" La mayoría de las ideas fundamentales de la ciencia son esencialmente sencillas y por regla general pueden ser expresadas en un lenguaje comprensible para todos"

Albert Einstein

CAPACITACIÓN ESPERMÁTICA EN EQUINOS: BASES MOLECULARES, NUEVOS MÈTODOS DE DETECCIÓN Y EFECTOS SOBRE LA FERTILIDAD

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INTRODUCCIÓN

INSEMINACIÓN ARTIFICIAL

INTRODUCCIÓN

Características del esperma

- Célula términal
- Mayor suceptibilidad a daño por ROS

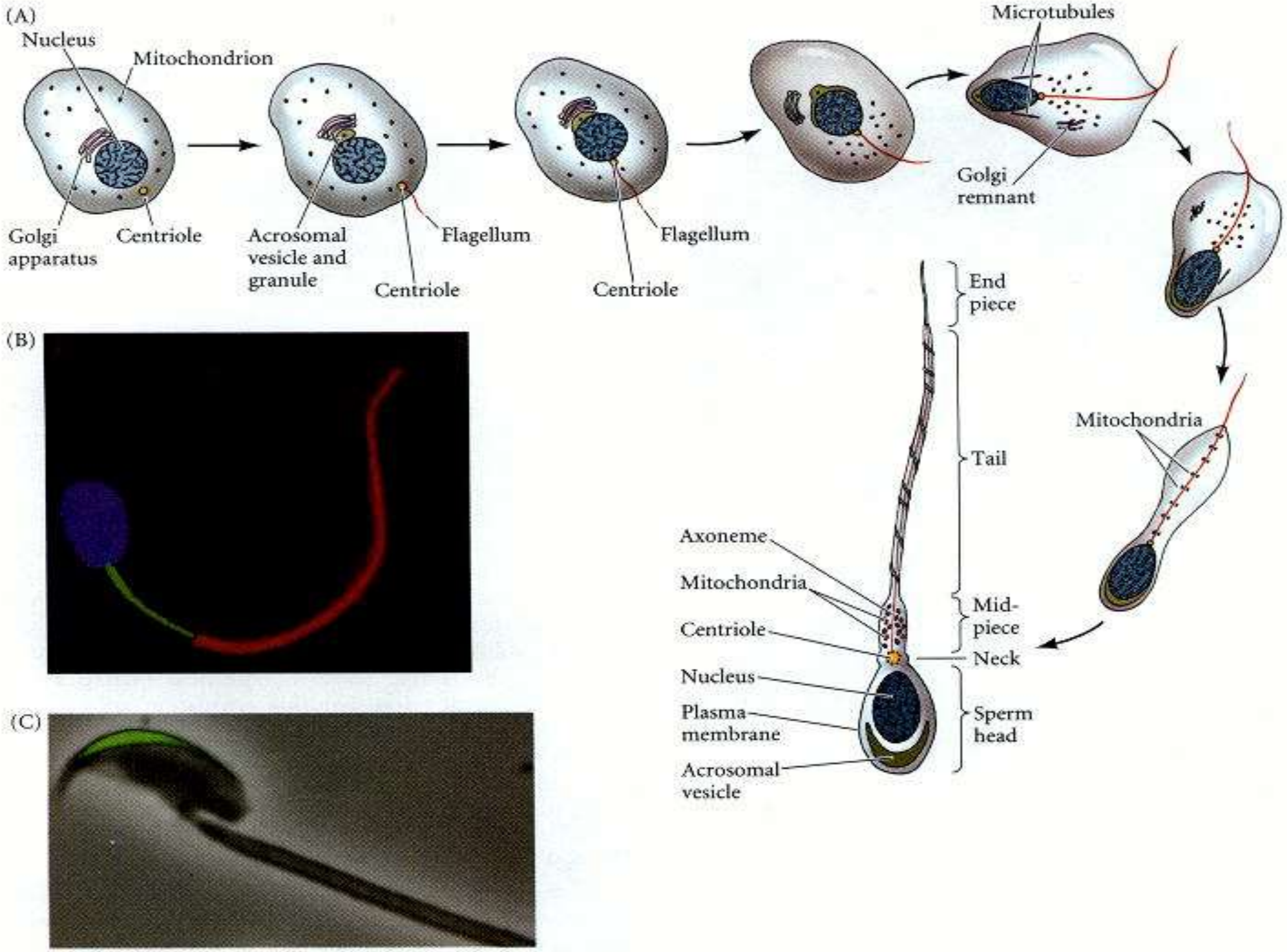
criop … vación

- Estrés oxidativo
- Daños a DNA, membrana y proteínas
- Capacitación e hipermotilidad prematura
- Apoptosis

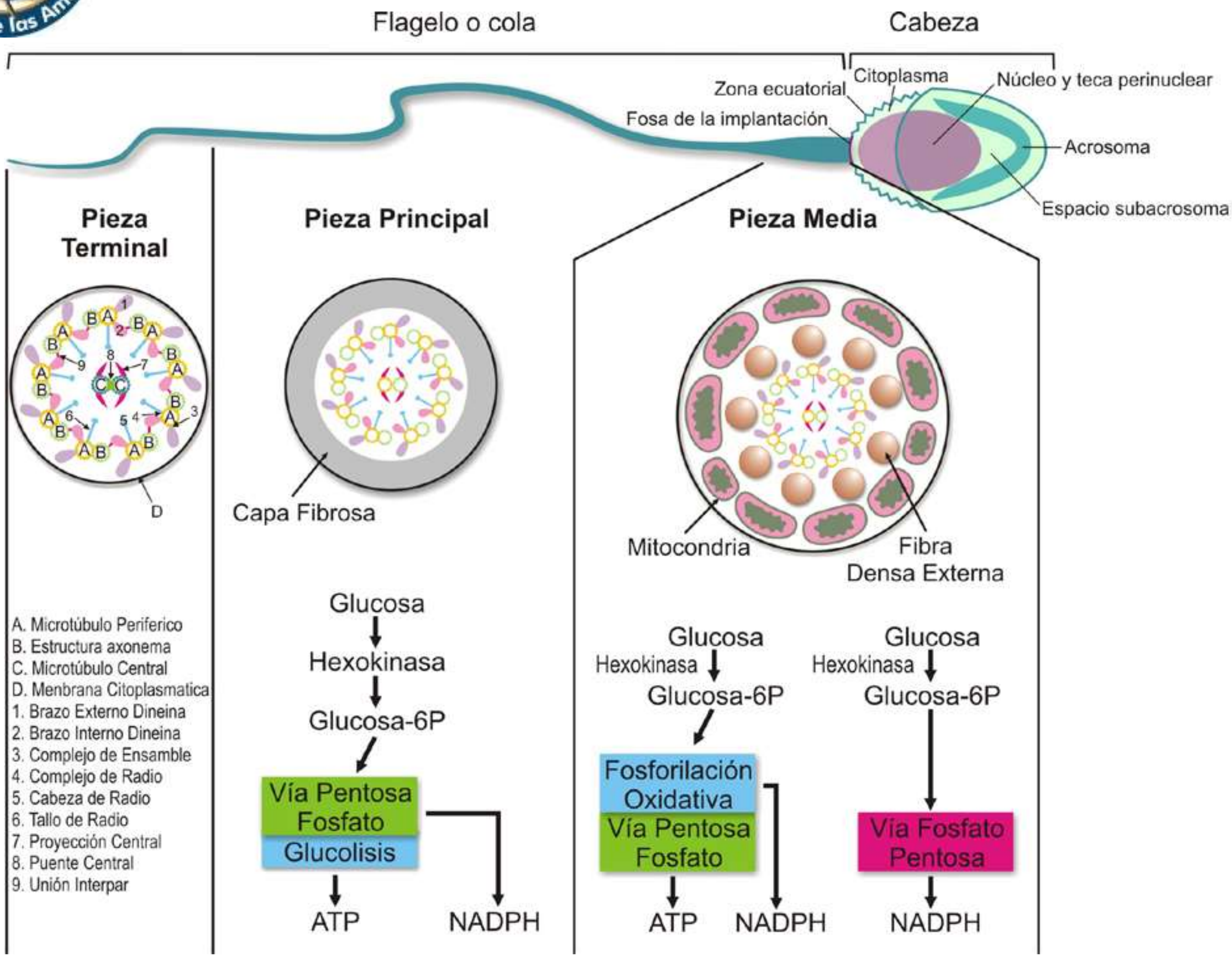
Baj … cia de p … sos

- Bajas tasas de fertilidad
- Bajas tasas de gestación

INTRODUCCIÓN

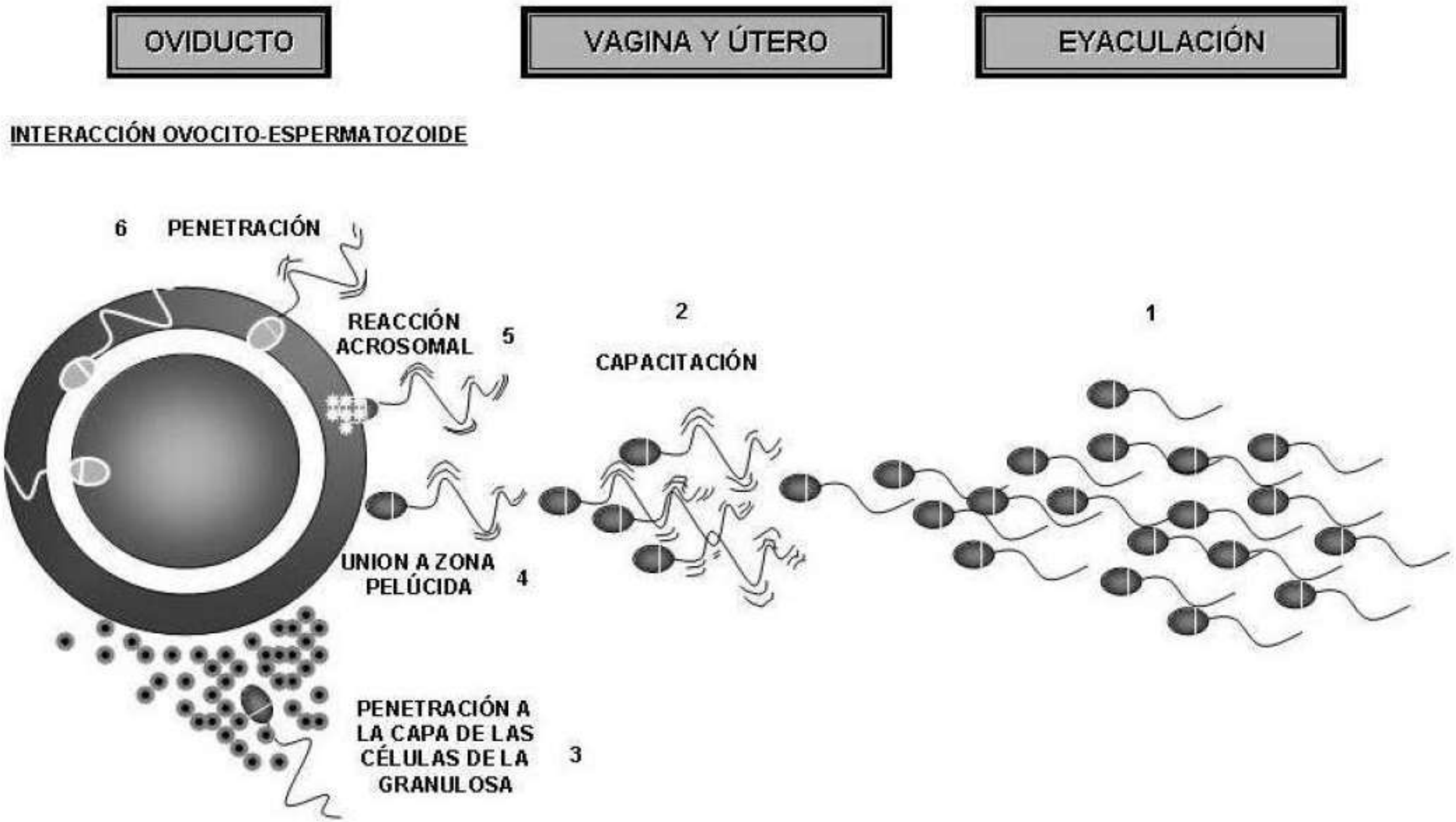


INTRODUCCIÓN



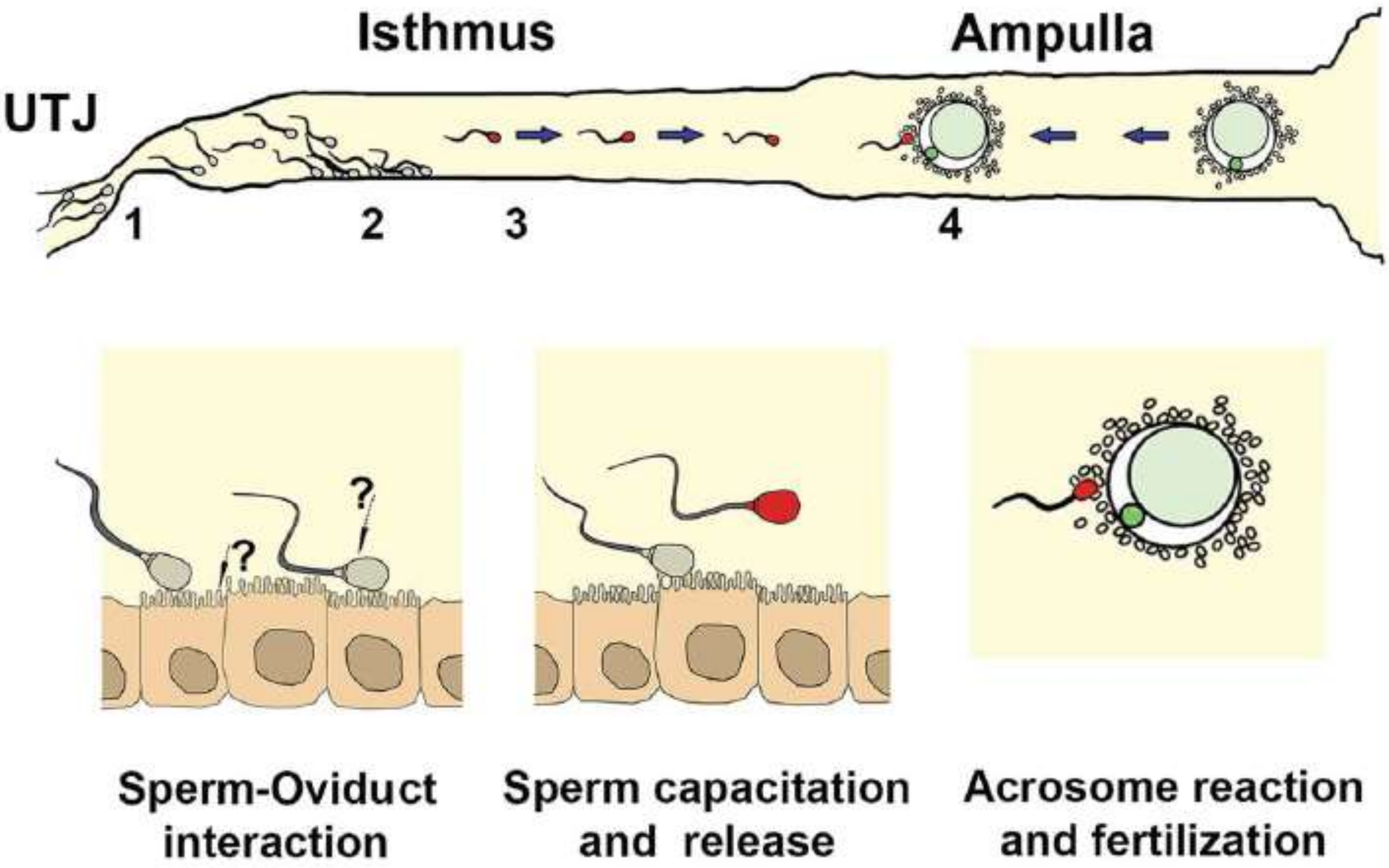
INTRODUCCIÓN

1. Maduración epididimal
2. Atravesar el útero y alcanzar el oviducto.
3. Capacitarse.
4. Unirse al oocito.
5. Sufrir reacción acrosomal.
6. Atravesar la zona pelúcida y lograr la fecundación.

