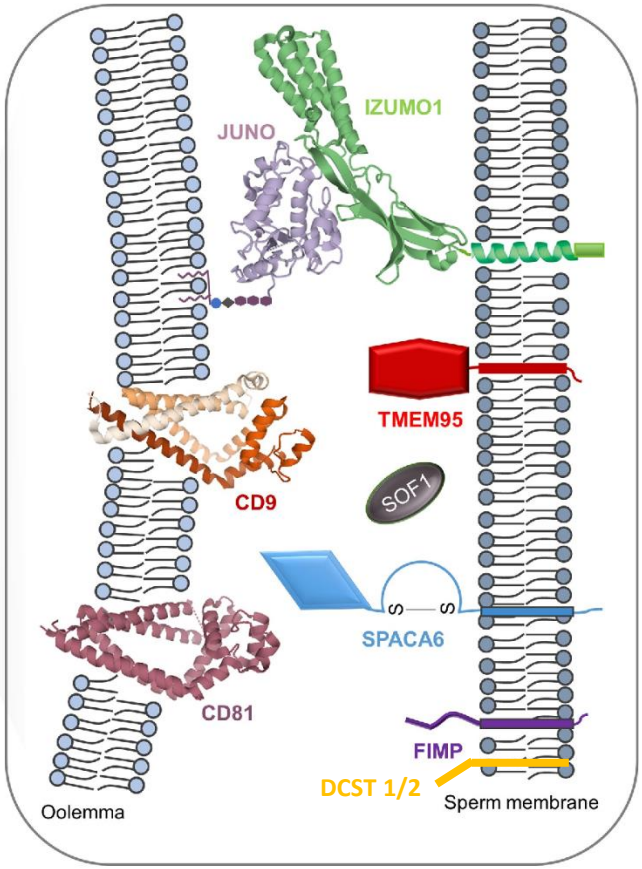


Key molecules of gamete recognition

❖ *TMEM95, SOF1, FIMP, DCST1/2*

- sperm specific proteins
- CRISPR –depletion/gene targetting causes male mice sterility
- spermatozoa can not fuse with oolema
- no effect on Izumo localization





- roles in molecular recognition?

PNAS

Noda et al 2020

Sperm proteins SOF1, TMEM95, and SPACA6 are required for sperm–oocyte fusion in mice

Taichi Noda[a,1], Yonggang Lu[a,1], Yoshitaka Fujihara[a,2], Seiya Oura[a,b], Takayuki Koyano[c], Sumire Kobayashi[a,b], Martin M. Matzuk[d,e,f], and Masahito Ikawa[a,f,3]

[a]Research Institute for Microbial Diseases, Osaka University, 565-0871 Osaka, Japan; [b]Graduate School of Pharmaceutical Sciences, Osaka University, 565-0871 Osaka, Japan; [c]Division of Molecular Genetics, Shigei Medical Research Institute, 701-0202 Okayama, Japan; [d]Center for Drug Discovery, Baylor College of Medicine, Houston, TX 77030; [e]Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX 77030; and [f]The Institute of Medical Science, The University of Tokyo, 108-8639 Tokyo, Japan

Fujihara et al 2020

Spermatozoa lacking Fertilization Influencing Membrane Protein (FIMP) fail to fuse with oocytes in mice

Yoshitaka Fujihara[a,b,c], Yonggang Lu[a], Taichi Noda[a], Asami Oji[a,d], Tamara Larasati[a,e], Kanako Kojima-Kita[a,e], Zhifeng Yu[a], Ryan M. Matzuk[b], Martin M. Matzuk[b,1], and Masahito Ikawa[a,e,f,1]

[a]Research Institute for Microbial Diseases, Osaka University, Suita, 565-0871 Osaka, Japan; [b]Center for Drug Discovery and Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX 77030; [c]Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center, Suita, 564-8565 Osaka, Japan; [d]Laboratory for Developmental Epigenetics, RIKEN Center for Biosystems Dynamics Research, Kobe, 650-0047 Hyogo, Japan; [e]Graduate School of Medicine, Osaka University, Suita, 565-0871 Osaka, Japan; and [f]The Institute of Medical Science, The University of Tokyo, Minato-ku, 108-8639 Tokyo, Japan

eLife

Lams-Torazo al 2020

TMEM95 is a sperm membrane protein essential for mammalian fertilization

Ismael Lamas-Toranzo[1†], Julieta G Hamze[2†], Enrica Bianchi[3], Beatriz Fernández-Fuertes[4,5], Serafín Pérez-Cerezales[1], Ricardo Laguna-Barraza[1], Raúl Fernández-González[1], Pat Lonergan[4], Alfonso Gutiérrez-Adán[1], Gavin J Wright[3], María Jiménez-Movilla[2*], Pablo Bermejo-Álvarez[1*]

[1]Animal Reproduction Department, INIA, Madrid, Spain; [2]Department of Cell Biology and Histology, Medical School, University of Murcia, IMIB-Arrixaca, Murcia, Spain; [3]Cell Surface Signalling Laboratory, Wellcome Trust Sanger Institute, Cambridge, United Kingdom; [4]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland; [5]Department of Biology, Faculty of Sciences, Institute of Food and Agricultural Technology, University of Girona, Girona, Spain

Inoue et a 2021

Evolutionarily conserved sperm factors, DCST1 and DCST2, are required for gamete fusion

Naokazu Inoue[1*], Yoshihisa Hagihara[2], Ikuo Wada[1]

[1]Department of Cell Science, Institute of Biomedical Sciences, School of Medicine, Fukushima Medical University, Fukushima, Japan; [2]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Ikeda, Japan

Noda et a 2020

communications biology

ARTICLE

https://doi.org/10.1038/s42003-022-03289-w OPEN

Sperm membrane proteins DCST1 and DCST2 are required for sperm-egg interaction in mice and fish

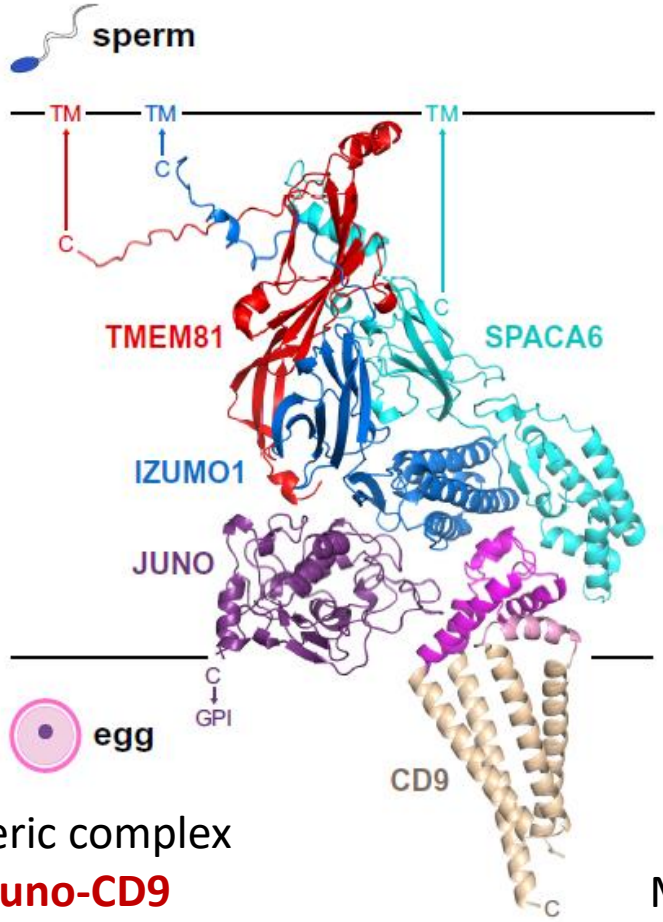
Taichi Noda[1,2,3], Andreas Blaha[4,5], Yoshitaka Fujihara[1,6], Krista R. Gert[4,5], Chihiro Emori[1], Victoria E. Deneke[4], Seiya Oura[1,7], Karin Panser[4], Yonggang Lu[1], Sara Berent[4], Mayo Kodani[1,7], Luis Enrique Cabrera-Quio[4,5], Andrea Pauli[4✉] & Masahito Ikawa[1,7,8✉]

Key molecules of gamete recognition

- AlphaFold Multimer (an extension of AlphaFold)
  - a deep learning model for predicting the 3D structure of proteins

trimeric complex

IZUMO1-TMEM81-SPACA6



dimeric complex

Juno-CD9

MAIA?

