

Fecundación en mamíferos  
Eventos Celulares y Moleculares.  
Miguel Llanos  
Universidad de Chile  
22-10-04

# Interacción Espermatozoide-Oocito e Inducción de la RA

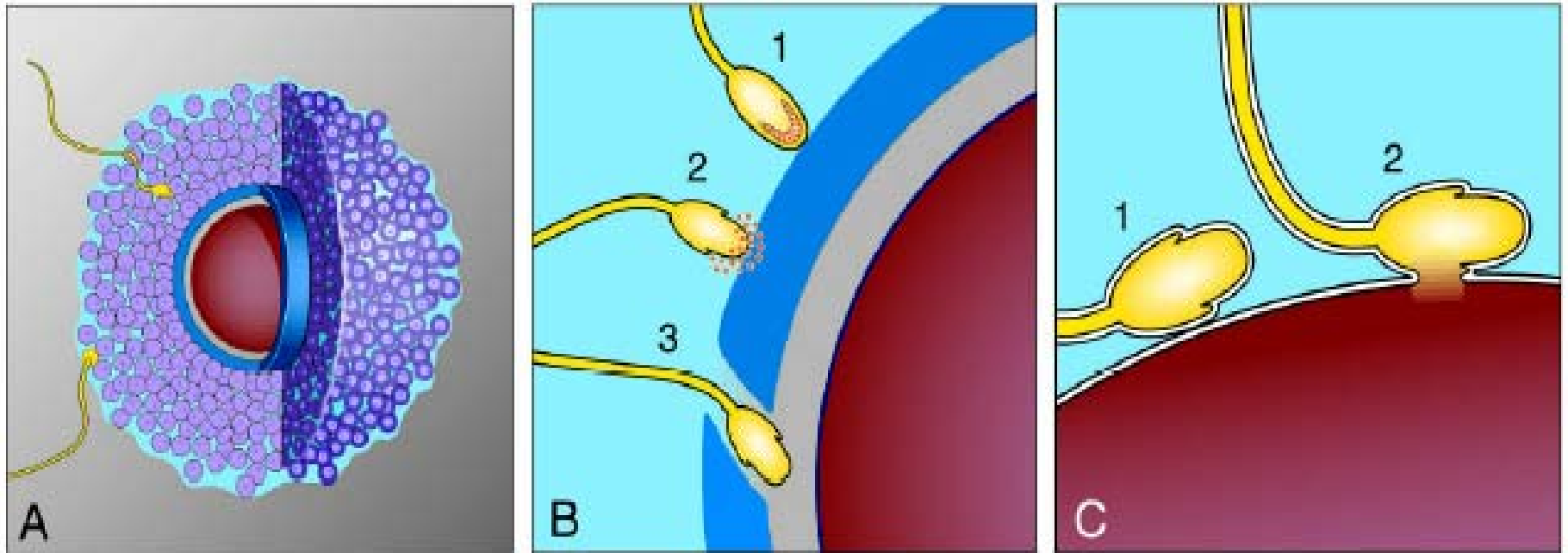
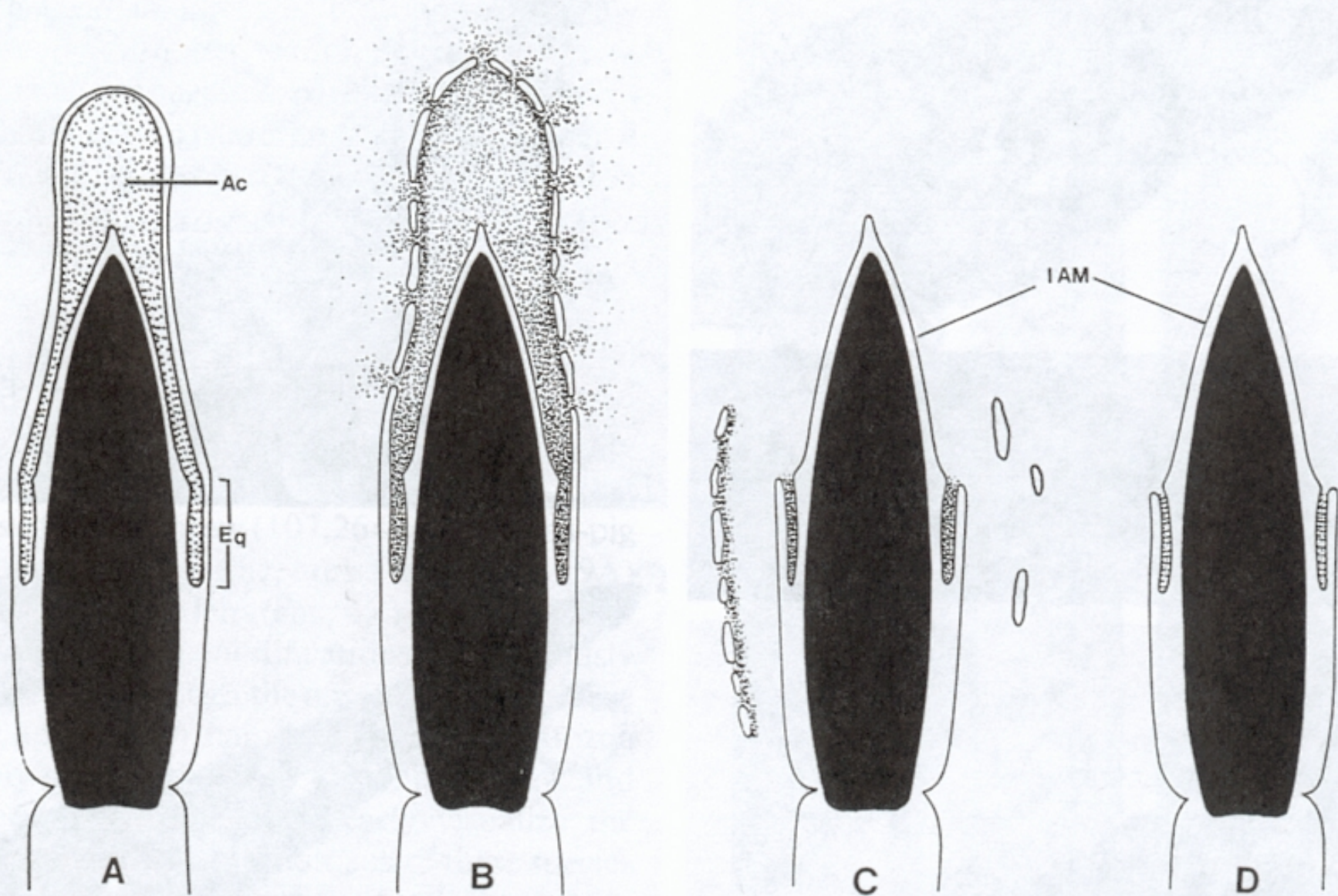


Fig. 1. (A) Sperm penetration of cumulus cells (purple) to reach zona (navy blue). (B) Egg depicted with cumulus cells removed; sperm 1 binds to the zona pellucida (navy blue); sperm 2 undergoes exocytosis, releasing acrosomal contents (orange-red); sperm 3 penetrates the

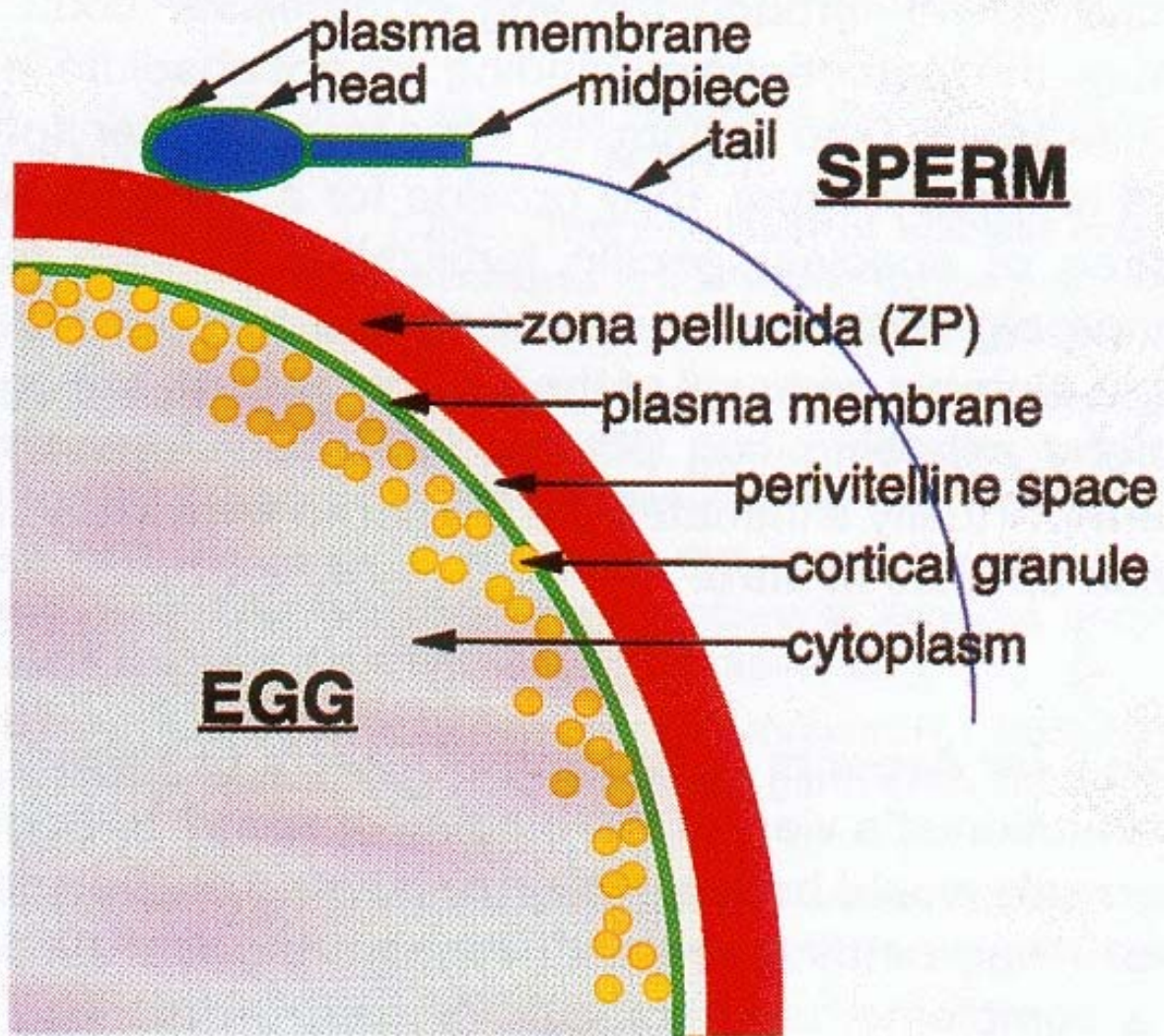
zona pellucida and begins entry into perivitelline space (gray). (C) Sperm 1 binds to the egg plasma membrane by the side of its head, in a central region (equatorial region); sperm 2 fuses with the egg plasma membrane.



**FIG. 16.** Diagrams illustrating the progression of the acrosome reaction. **(A)** Before the reaction. **(B)** The reaction in progress; multiple fusions between the plasma and outer acrosomal membrane allow the release or exposure of acrosomal contents (enzymes). **(C–D)** The reaction is completed; vesiculated membranes are held together by a “sticky” acrosomal matrix or disperse. *Ac*, Acrosomal cap; *Eq*, equatorial segment; *IAM*, inner acrosomal membrane. (Modified slightly from ref. 530.)