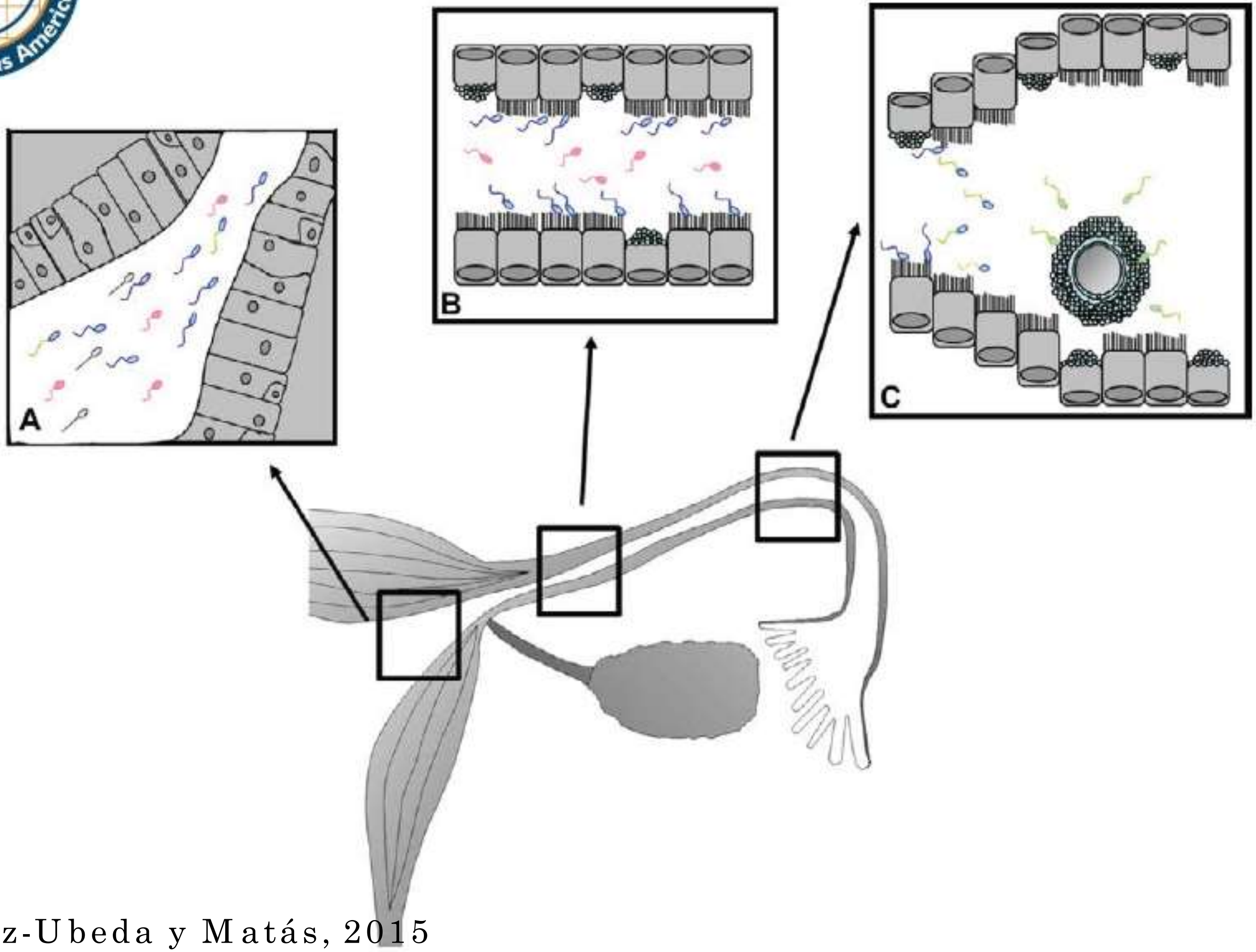


OVIDUCTO
VAGINA Y ÚTERO
EYACULACIÓN
INTERACCIÓN OVOCITO-ESPERMATOZOIDE
6 PENETRACIÓN
REACCIÓN ACROSOMAL 5
2
CAPACITACIÓN
1
UNION A ZONA PELÚCIDA 4
PENETRACIÓN A LA CAPA DE LAS CÉLULAS DE LA GRANULOSA 3

Ríos, 2010



Gervasi and Visconti, 2015



López-Ubeda y Matás, 2015

UN POCO DE HISTORIA

- Chang y Austin 1951

- Desarrollo de la FIV

- Conejos y ratas (espermas eyaculados y epididimarios, antes y despúes de la ovulación)

- Austin → término capacitación

Dr Min Chueh Chang

the sperm must undergo physiological changes in the female tract to acquire the ability to penetrate the egg

CAPACITACIÓN ESPERMÁTICA

Chang MC. 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature*. 168:697-698.

Chang MC. 1959. Fertilization of rabbit ova in vitro. *Nature*. 184(Suppl 7):466-467.

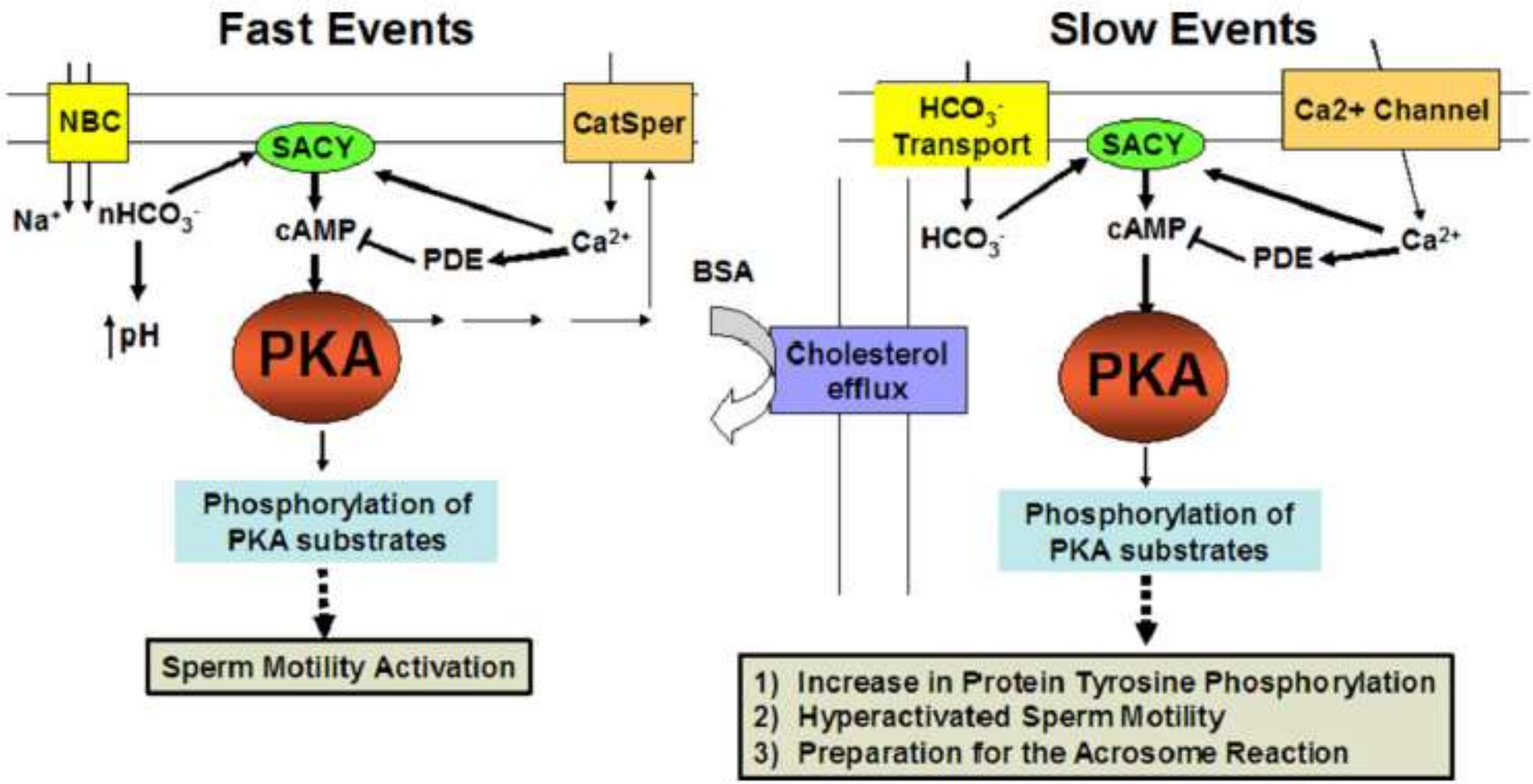
Chang MC. 1971. Second annual Carl G. Hartman Lecture. Experimental studies of mammalian spermatozoa and eggs. *Biol Reprod*. 4:3-15.

Chang MC. 1984. The meaning of sperm capacitation. A historical perspective. *J Androl*. 5:45-50.

Austin CR. 1951. Observations on the penetration of the sperm in the mammalian egg. *Aust J Sci Res B*. 4:581-596.

Austin CR. 1952. The capacitation of the mammalian sperm. *Nature*. 170:326.