Deneke et al. 2024

Cell

CellPress
OPEN ACCESS

Short article

A conserved fertilization complex bridges sperm and egg in vertebrates

Victoria E. Deneke,[1,9,*] Andreas Blaha,[1,2,9] Yonggang Lu,[3,4] Johannes P. Suwita,[1,2] Jonne M. Draper,[1] Clara S. Phan,[1] Karin Panser,[1] Alexander Schleiffer,[1] Laurine Jacob,[1] Theresa Humer,[1,2] Karel Stejskal,[5] Gabriela Krssakova,[1] Elisabeth Roitinger,[5] Dominik Handler,[5] Maki Kamoshita,[4] Tyler D.R. Vance,[6] Xinyin Wang,[6] Joachim M. Surm,[7] Yehu Moran,[7] Jeffrey E. Lee,[6] Masahito Ikawa,[4,8] and Andrea Pauli[1,10,*]

[1]Research Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), 1030 Vienna, Austria
[2]Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, Vienna, Austria
[3]Premium Research Institute for Human Metaverse Medicine (WPI-PRIMe), Osaka University, Osaka 565-0871, Japan
[4]Department of Experimental Genome Research, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan
[5]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), 1030 Vienna, Austria
[6]Department of Laboratory Medicine and Pathobiology, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada
[7]Department of Ecology, Evolution and Behavior, Alexander Silberman Institute of Life Sciences, Faculty of Science, The Hebrew University of Jerusalem, Jerusalem, Israel
[8]Laboratory of Reproductive Systems Biology, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan
[9]These authors contributed equally
[10]Lead contact
*Correspondence: victoria.deneke@imp.ac.at (V.E.D.), andrea.pauli@imp.ac.at (A.P.)
https://doi.org/10.1016/j.cell.2024.09.035

Elofsson et al 2024

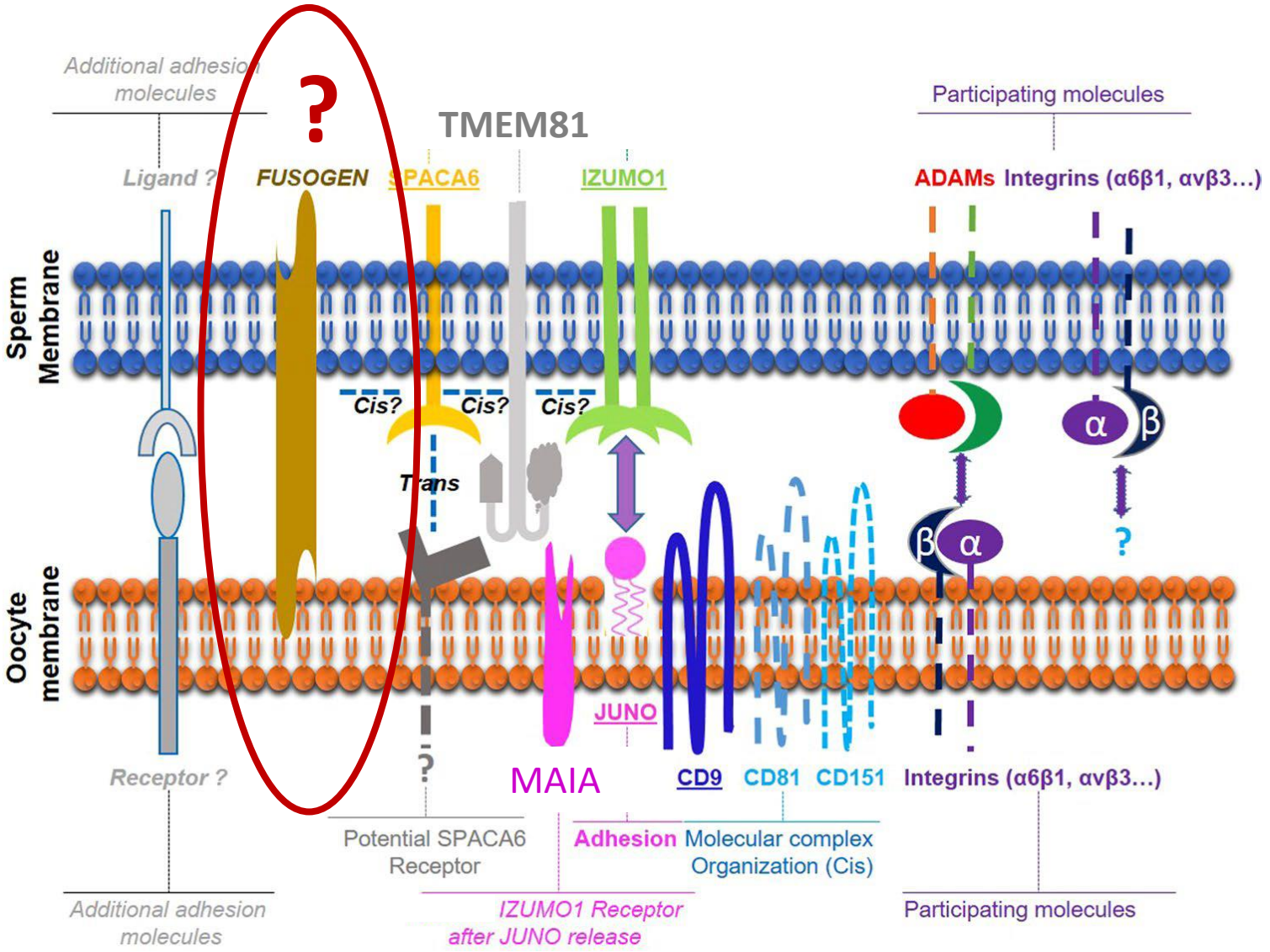
eLife

Deep learning insights into the architecture of the mammalian egg-sperm fusion synapse

Arne Elofsson[1]*, Ling Han[2], Enrica Bianchi[3†], Gavin J Wright[3], Luca Jovine[2]*

[1]Science for Life Laboratory and Department of Biochemistry and Biophysics, Stockholm University, Solna, Sweden; [2]Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; [3]Department of Biology, Hull York Medical School, York Biomedical Research Institute, University of York, York, United Kingdom