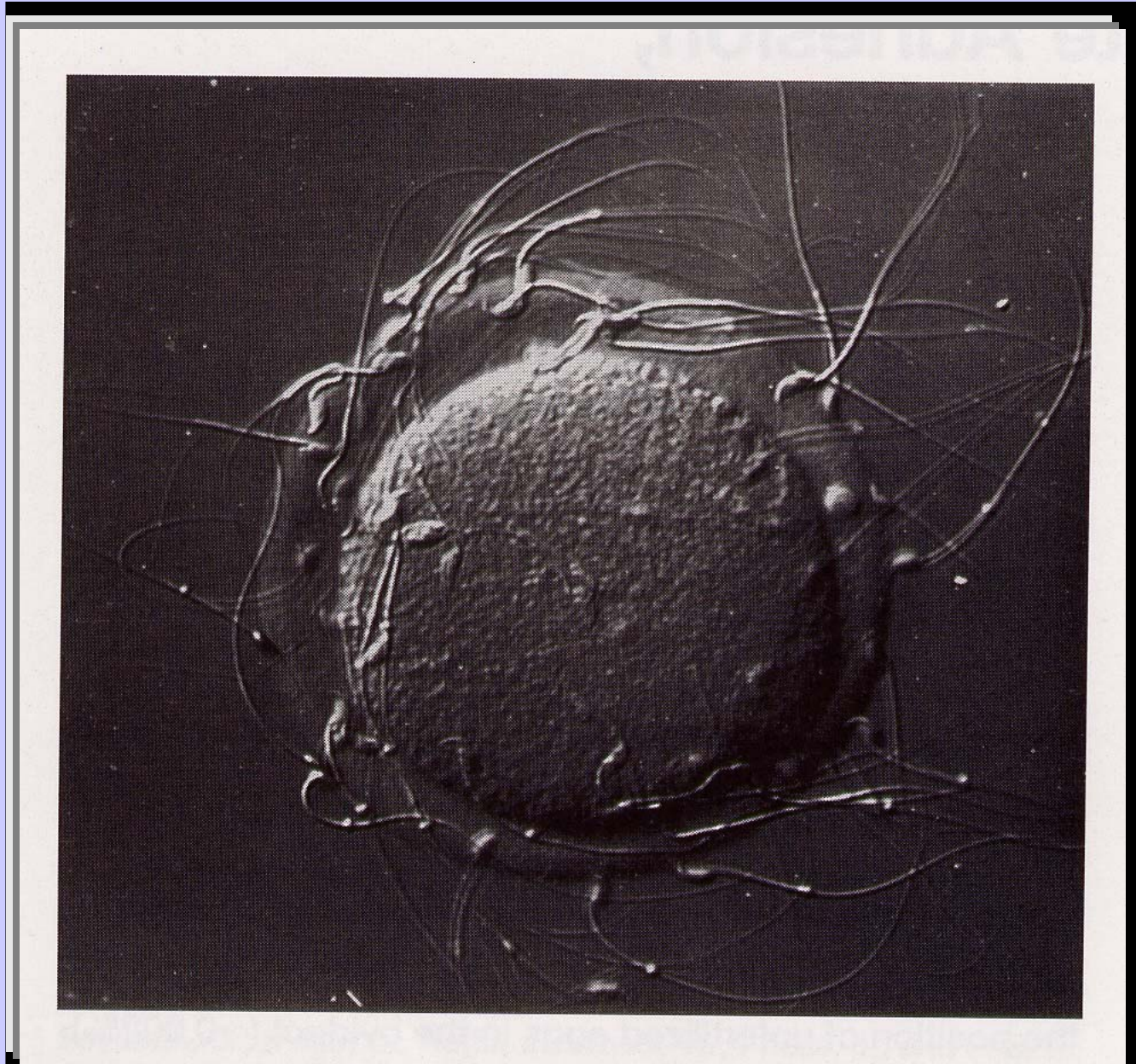


## Binding of Acrosome-Intact Sperm to Egg ZP



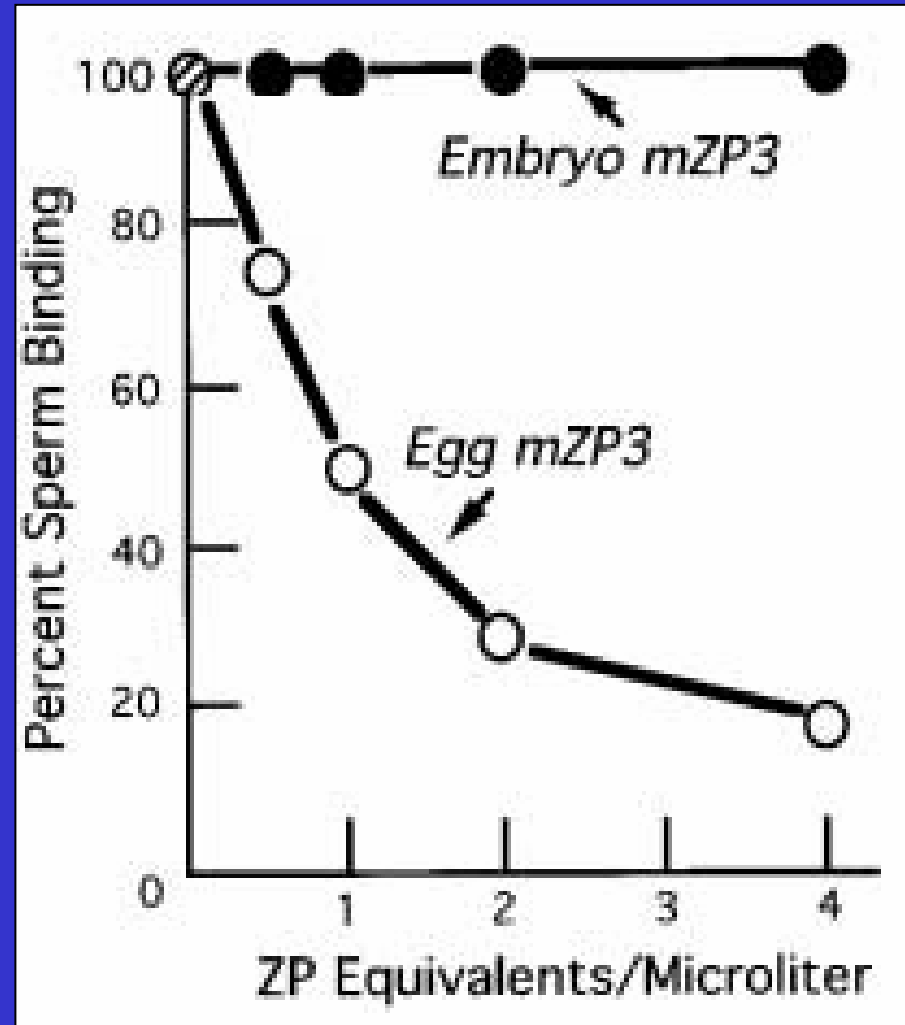
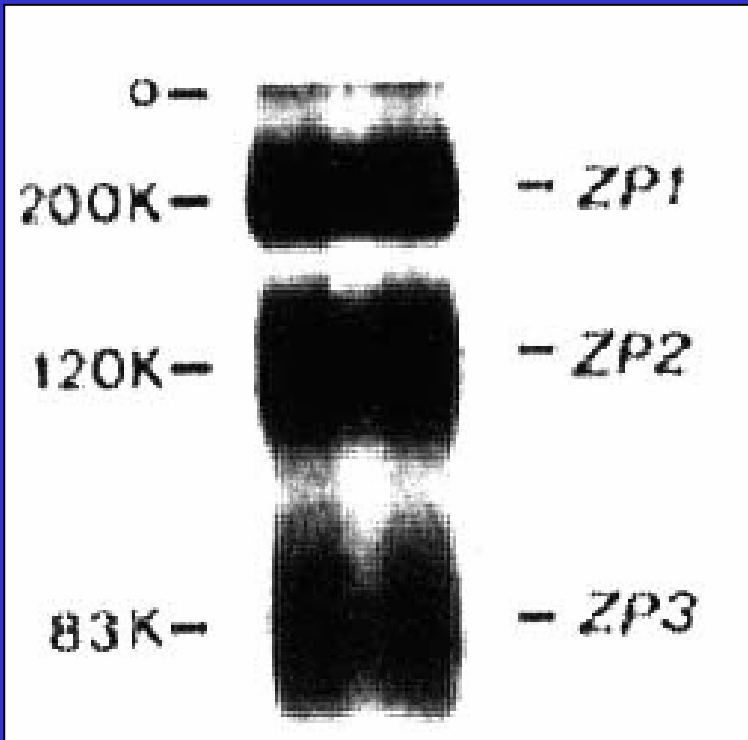


# Interacción Espermatozoide-Zona (Nomarski)



## Competencia mZP3 soluble y unión al oocito

### Glicoproteínas de la ZP





## Binding of Sperm to Zona Pellucida Filaments

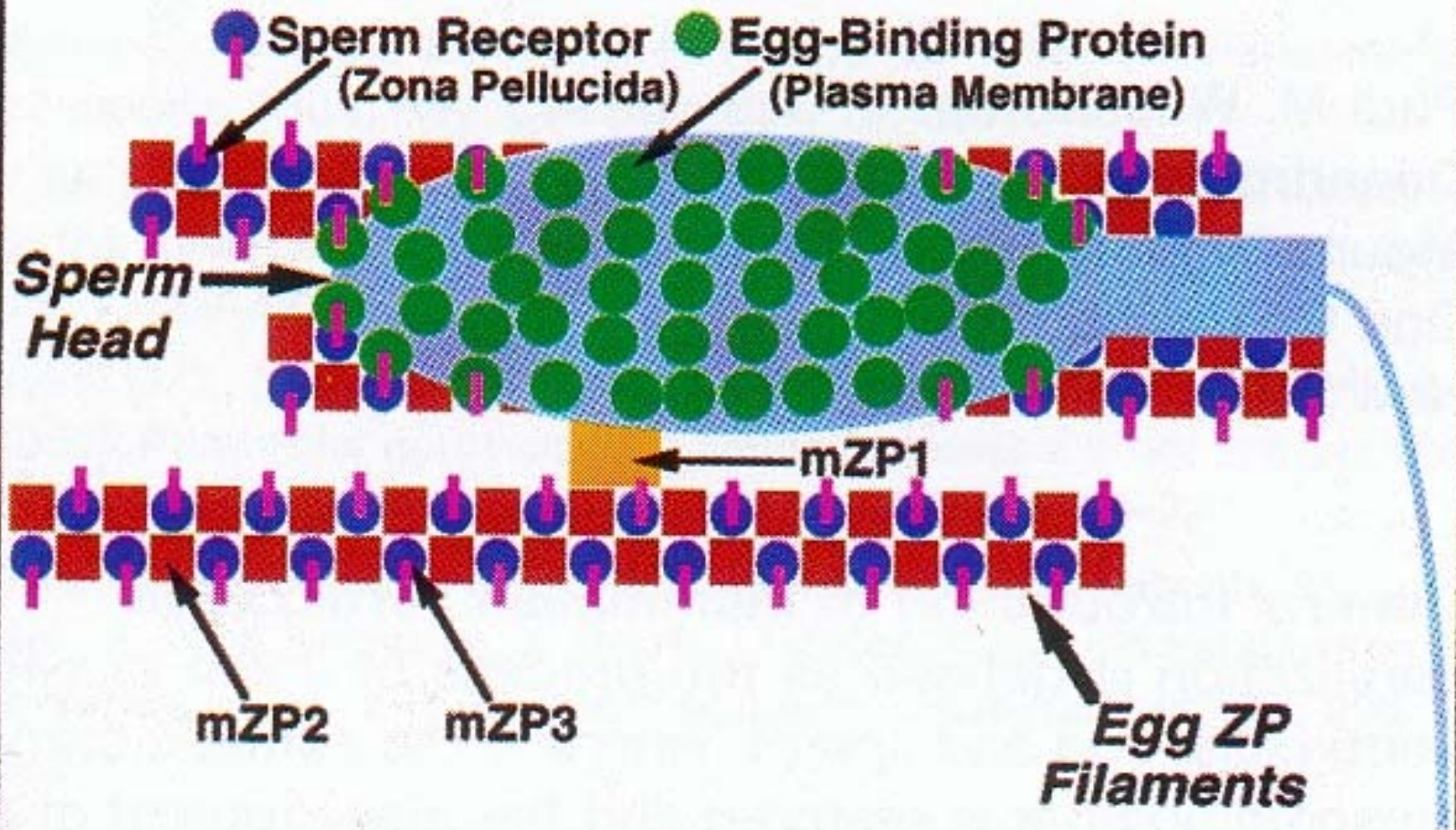


Table 1. Gamete surface proteins and zona pellucida proteins with reported gene knockout.

Gamete protein	KO phenotype: Major features
Sperm protein	
Galactosyl transferase	Fertile males; ZP3-induced acrosome reaction is defective; increase in sperm binding to zona (10)
Egg protein	
ZP1, ZP2	Infertile females; structurally defective zona (43, 44)
ZP3	Infertile females; no zona made (45, 46)



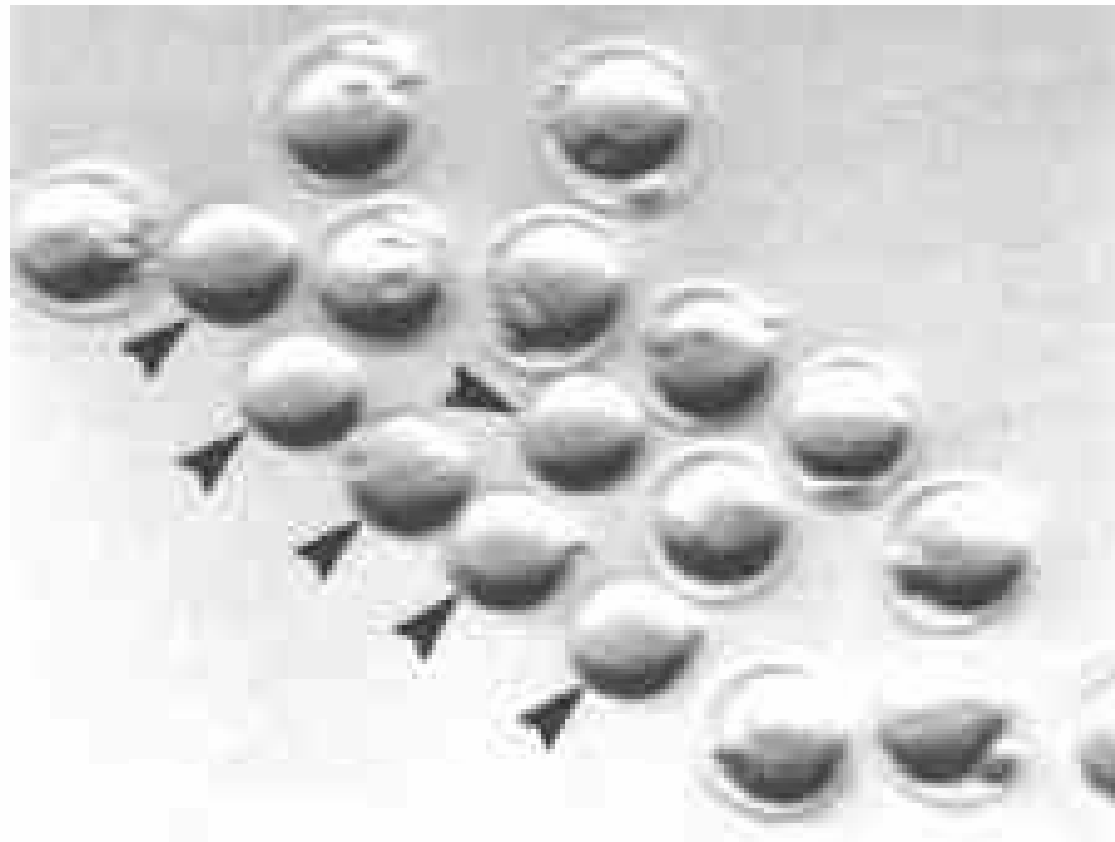


Fig. 8. Ovulated eggs obtained from mice homozygous null for *mZP3* lack a ZP. Shown are six ovulated eggs lacking a ZP recovered from *mZP3*<sup>-/-</sup> mice (arrowheads) and twelve ovulated eggs which have a ZP recovered from wild-type (*mZP3*<sup>+/+</sup>) mice. The light photomicrograph was taken using Nomarski differential interference contrast microscopy. (magnification ~13.5X).

# **INDUCTORES FISIOLÓGICOS DE LA REACCIÓN ACROSÓMICA**

- **LA ZONA PELUCIDA DEL OOCITO (ZP3)**
- **LA HORMONA ESTEROIDAL PROGESTERONA**

# ESPERMATOZOIDE

Capacitación

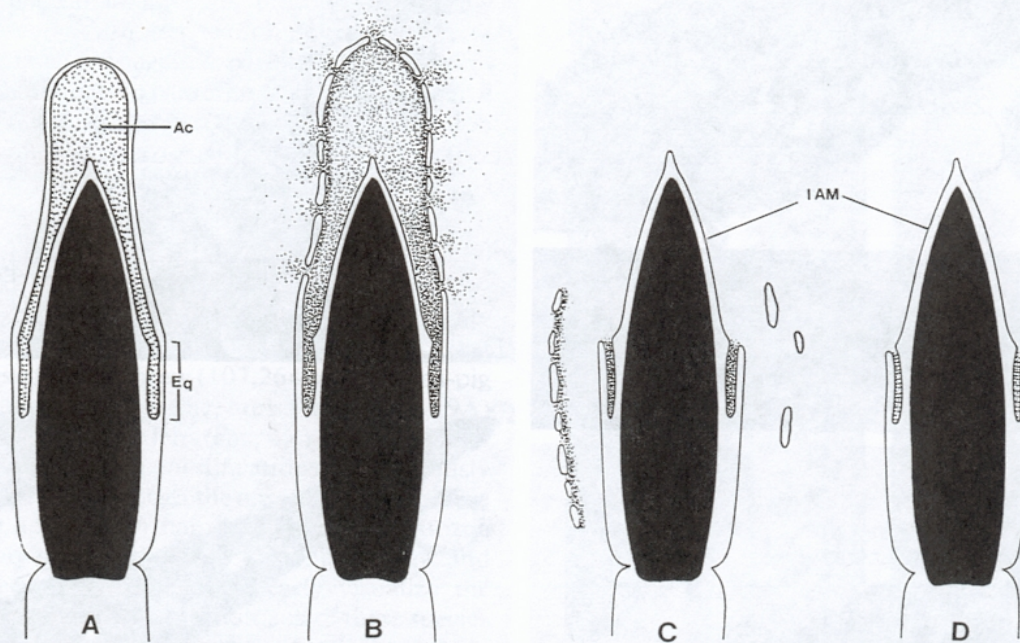
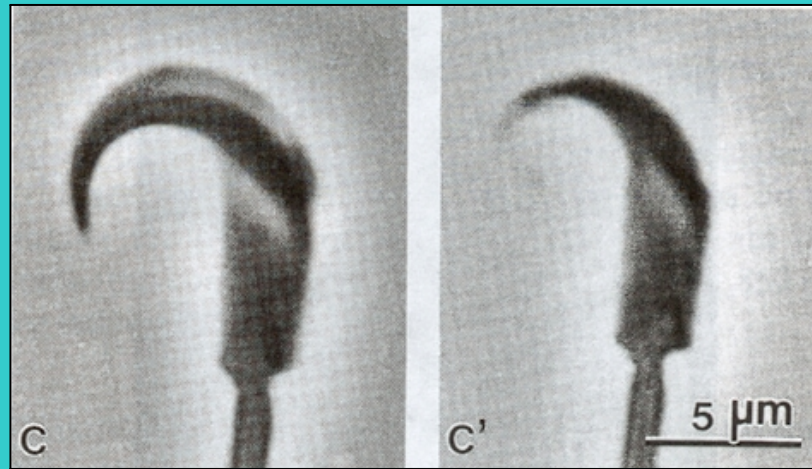


