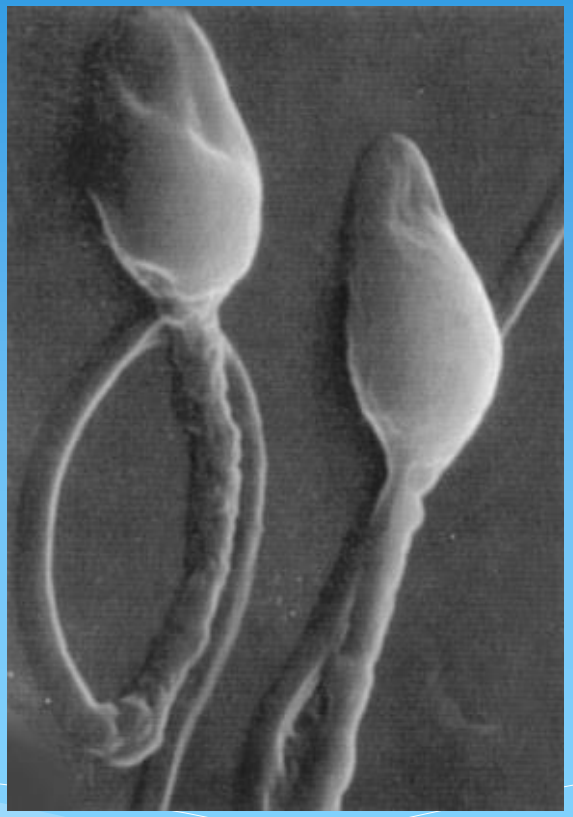
* ***The following material can be used exclusively for the teaching activity of the MS Programme in Reproductive Biotechnologies- University of Teramo***

UNIVERSITY of
TERAMO

MS Reproductive Biotechnologies

SPERM CELL BIOLOGY

Prof. Luisa Gioia



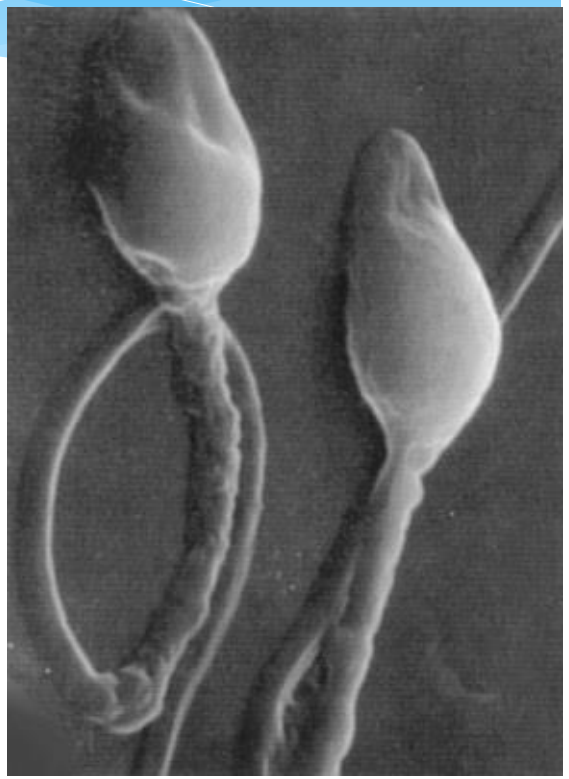
THE FERTILIZING SPERM'S LIFE-STORY

ANDROLOGY

Formation (testis)

Maturation (epididymis)

Storage (epididymis)



- Deposition in female tract
- Capacitation
- Bind to zona pellucida (ZP)
- Fertilization
  - Acrosome reaction (AR)
  - Penetration through ZP
  - Fusion with egg
  - Activation of egg

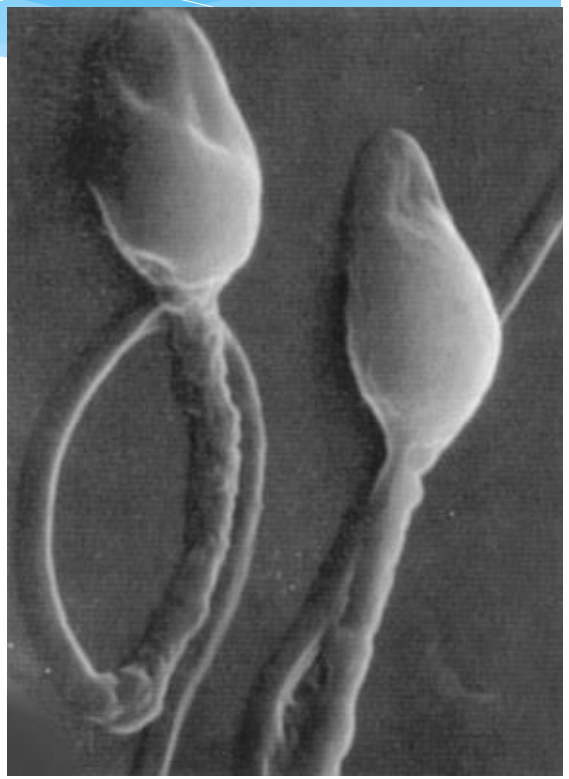
Decondensation of sperm head

Formation of male PN

Syngamy and initiation of cell cycle

THE FERTILIZING SPERM'S LIFE-STORY

Formation (testis)
Maturation (epididymis)
Storage (epididymis)