Key molecules of gamete recognition



Key molecules of gamete recognition

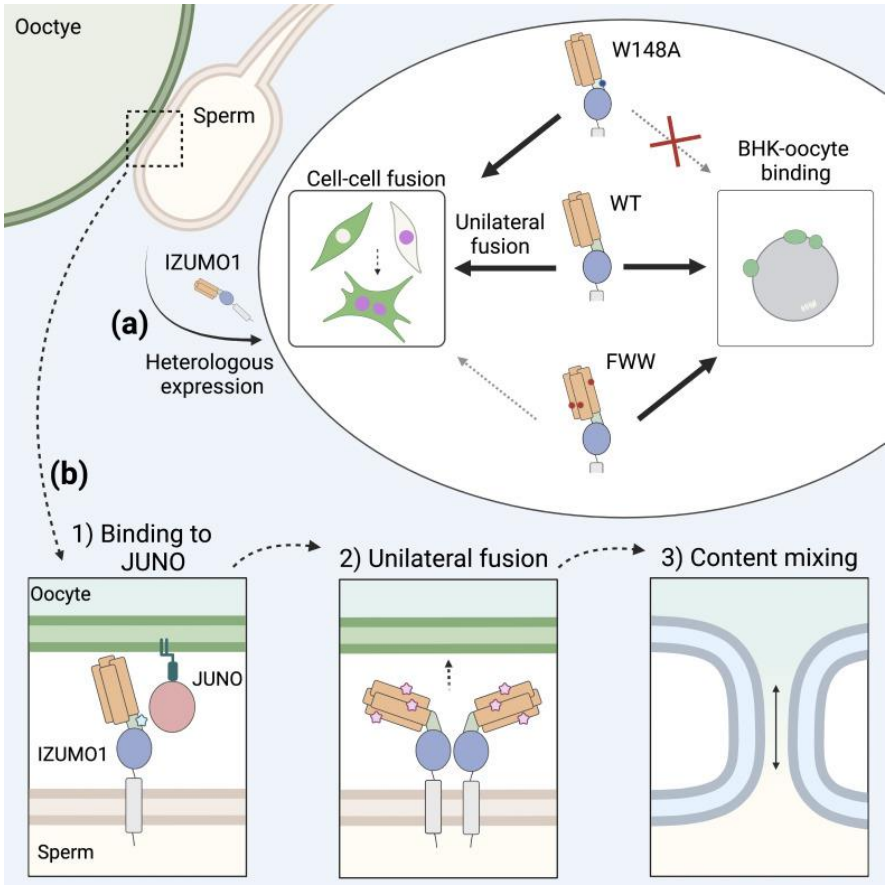
Brukman et al. 2023

JCB Journal of Cell Biology

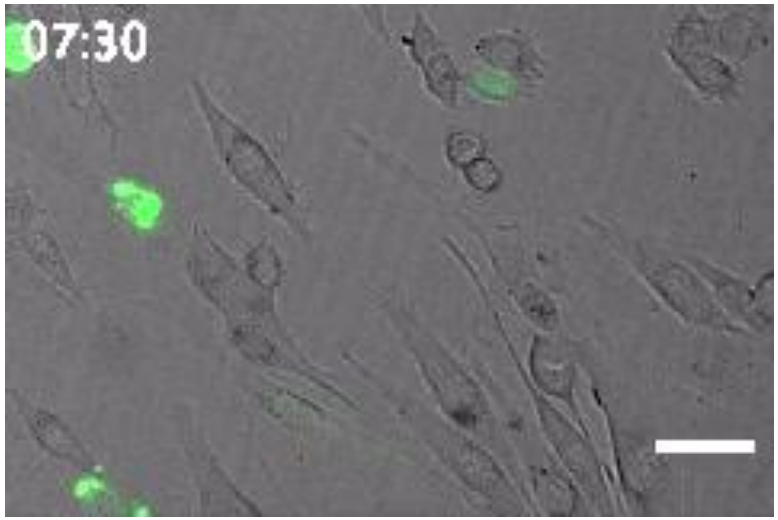
REPORT

A novel function for the sperm adhesion protein IZUMO1 in cell–cell fusion

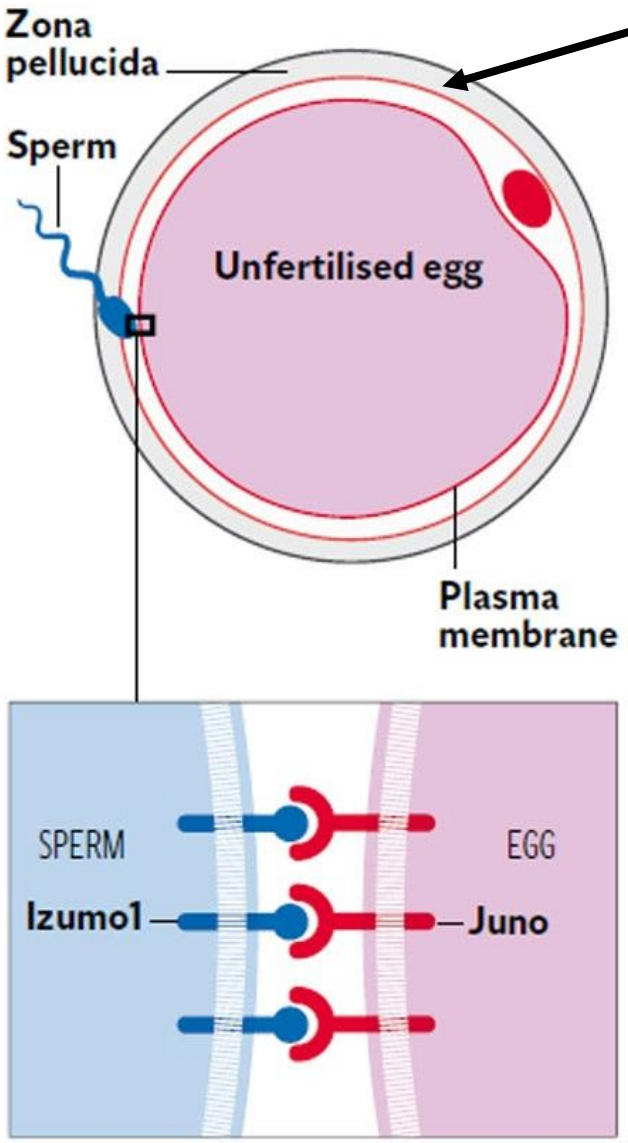
Nicolas G. Brukman[1*], Kohdai P. Nakajima[2*], Clari Valansi[1], Kateryna Flyak[1], Xiaohui Li[1], Tetsuya Higashiyama[2,3,4], and Benjamin Podbilewicz[1]



- Izumo is capable to induce fusion of primary cells via viral-like mechanism
- fusogenic activity of Izumo follows adhesion mediated by Juno-Izumo interaction
- Juno-binding and fusion are mediated by two differrent Izumo domains



Interspecies gamete interaction



prevents cross-species fertilization

The nature of Juno-Izumo interaction is conserved accross mammalian species

Naz 2014
Inoue 2013
Bianchi and Wright 2015
Yanagimatchi 1976

	ZP-free eggs
Mouse	Mouse /Human
Mouse/Pig/Hamster	Hamster
Human	Human/**Hamster**

Gamete misrecognition

CLINICAL IMPLICATIONS:

Altered expression

Mutation of receptors

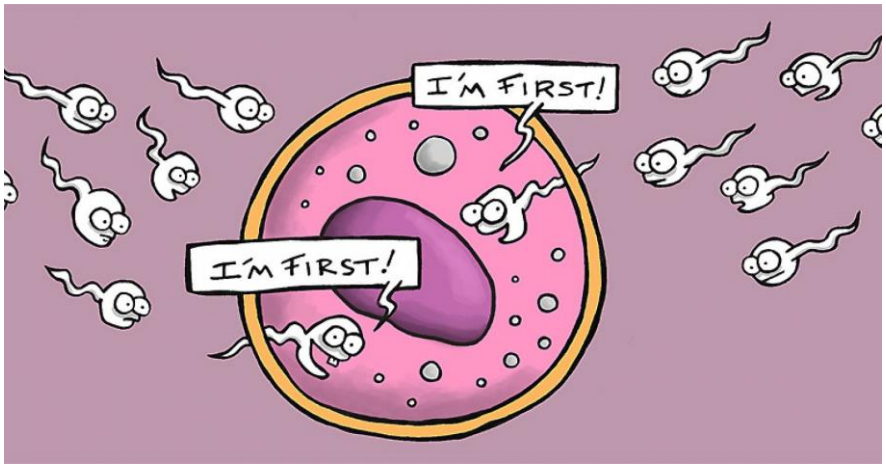
Interfering agents

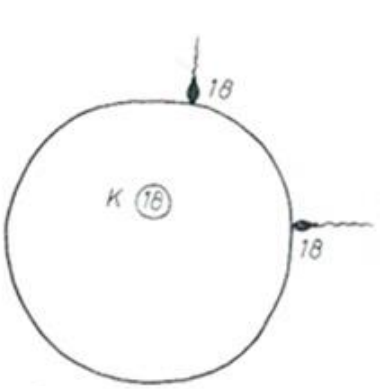
Idiopathic infertility
and fertilization failure

Polyspermy

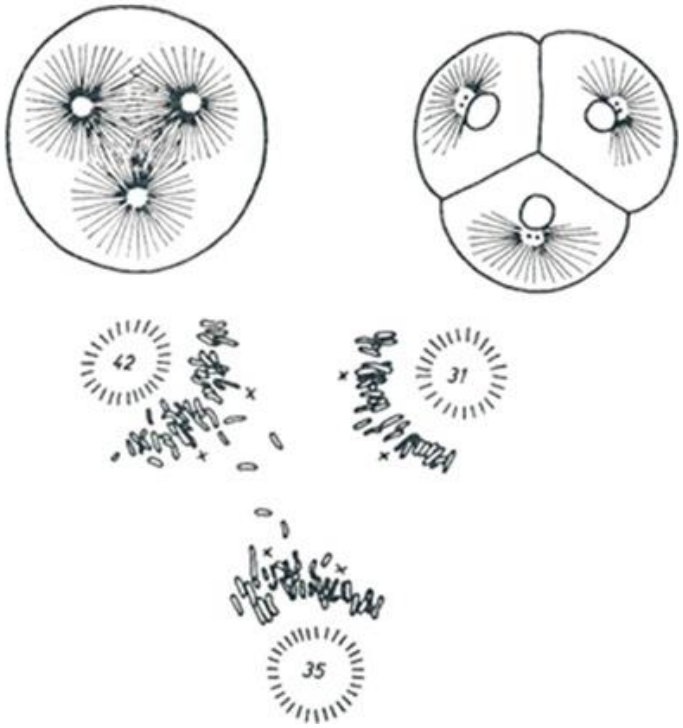
- lethal condition when the egg is penetrated by more than one spermatozoa

- more then two copies of each gene cause genetic inbalances and resulting embryo is non-viable