EVENTOS LENTOS Y RÁPIDOS



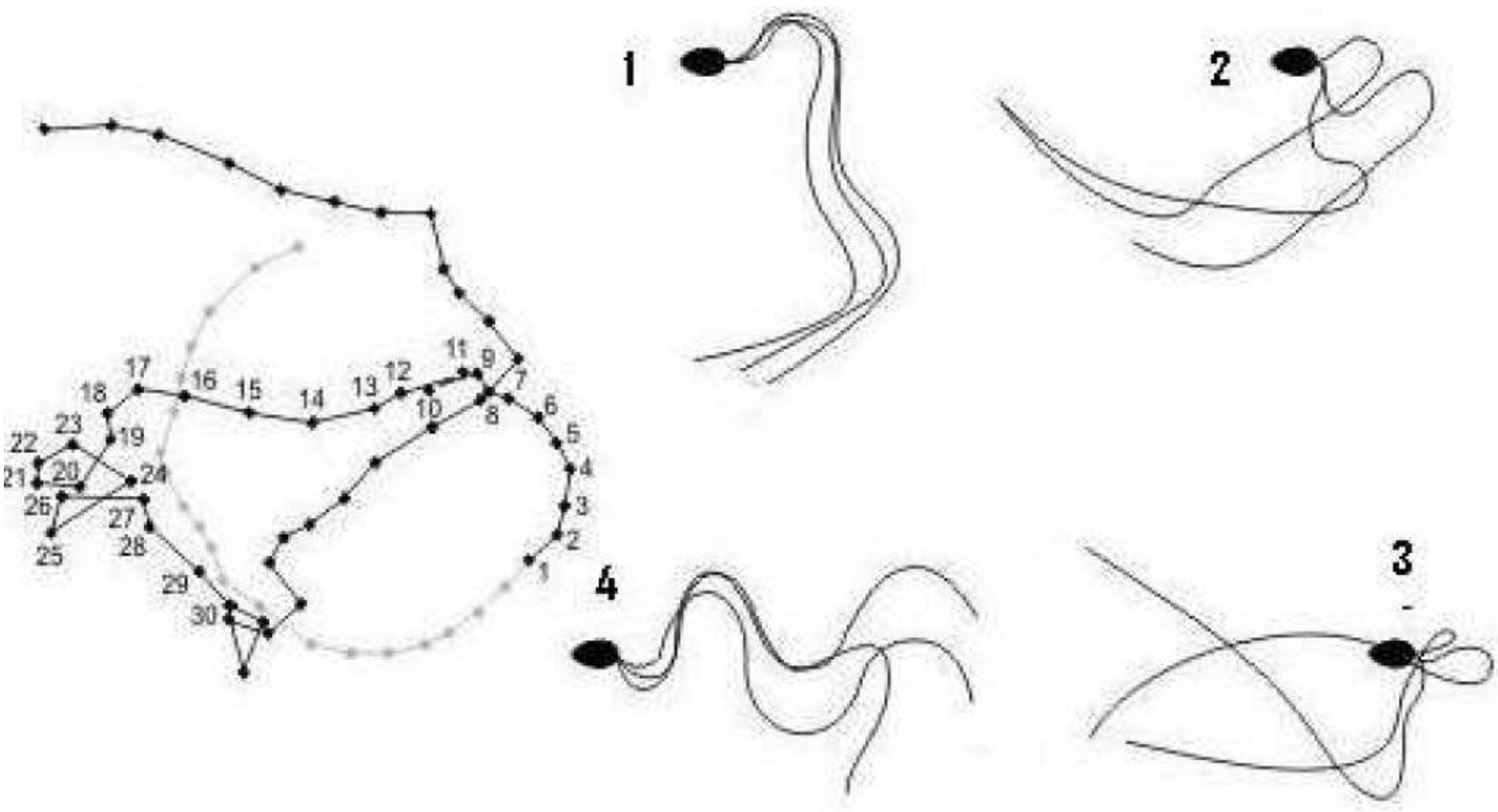
Visconti , 2009

EVENTOS LENTOS Y RÁPIDOS

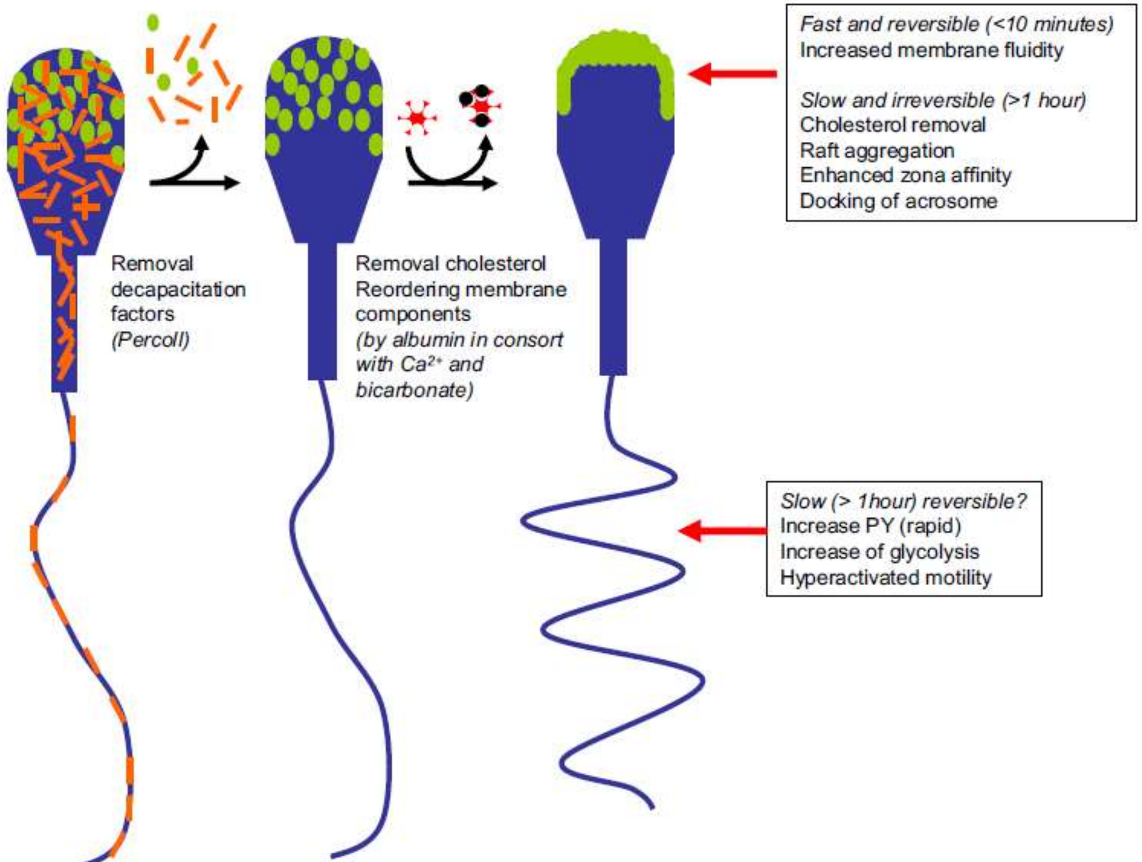
Rápidos: Activación de la movilidad en respuesta a $HCO3^{-}$

Lentos: Hiperactivación, eflujo de colesterol, fosforilación de tirosinas, preparación para reacción acrosómica

MOVIMIENTO FLAGELAR E HIPERACTIVACIÓN

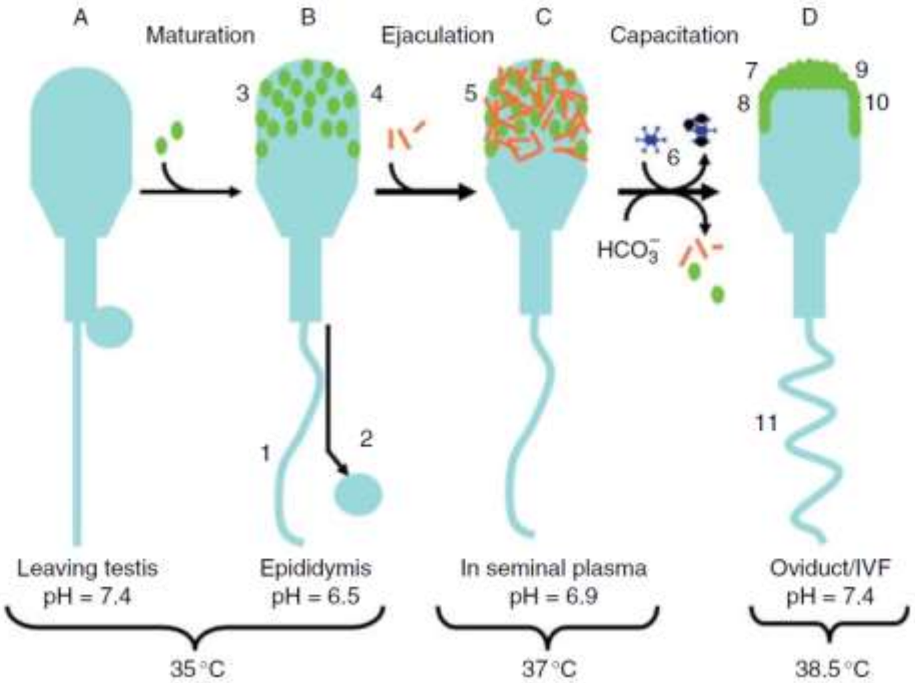


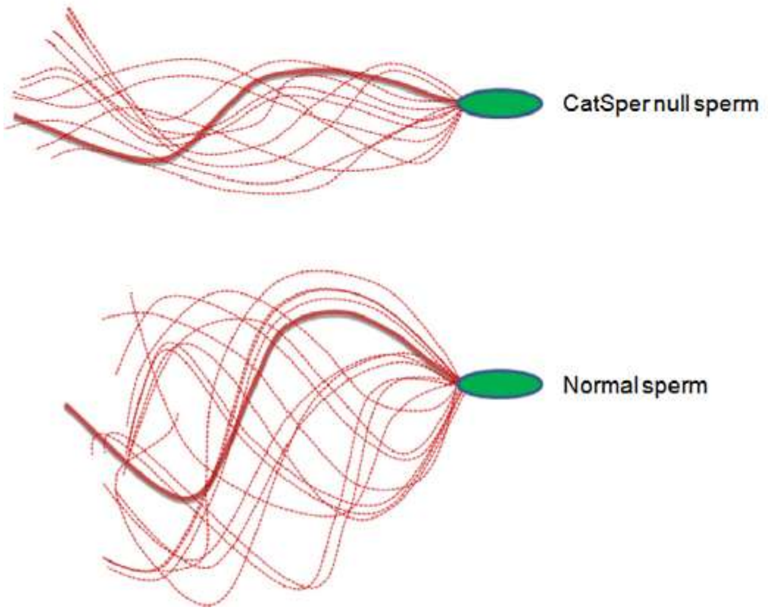
CAPACITACIÓN ESPERMÁTICA



Leahy and Gadella, 2011

CAPACITACIÓN ESPERMÁTICA





Leahy and Gadella, 2011

EVENTOS POST-CAPACITACIÓN

Hiperactivación

↓

Unión a la zona pelúcida

↓

Reacción acrosomal

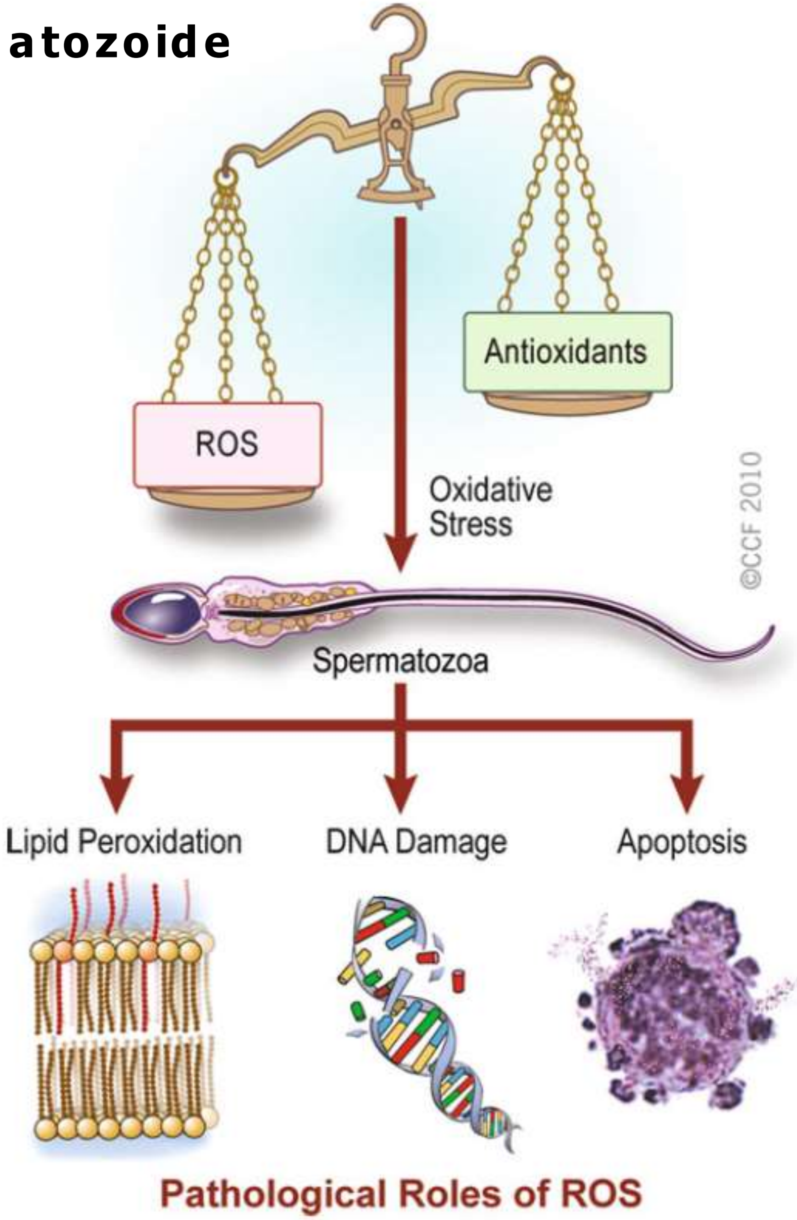
↓

Penetración de la zona pelúcida

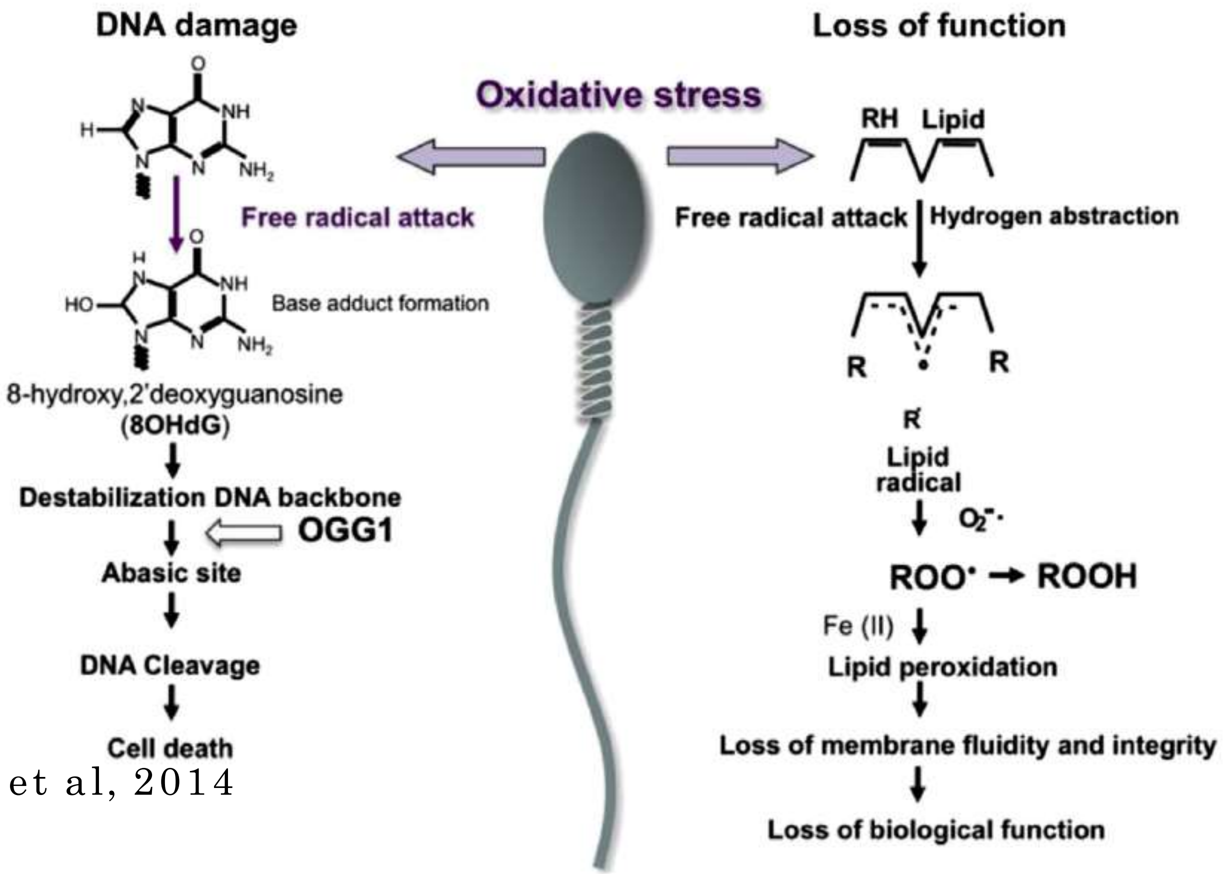
↓

Fusión oocito-espermatozoide

Estrés oxidativo y daño al espermatozoide



ESTRÉS OXIDATIVO EN ESPERMATOZOIDE



Aitken et al, 2014

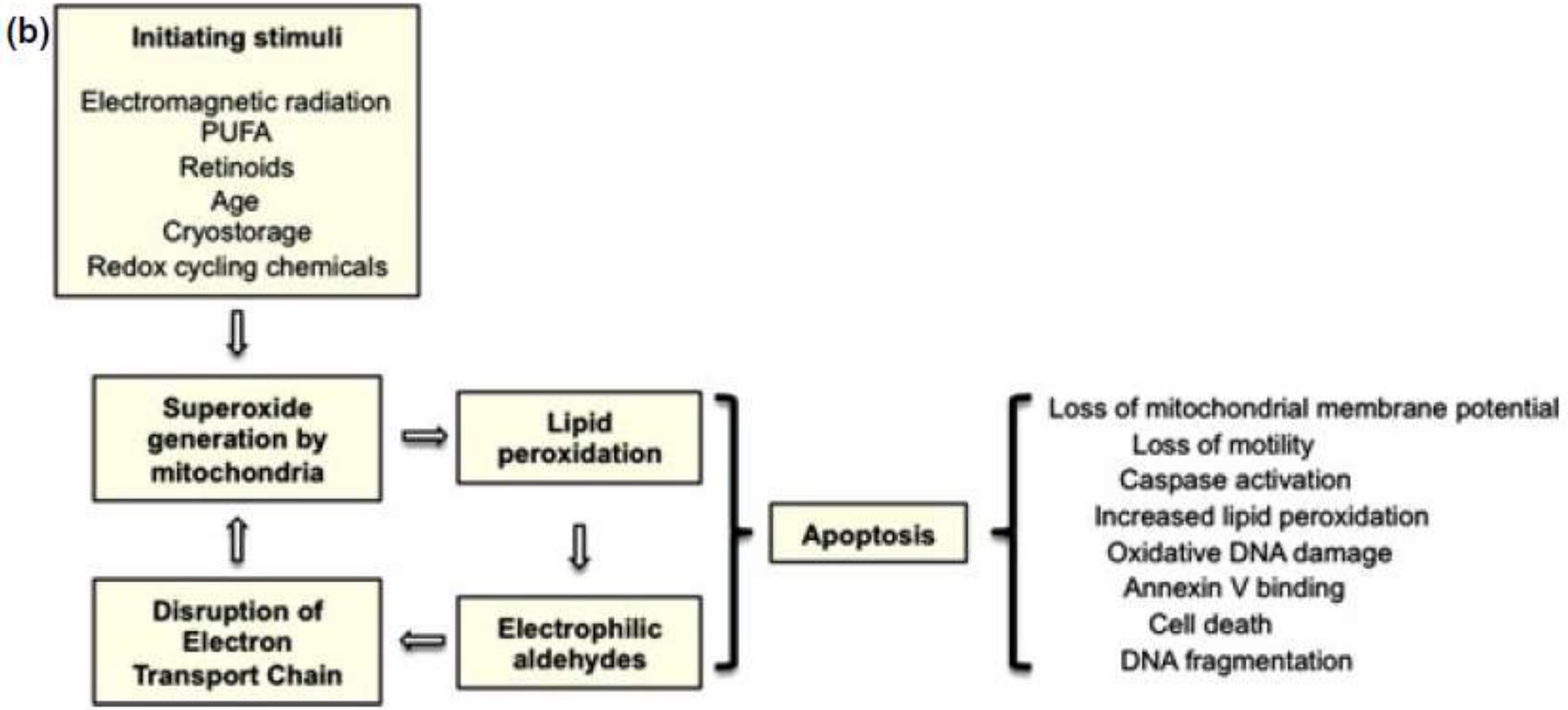
ESPECIES REACTIVAS DE OXÍGENO Y NITRÓGENO