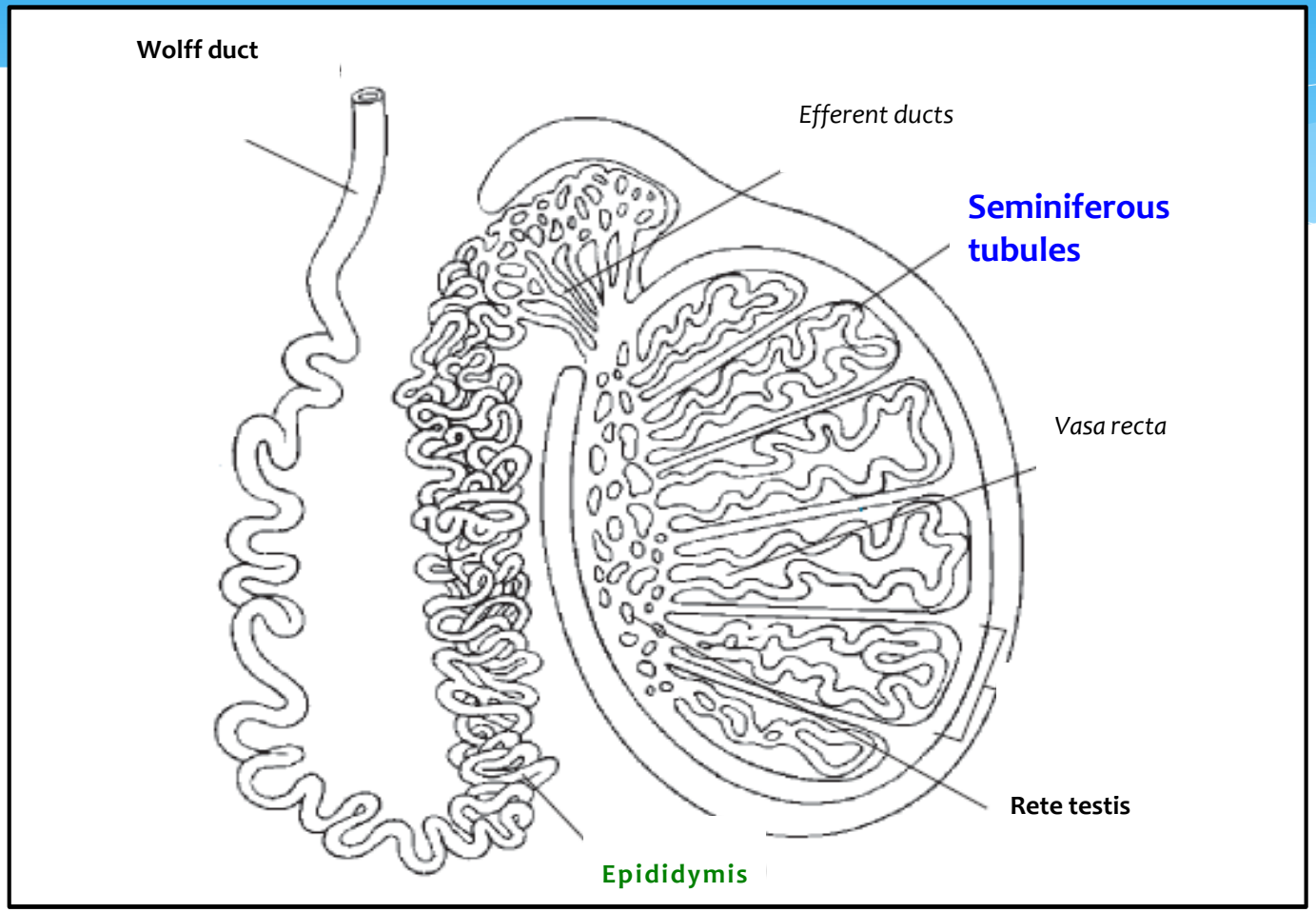


Recalling the key aspects on…..

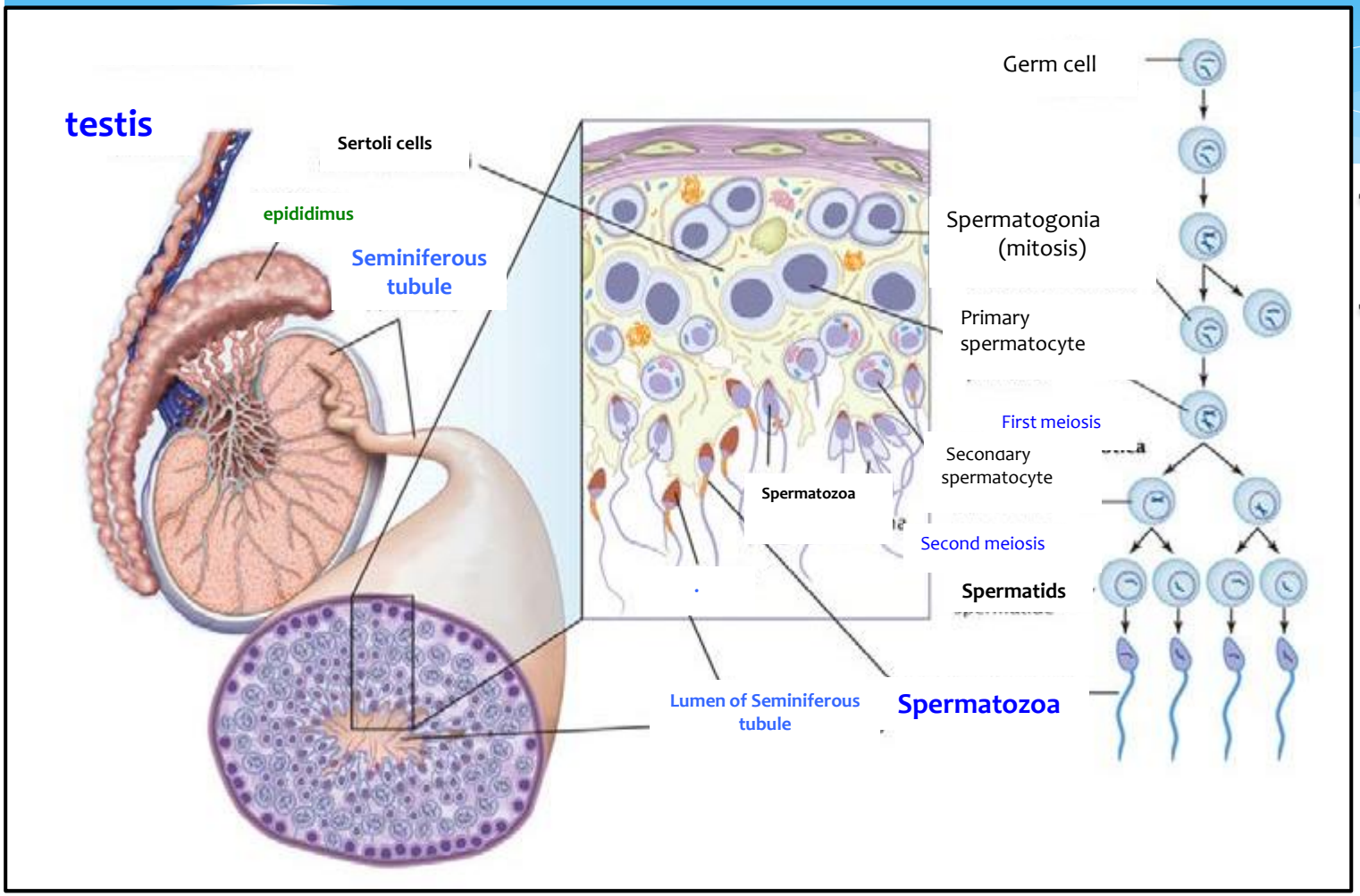
Spermatogenesis

* **Purpose:** to establish and maintain daily output of fully differenciated spermatozoa

Spermatogenesis starts at the onset of puberty and occurs within the seminiferous tubules