Acrosoma



Fecundación

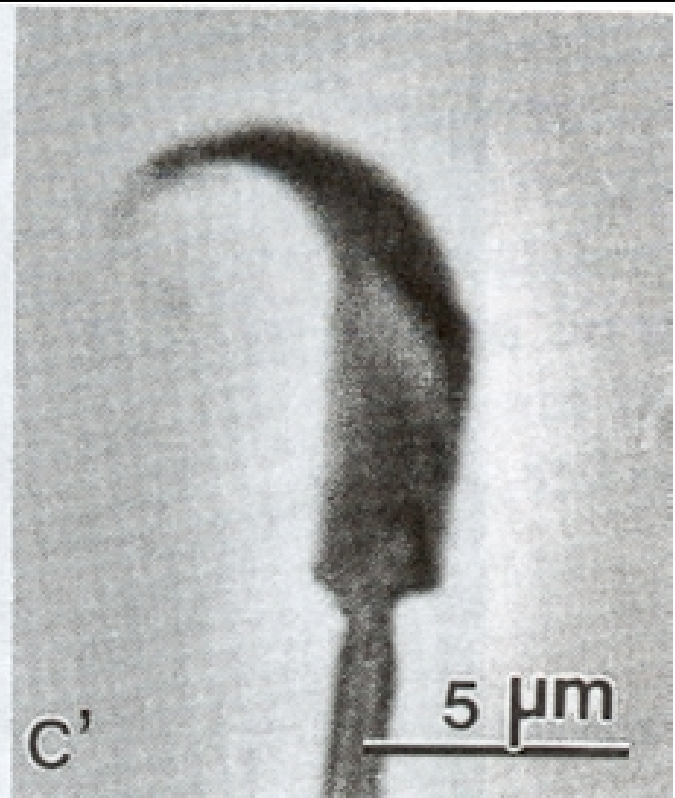




**FIG. 16.** Diagrams illustrating the progression of the acrosome reaction. (A) Before the reaction. (B) The reaction in progress; multiple fusions between the plasma and outer acrosomal membrane allow the release or exposure of acrosomal contents (enzymes). (C–D) The reaction is completed; vesiculated membranes are held together by a “sticky” acrosomal matrix or disperse. *Ac*, Acrosomal cap; *Eq*, equatorial segment; *IAM*, inner acrosomal membrane. (Modified slightly from ref. 530.)



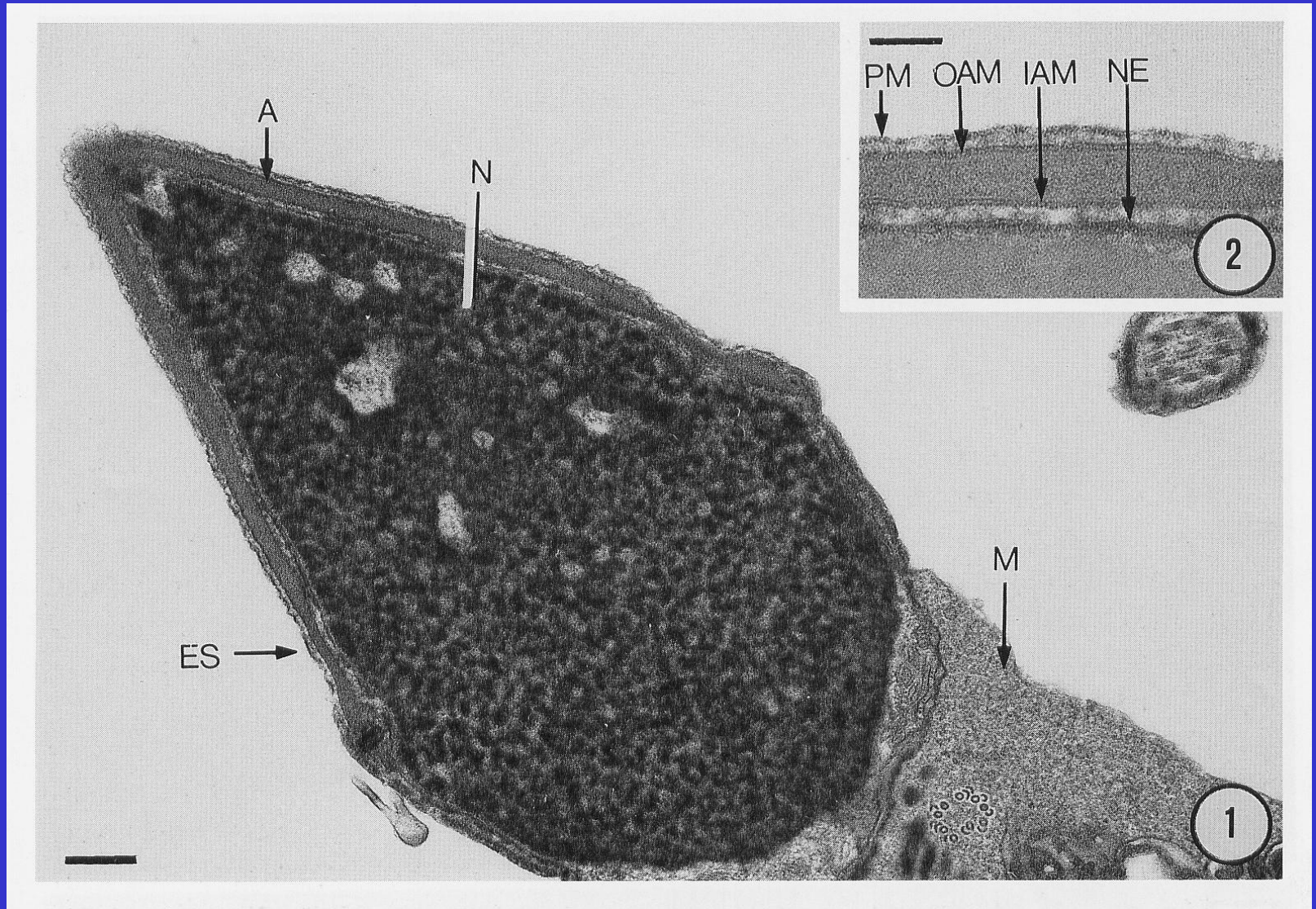
Intacto



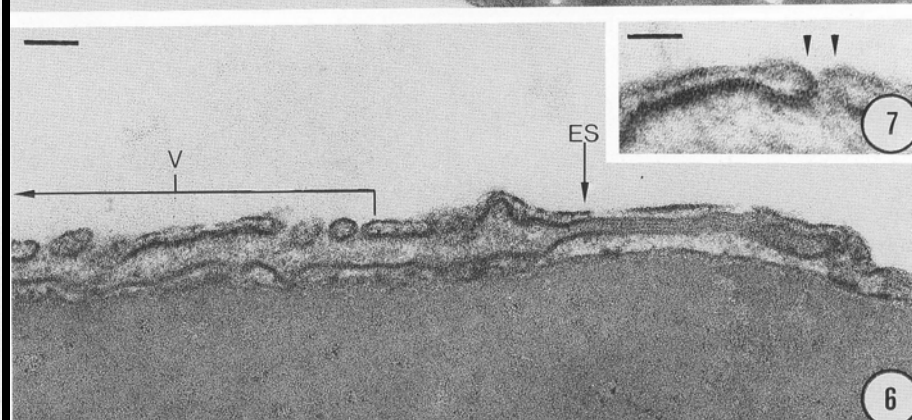
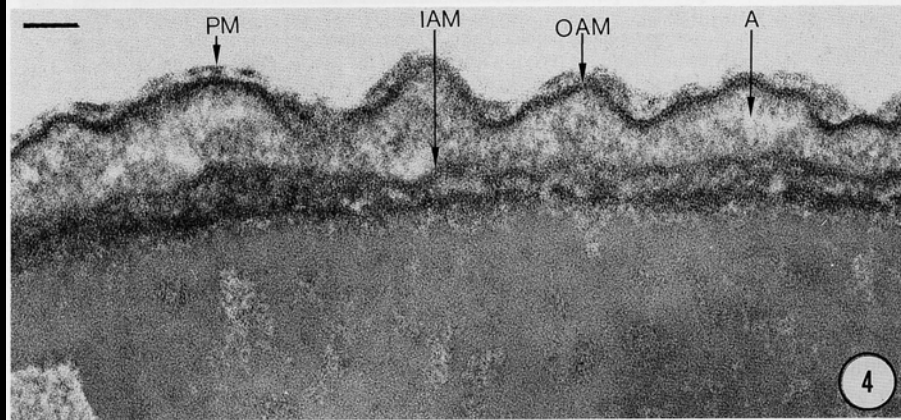
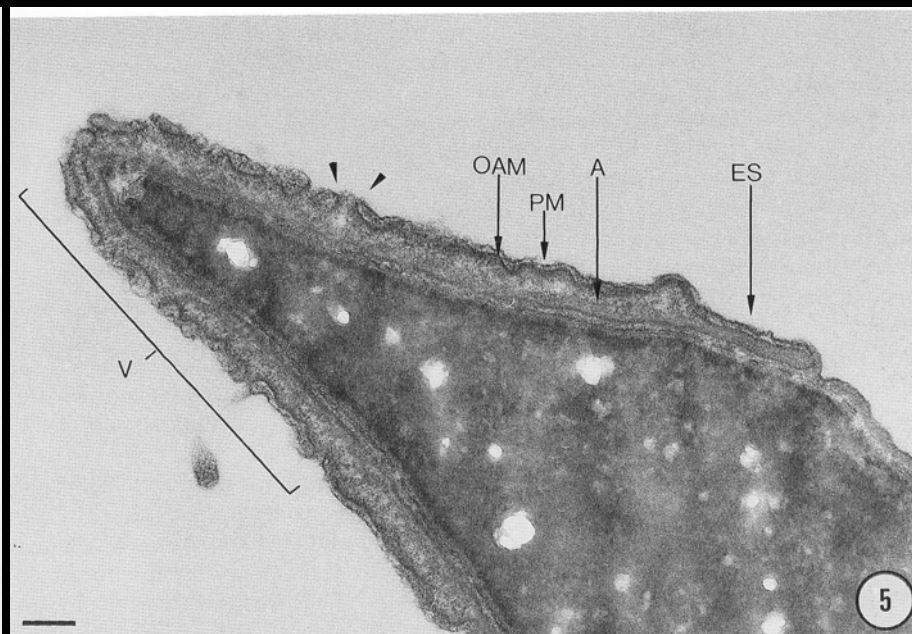
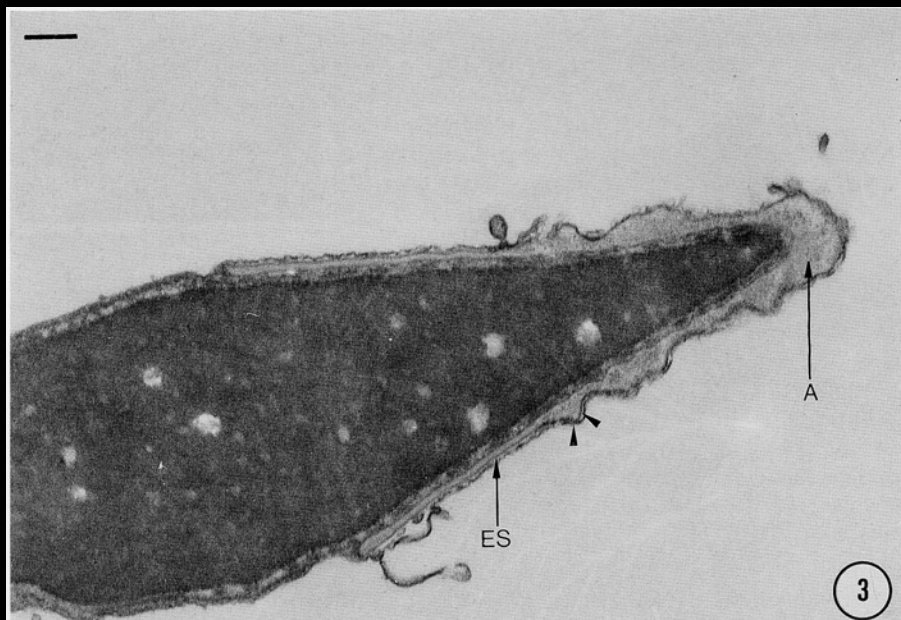
Reaccionado