TABLE 12.1 Reactive Oxygen and Nitrogen Species (ROS and RNS)

Radicals	Nonradicals
Superoxide ($O_2^{\bullet -}$)	Hydrogen peroxide (H_2O_2)
Hydroperoxyl ($HOO^{\bullet}$)	Alkyl hydroperoxides (LOOH)
Peroxyl ($LOO^{\bullet}$)	Singlet oxygen ($^1O_2$)
Alkoxyl ($LO^{\bullet}$)	Ozone (O_3)
Hydroxyl ($^{\bullet}OH$)	Hypochlorous acid (HOCl)
Nitric oxide (nitrogen monoxide; $^{\bullet}NO$)	Peroxynitrite ($ONOO^-$)
Nitrogen dioxide ($^{\bullet}NO_2$)	

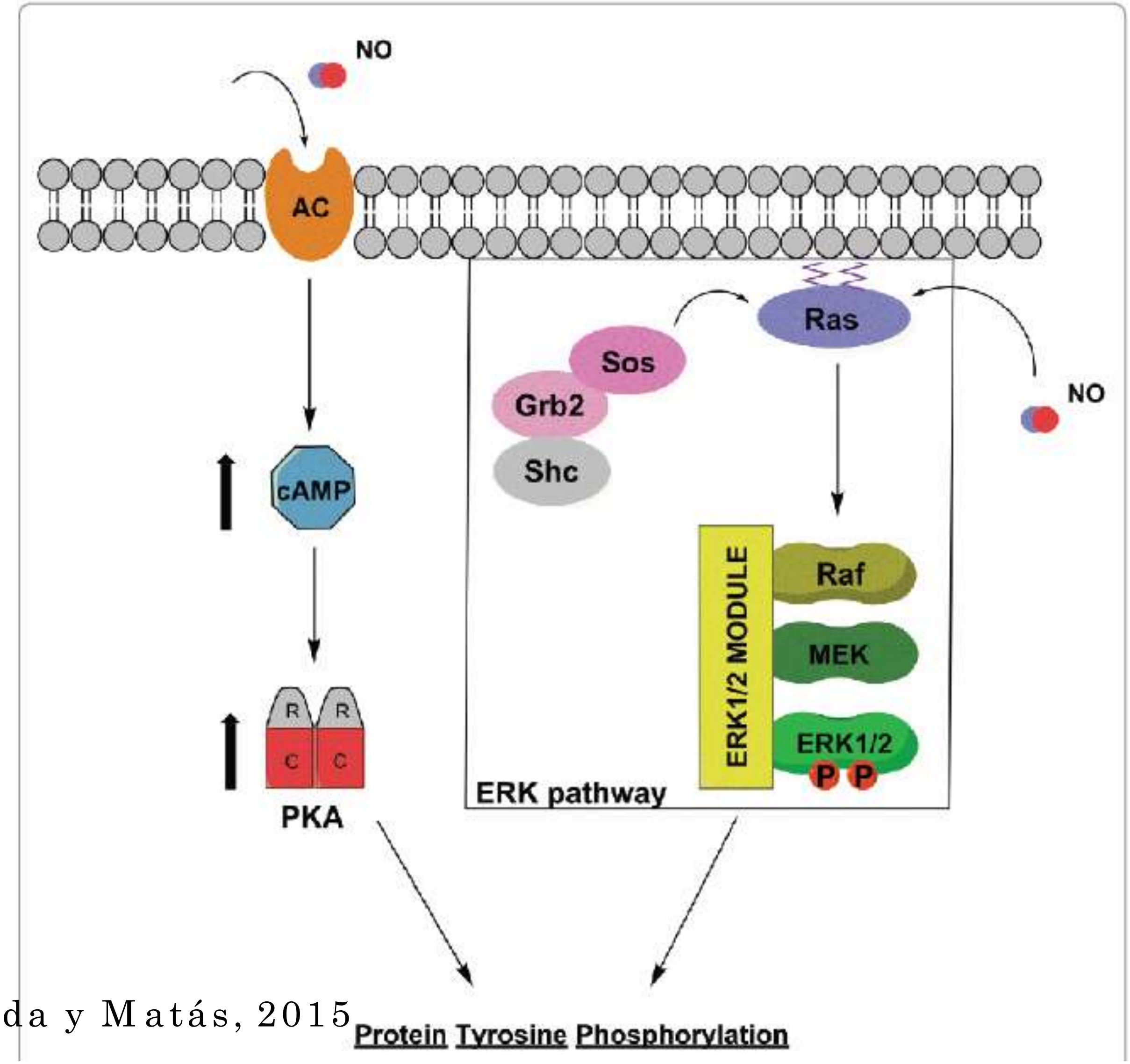
Hermes-Lima, 2004

DAÑO AL ESPERMATOZOIDE

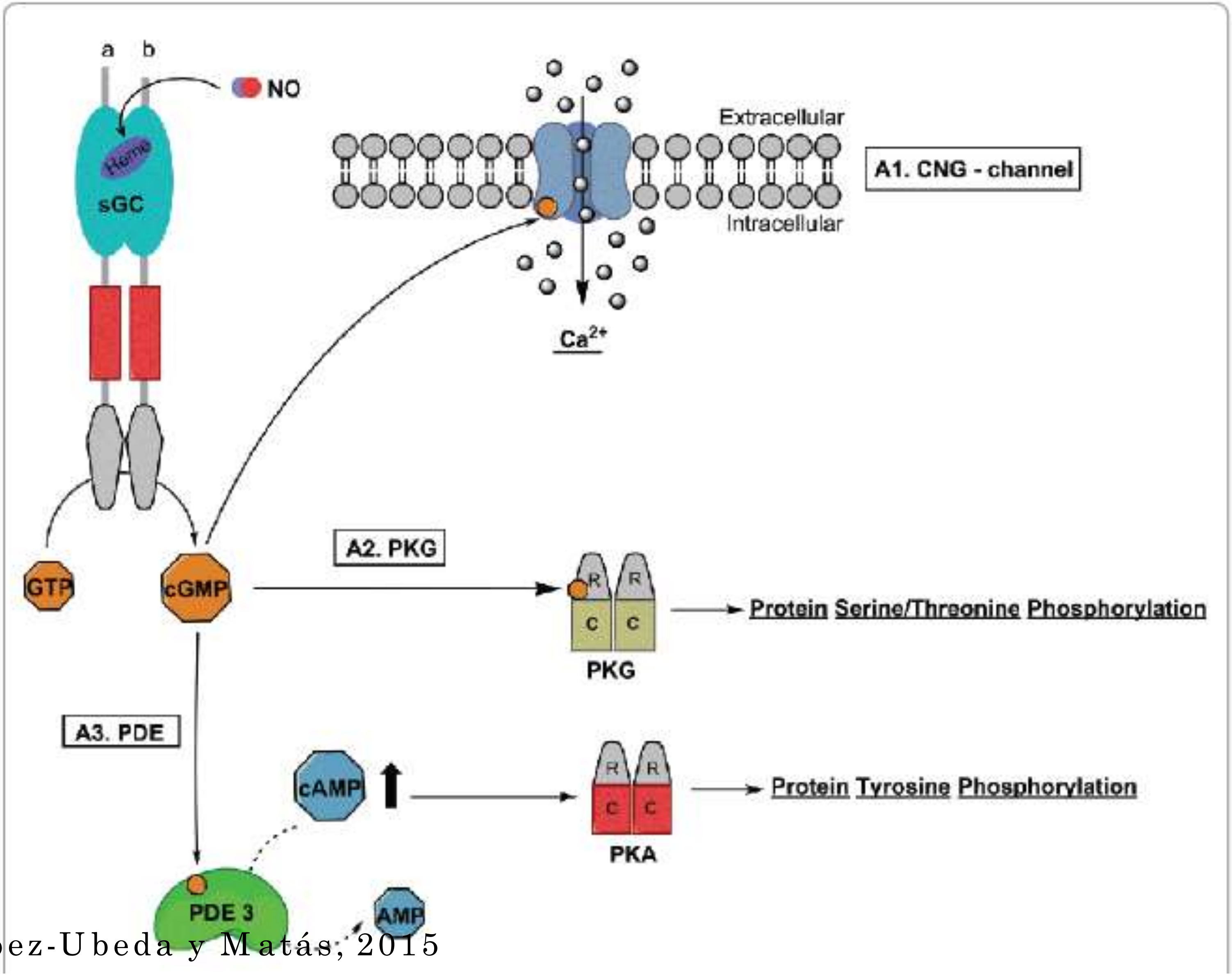


Aitken et al, 2012

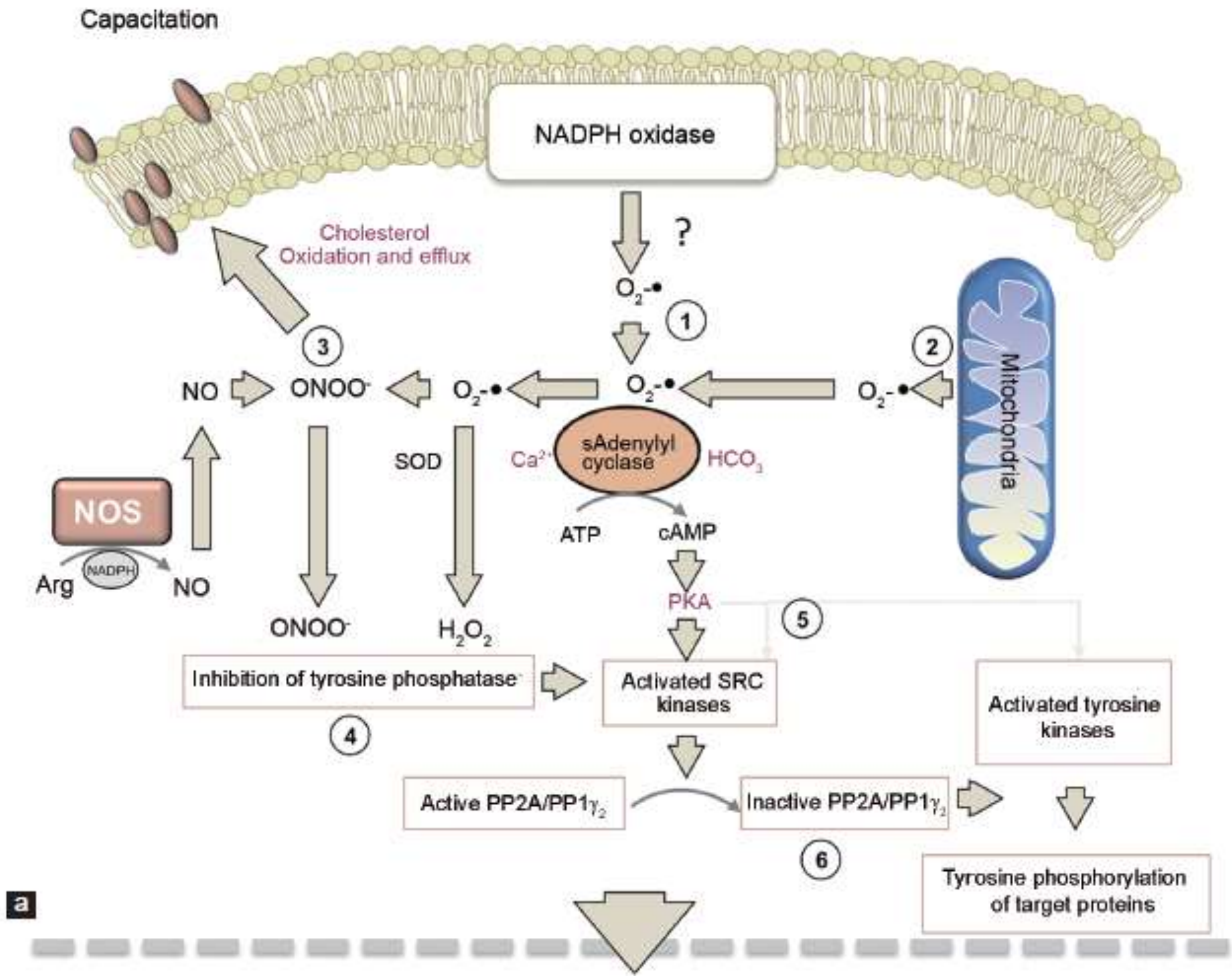
NO y CAPACITACIÓN



López-Ubeda y Matás, 2015



López-Ubeda y Matás, 2015

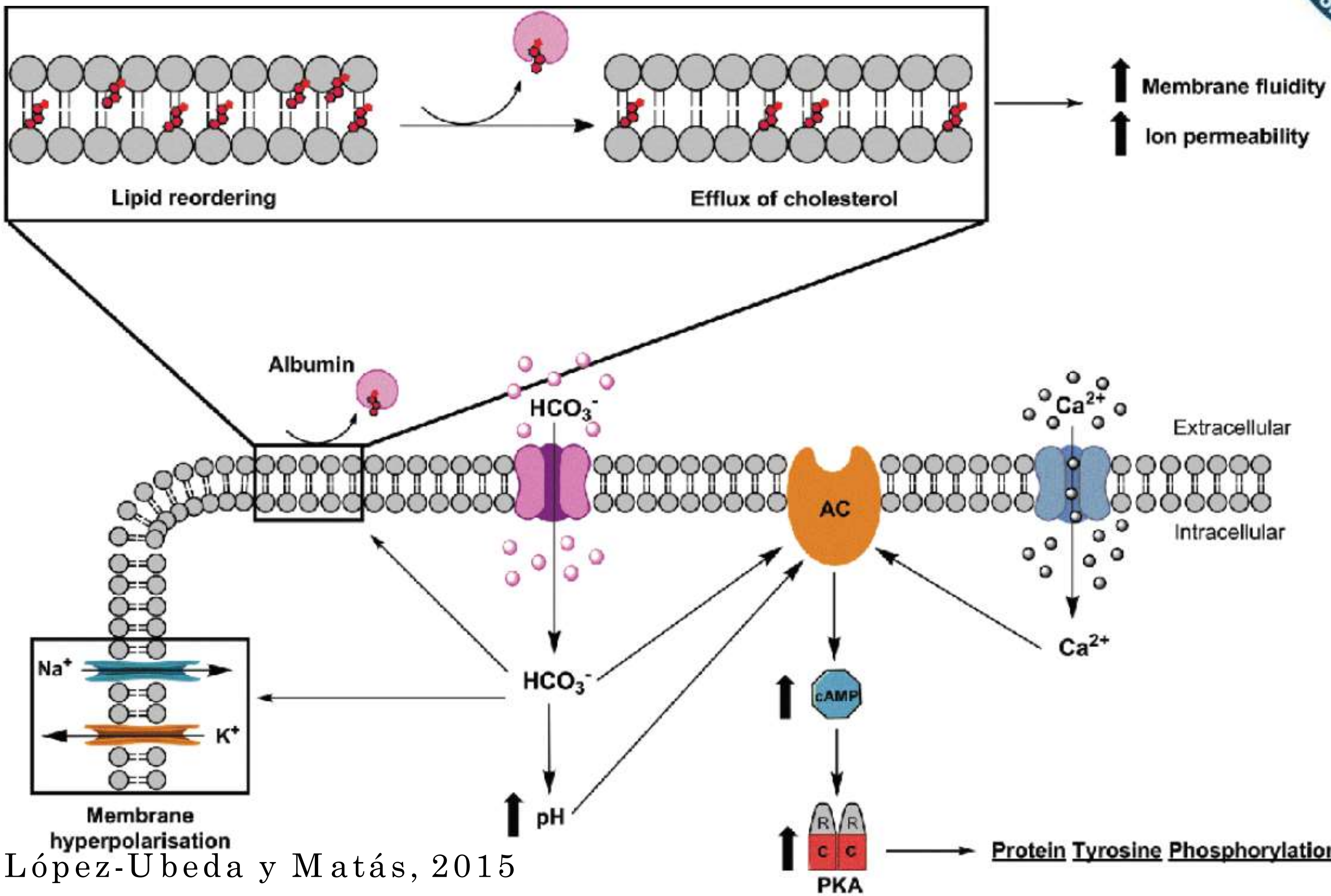

Capacitation
NADPH oxidase
?
O_2-•
1
Cholesterol
Oxidation and efflux
3
2
NO
ONOO$^-$
O_2-•
O_2-•
O_2-•
Mitochondria
SOD
Ca^{2+}
sAdenylyl cyclase
HCO_3
NOS
Arg
NADPH
NO
ATP
cAMP
PKA
5
ONOO$^-$
H_2O_2
Inhibition of tyrosine phosphatase
Activated SRC kinases
Activated tyrosine kinases
4
Active PP2A/PP1γ_2
Inactive PP2A/PP1γ_2
6
Tyrosine phosphorylation of target proteins
a

$HCO3^-$ y CAPACITACIÓN

- 3-4 mM (Epididimo)- > 20 mM (oviducto)
- Incremento del pH celular
- Estimulación de la AC → Aumento del cAMP → PKA → PY
- Cambios en potencial de membrana, alto K, bajo $HCO3^-$, bajo Na^+ →