Spermatogenesis



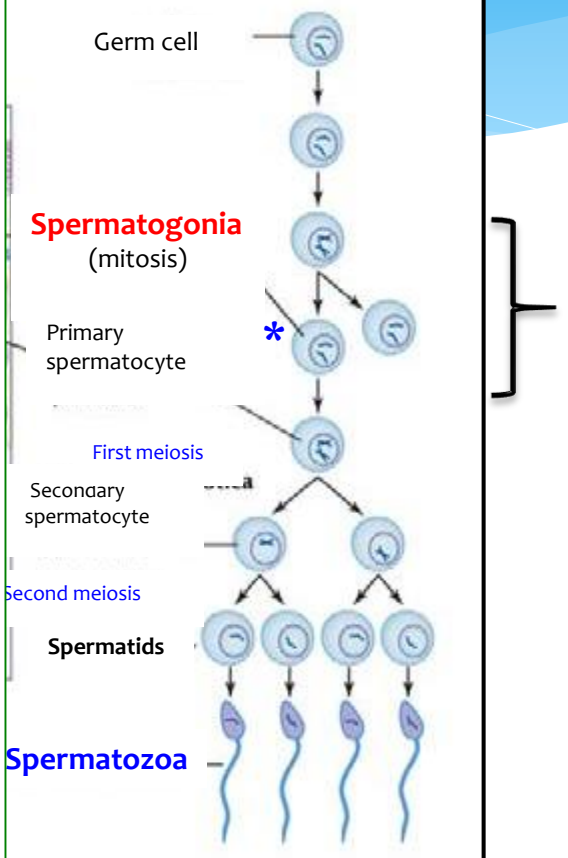
Spermatogenesis:
1.proliferative phase

Pay attention!

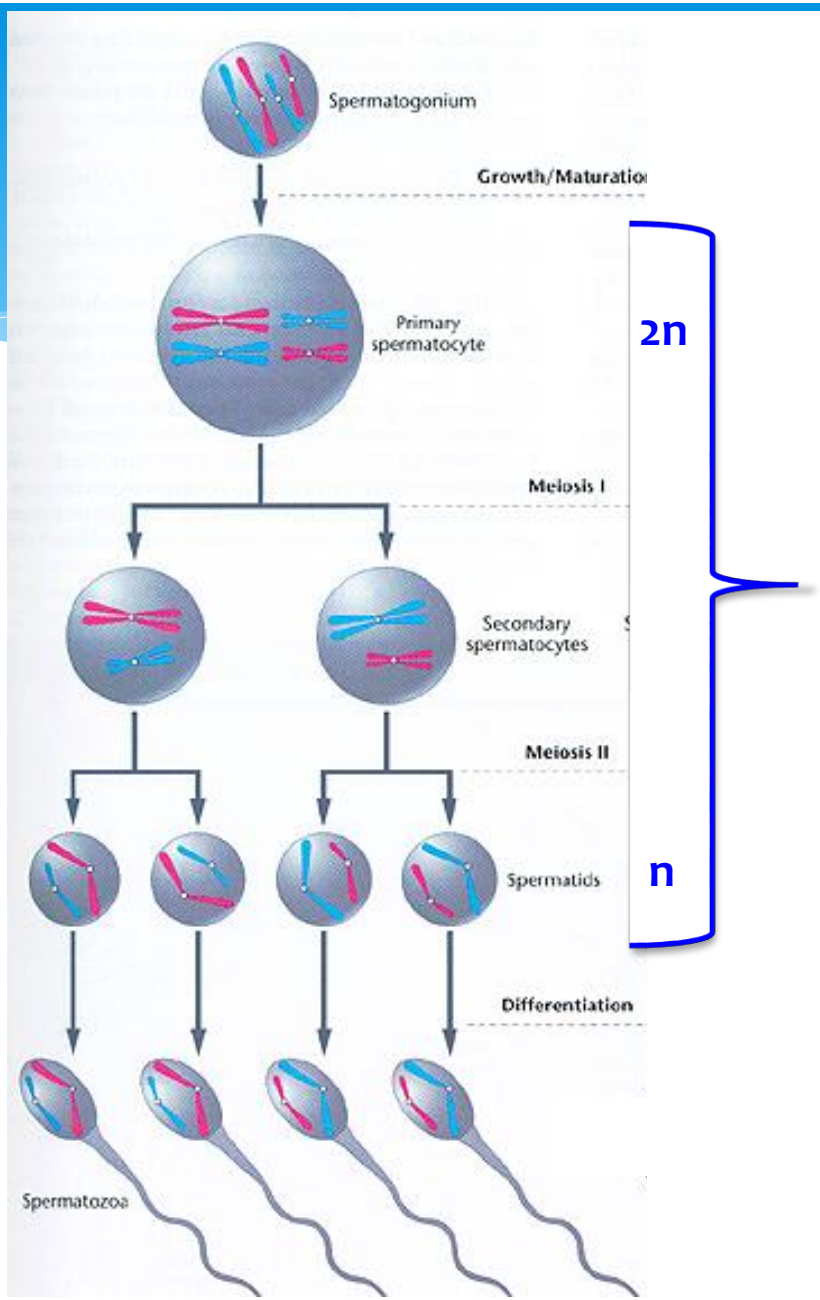
To sustain sperm production, spermatogenic stem cells undergo perpetual renewal.

Thus, within the seminiferous tubules ***an A-type spermatogonia could commit either to renewal or to differentiation into B-type spermatogonia* competent to enter meiosis.***

Continuous production of spermatozoa is maintained through the reproductive lifespan of Mammals