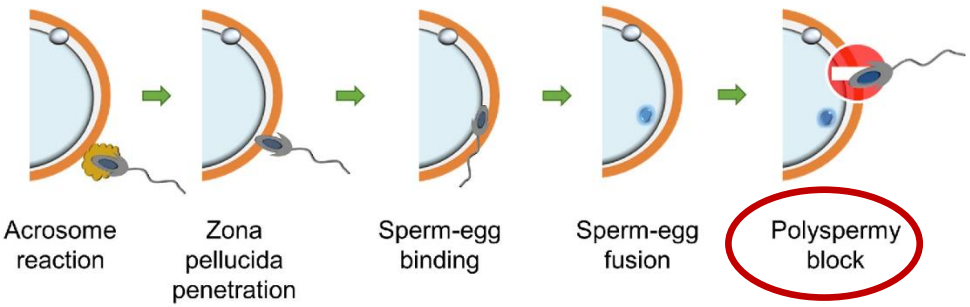



Theodor Boveri

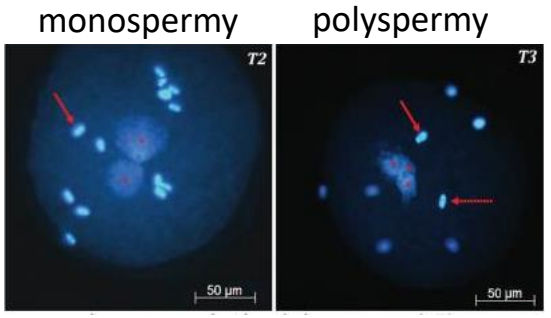


Polyspermy prevention

- *In vivo*
  - **Fish:** the sperm can only enter the egg just via the narrow opening, the micropyle, the rest of the egg being covered by impermeable chorion
  - **Sea urchin:** limitation on the number of sperm that are able to penetrate the extracellular coats and fuse with the egg
  - **Mammals**
    - **passage** through the female reproductive system
    - limited **viability** of sperm in oviductal reservoirs
    - structural organisation and postfertilization modifications of **zona pellucida**
    - rearrangement of **oolema**



- *In vitro*
  - optimising sperm concentration and coincubation time with the egg
  - ICSI



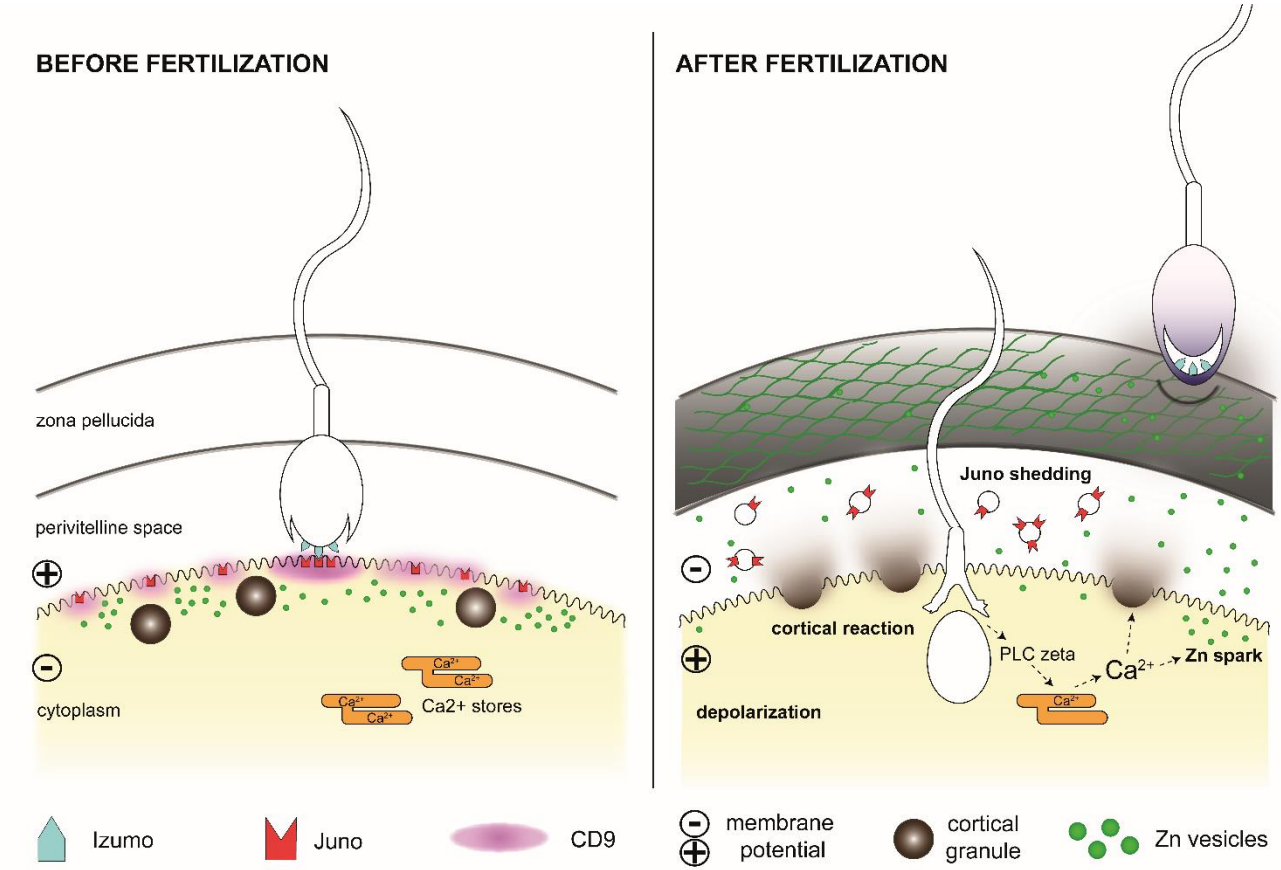
Polyspermy block overview

- Cortical reaction
- Zinc spark

} Modification of Zona Pellucida

- Membrane depolarization
- Juno removal

} Modification of oolema



Trebichalska and Holubcova, JARG 2020

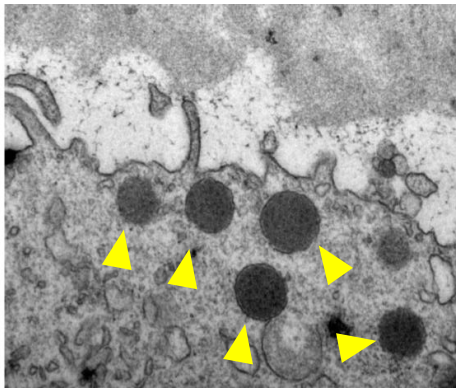
Polyspermy block on ZP

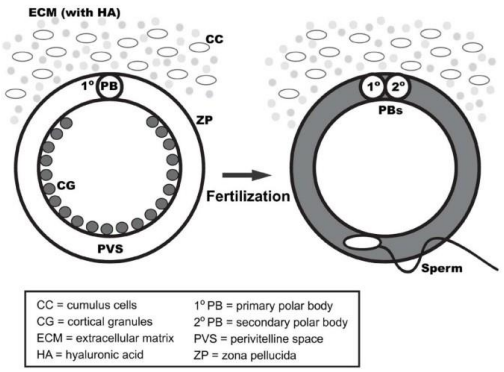
Mio et al 2012

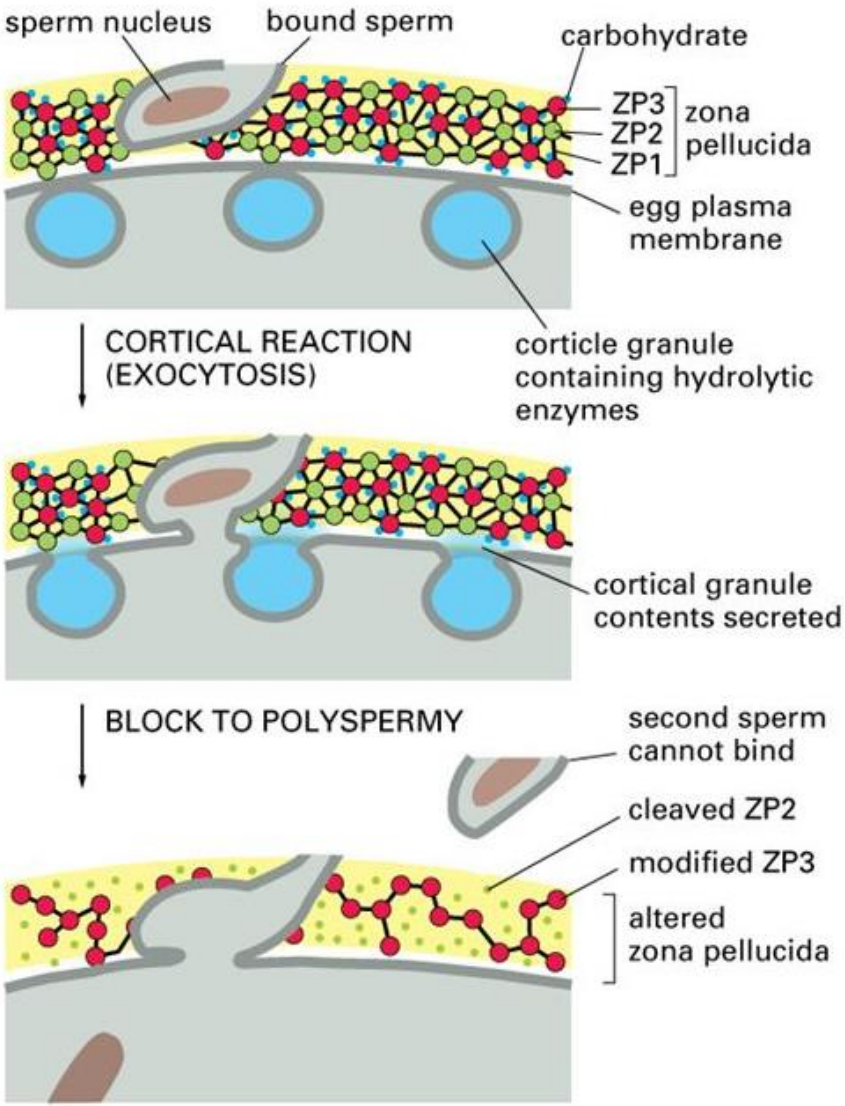
Polyspermy block on ZP

❖ CORTICAL REACTION

- slow and permanent block to polyspermy
- sperm penetration triggers exocytosis of **cortical granules**
- the content of granules modifies structure of zona pellucida and makes it impermeable for incoming sperms = *zona hardening*