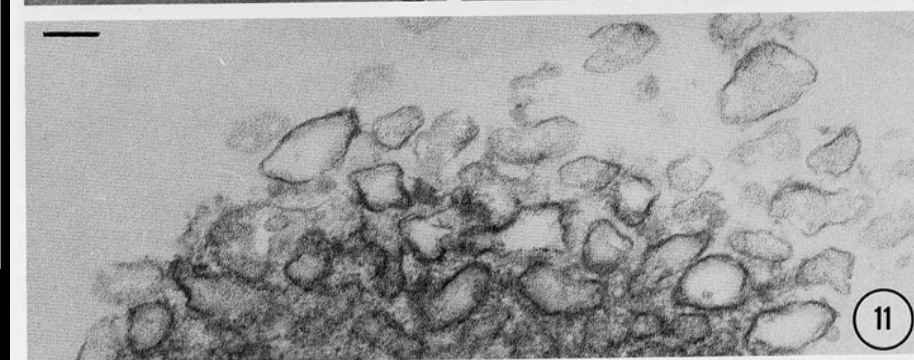
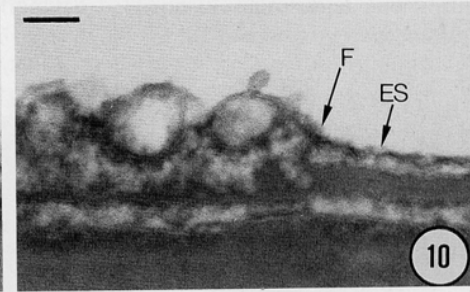
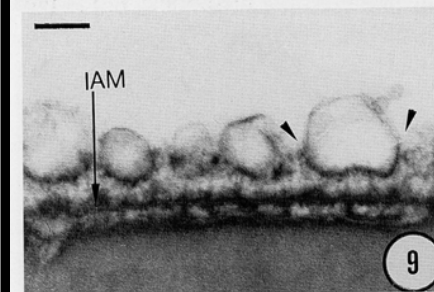
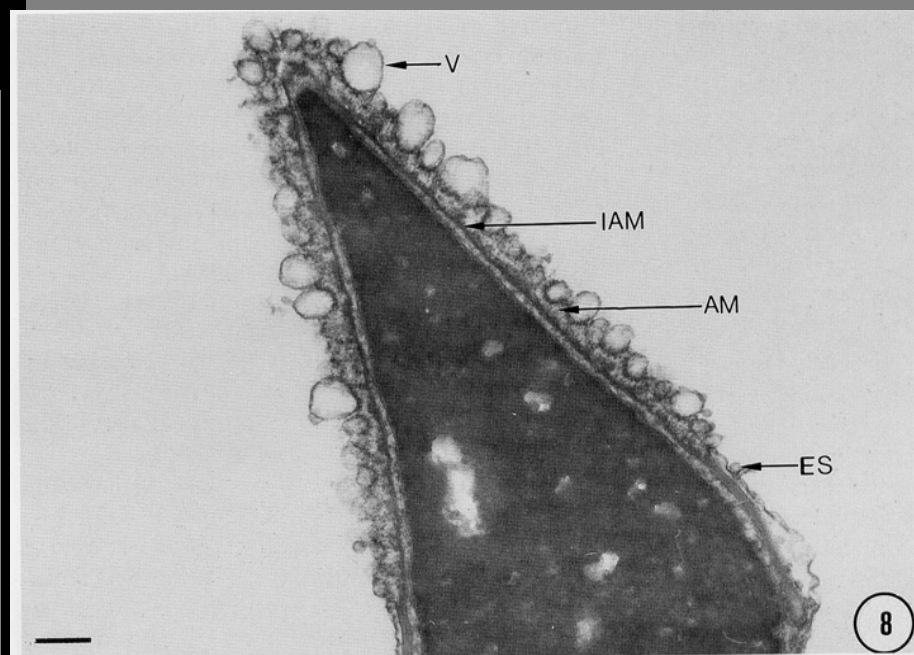
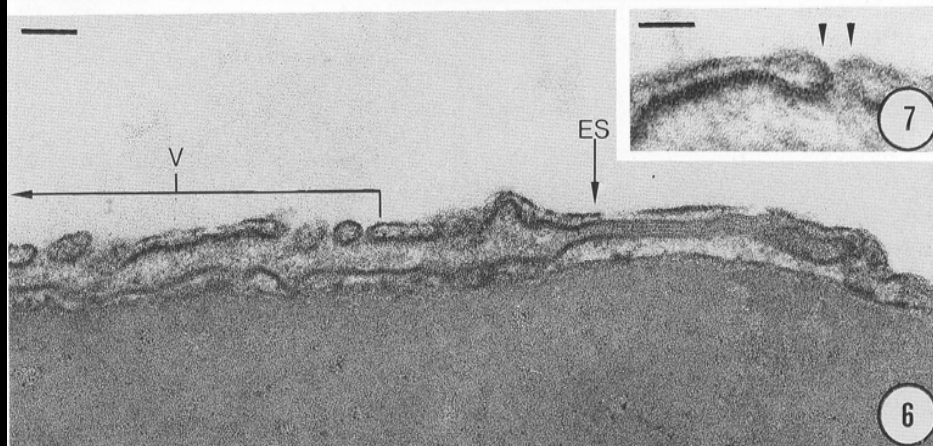
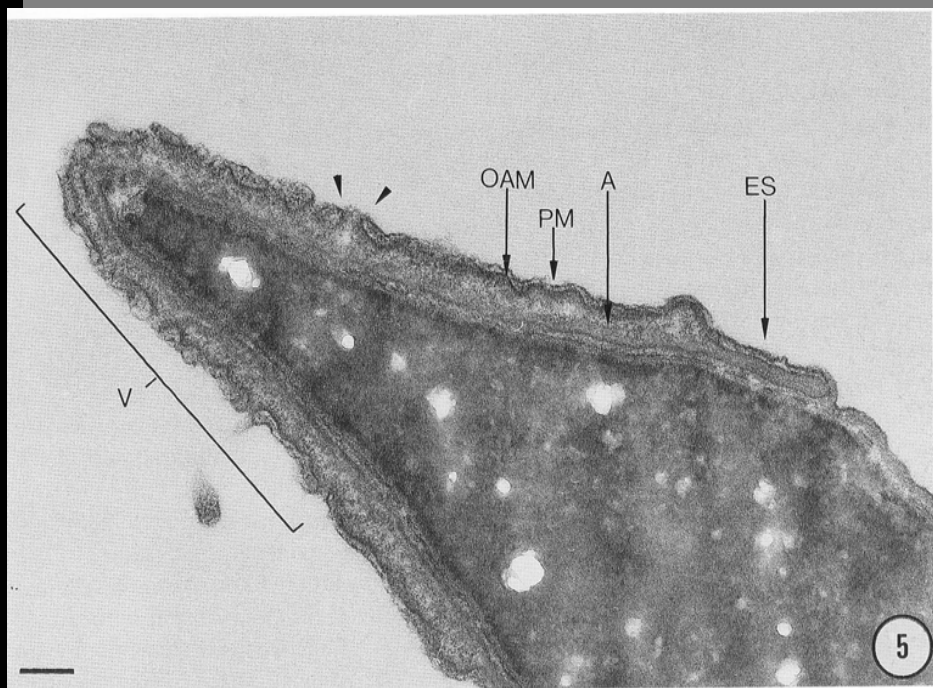


# Comienzo Secuencia RA Estimulada por hFF Pre-ovulatorio en Espermatozoides Humanos

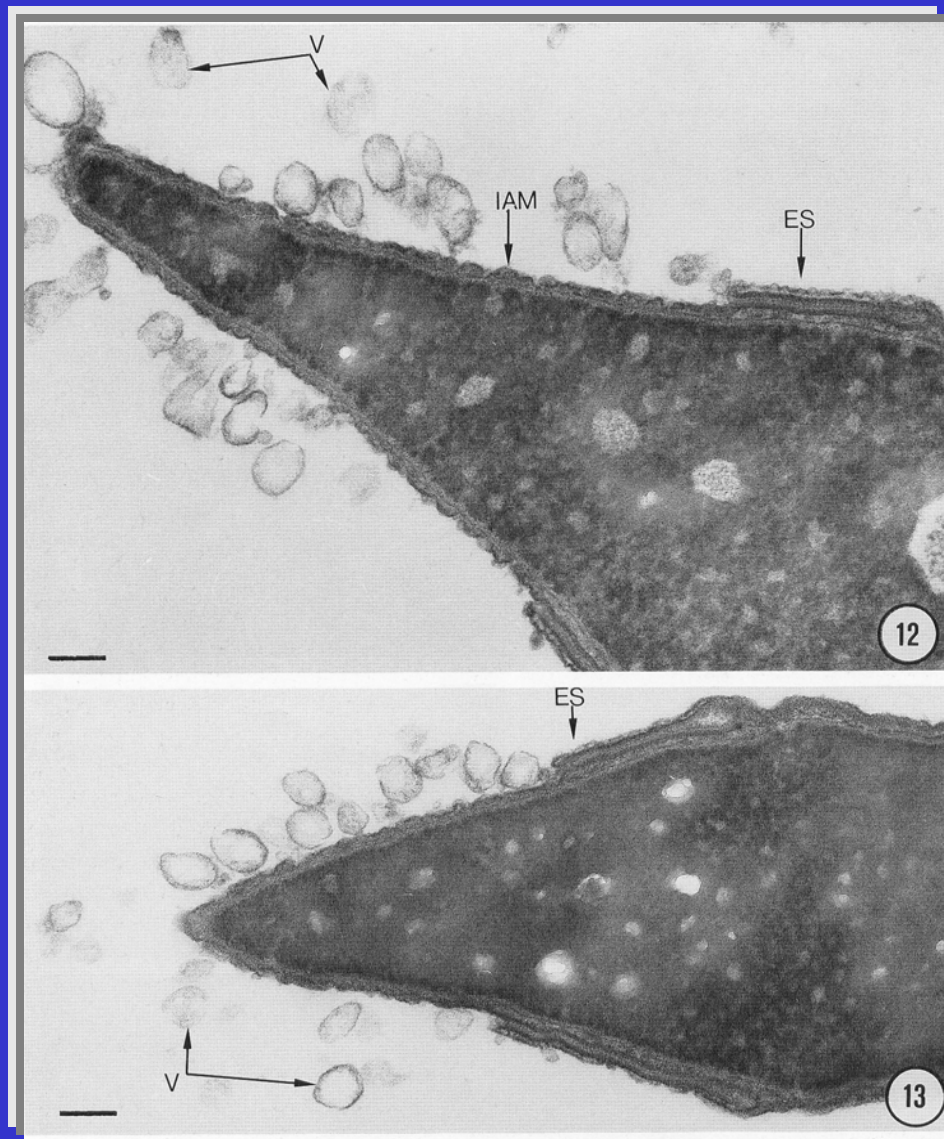
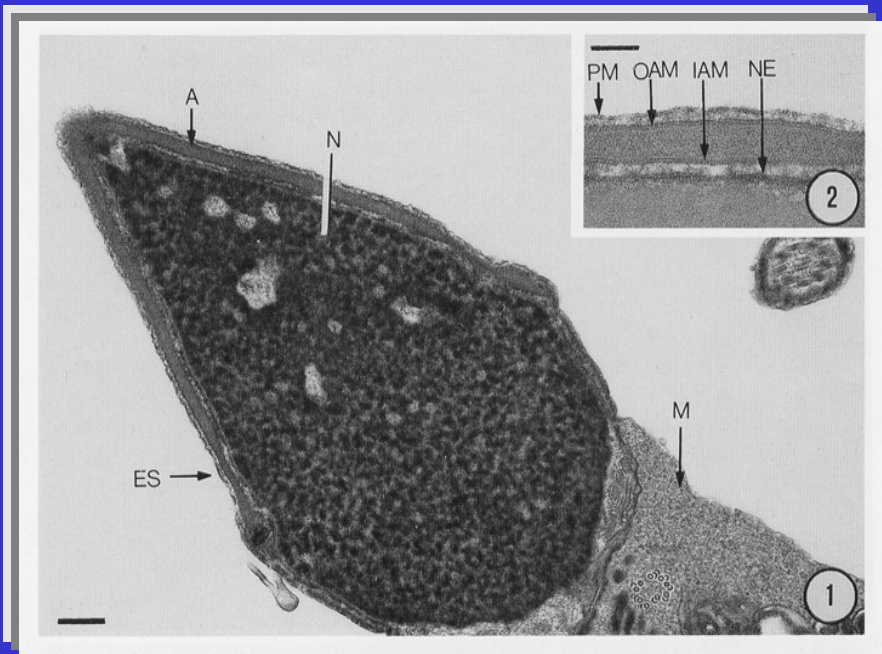






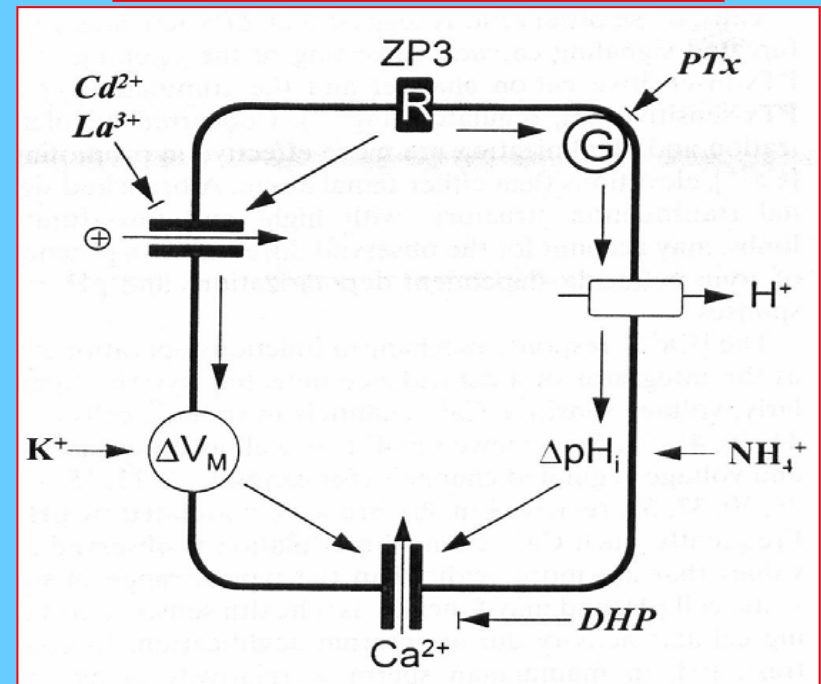
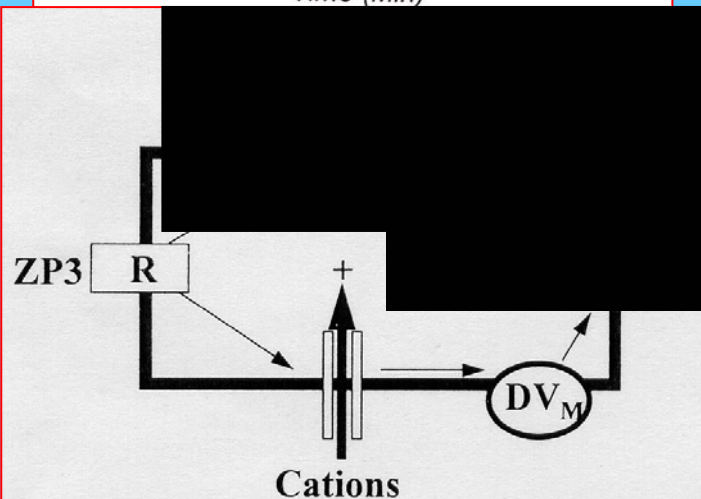
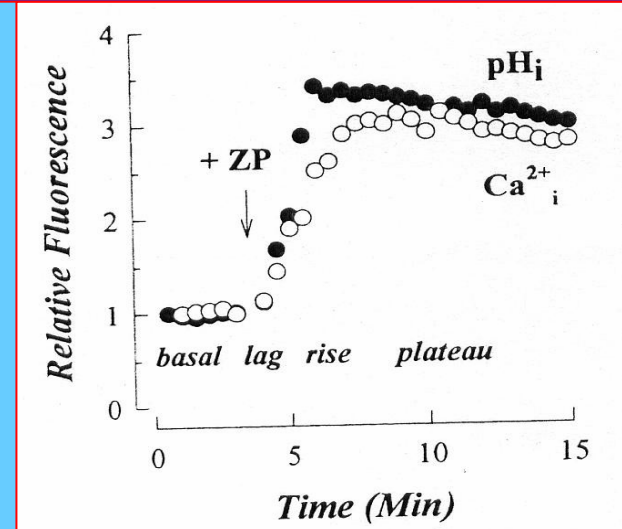
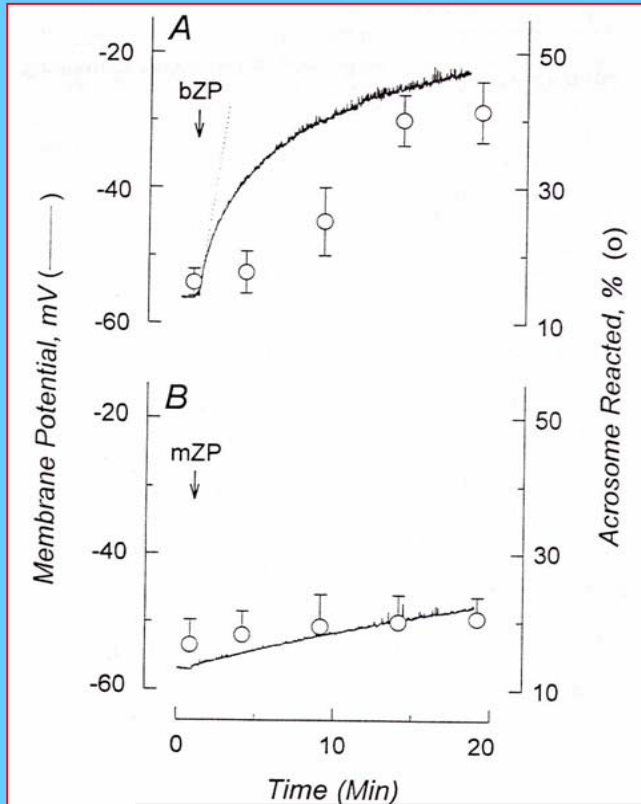