- Bajo K, alto $HCO3^-$, alto Na^+
- PKA → PLD → Polimerización de actina F → Reacción acrosómica

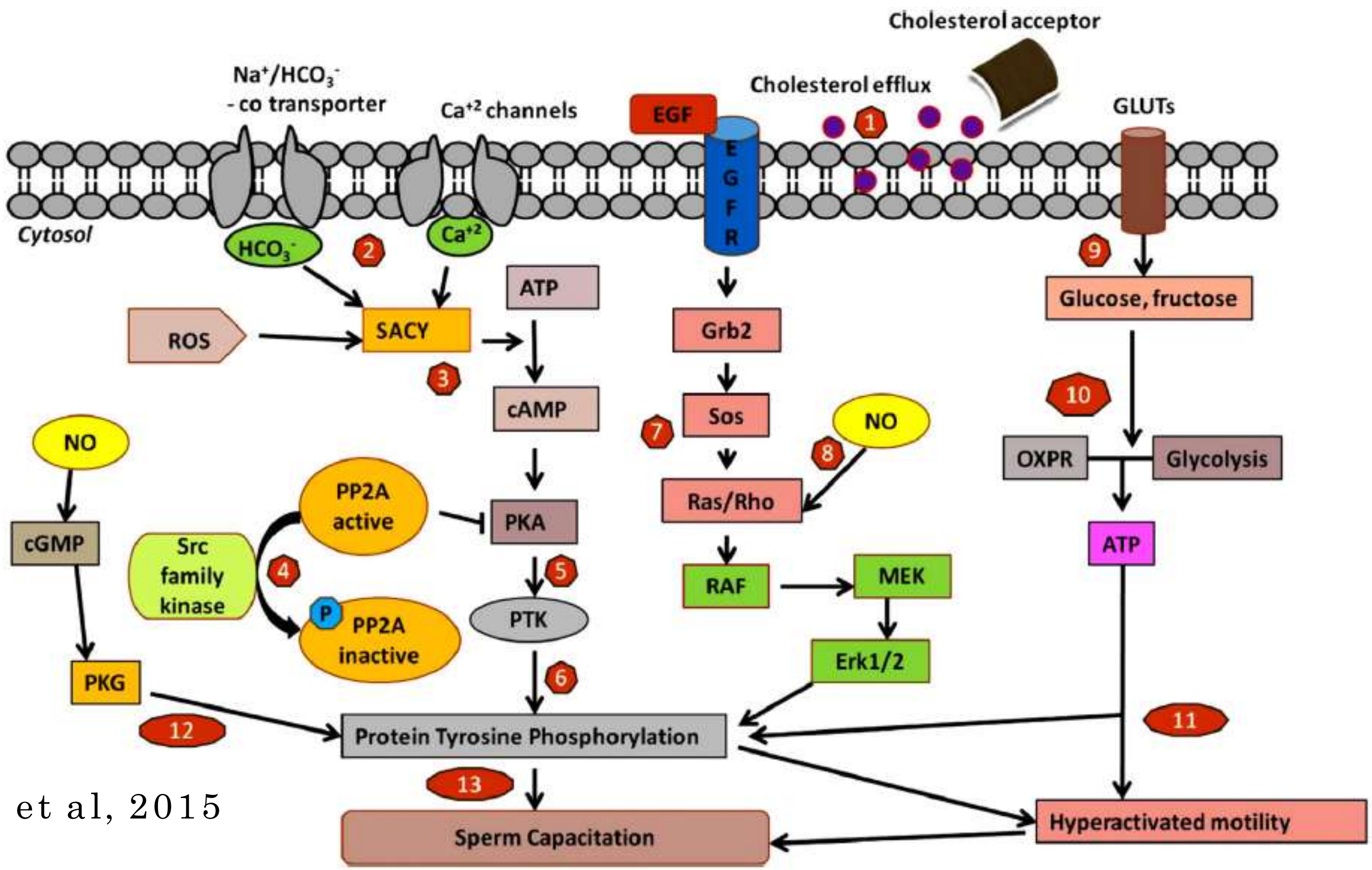
HCO_3^- y CAPACITACIÓN

- Modifica estructura de lípidos de membrana
- A través de vía sAC/ cAMP /PKA
- Reordenamiento de PS, EM, PC.
- Reubicación del colesterol → remoción del colesterol → aumento fluidez → aumento permeabilidad de iones. En sinergia con albúmina.
- Oxiesteroles mas hidrofóbicos (vía ROS). Mejor remoción



López-Ubeda y Matás, 2015

SEÑALIZACIÓN EN CAPACITACIÓN



Naresh et al, 2015

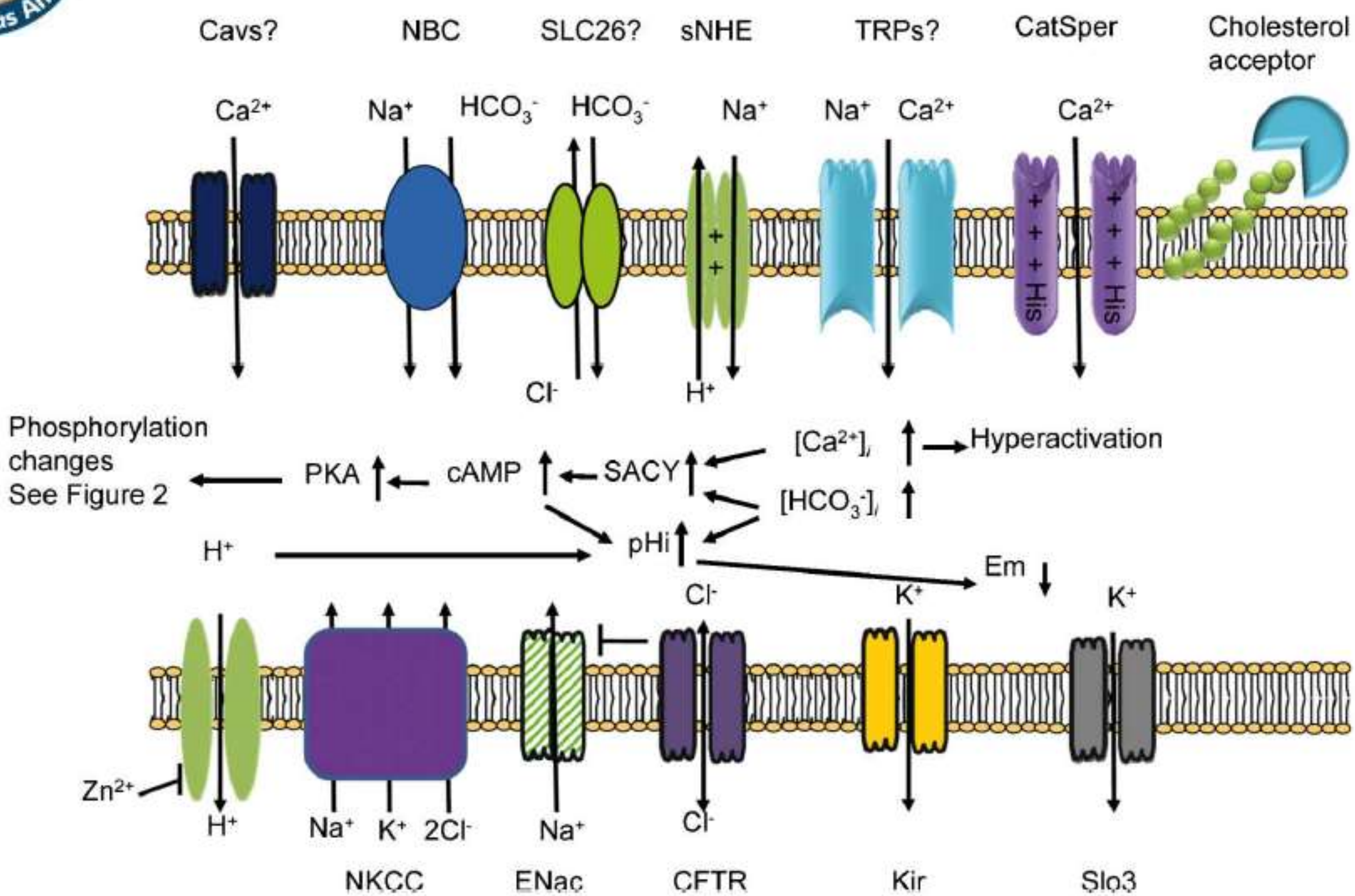
Ca^{++} y CAPACITACIÓN

- 1915, Loeb, Ca^{++} → medio extracelular → fertilización
- Eflujo de colesterol → influjo de Ca^{++} → prerequisito RA
- Ca^{++} → activa AC
- Sinergia de Ca^{++} y bicarbonato → activación de capacitación → depende especie (cerdo vs ratón)
- Ca^{++} → entrada por CatSperm
- pH ácido → regulador negativo de entrada de Ca^{++}

NBC	/	HCO_3^-/Na^+ influx	induces hyperpolarization through the HCO_3^- influx; increase protein tyrosine phosphorylation through Na^+ influx	induce	mouse	[48]

Name of channel/ stimuli	Localization on spermatozoa/availability	Role in ion flow	Role in sperm physiology	Effects on capacitation	Species	Contributors
CatSper	Principal piece of sperm tail	Ca^{2+} influx	Ca^{2+} uptake, hyperactivated motility	induce	human	[69]