Spermatogenesis:
2.Meiotic phase

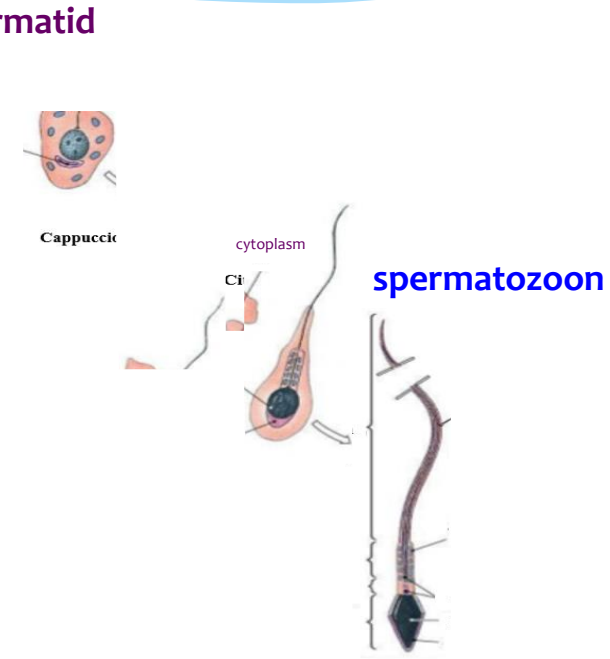


Sperm cell meiosis

Spermatogenesis:
3.**Differentiative phase or spermiogenesis**

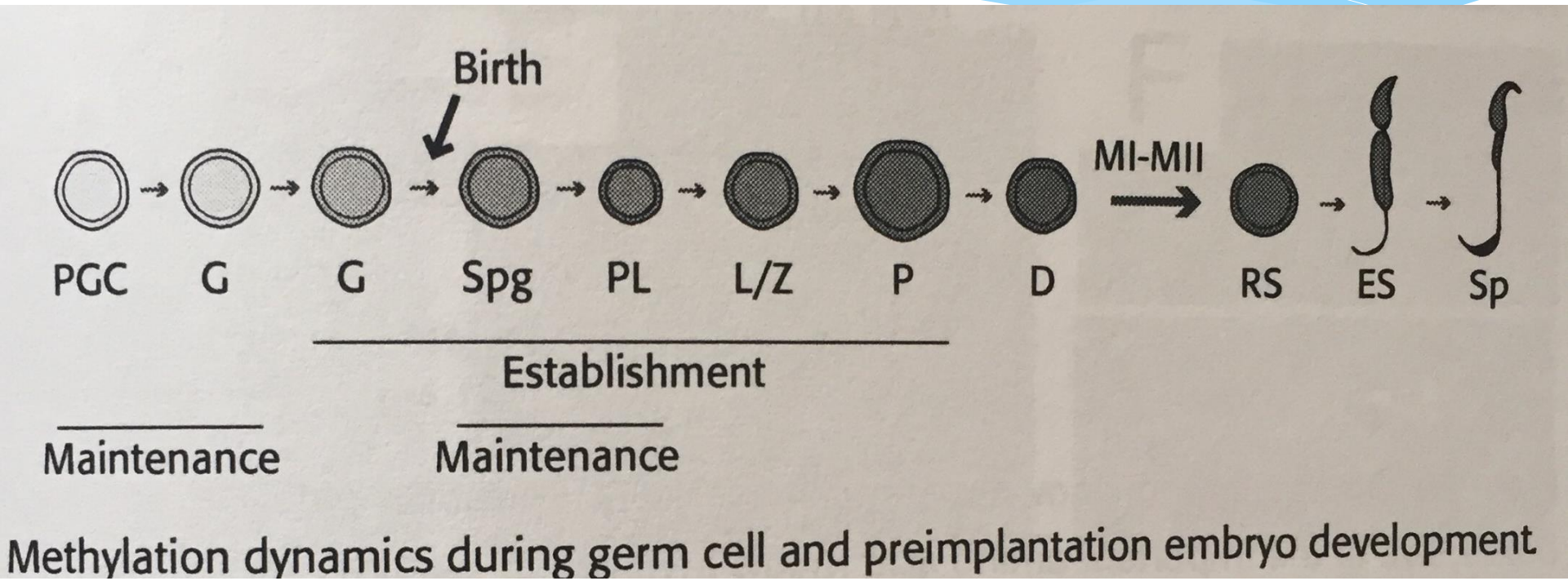
* During the late stages of spermatogenesis (spermiogenesis), the haploid somatic-cell like round spermatid is transformed into a **specialized, nearly cytoplasm-free spermatozoon** capable of acquiring progressive motility and fertilizing a mature oocyte in the site of fertilization

- **Genomic methylation pattern** is also acquired during male germ cell development



Progression of **genomic methylation pattern acquisition** during male germ cell development, as represented by the intensity of the gray shading.

De-novo and mainteinance methylation events are indicated under the appropriate germ cell types.



From Lucifero et al 2004

THE FERTILIZING SPERM'S LIFE-STORY

-In epididymus sperm cells are immotile.
-Just before ejaculation, cointained in seminal plasma, motility is activated.
-After ejaculatation, inside the uterus spermatozoa are motile
-Then, inside the oviductal isthmus sperm motility is inactivated

- ✔ **Formation (testis)**
- ✔ **Maturation (epididymis)**
- ✔ **Storage (epididymis)**

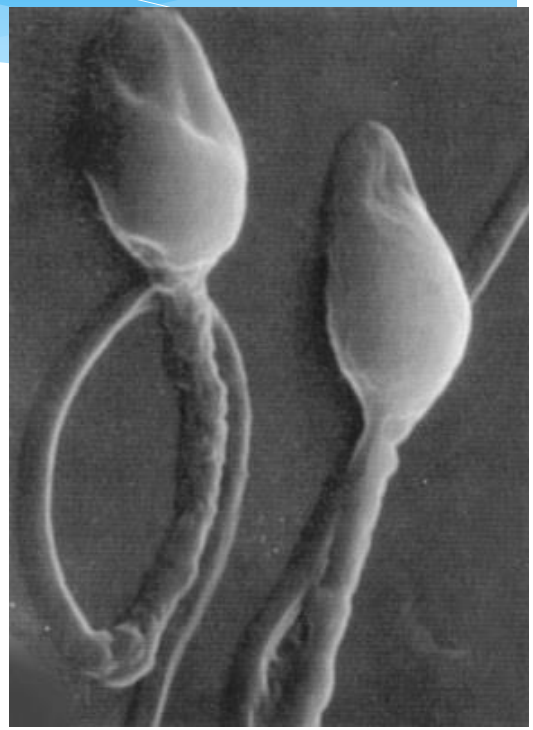
Coitus →

- **•Deposition in female tract**
- **•Capacitation**
- •Bind to zona pellucida (ZP)
- •Fertilization
  - Acrosome reaction (AR)
  - Penetration through ZP
  - Fusion with egg
  - Activation of egg