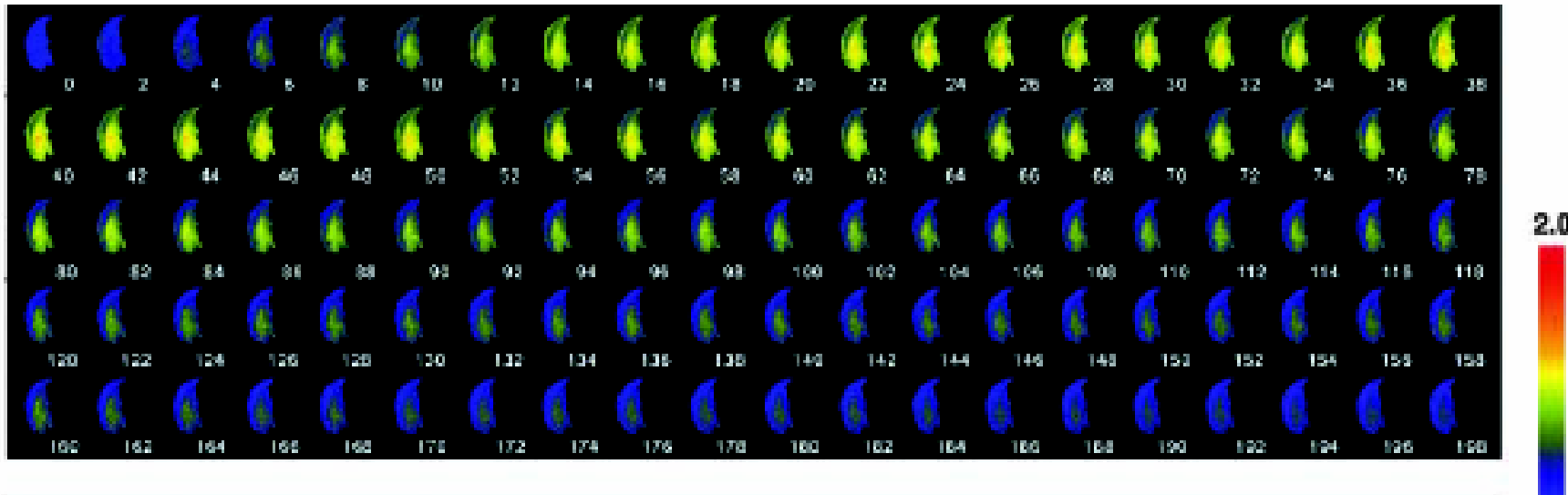
# Efectos de ZP en el Espermatozoide Involucrados en la Inducción de la RA



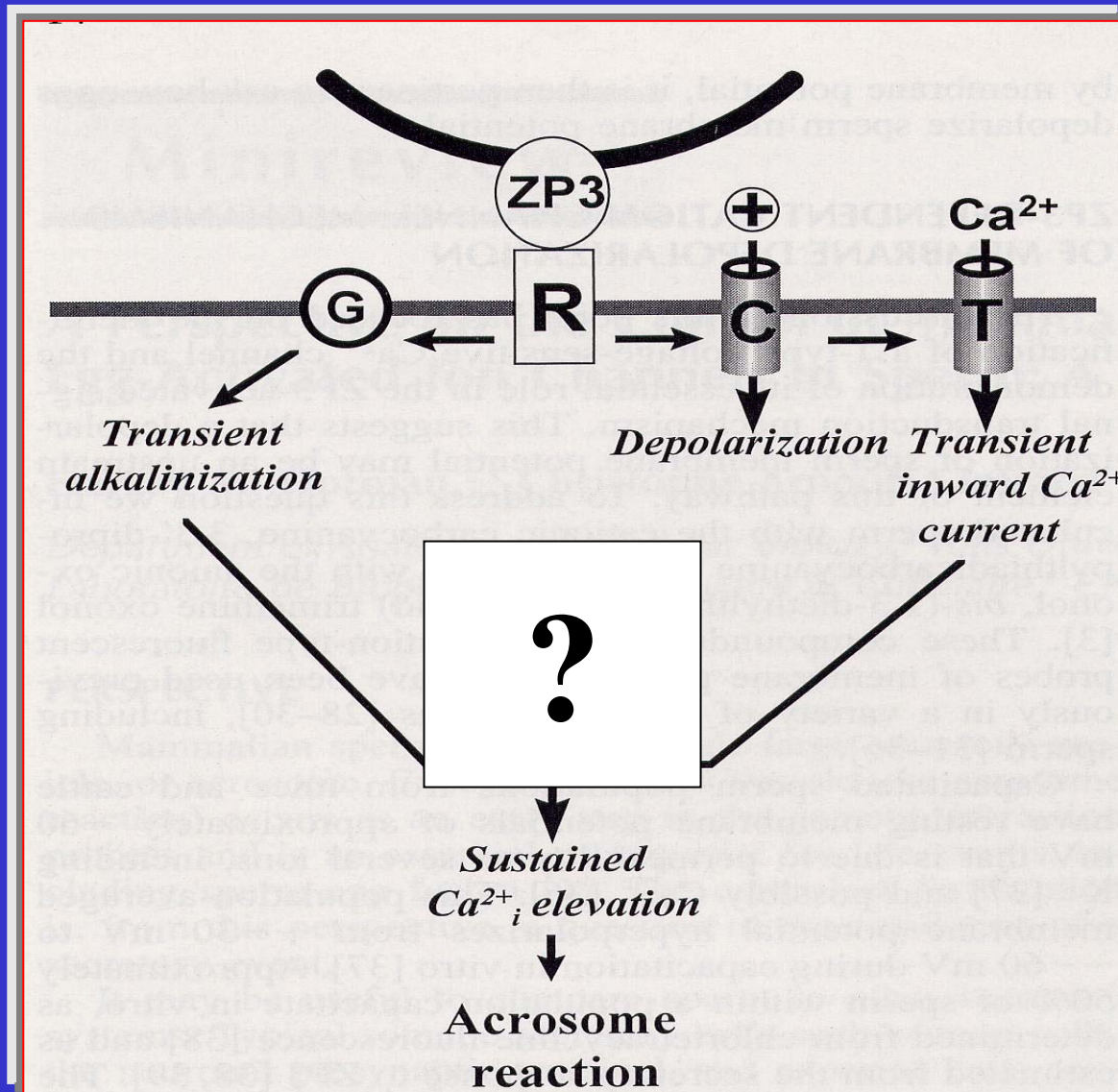
Elevación de calcio intraespermático por acción de ZP soluble  
Imágenes microscopía confocal. Tomas en intervalos de 2 seg.  
Niveles de fluorescencia se transforman a pseudocolor corres-  
A un valor de concentración de calcio libre.

**A**

**ZP:+/+**

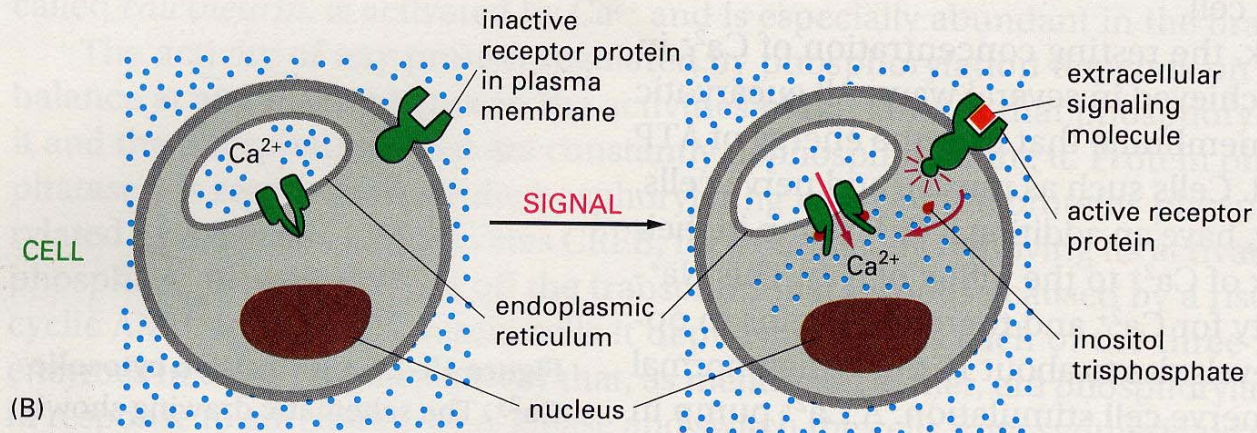
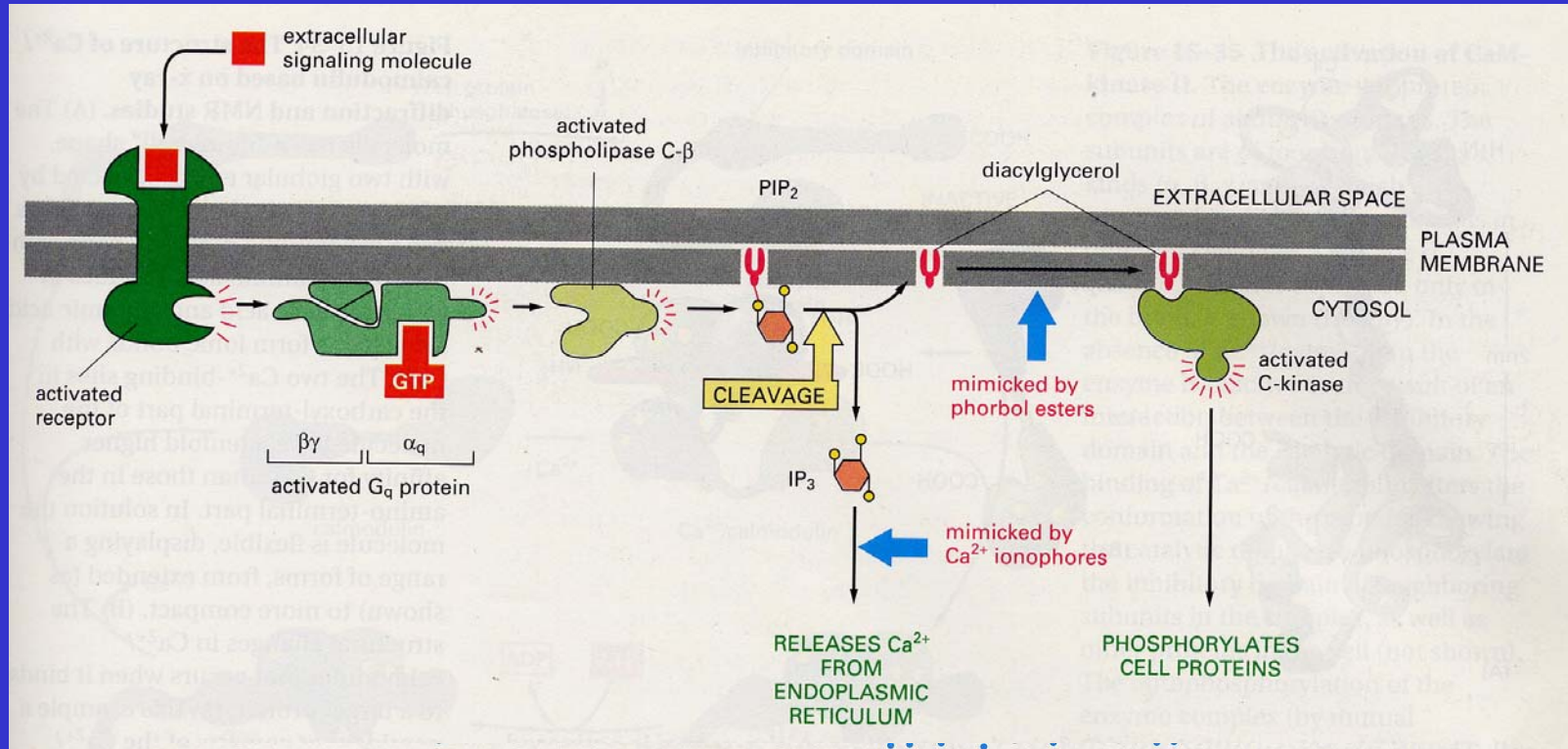