Shi-Kai Jin and Wan-Xi Yang, 2017



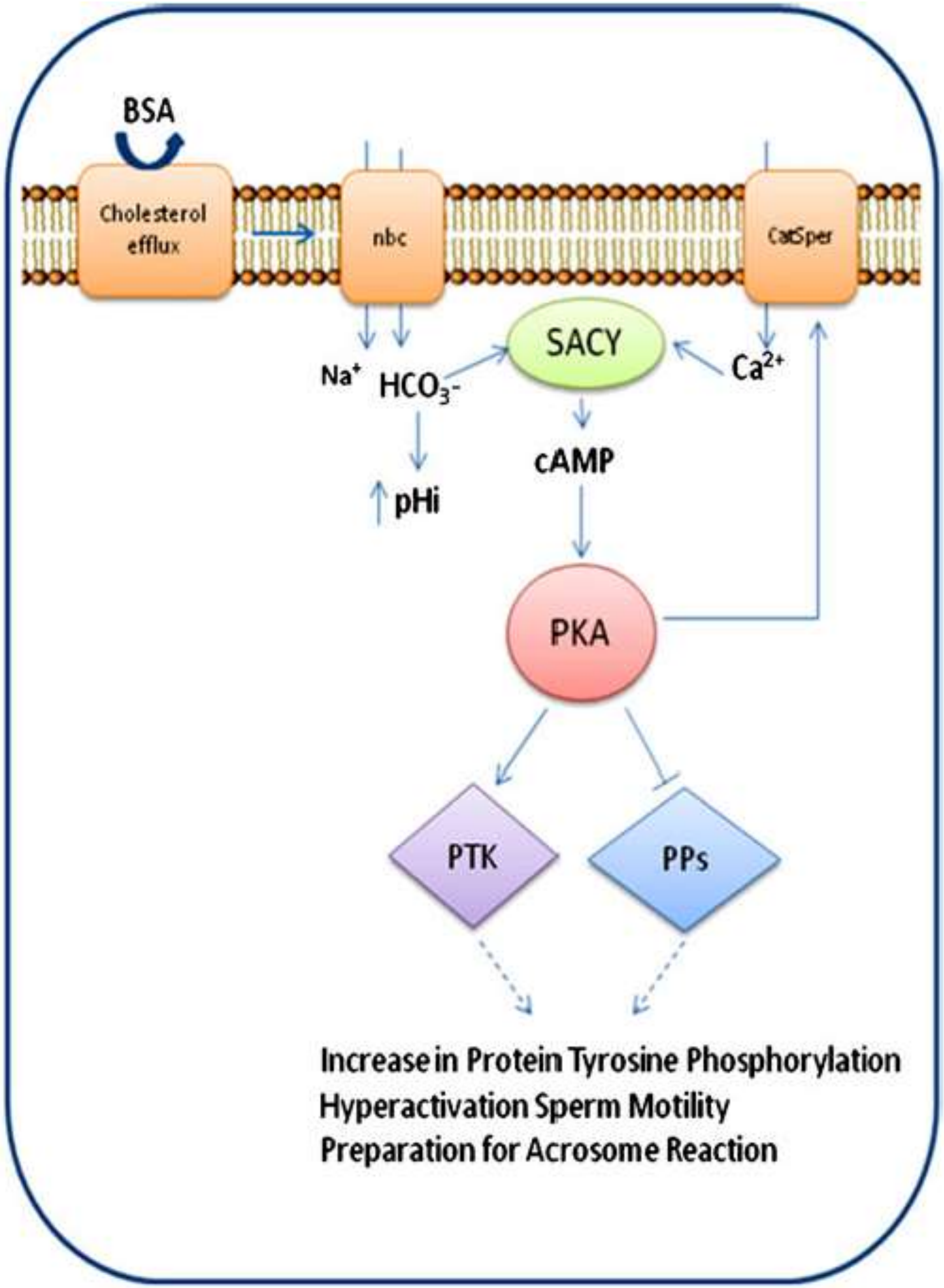
Visconti et al, 2011

Signal	Loss of function knockout mouse model	Phenotype	References
cAMP	Protein kinase A catalytic subunit α2 (PKA Cα2)	Infertility. Impaired motility, no $HCO3^-$-mediated Ca^{2+} increase, no increase of tyrosine phosphorylation.	Nolan M et al., 2004
	Soluble adenylyl cyclase (ADCY10, or sAC)	Infertility. Lack of forward motility. Infertility, even after rescuing motility with cAMP analogues. Infertility. Deficit in cAMP production, low levels of ATP and impaired hyperactivation.	Espósito G et al., 2004 Hess KC et al., 2005 Xie F et al., 2006
	A-kinase-anchoring-protein 4 (AKAP4)	Infertility. Deficiency of fibrous sheath and lack of motility. Increase of PKA RII in soluble fraction, decreased protein phosphatase 1 γ2 activity.	Miki et al., 2002 Huang Z et al., 2005
pH	Sperm specific Na^+/H^+ exchanger (sNHE)	Infertility. Complete loss of motility. cAMP rescued motility and fertility. sNHE is required for expression of sAC and both proteins complex in the membrane.	Wang D et al., 2003 Wang D et al., 2007
	H^+ channel HV1	No fertility defects (HV1 currents are active in human sperm but no currents were found in mouse sperm).	Ramsey S et al., 2009

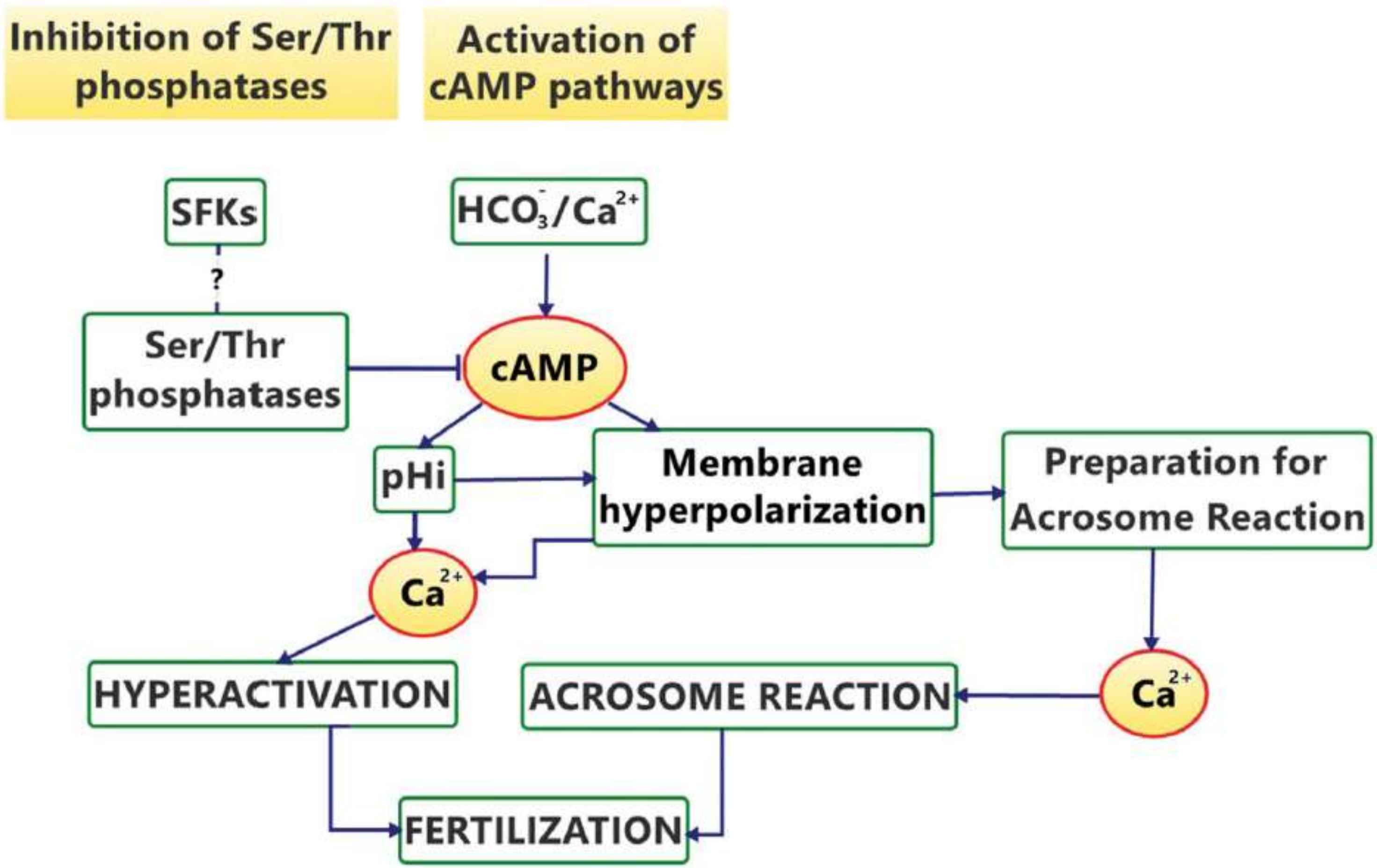
Gervasi and Visconti, 2015

Membrane potential (V_m)	Sperm specific Na^+ channel (SLO3)	Infertility. Impaired progressive motility, depolarization of the membrane after capacitation, inability to undergo acrosome reaction.	Santi CM et al., 2010 Zeng XH et al., 2011
	Na^+/K^+ ATPase α4 subunit	Infertility. Reduced sperm motility and hyperactivation. Increased intracellular Na^+ and depolarization of the membrane.	Jimenez T et al., 2011
Ca^{2+}	CAV2.3 channels	Subfertility. Altered Ca^{2+} response and reduced acrosome reaction.	Cohen R et al., 2014
	CATSPER channels	CATSPER1: Infertility. Impaired motility and lack of cAMP-induced Ca^{2+} influx. CATSPER2: Infertility. Normal forward motility, lack of hyperactivated motility. CATSPER3 and Catsper 4: Infertility. Lack of hyperactivated motility.	Ren D et al., 2001 Quill TA et al., 2003 Qi H. et al., 2007

Gervasi and Visconti, 2015



Signorelli et al, 2011



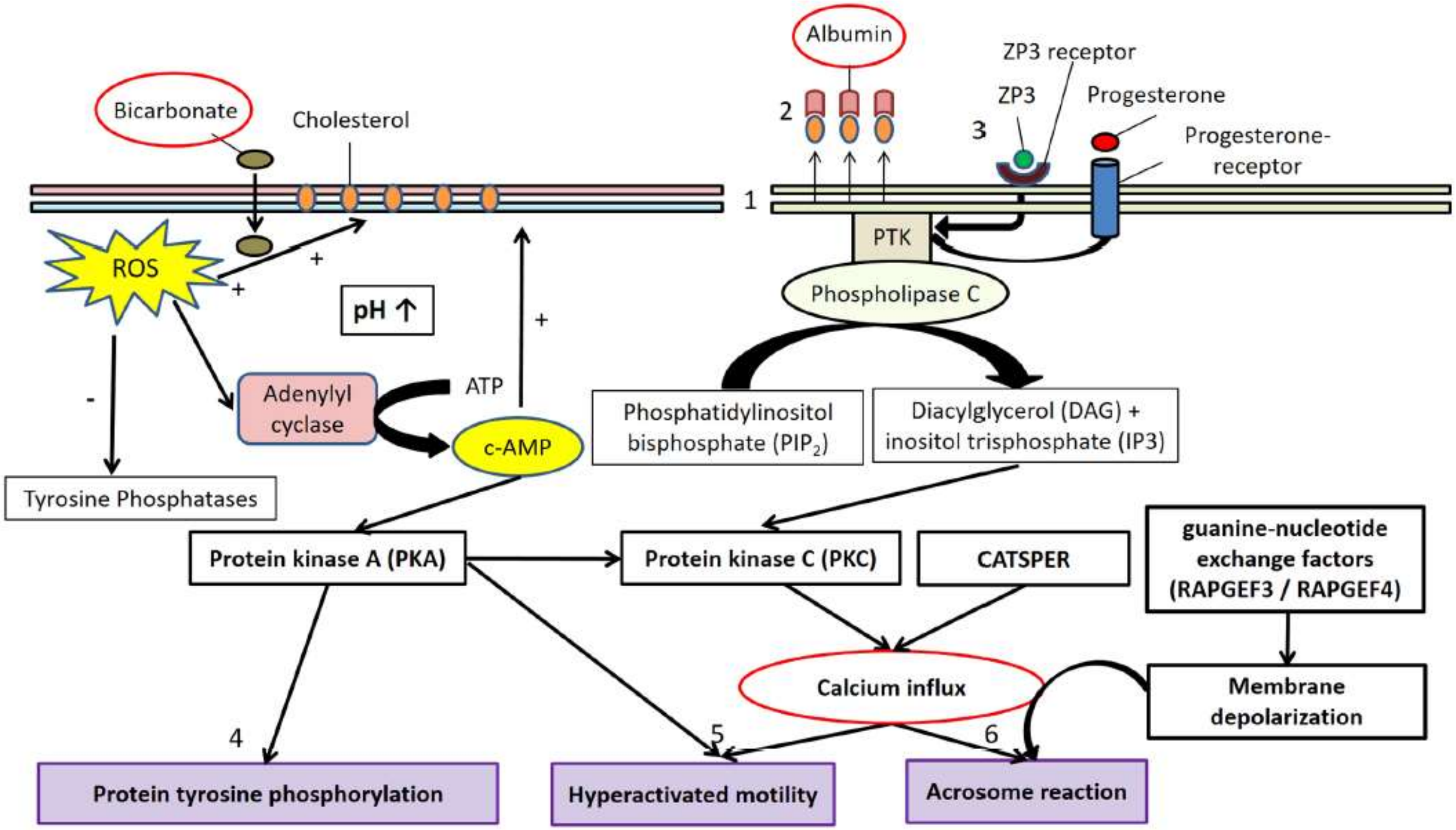
Gervasi and Visconti, 2015

Table 2 Expression of the family PPP of PSP in various tissues and in testis and sperm (*ND* not determined) modified from Fardilha et al. (2011)

Phosphatase	Other tissues	Testis	Sperm
PP1α	Ubiquitous; more predominantly in brain and lung (Shima et al. 1993a)	Yes (Shima et al. 1993b)	Yes (Suzuki et al. 2010; Fardilha et al. 2011)
PP1β	Ubiquitous; more predominantly in brain, intestine and lung (Shima et al. 1993a)	Yes (Shima et al. 1993b)	No
PP1γ1	Ubiquitous; more predominantly in brain (Shima et al. 1993a)	Yes (Shima et al. 1993b)	No
PP1γ2	Low abundance (Strack et al. 1999)	Yes (Kitagawa et al. 1990; Sasaki et al. 1990; Shima et al. 1993b)	Yes (Smith et al. 1996; Shima et al. 1993b; Vijayaraghavan et al. 1996)
PP2A	Ubiquitous; more predominantly in brain (Khew-Goodall and Hemmings 1988)	Yes (Kloeker et al. 2003)	Yes (Tash et al. 1988; P. Morales, unpublished data)
PP2B	Ubiquitous; more predominantly in brain (Wallace et al. 1980)	Yes (Ueki et al. 1992; Chang et al. 1994)	Yes (Vijayaraghavan et al. 1996; Huang et al. 2005; P. Morales, unpublished data)
PP4	Ubiquitous; more predominantly in lung, liver and kidney (Hu et al. 2001)	Yes, highly abundant (Hu et al. 2001; Kloeker et al. 2003)	ND
PP5	Ubiquitous; more predominantly brain (Becker et al. 1994; Chinkers 1994)	Yes (Becker et al. 1994)	ND
PP6	Ubiquitous; more predominantly heart and skeletal muscle (Bastians and Ponstingl 1996)	Yes, highly abundant (Bastians and Ponstingl 1996; Kloeker et al. 2003)	ND
PP7	Ubiquitous; more predominantly sensory organs (Andreeva and Kutuzov 2009)	Yes, highly abundant (Andreeva and Kutuzov 2009)	ND