Decondensation of sperm head

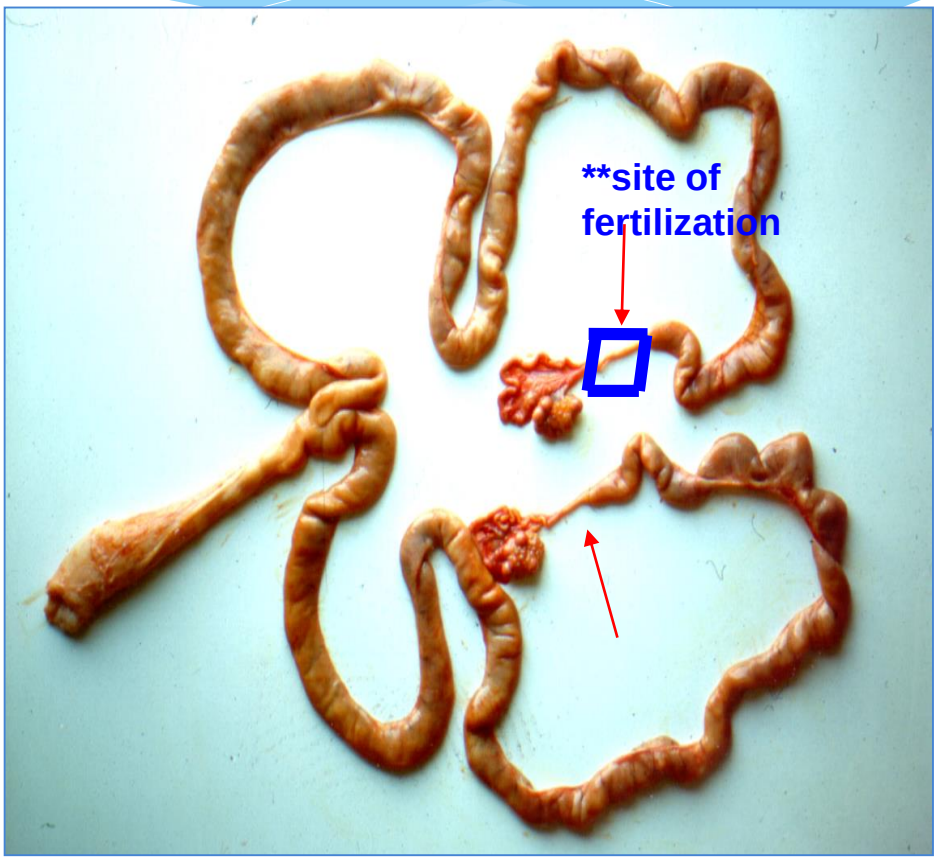
Formation of male PN

Syngamy and initiation of cell cycle



After coitus, ejaculated sperm are released into female tract*. Before gaining the ability to fertilize the oocyte, they must reside there a minimum period and undergo the process of **CAPACITATION**

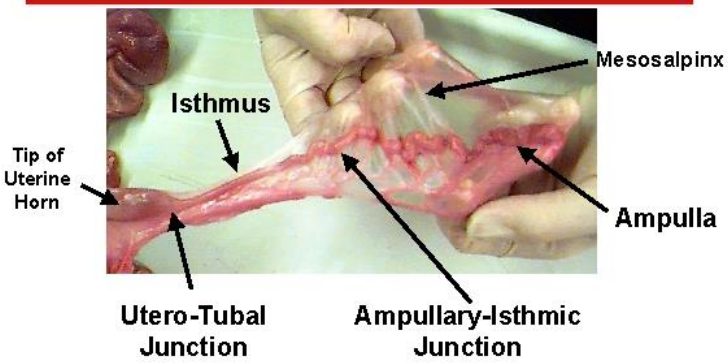
Capacitated spermatozoa meet the ovulated MII oocyte at the ***site of fertilization**, *that is in the oviduct in the swine*

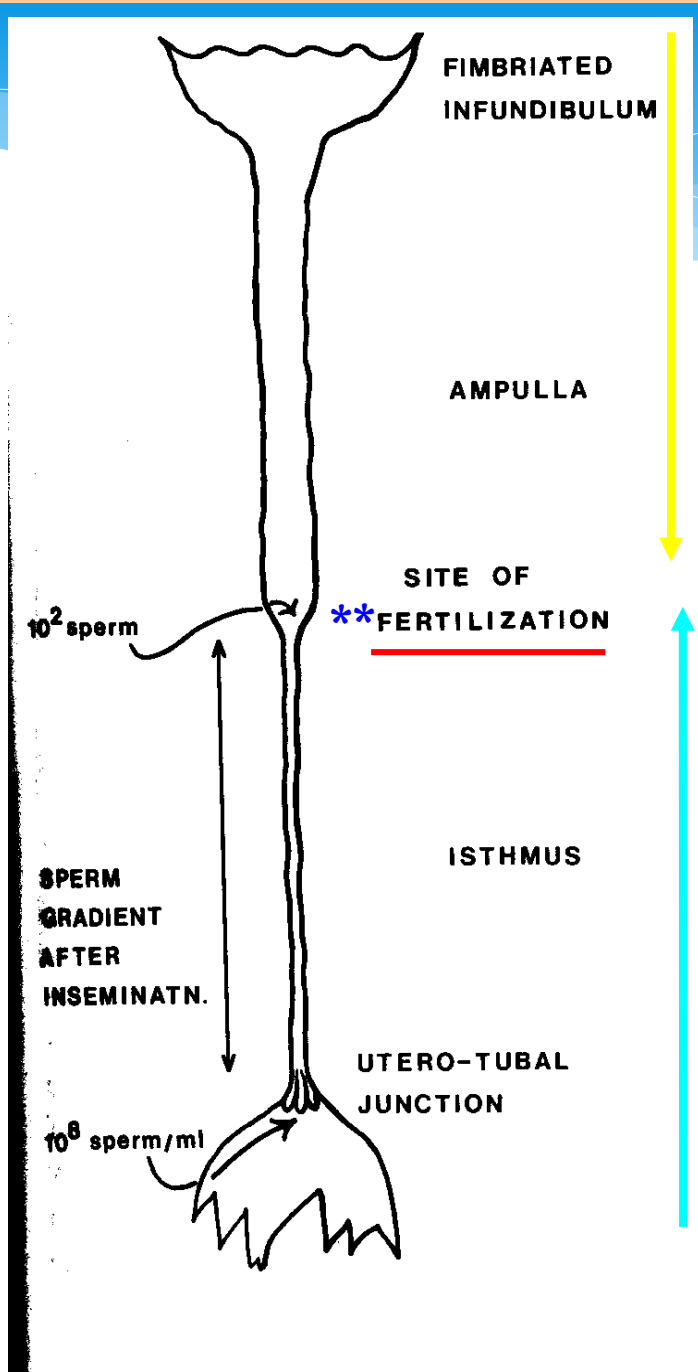


*In swine, the **uterus** is the site of semen deposition

A low number of spermatozoa reaches the site of fertilization*

Utero-Tubal Junction





Pig **oocyte** ovutated from ovary reaches the site of fertilization within a few minutes, then waits the sperm

After fertilization, the **zygote** goes slowly through the oviductal isthmus and within days it reaches the uterus, where it implants and develops

Pig **spermatozoa** go in the opposite direction, that is from uterus toward the site of fertilization. Here they wait for approximately 40 hours

TIME OF GAMETE SURVIVAL

Species	Oocyte (h)	Sperm (h)
Bovine	10 – 12	24 – 48
Ovine	10 – 15	24 – 48
Swine	8 – 12	24 – 42
Horse	8 – 10	140
Human	8 – 10	24 – 72