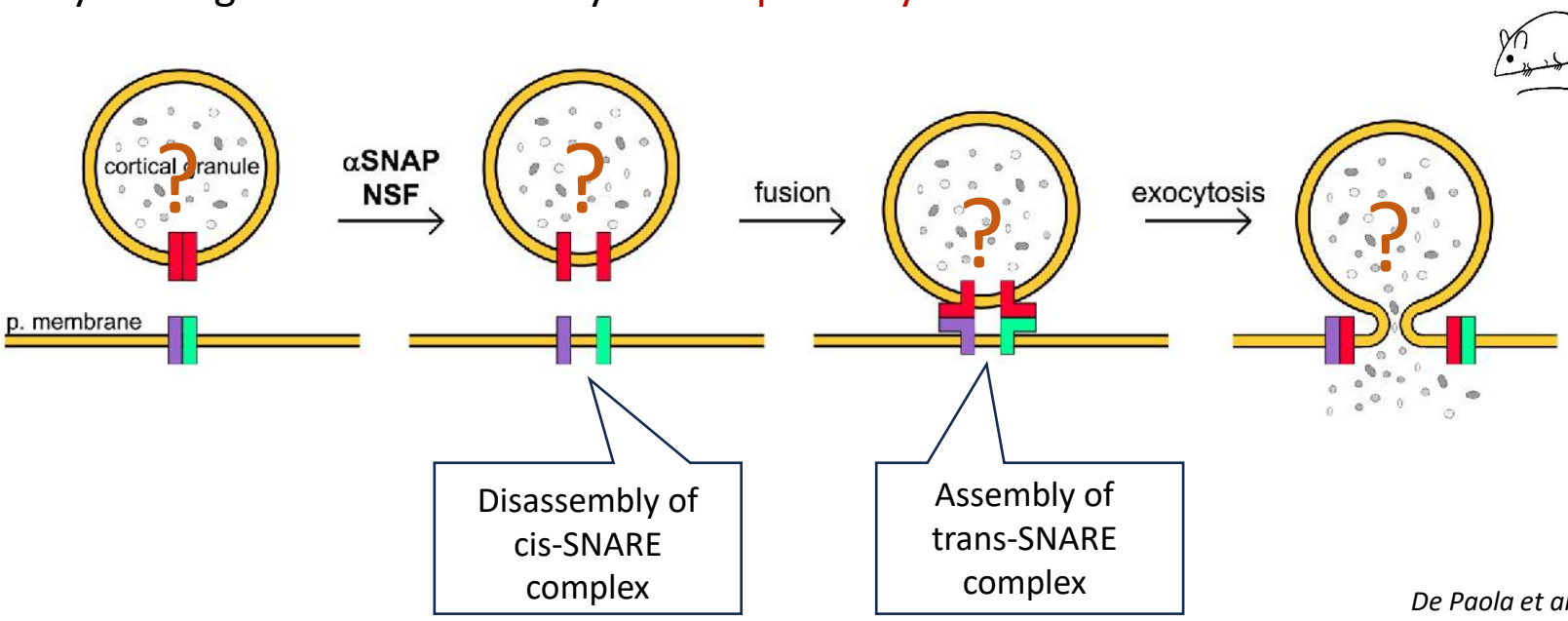


Polyspermy block on ZP

❖ CORTICAL REACTION

SNARE proteins
- membrane associated proteins oriented to cytosol
- involved in fusion of vesicles with targeted membrane

- exocytosis og GCs is mediated by SNARE pathway



De Paola et al 2015

- SNARE association with complexin prevents spontaneous secretion of CGs
- exocytosis followed by clatrin dependent endocytosis

- premature exocytosis in *in vitro* matured and vitrified/thawed human oocytes !

Polyspermy block on ZP

❖ CORTICAL REACTION

❑ OVASTACIN

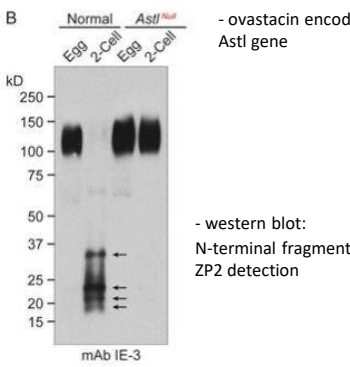
- belongs to astacin family of metaloproteases
- key enzyme of cortical granule content
- cleaves ZP2, a building component of ZP and primary sperm-binding ligand
- ZP2 destruction establishes definitive post fertilization block

Burkart et al 2012

JCB: Report

Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy

Anna D. Burkart, Bo Xiong, Boris Baibakov, Maria Jiménez-Movilla, and Jurrien Dean



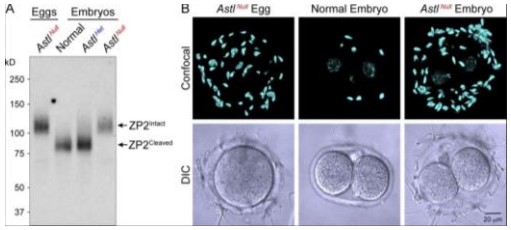
- ovastacin encoded by Astl gene

- western blot: N-terminal fragment of ZP2 detection

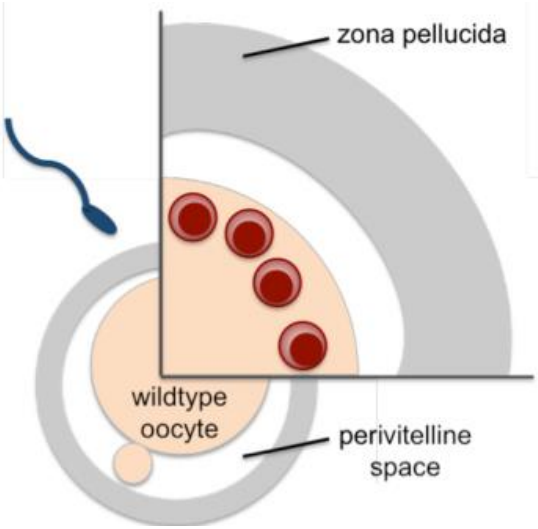
Jurrien Dean

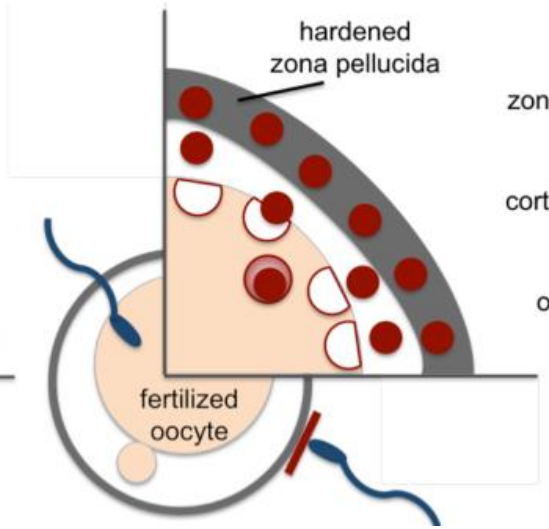
Astl-/-

- ovastacine-deficient eggs bind sperm after fertilization



- ovastacin is partially active before fertilization-triggered exocytosis and pre-hardens the ZP