Signorelli et al, 2011

CAPACITACIÓN EN EQUINOS



Leemans et al, 2019

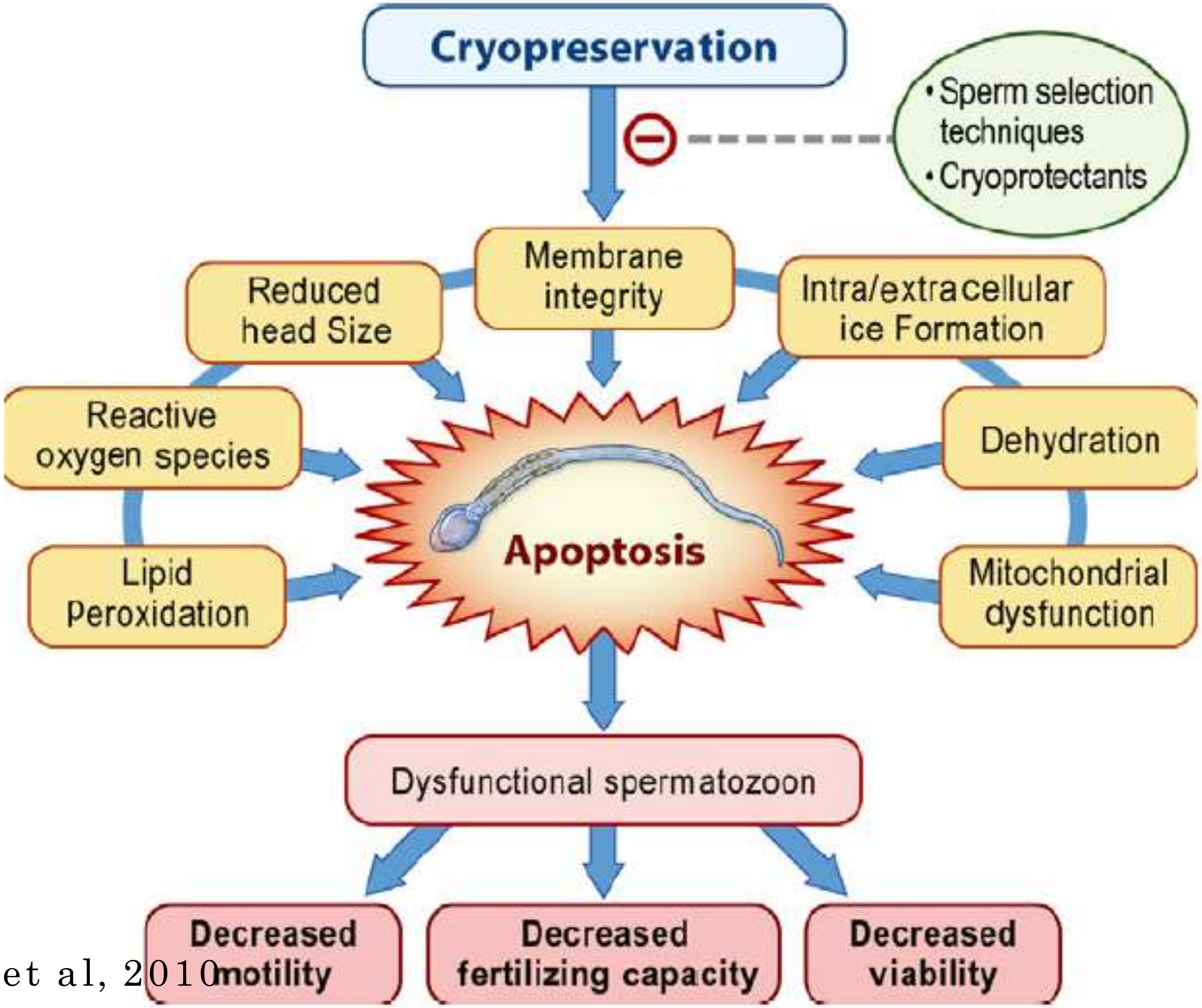
Table 1 Overview of capacitation triggers and their *in vitro* capacitation effect in different mammalian species.

Capacitating trigger	Capacitation effect	Species	Reference
Ca^{2+}	Membrane fluidity	Mouse	Visconti *et al.* (1995*a*,*b*)
	Protein tyrosine phosphorylation	Man	Osheroff *et al.* (1999)
	Hyperactivated motility	Pig	Flesch & Gadella (2000)
	Acrosome reaction	Cow	Byrd (1981), Breininger *et al.* (2010)
		Horse	McPartlin *et al.* (2008)
HCO_3^-	Membrane fluidity	Mouse	Visconti *et al.* (1995*a*,*b*)
	Protein tyrosine phosphorylation	Hamster	Visconti *et al.* (1999*b*)
	Hyperactivated motility	Man	Osheroff *et al.* (1999)
	Acrosome reaction	Pig	Flesch & Gadella (2000)
		Cow	Breininger *et al.* (2010)
		Horse	Rathi *et al.* (2003), McPartlin *et al.* (2008)
Albumin	Cholesterol depletion	Mouse	Visconti *et al.* (1995*a*,*b*)
		Pig	Flesch & Gadella (2000)
		Cow	Byrd (1981)
		Horse	McPartlin *et al.* (2008)
Methyl β-cyclodextrin	Cholesterol extraction	Mouse	Visconti *et al.* (1999*a*)
		Pig	van Gestel *et al.* (2005*b*)
		Horse	Bromfield *et al.* (2014)
Heparin	Membrane fluidity	Cow	Parrish *et al.* (1988), Gualtieri *et al.* (2005), Breininger *et al.* (2010)
	Hyperactivated motility		
	Protein tyrosine phosphorylation		

Leemans et al, 2019

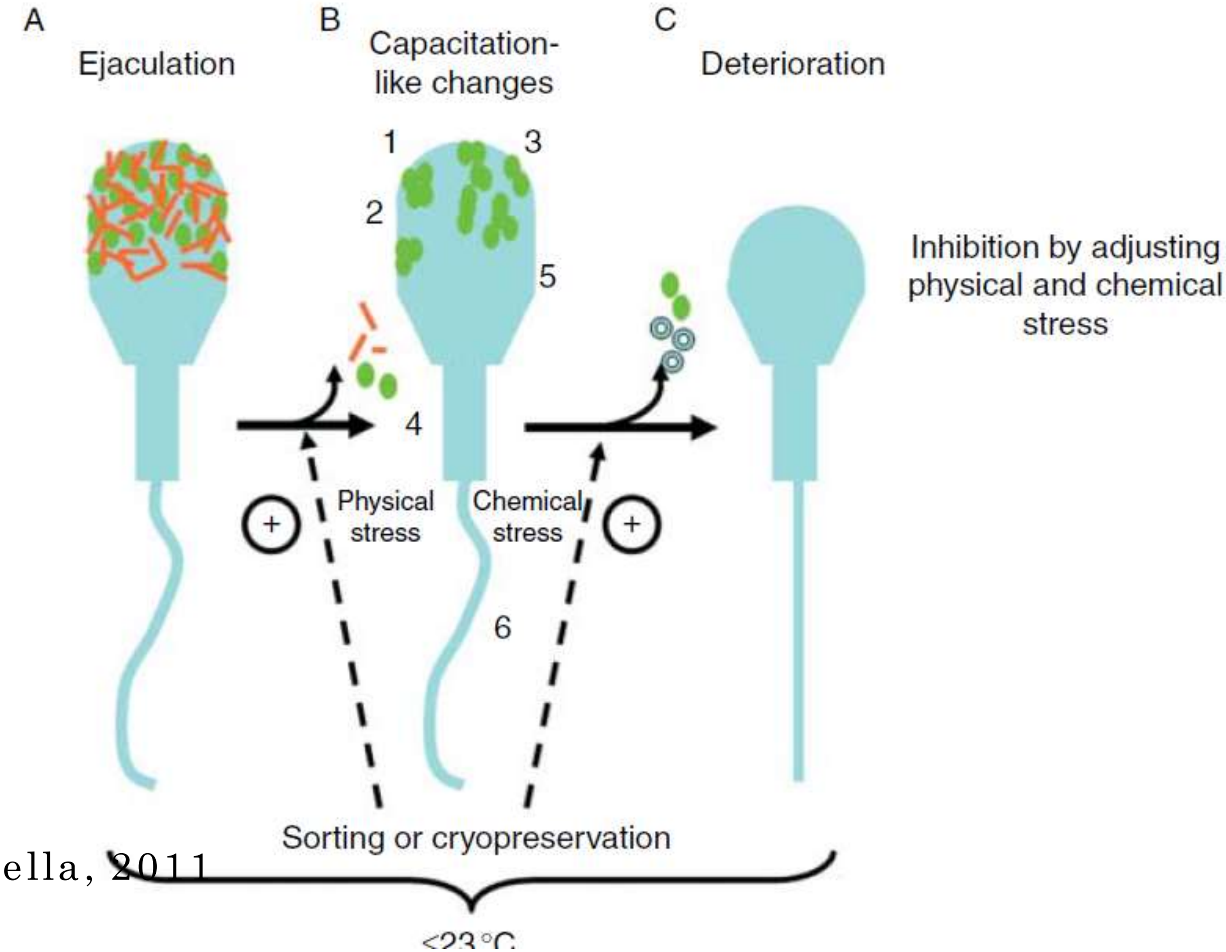
	Acrosome reaction		
Progesterone	Acrosome reaction	Horse	Cheng *et al.* (1998*a*,*b*)
	Hyperactivated motility	Man	Lishko *et al.* (2011)
Ca^{2+} ionophore A23187	Acrosome reaction	Mouse	Tateno *et al.* (2013)
		Man	Bielfeld *et al.* (1994), Liu *et al.* (2011)
		Pig	
		Cow	Birck *et al.* (2009)
		Horse	Fraser *et al.* (1995)
	Hyperactivated motility	Mice	Balao da Silva *et al.* (2013), Tateno *et al.* (2013)
Lysophospha-tidylcholine	Acrosome reaction	Horse	Graham (1996)
c-AMP and caffeine	Protein tyrosine phosphorylation	Cow	Breininger *et al.* (2010)
		Horse	Pommer *et al.* (2003)
	Hyperactivated motility	Pig	Funahashi & Nagai (2001)
ROS	Protein tyrosine phosphorylation	Cow	Breininger *et al.* (2010)
		Horse	Baumber *et al.* (2003)
Alkaline medium pH	Protein tyrosine phosphorylation	Horse	Gonzalez-Fernandez *et al.* (2012)
	Hyperactivated motility	Cow	Marquez & Suarez (2007)
Procaine	Hyperactivated motility	Guinea pig	Mujica *et al.* (1994)
		Horse	McPartlin *et al.* (2009), Leemans *et al.* (2015*a*)

Leemans et al, 2019



Said et al, 2010

CRIOCAPACITACIÓN



Leahy and Gadella, 2011

CRIOCAPACITACIÓN

*Sperm changes associated with capacitation and cryopreservation**

Capacitation	Cryopreservation
CTC fluorescence pattern B	CTC fluorescence pattern B
Plasma membrane reorganization and fluidization	Plasma membrane reorganization and destabilization
Elevated intracellular calcium	Elevated intracellular calcium
Generation of reactive oxygen species	Generation of reactive oxygen species
cAMP-mediated protein tyrosine phosphorylation	Appearance of tyrosine-phosphorylated proteins
Able to fertilize oocytes in vitro	Able to fertilize oocytes in vitro

* CTC indicates chlortetracycline; cAMP, cyclic adenosine monophosphate.

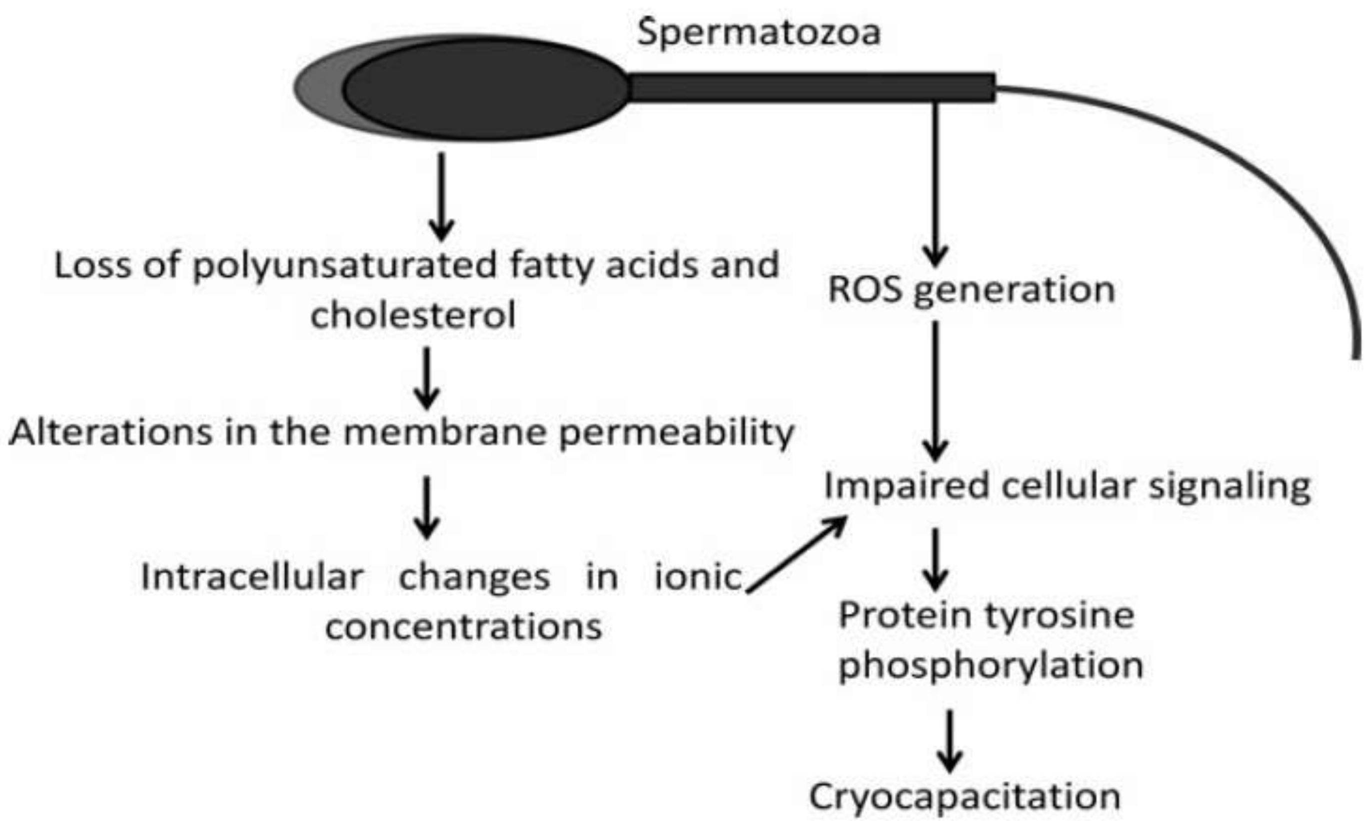
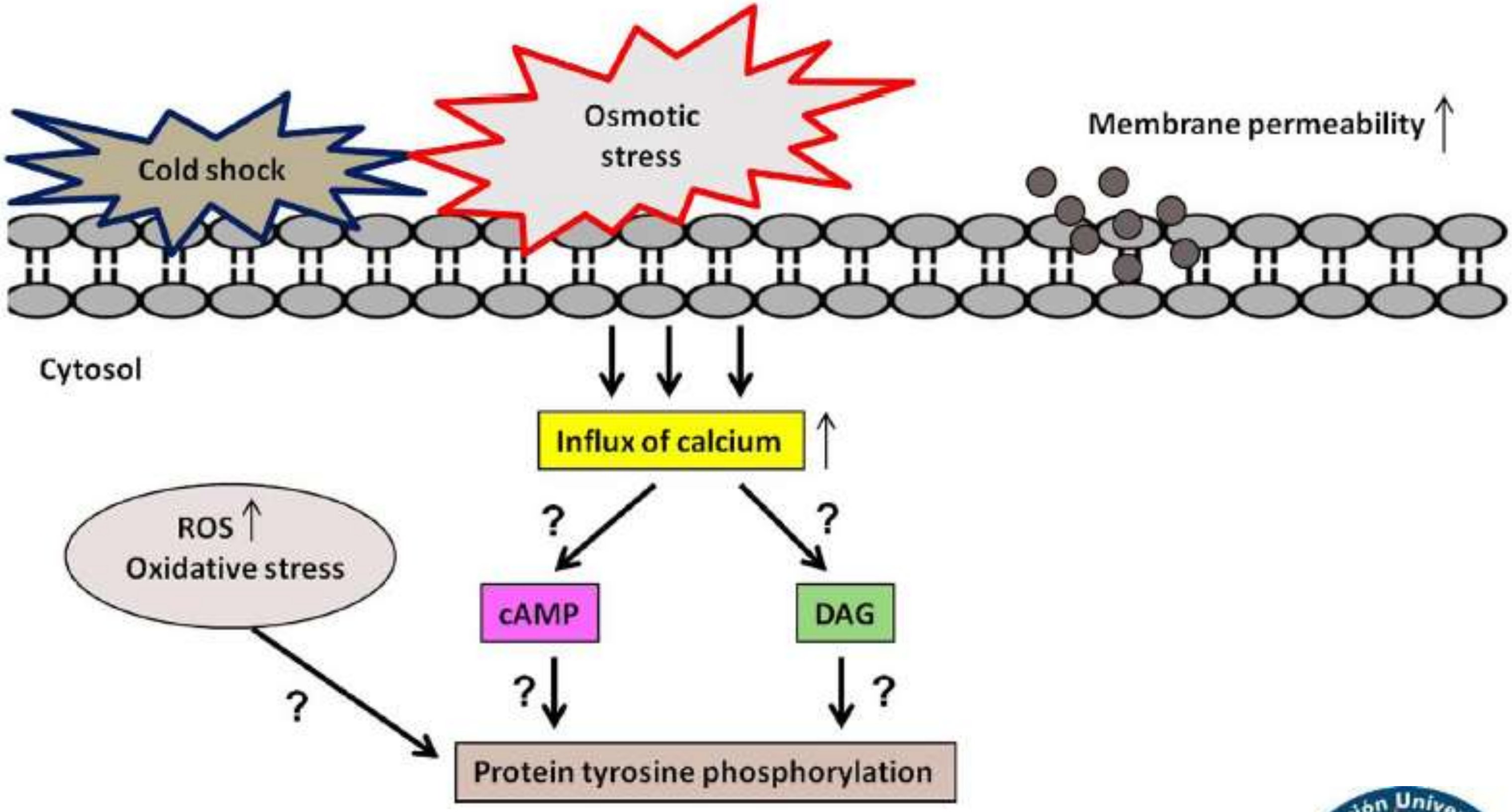