The coordination between the transport of the gametes is essential for the success of fertilization

* **How spermatozoa are induced to reactivate motility and leave the reservoir (utero-tubal junction) to get the site of fertilization?**

At ovulation, **P4** is released to the isthmus of oviduct by a **counter-current system** re-activating the motility of the spermatozoa contained in the *reservoir* (utero-tubal junction)

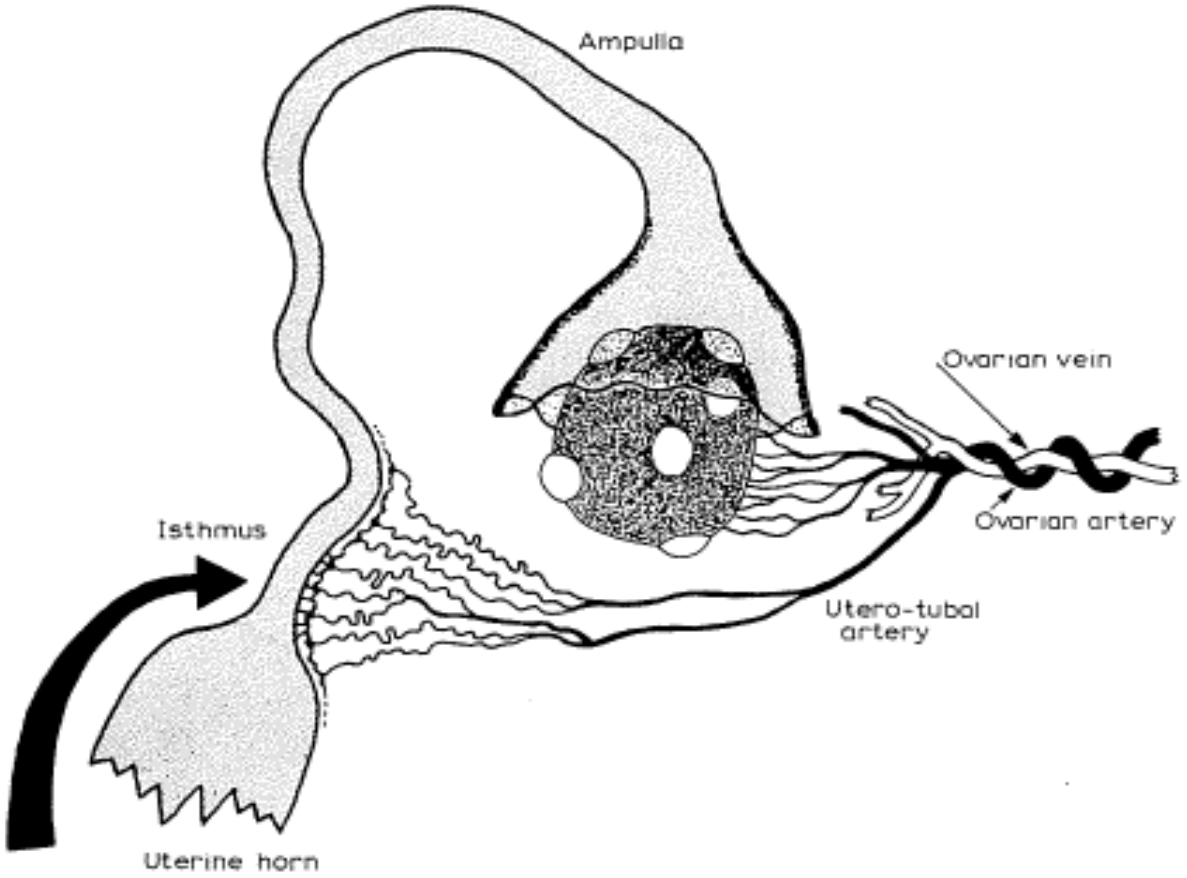
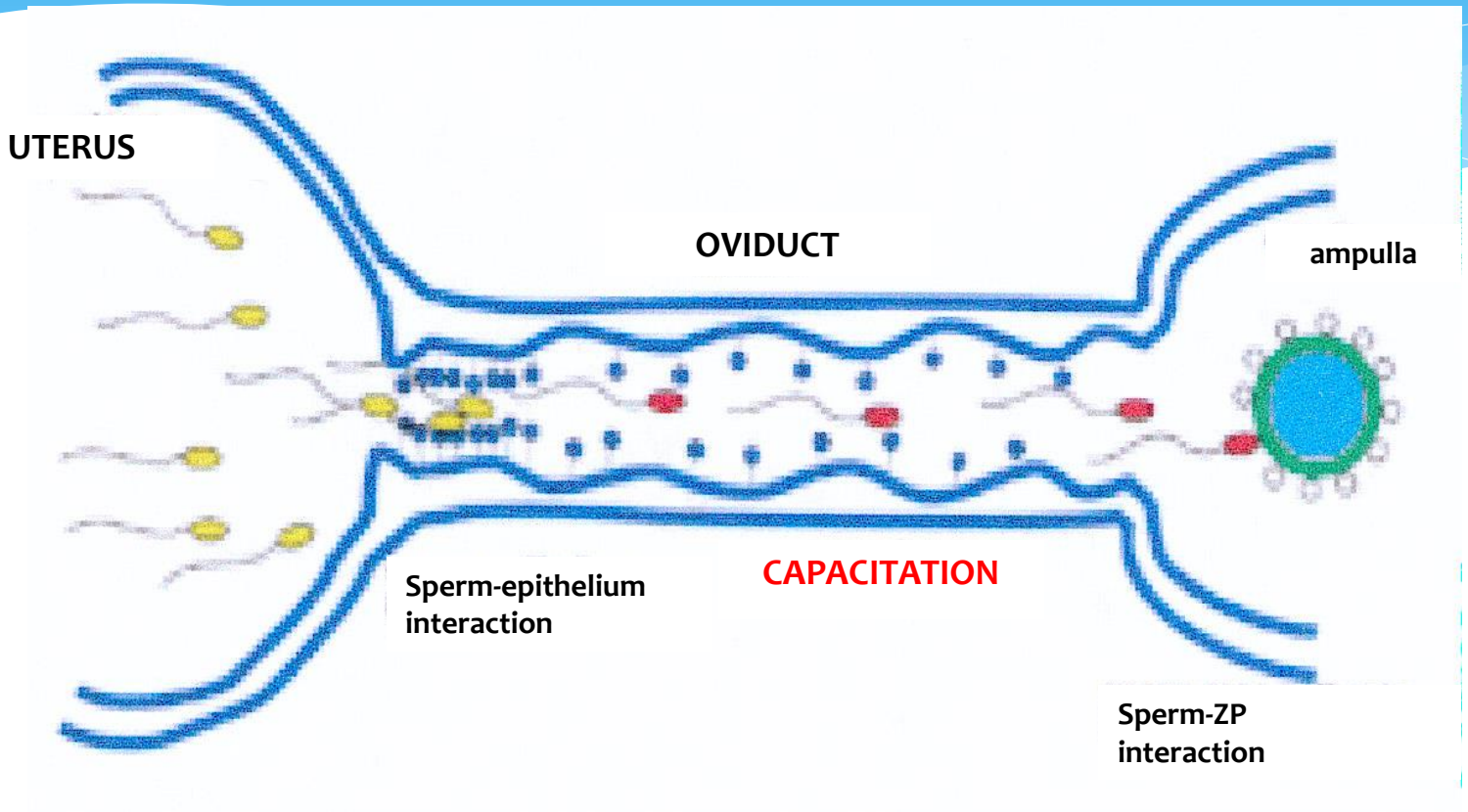