Polyspermy block on ZP

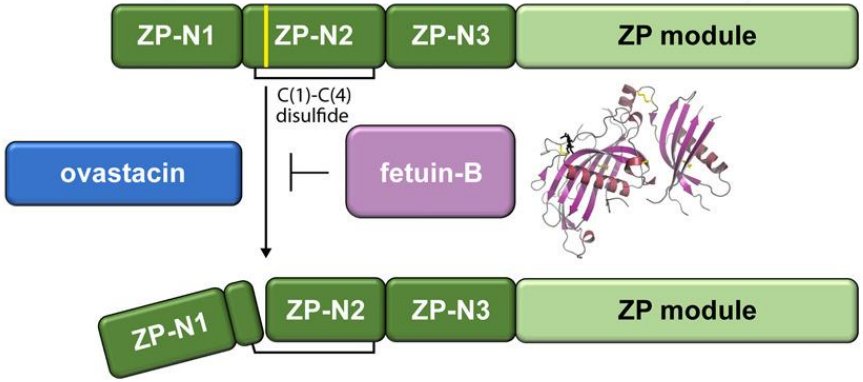
❖ CORTICAL REACTION

Dietzel et al 2013

❑ FETUIN -B

- liver-derived plasma protein
- antagonize basal activity of constantly leaking ovastacin from unfertilized oocytes

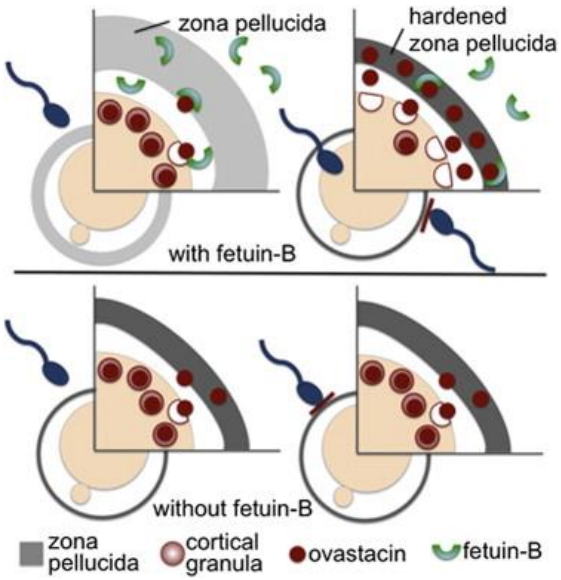
- cortical reaction overwhelms fetuin-B buffering capacity thereby intiating Zona hardening

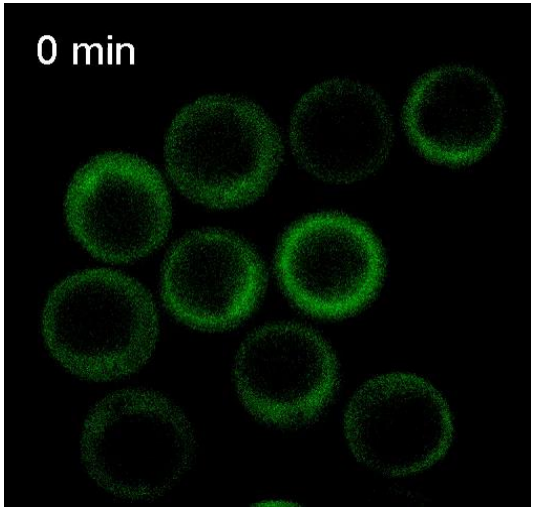


- serum levels fluctuate during menstrual cycle

CLINICAL IMPLICATIONS:

- Modulation of female fertility?
- Supplementation of IVF medium for prevention of ZP hardening?



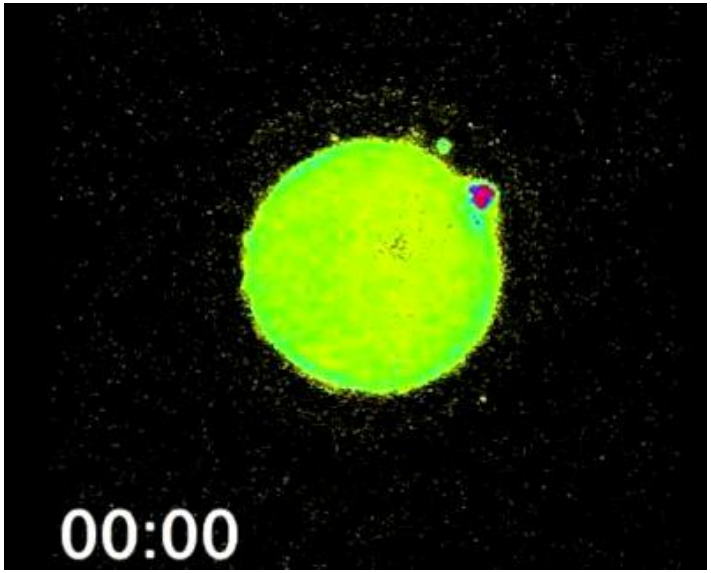


Cappa et al 2018

Polyspermy block on ZP

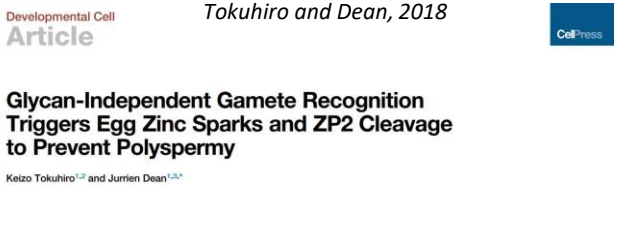
❖ ZINC SPARK

- Zn accumulation during maturation sustains MII arrest
- **rapid exocytosis of Zn containing vesicles immediately upon sperm entry**
- **Zn stabilizes ZP proteins and causes its hardening**



Zinc spark = early hallmark of fertilization

Tokuhiro and Dean, 2018


Developmental Cell
Article

Glycan-Independent Gamete Recognition Triggers Egg Zinc Sparks and ZP2 Cleavage to Prevent Polyspermy

Keizo Tokuhiro[1,2] and Jurrien Dean[1,3,*]

Jurrien Dean

- **Zn release**
  = fast and transient block to polyspermy

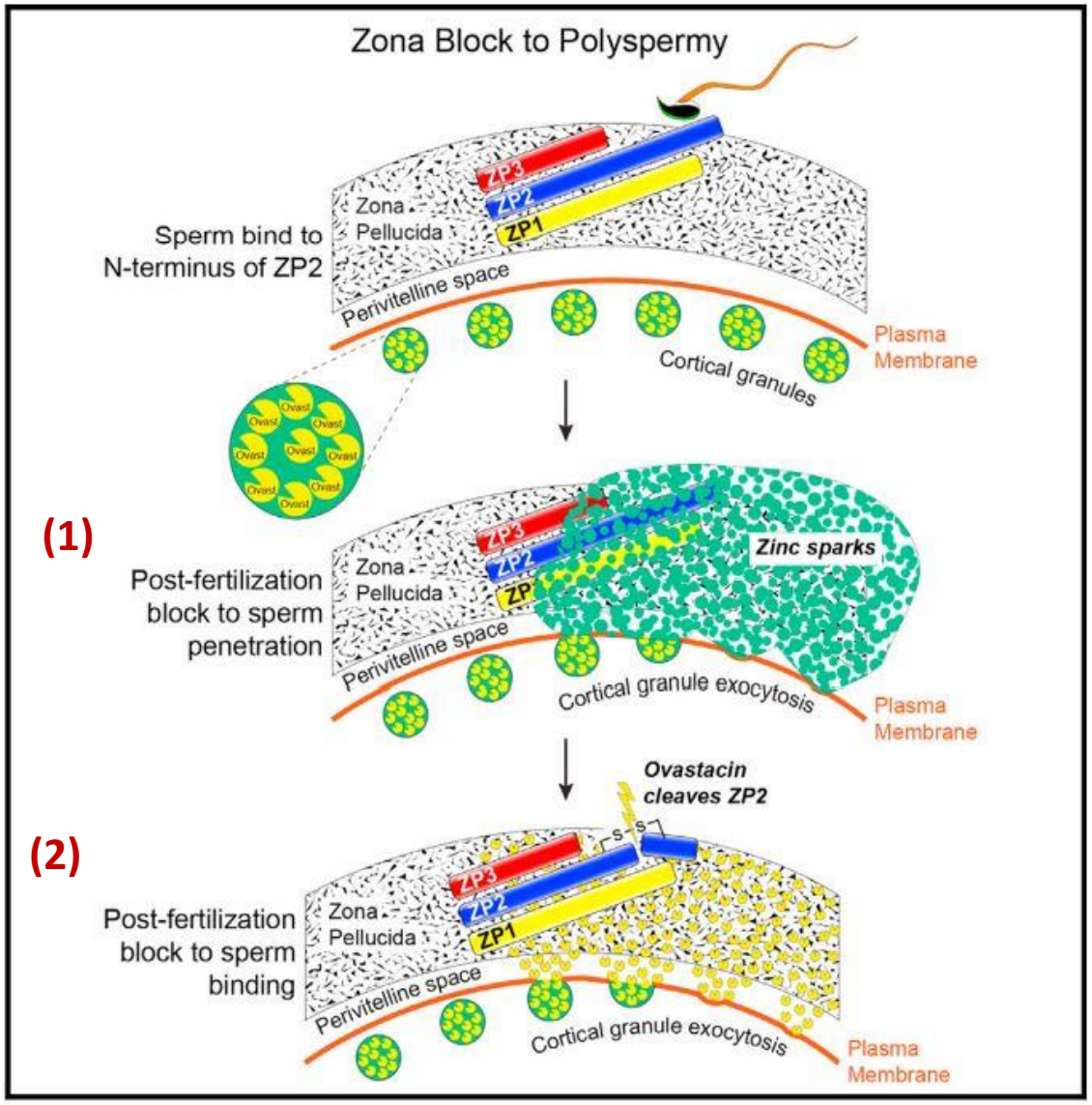
CLINICAL IMPLICATIONS:

- quantification of Zn spark after conventional IVF
  -> best quality embryo selection
  -> oocytes exerting a low signal -> rescue ICSI?
- Zn-specific chelatators for AOA?
- Supplementation of IVM medium?