# RA y canales T de calcio. Modelo



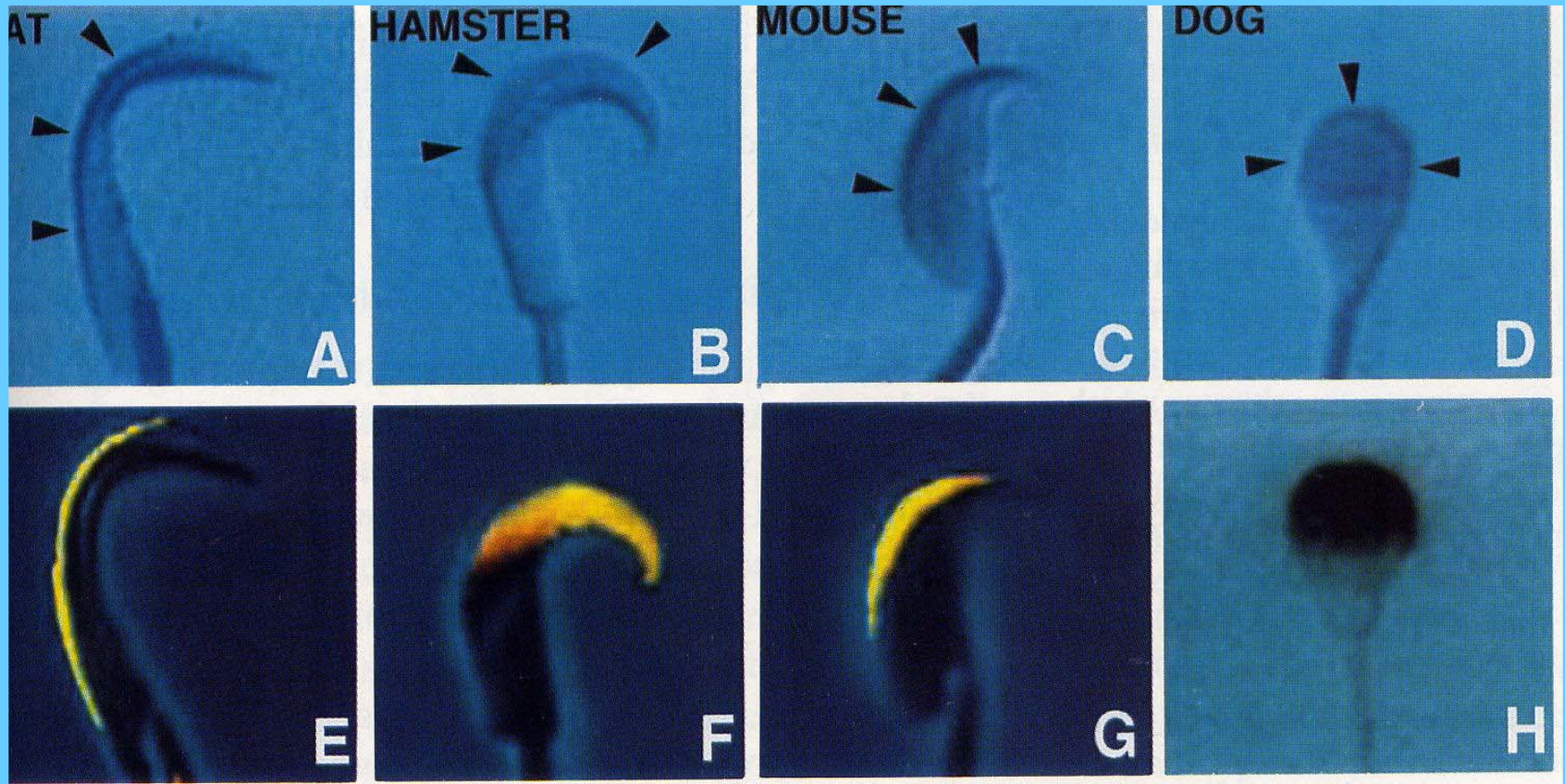


# Agonista-Receptor-Prot. G-PLC-IP3-Calcio Intracelular

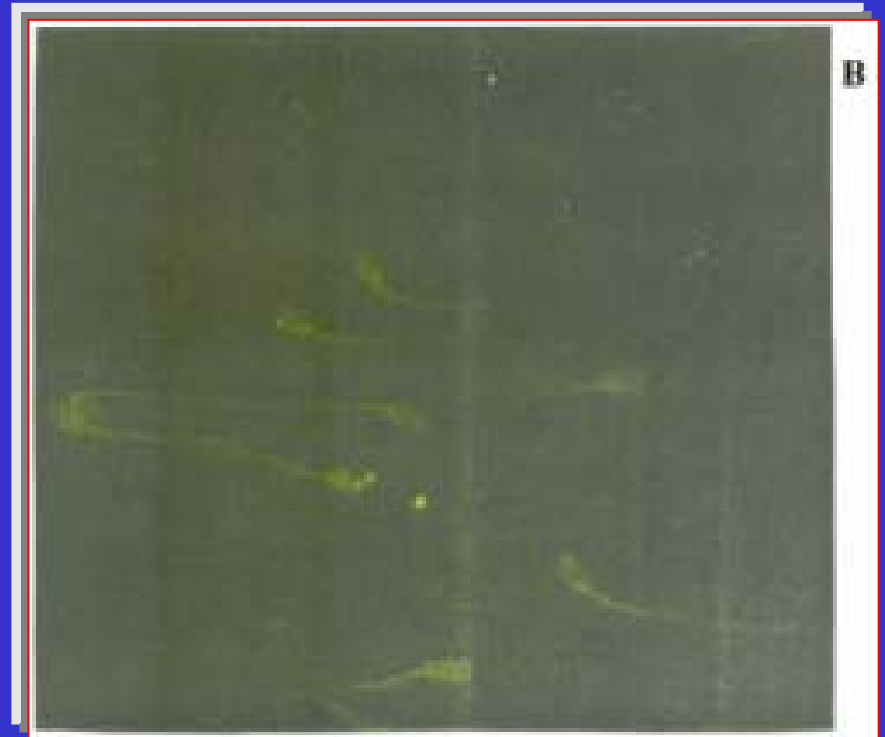
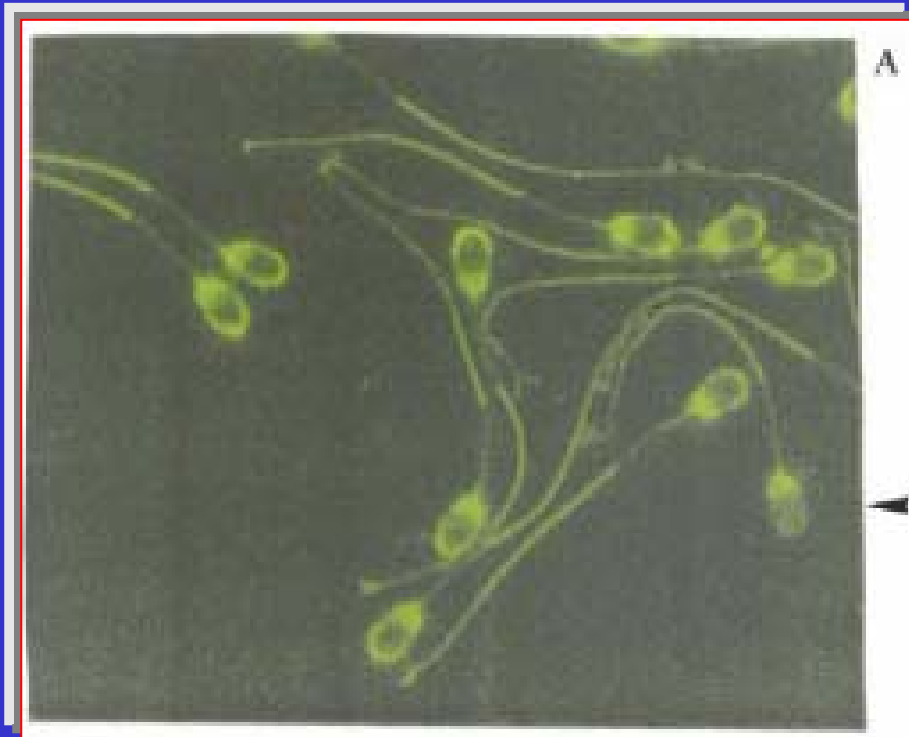