Figure 4. A semi-diagrammatic representation of the arterial blood supply to the ovary and isthmus of the pig Fallopian tube. A portion of the ovarian vein is also shown. A counter-current transfer of follicular hormones was demonstrated from the ovarian vein to the corresponding artery and thus into the utero-tubal branch [37].

Interactions



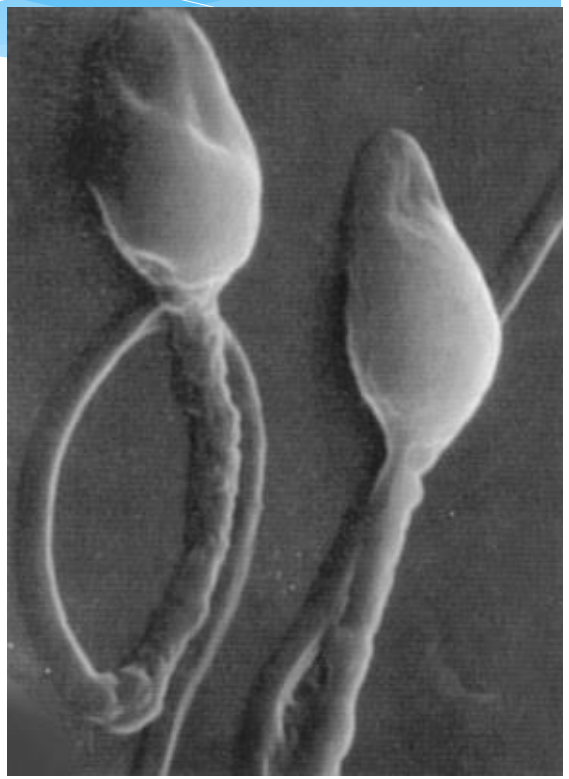
CAPACITATION

THE FERTILIZING SPERM'S LIFE-STORY

Formation (testis)

Maturation (epididymis)

Storage (epididymis)



- Deposition in female tract
- **Capacitation**
- Bind to zona pellucida (ZP)
- Fertilization
  - Acrosome reaction (AR)
  - Penetration through ZP
  - Fusion with egg
  - Activation of egg

Decondensation of sperm head

Formation of male PN

Syngamy and initiation of cell cycle

CAPACITATION

Induces a series of physiological changes in the sperm, which acquires the ability of responding to an agonist (e.g. ZP)

Events occurring in sperm head	Events occurring in sperm tail
<u>Events that initiate capacitation:</u> changes in the lipid organization of the sperm plasma membrane that are essential to **enhance the fusogenicity of sperm membranes** **Event that is a consequence of capacitation: AR**	* Motility changes (**hyperactivated motility**) Glycolisis is important in the event leading to hyperactivation

CAPACITATION

Spermiogenesis/ sperm maturation/ storage

Sperm membrane must remain stable

time of fertilization

Sperm membrane must be in a metastable fusible state

A preparatory **process of membrane destabilization** must take place prior to sperm encountering the egg

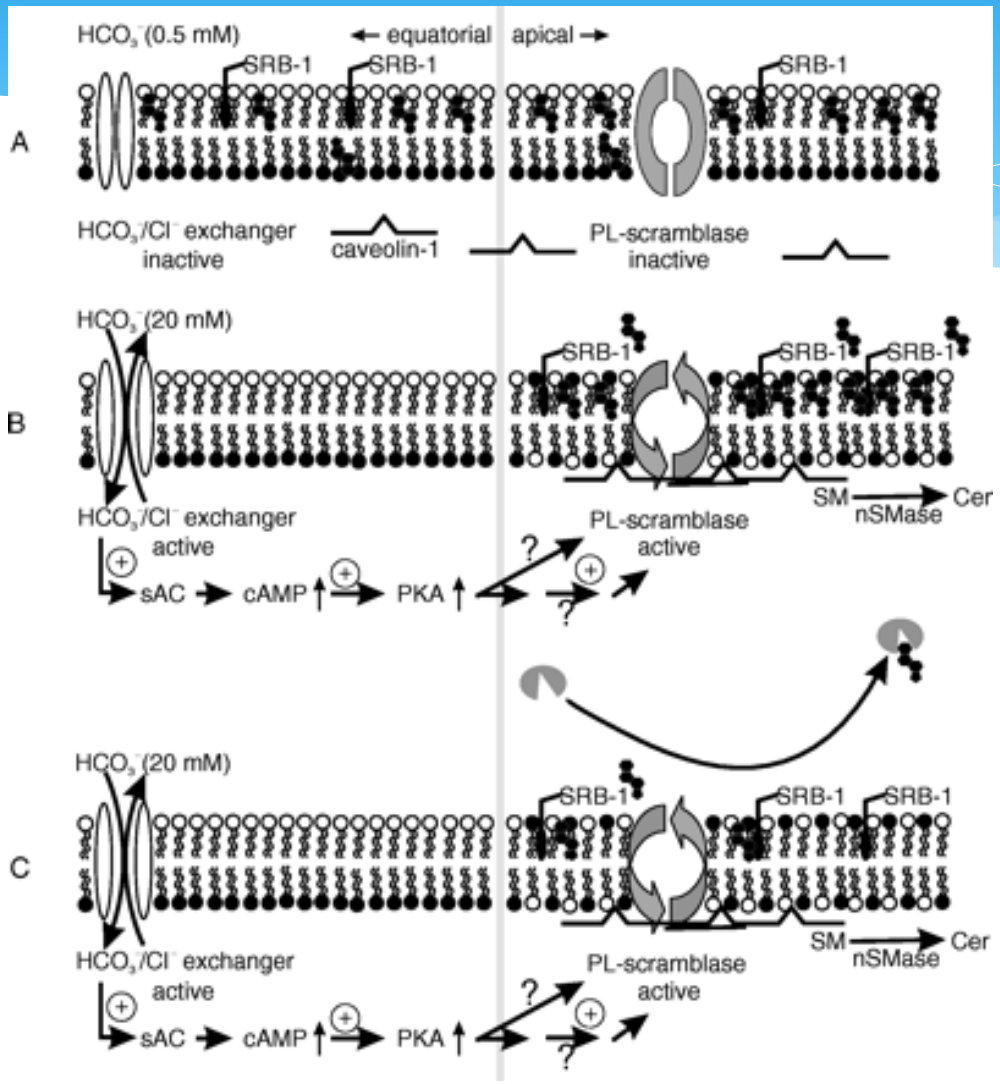
Molecular basis of CAPACITATION in mammalian sperm