Polyspermy block on ZP

Zinc spark
= early transient ZP block
-strat in 2-3 min

Ovastacin cleavage of ZP2
= late permanent ZP block
- start in >30 min

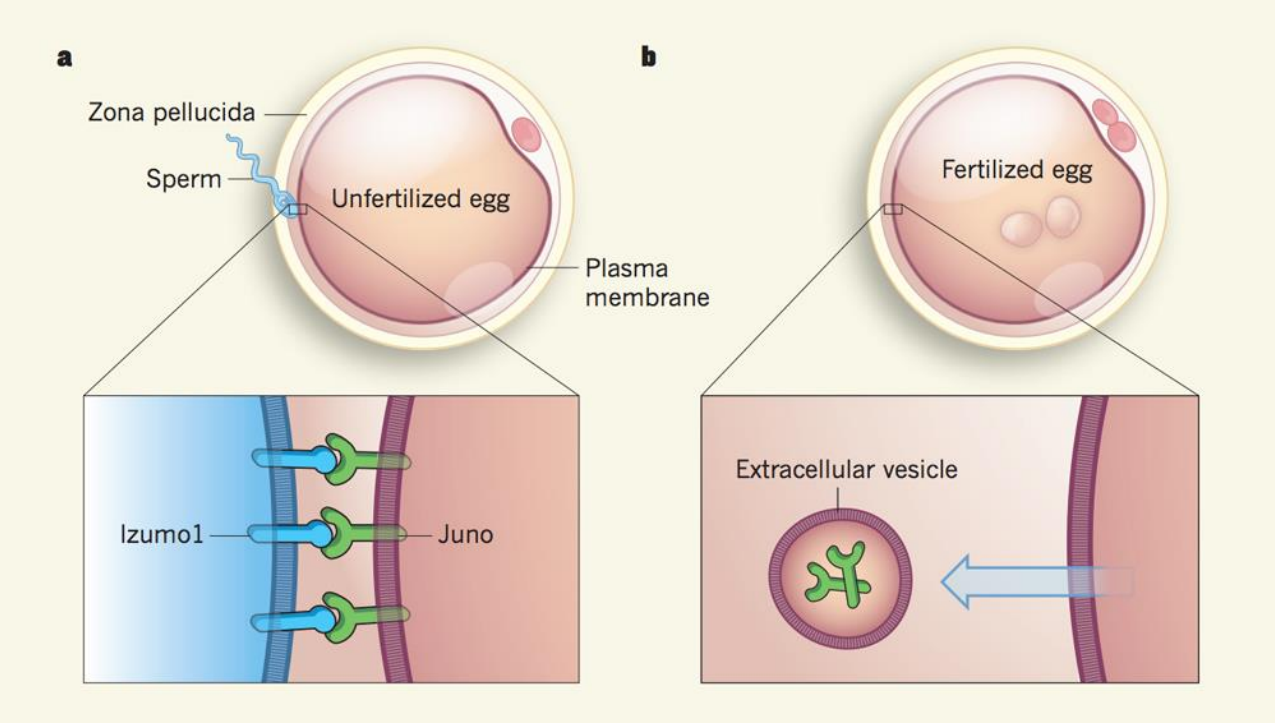


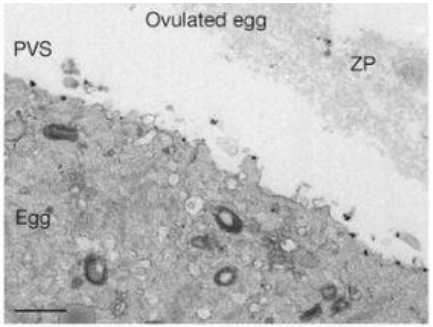
Tokuhiro and Dean, 2018

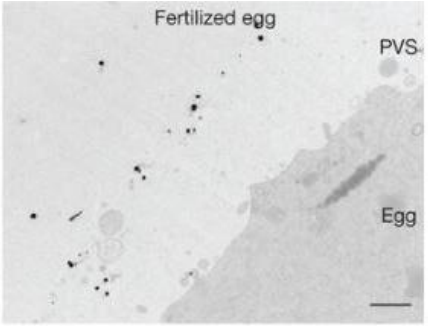
Polyspermy block on oolema

❖ JUNO SHEDDING

- rapid permanent oolema block
- Juno receptor is removed from oolema after normal fertilization (but not activation and ICSI)
- absence of Izumo receptor makes the oocyte refractory to late-coming sperms







Bianchi et al 2014

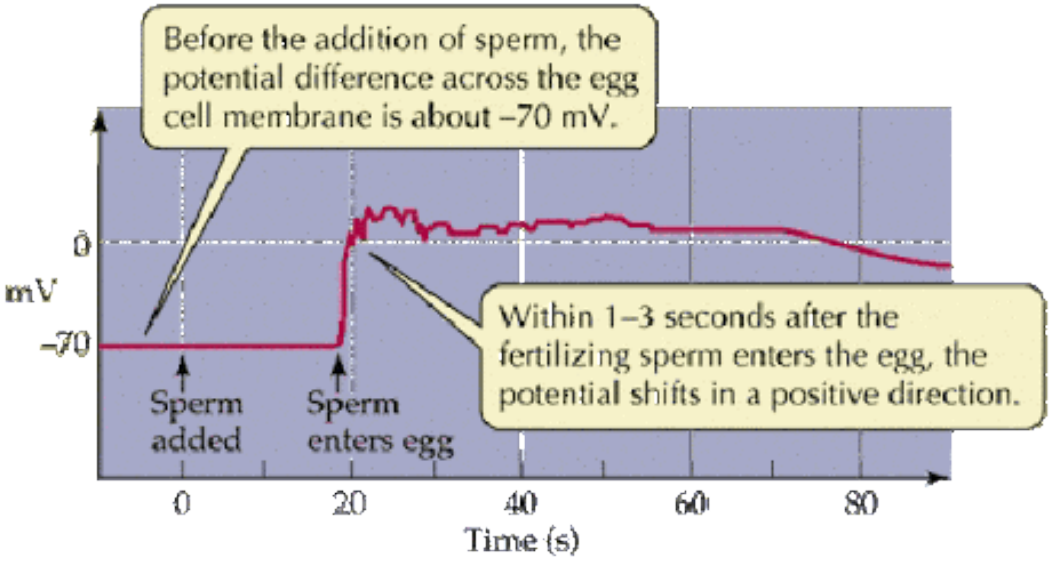
Polyspermy block on oolema

❖ MEMBRANE DEPOLARIZATION

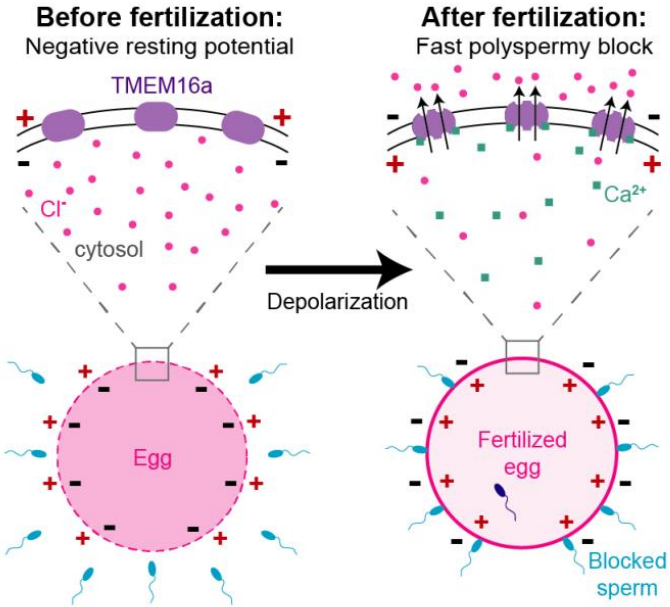
- fast and short-lasting block to polyspermy
- transient alteration of electric charges on egg´s surface
- discovered and studied in marine animals, but unfounded in mammals