# Localización de Receptores de $IP_3$ en Espermatozoides de Mamífero.

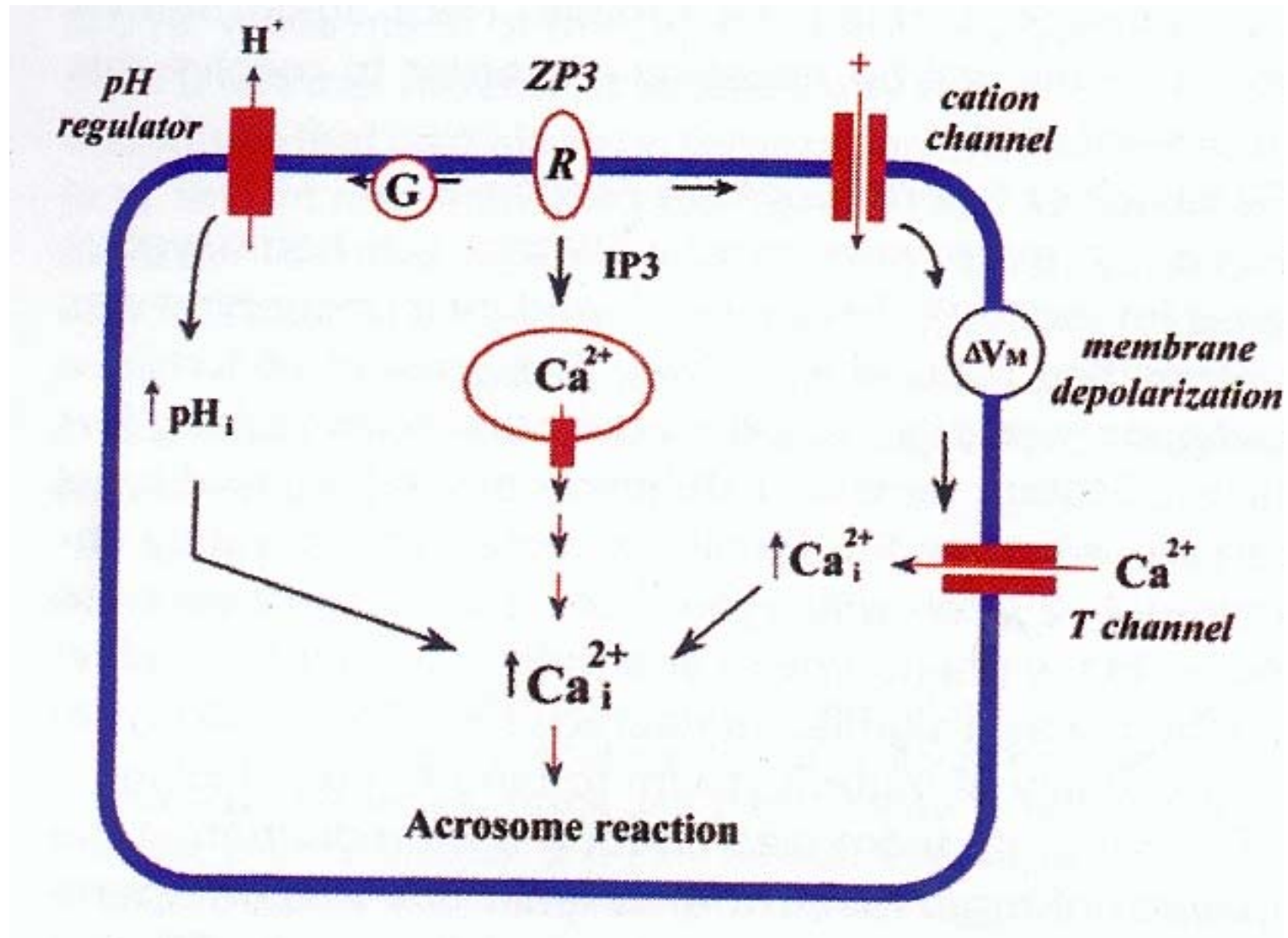


# Immunolocalización de receptores de IP3 en espermatozoides de Bovino

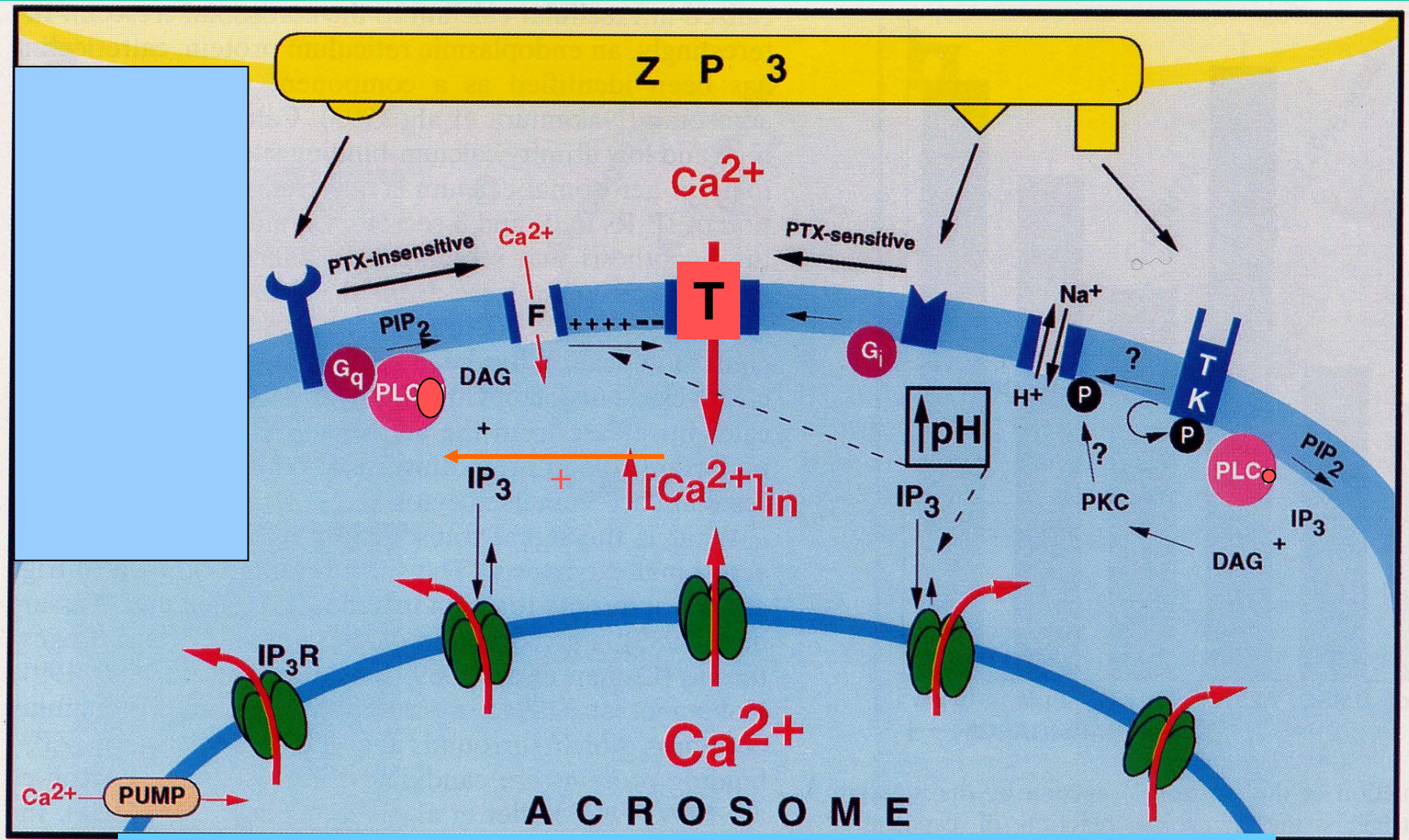


# Calcio y RA inducida por ZP3.

## Secuencias. Modelo



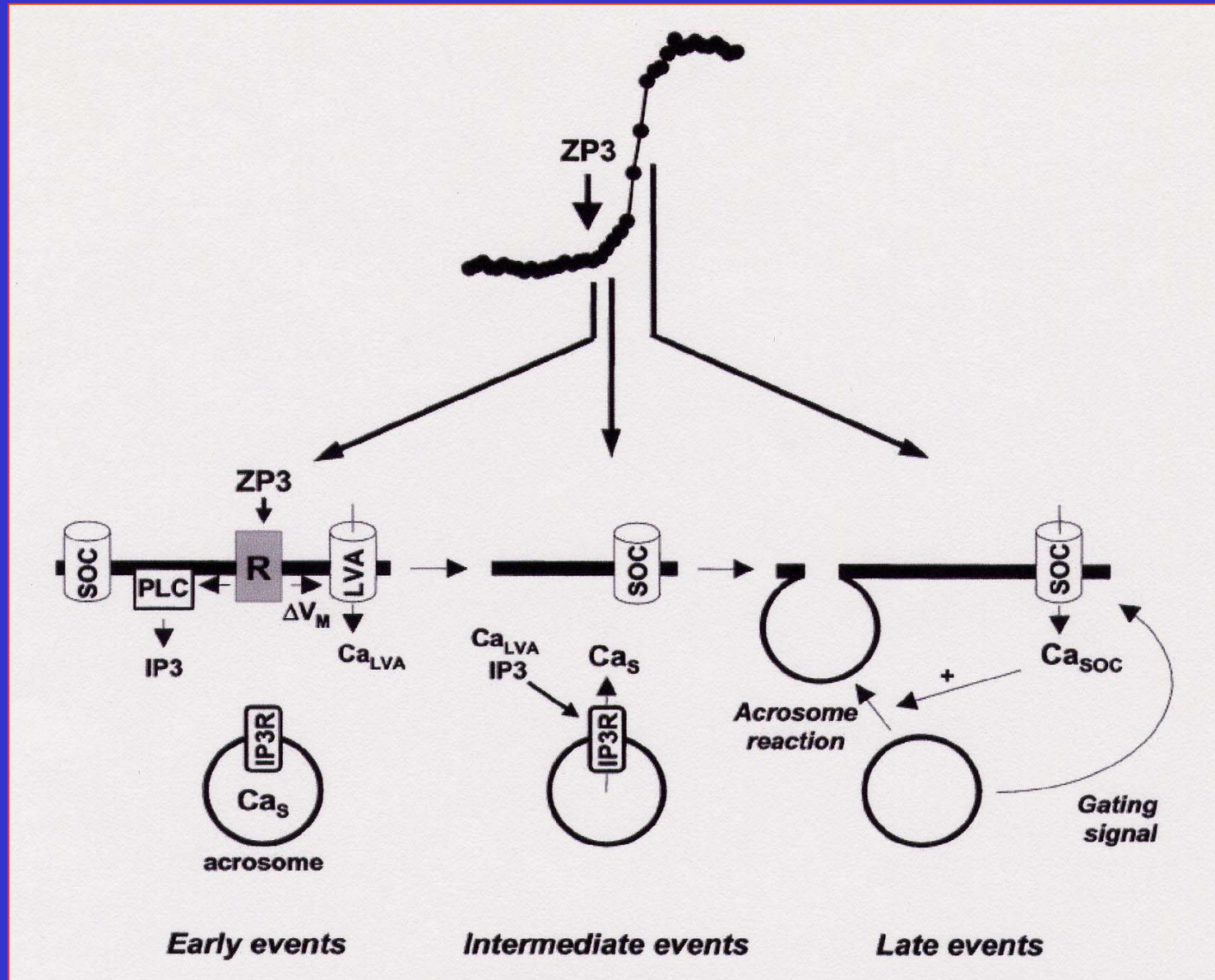




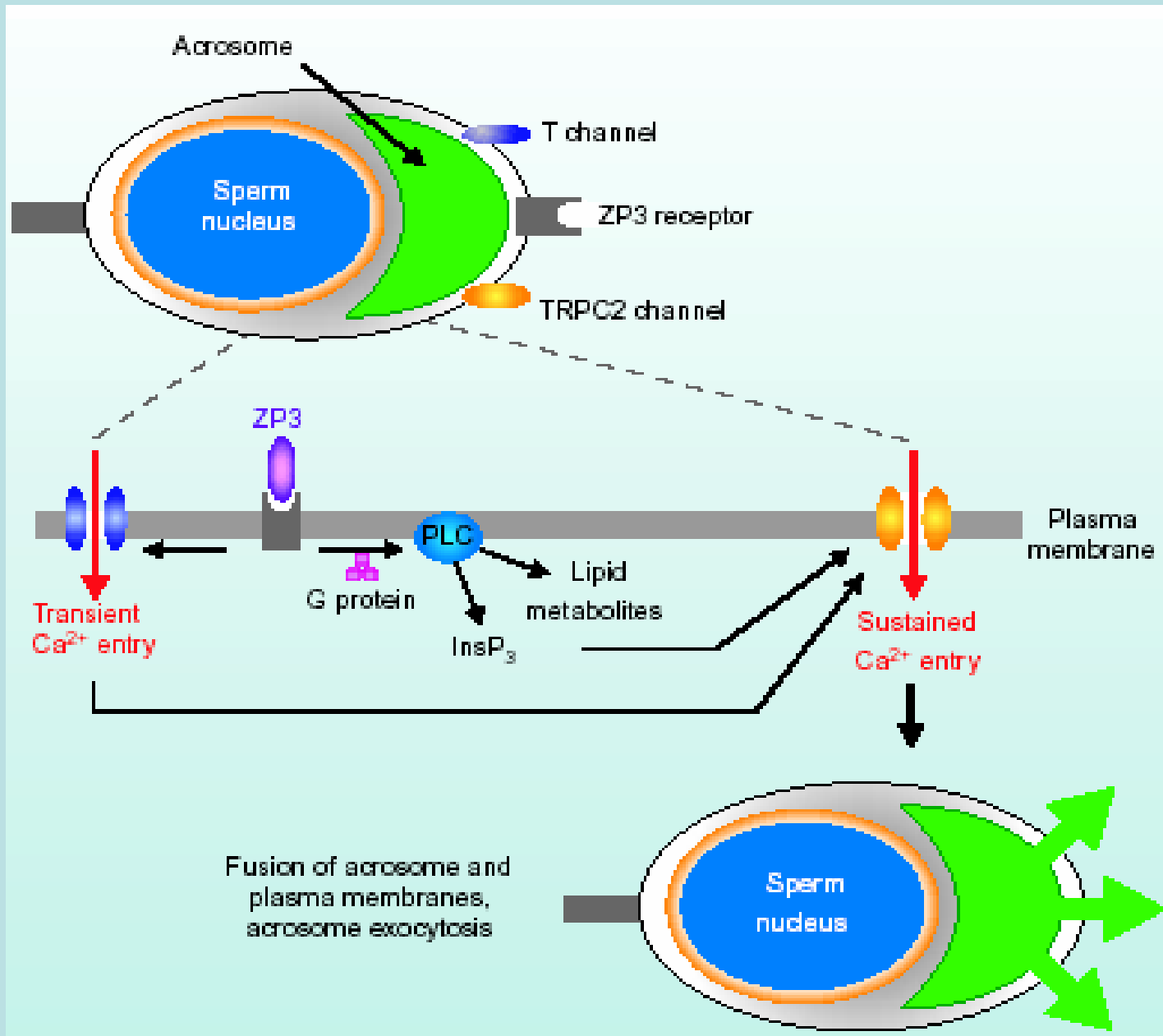
Como Depósito de Calcio Intraespermático



# Eventos Asociados a la Elevación de Calcio Intraespermático Involucrados en el Desarrollo de la RA



# Estimulación de eventos de la RA del espermatozoide por acción de ZP3





# Interacción Espermatozoide-Oocito e Inducción de la RA

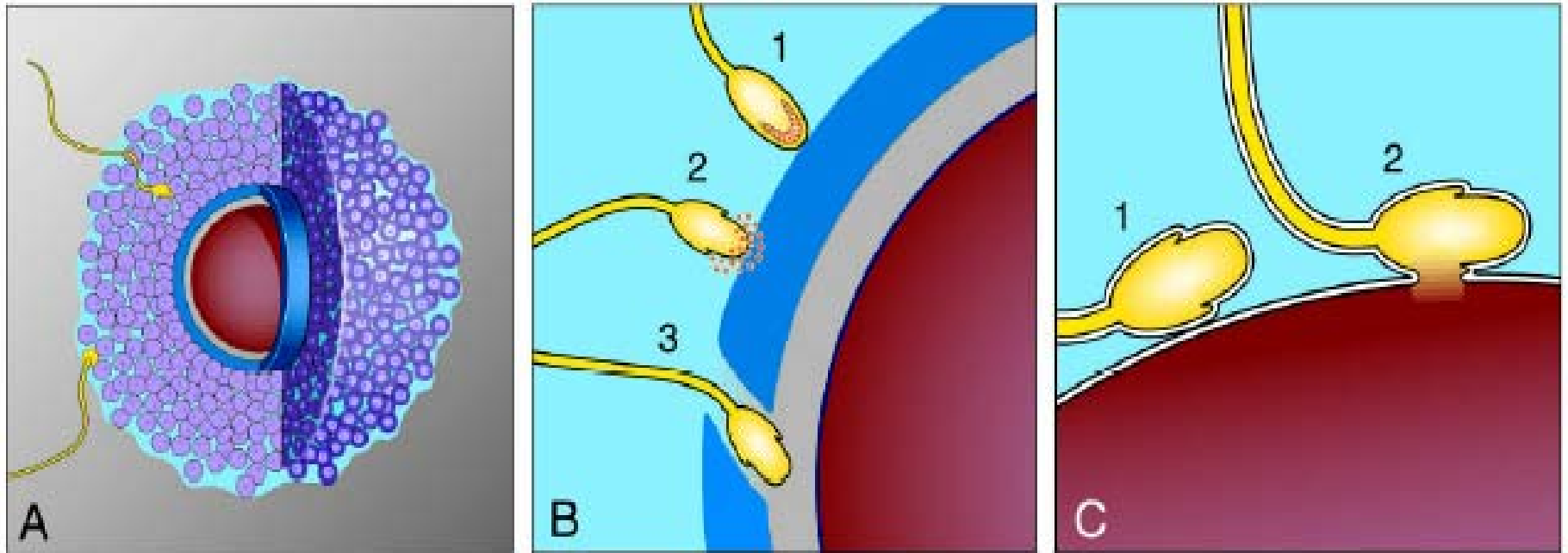
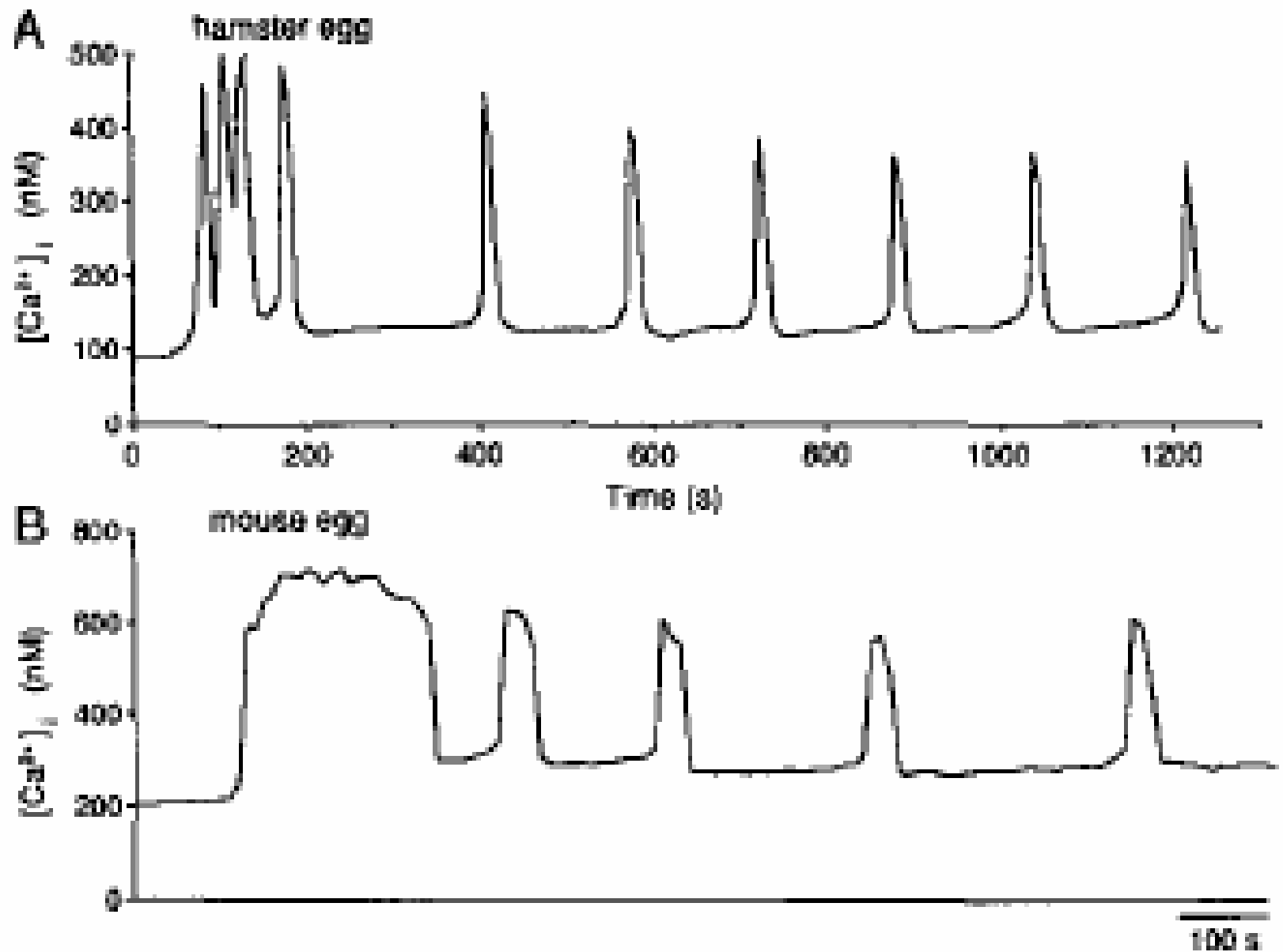


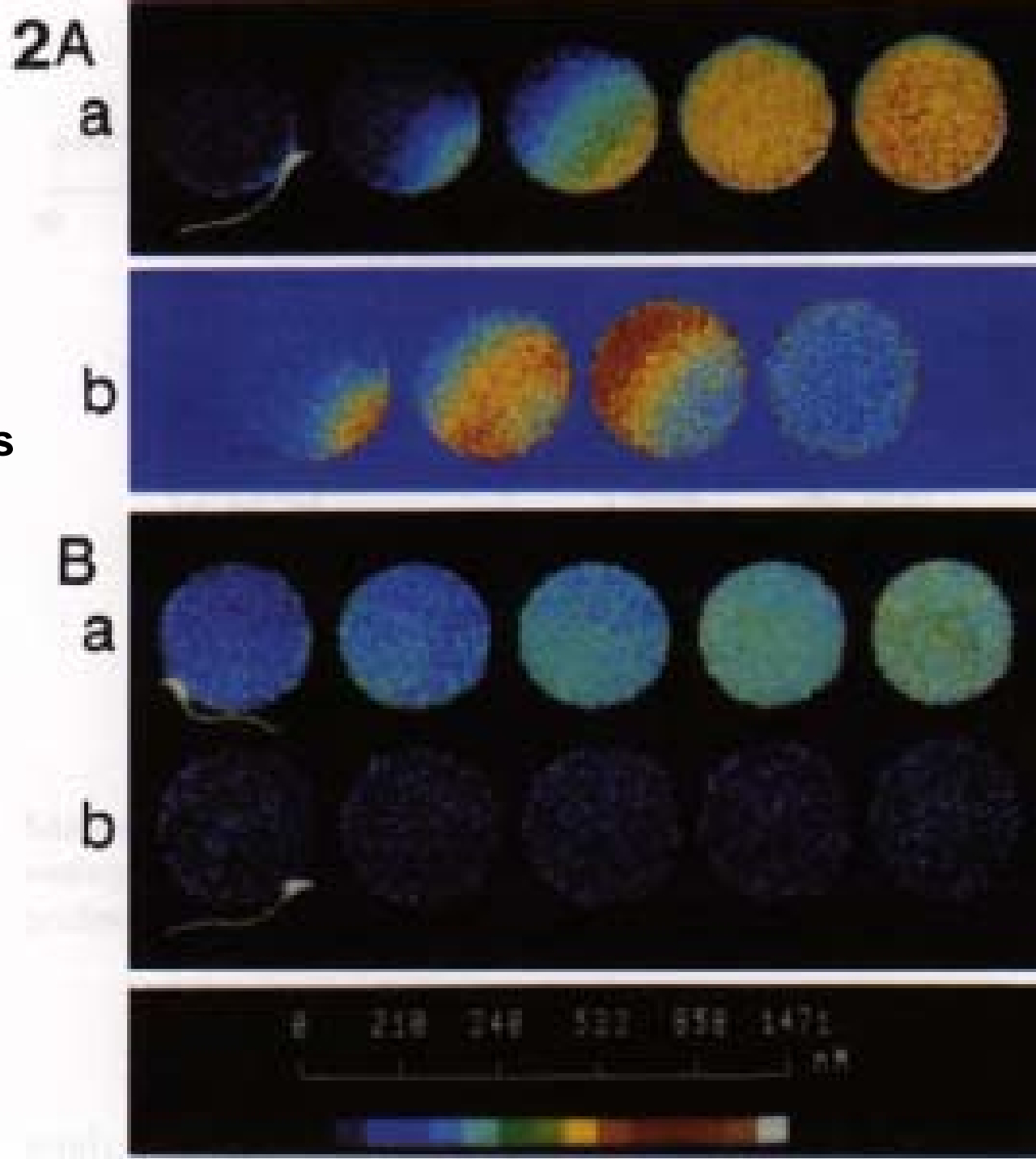
Fig. 1. (A) Sperm penetration of cumulus cells (purple) to reach zona (navy blue). (B) Egg depicted with cumulus cells removed; sperm 1 binds to the zona pellucida (navy blue); sperm 2 undergoes exocytosis, releasing acrosomal contents (orange-red); sperm 3 penetrates the

zona pellucida and begins entry into perivitelline space (gray). (C) Sperm 1 binds to the egg plasma membrane by the side of its head, in a central region (equatorial region); sperm 2 fuses with the egg plasma membrane.