Fig. 1. Diagrammatic representation of cryopreservation induced cryocapacitation

SEÑALIZACIÓN EN CRIOAPACITACIÓN



Naresh et al, 2015

CÓMO MEDIMOS CRIOCAPACITACIÓN

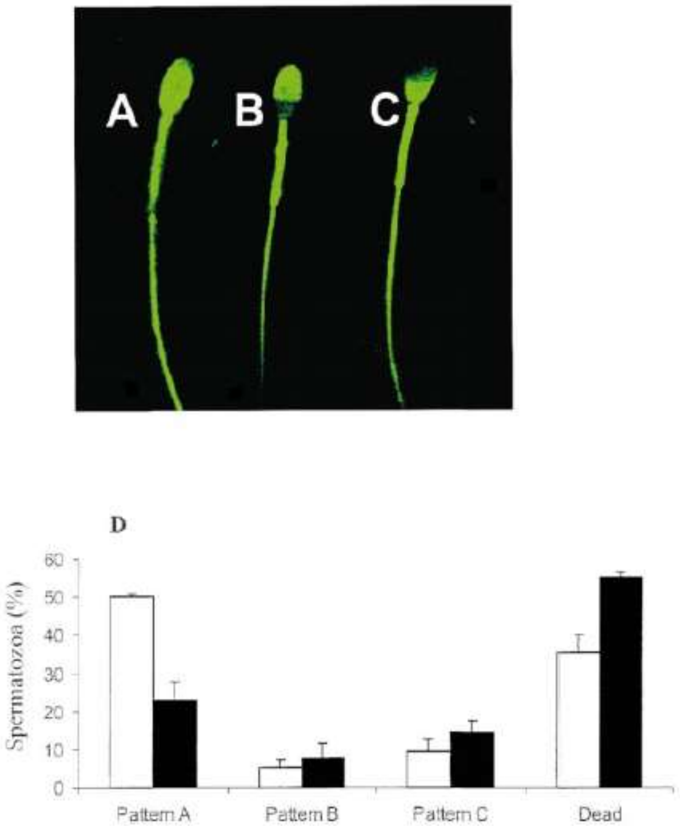


FIG. 1. The three patterns obtained for CTC-stained viable spermatozoa. **A**) Pattern A: whole sperm head shows bright fluorescence, with or without a brighter equatorial band; this is indicative of a noncapacitated spermatozoa. **B**) Pattern B: the acrosomal region of the sperm head fluoresce brightly but the postacrosomal region does not; this denotes a capacitated, acrosome-intact spermatozoa. **C**) Pattern C: the acrosomal region of the sperm head is nonfluorescent, with or without a fluorescent, postacrosomal region; this indicates a capacitated, acrosome-reacted spermatozoa. Sperm head length ~7.0 μm. **D**) A bar chart demonstrating the mean (± SD) percentage of viable sperm fluorescing in the various CTC staining patterns after 0 h (unshaded) and 5 h (shaded) of incubation.

CÓMO MEDIMOS CRIOAPACITACIÓN

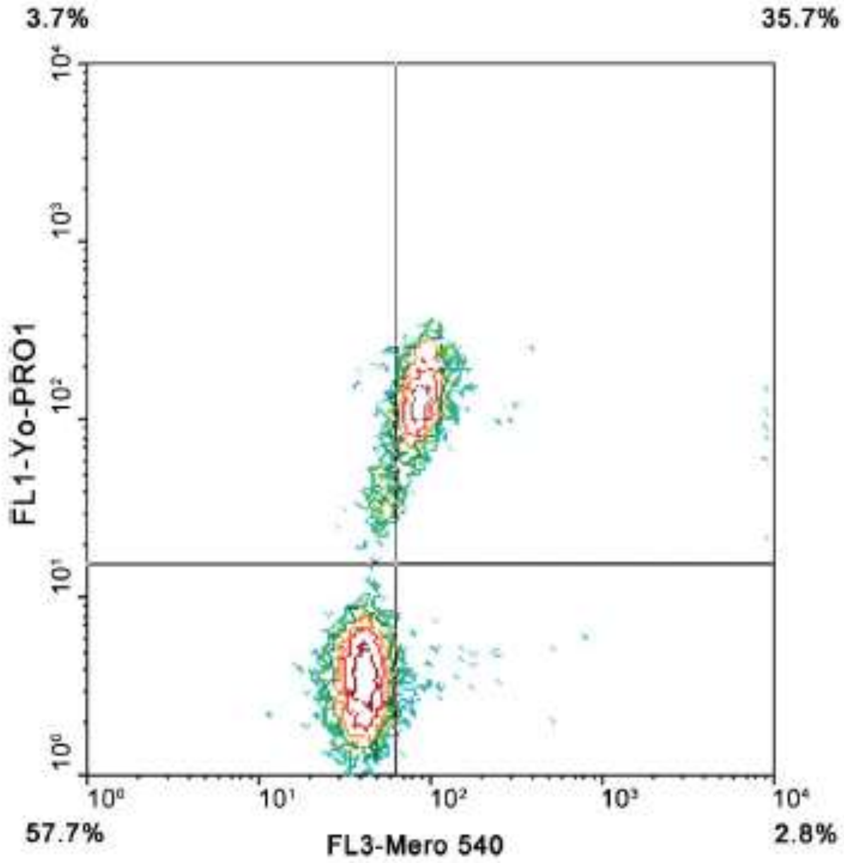


Figure 8 Plasma membrane asymmetry in a bull sperm sample measured by Merocyanine 540/YO-PRO-1 labeling. Contour plot shows viable cells with stable plasma membrane (lower left quadrant), viable cells with destabilized plasma membrane (lower right quadrant) and dead spermatozoa (upper left and right quadrants).

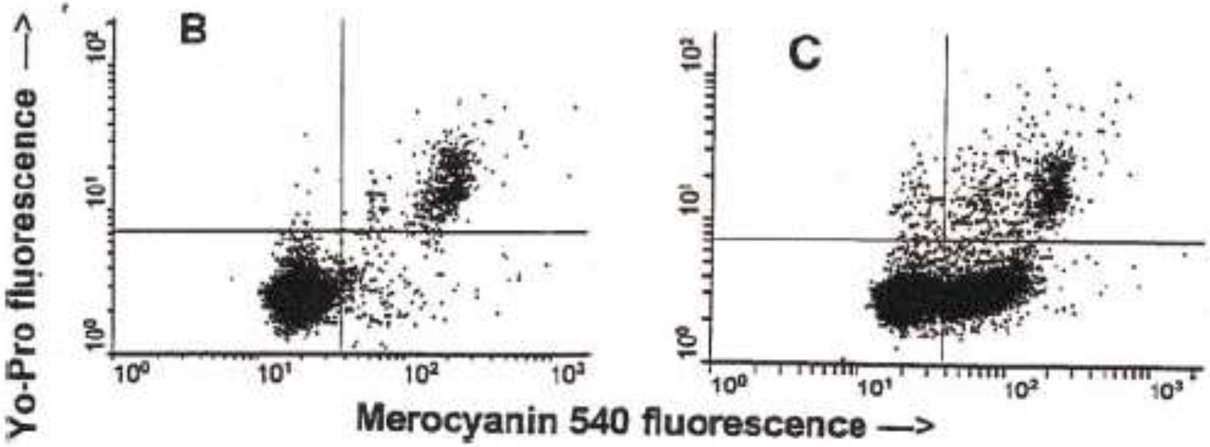
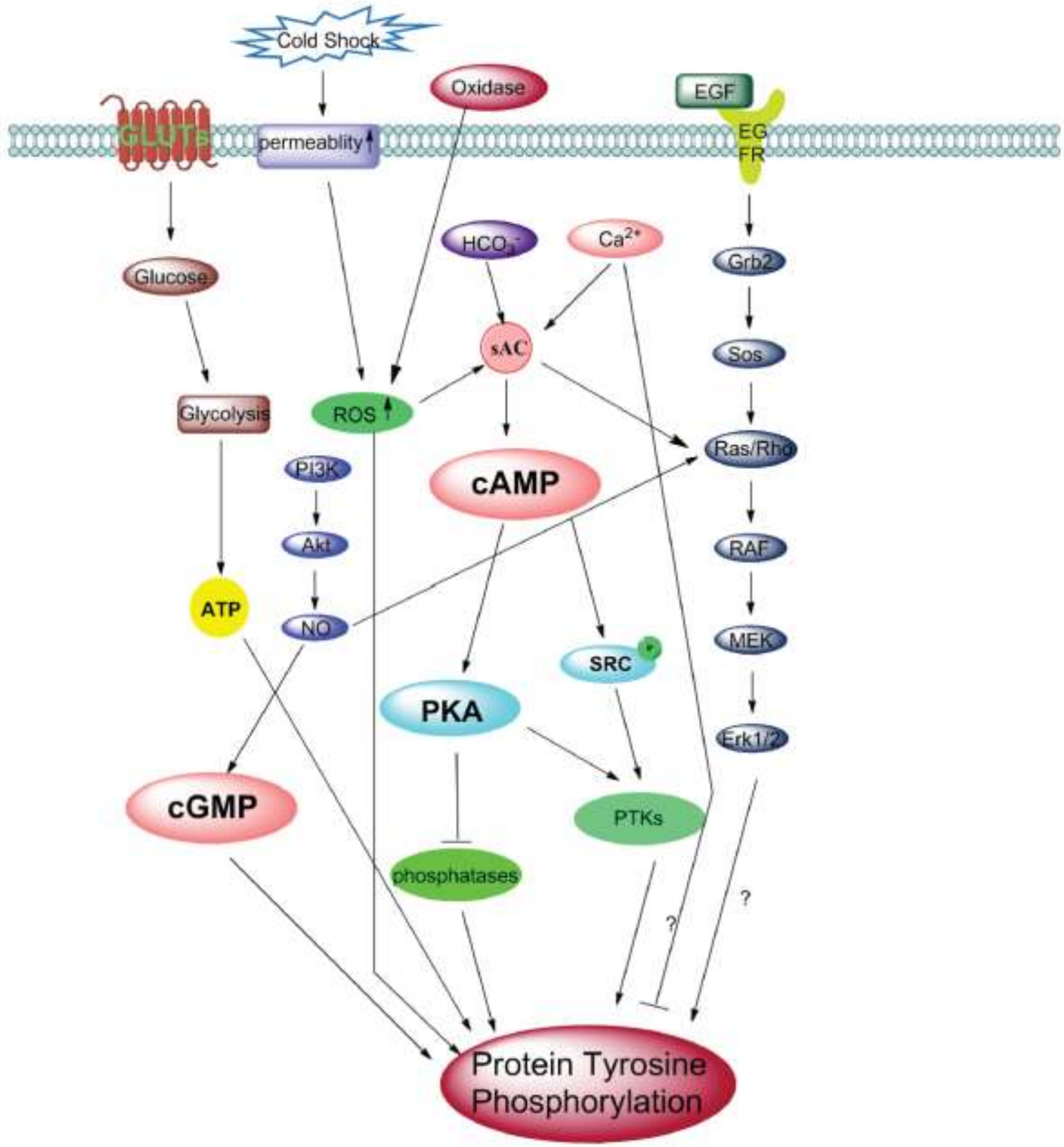


FIG. 2. The two patterns obtained for viable sperm stained with merocyanine 540. **A**) The highly fluorescent sperm heads demonstrate capacitated spermatozoa, and the poorly fluorescent heads demonstrate noncapacitated spermatozoa. Sperm head length ~7.0 µm. Scatter plots for the amount of fluorescence detected flow cytometrically for sperm from a single ejaculate stained with merocyanine 540 and Yo-Pro-1 after 0 h (**B**) and 5 h (**C**) of incubation in Tyr+bic medium. The X-axis depicts the amount of fluorescence emitted by merocyanine 540 probe that was bound to individual sperm cells as measured in arbitrary units by the FL-1 detector, and the Y-axis depicts the amount of fluorescence in arbitrary units emitted by the Yo-Pro-1 probe that was bound to individual sperm cells by the FL-3 detector. In both plots, sperm cells that fluoresced more intensely with Yo-Pro-1 than the superimposed horizontal line were considered to be nonviable, whereas the viable sperm cells with higher merocyanine 540-depended fluorescence than the superimposed vertical line were considered to be capacitated.


Cold Shock
Oxidase
EGF
GLUTs
permeablity
EG FR
HCO3-
Ca2+
Glucose
Grb2
sAC
Sos
Glycolysis
ROS
Ras/Rho
PI3K
cAMP
Akt
RAF
ATP
NO
MEK
SRC
PKA
Erk1/2
cGMP
PTKs
phosphatases
?
?
Protein Tyrosine Phosphorylation

CONCLUSIONES

- El espermatozoide necesita de las ROS, $HCO3^{-}$ y Ca^{++} para la capacitación

- La criopreservación causa un aumento del estrés oxidativo

- El estrés oxidativo y el shock frio causan criocapacitación

- La criocapacitación reduce la longevidad del espermatozoide y por ende su capacidad fertilizante