Sea urchin

Xenopus laevis



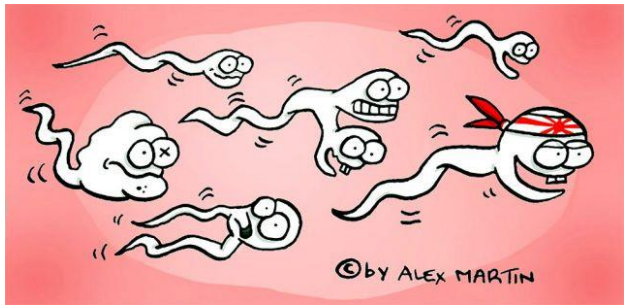
Purves et al 1998



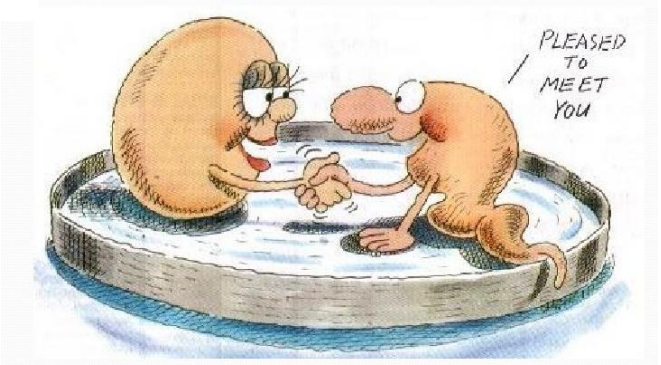
Wozniak et al 2018

Practical implications

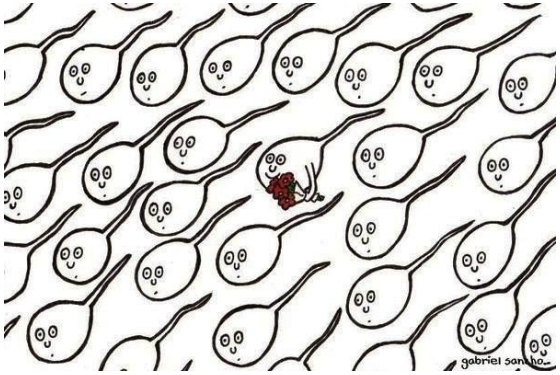
- **Improved diagnostics and informed consulting**



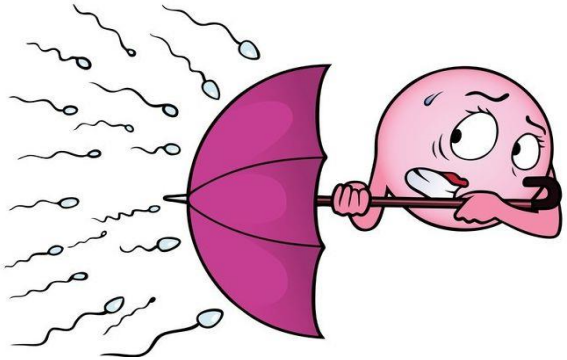
- **Better gamete culture and/or in vitro fertilization conditions**



- **Biomarker-based assay for sperm selection**



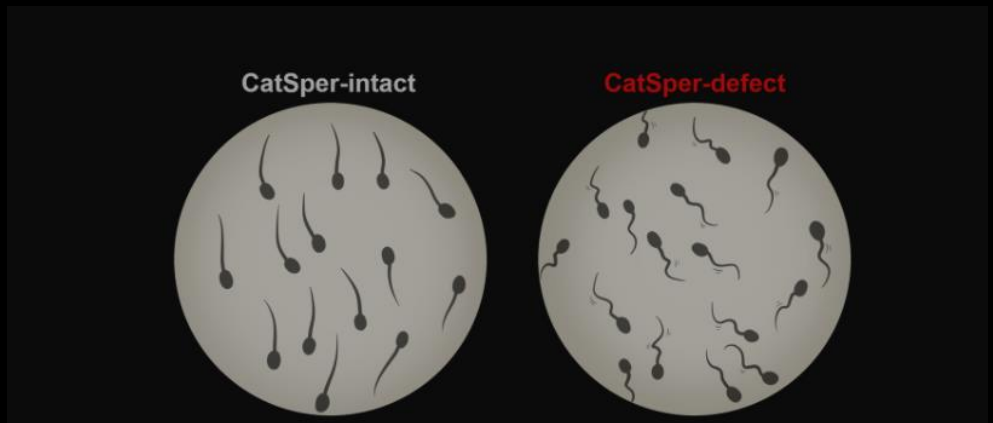
- **Short-acting non-hormonal contraceptive**



Practical implications

CatSperm test

1. Add ejaculate
2. Incubate
3. Determine motility

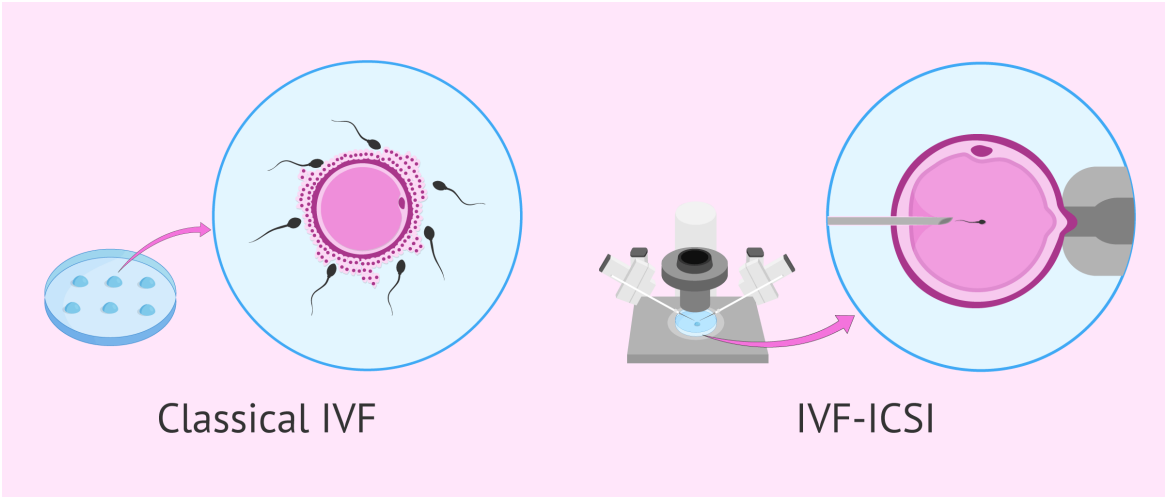


The evaluation is very simple: If CatSper function is intact, sperm become immotile in the Cat**Flux** Test Buffer. In contrast, motile sperm in the Test Buffer is indicative of CatSper-related infertility.

CatFlux buffer™
- low Ca2+ and progesterone

CatSperm channel functional
→ sperm become immotile

Conventional IVF vs. ICSI



THE LANCET

1992

Gianpiero Palermo

SHORT REPORT

Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte

GIANPIERO PALERMO HUBERT JORIS
PAUL DEVROEY ANDRE C. VAN STEIRTEGHEM

Intracytoplasmic sperm injection (ICSI) is a promising assisted-fertilisation technique that may benefit women who have not become pregnant by in-vitro fertilisation (IVF) or subzonal insemination (SUZI) of oocytes. We have used ICSI to treat couples with infertility because of severely impaired sperm characteristics, and in whom IVF and SUZI had failed. Direct injection of a single spermatozoon into the ooplasm was done in 47 metaphase-II oocytes: 38 oocytes remained intact after injection, 31 became fertilised, and 15 embryos were replaced in utero. Four pregnancies occurred after eight treatment cycles—two singleton and one twin pregnancy, and a preclinical abortion. Two healthy boys have been delivered from the singleton pregnancies and a healthy boy and girl from the twin pregnancy.

Lancet 1992; **340**: 17–18.

ICSI (IntraCytoplasmic Sperm Injection)

- developed for sperm factor infertility
- bypassess natural sperm selection and gamete interaction
- overused in clinical practice
- increased cost vs. effectiveness

ZP free oocyte ICSI

LinkedIn

AbdurRhman Saber • 1st
Senior Embryologist, QC head at Queens fertility and IVF centre
15h •