## Elevaciones transientes de calcio libre citoplasmático en el oocito en la fecundación



**Ondas de calcio al  
contacto de membranas  
gaméticas y rol de  
receptores para IP3  
intraovocitarios**





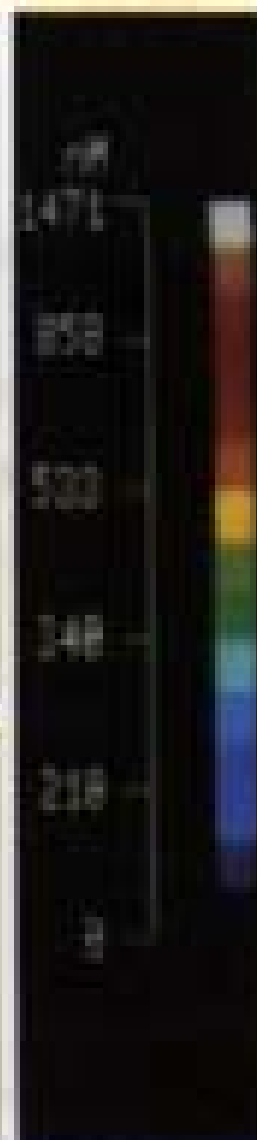
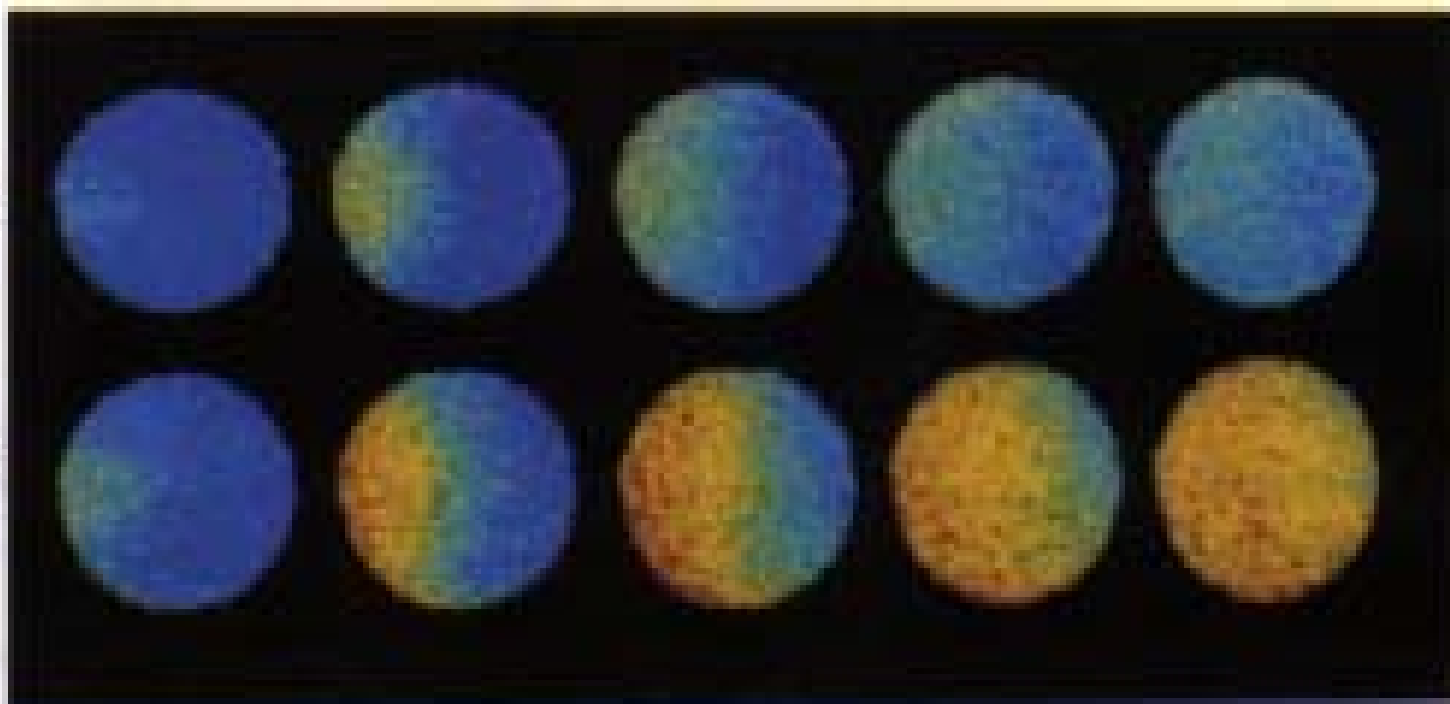
# Ondas de calcio libre intracitoplasmáticas e inyección intraovocitaria de IP3

4A

a

b

B



# Moléculas de señalización celular y liberación de calcio en el oocito en la fecundación

TABLE 1  
Signaling Components that Mediate  $\text{Ca}^{2+}$  Release in Eggs at Fertilization

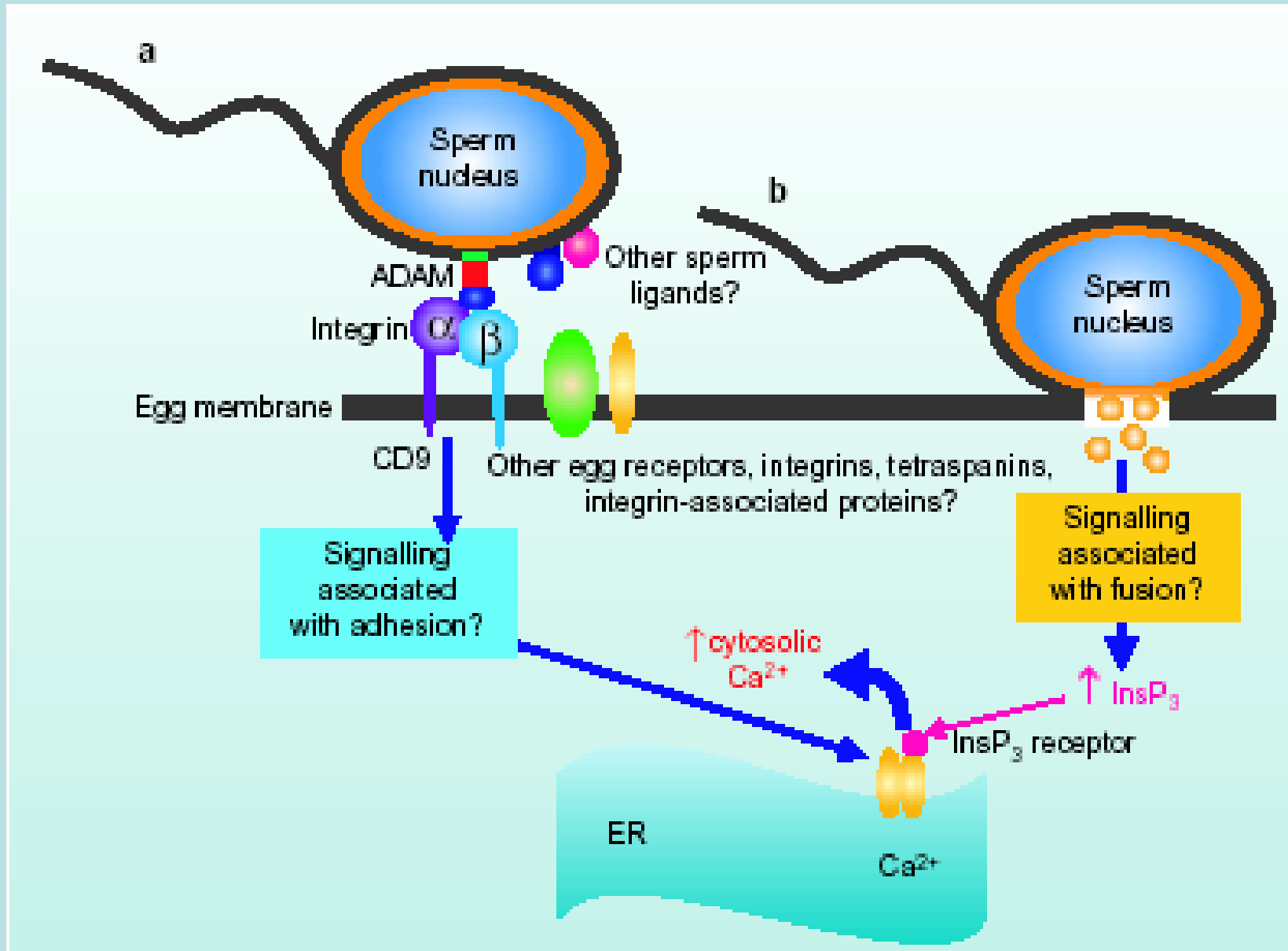
	Echinoderms	Ascidians	Amphibians	Mammals
Inositol trisphosphate <sup>a</sup>	+	+	+	+
Phospholipase $\text{C}\gamma$ <sup>b</sup>	+	+	?	?
Tyrosine kinase <sup>c</sup>	+	+	+	?

<sup>a</sup> Echinoderms: Whitaker and Irvine, 1984; Clapa and Whitaker, 1986; Chiba *et al.*, 1990; Mohri *et al.*, 1995; Carroll *et al.*, 1997, 1999; Lee and Shen, 1998; Shearer *et al.*, 1999; Iwasaki *et al.*, 2001. Ascidians: Toratani and Yokosawa, 1995; Albrieux *et al.*, 1997; Runft *et al.*, 2000. Amphibians: Busa *et al.*, 1985; Stith *et al.*, 1993; Nuccitelli *et al.*, 1993; Snow *et al.*, 1996; Runft and Jaffe, 1999; Yamamoto *et al.*, 2001. Mammals: Miyazaki, 1988; Miyazaki *et al.*, 1992, 1993; Kline and Kline, 1994.

<sup>b</sup> Echinoderms: Carroll *et al.*, 1997, 1999; Lee and Shen, 1998; Shearer *et al.*, 1999; Rongish *et al.*, 1999. Ascidians: Runft and Jaffe, 2000. Amphibians: Runft *et al.*, 1999; Sato *et al.*, 2000. Mammals: Dupont *et al.*, 1996; Mehlmann *et al.*, 1998, 2001; Jones *et al.*, 2000.

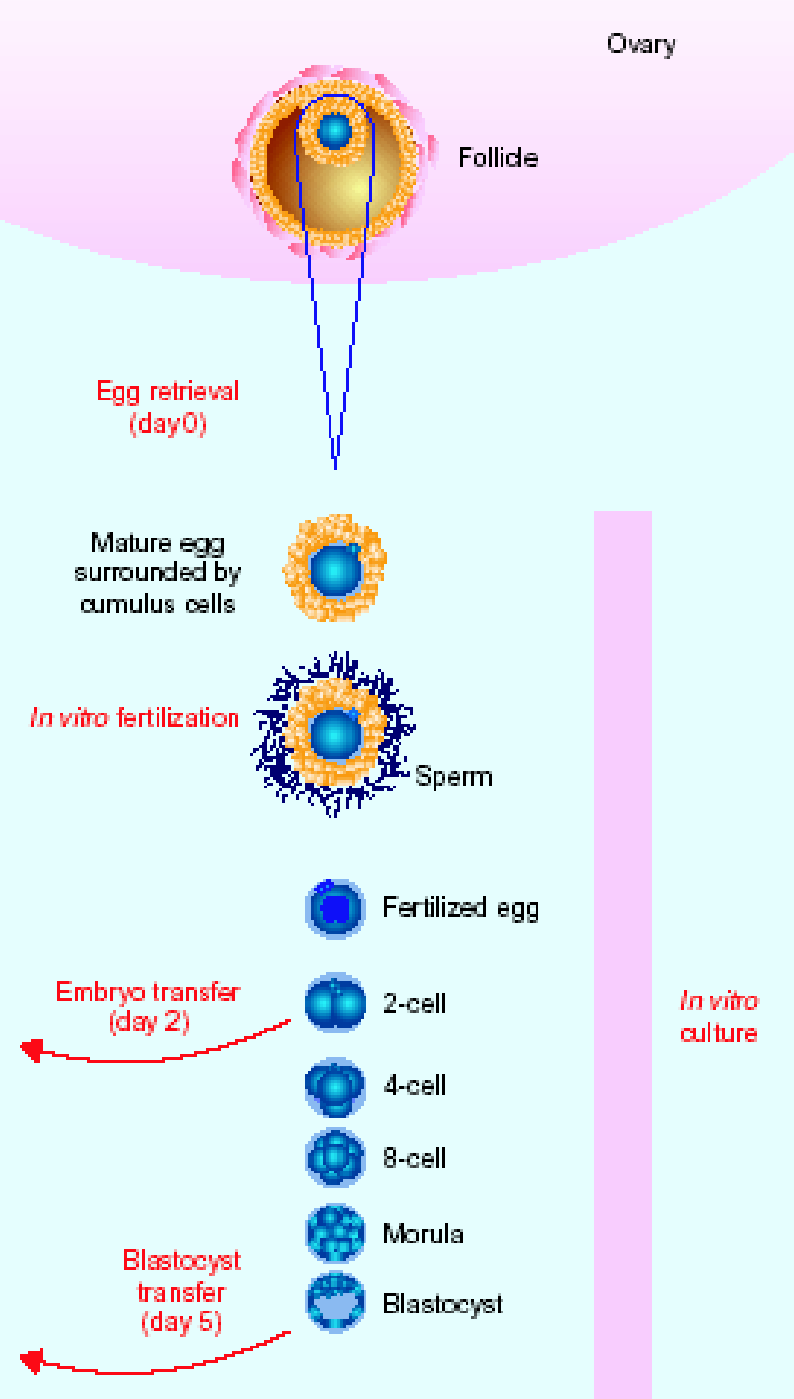
<sup>c</sup> Echinoderms: Clapa and Epel, 1991; Shen *et al.*, 1999; Giusti *et al.*, 1999a,b, 2000; Abassi *et al.*, 2000; Kinsey and Shen, 2000. Ascidians: Ueki and Yokosawa, 1997; Runft and Jaffe, 2000. Amphibians: Glahn *et al.*, 1999; K. Sato *et al.*, 1996, 1999, 2000. Mammals: Dupont *et al.*, 1996; Talmor *et al.*, 1998.

# Interacción de membranas gaméticas y activación del oocito

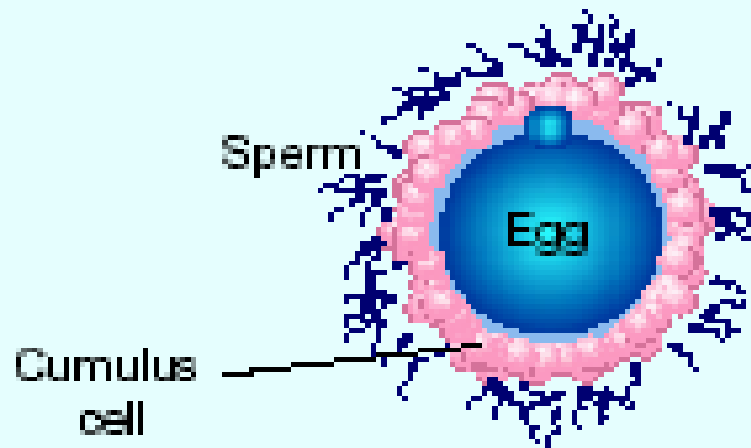




# Etapas asociadas a la fecundación in vitro.

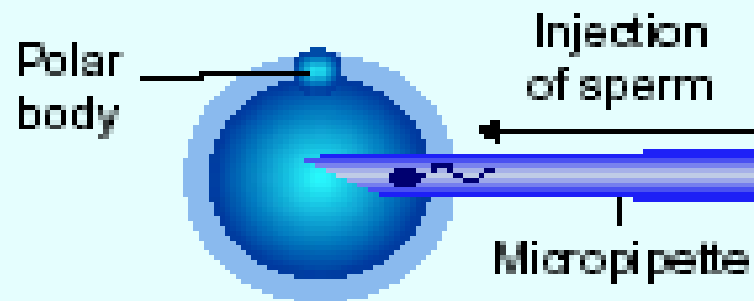


# Técnicas utilizadas en reproducción asistida I



Routine practice

*In vitro* fertilization



*Intracytoplasmic sperm injection*



*Cryopreservation*

# Técnicas utilizadas en reproducción asistida II