Sperm capacitation is a HCO^-_3-dependent process

HCO^-_3 transport in sperm causes	HCO^-_3 transport in sperm causes
1. stimulation of sperm AC 2. increase in cAMP 3. activation of PKA 4. phosphorylation of target proteins that initiate several signaling pathways (Harrison 2004; Harrison & Miller 2000)	* increase in intracellular pH

Sperm capacitation is a HCO^-_3-dependent process

HCO_3^--induced **scrambling** is a major direct effect that enables and facilitates albumin-mediated **cholesterol extraction**



* The component particularly responsible for inducing capacitation is **BICARBONATE (HCO_3^-)/CO_2**

* It acts by stimulating a special adenylyl cyclase (AC) abundant in sperm

cAMP increases

PKA activation

Tyr phosphorylation of several substrates

PKA-dependent activation of scramblase

Sperm plasma membrane changes

(alteration in lipid architecture)

Merocyanin staining

-bilayer redistribution of aminophospholipids
-lateral redistribution of seminolipid and cholesterol/ **cholesterol extraction** by Albumin

Gadella et al. 2002

CAPACITATION

Events occurring in sperm head

Events that initiate capacitation	Event that are a consequence of capacitation
* Changes in the lipid organization of the sperm plasma membrane that are essential to **enhance the fusogenicity of sperm membranes** * Increased tyrosine **phosphorilation of proteins** * Ion fluxes resulting in **alteration of sperm membrane potential**	* **AR**

Molecular basis of CAPACITATION in mammalian sperm

Sperm capacitation is a process in which early changes take place rapidly as 1 min, whereas full capacitation is accomplished within hours (depending on the species of interest)

* **HCO^-_3 and the cAMP pathway**
* **Changes that occur at the level of plasma membrane** (early events/late events)
* **Change of sperm plasma membrane potential**
* **Tyr Phosphorylation events**
* **Ca^{2+}**

Molecular basis of CAPACITATION in mammalian sperm

Changes that occur at the level of plasma membrane architecture

Change of sperm plasma membrane potential

rapid	slow
1. Associated with the activation of HCO3/AC/PKA pathway, that leads to the **activation of sperm phospholipid scramblase** causing a different lipid organization	* Related with the presence of cholesterol acceptors (i.e.**albumin**) that **facilitate cholesterol extraction** from sperm plasma membrane. This mechanism can account for the membrane fluidity changes

CAPACITATION

Events occurring in sperm tail

Events that initiate capacitation	Event that are a consequence of capacitation
* Increased tyrosine **phosphorylation of proteins**	* Motility changes (**hyperactivated motility**) Glycolisis is important in the event leading to hyperactivation

Molecular basis of CAPACITATION in mammalian sperm

- ✔ **HCO^-_3 and the cAMP pathway**
- ✔ **Changes that occur at the level of plasma membrane** (early events/late events)
- ✔ **Change of sperm plasma membrane potential**
- **Tyr Phosphorylation events**
- **Ca^{2+}**

Molecular basis of CAPACITATION in mammalian sperm

Tyr phosphorylation events

slow

* The rise in Tyr phosphorylation of proteins **gives rise to downstrem events in the sperm tail** (e. g. a rise in glycolysis and hyperactivated motility) **and in sperm head** (increased ZP affinity as well as acrosome fusogenicity)

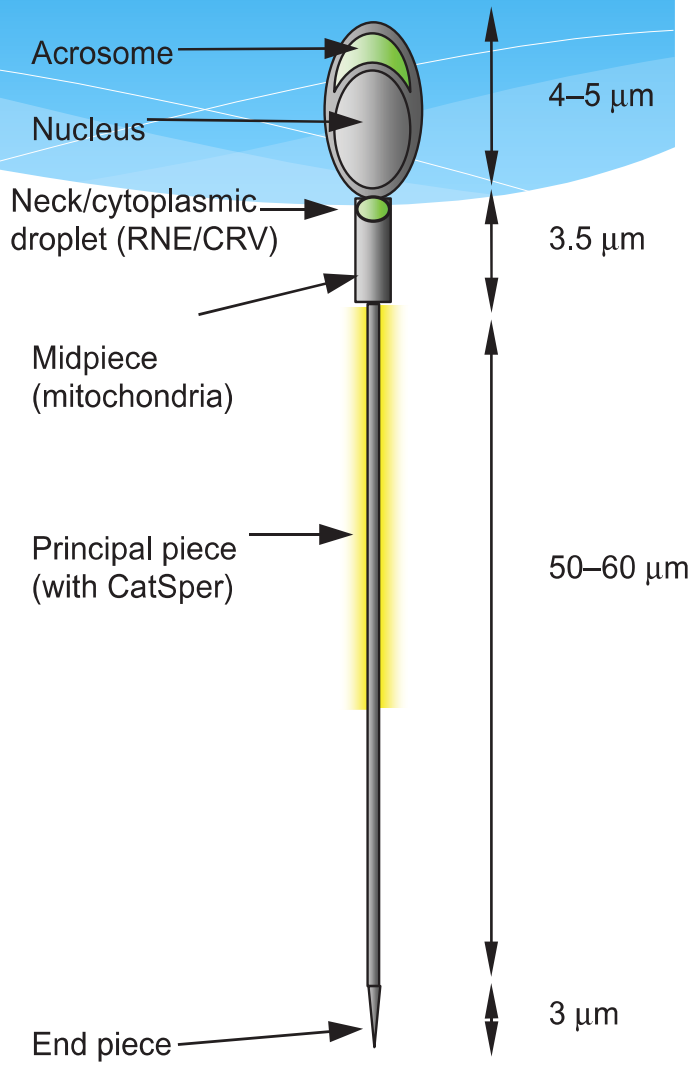
Molecular basis of CAPACITATION in mammalian sperm

- ✔ **HCO^-_3 and the cAMP pathway**
- ✔ **Changes that occur at the level of plasma membrane** (early events/late events)
- ✔ **Change of sperm plasma membrane potential**
- ✔ **Tyr Phosphorylation events**
- * **Ca^{2+}**

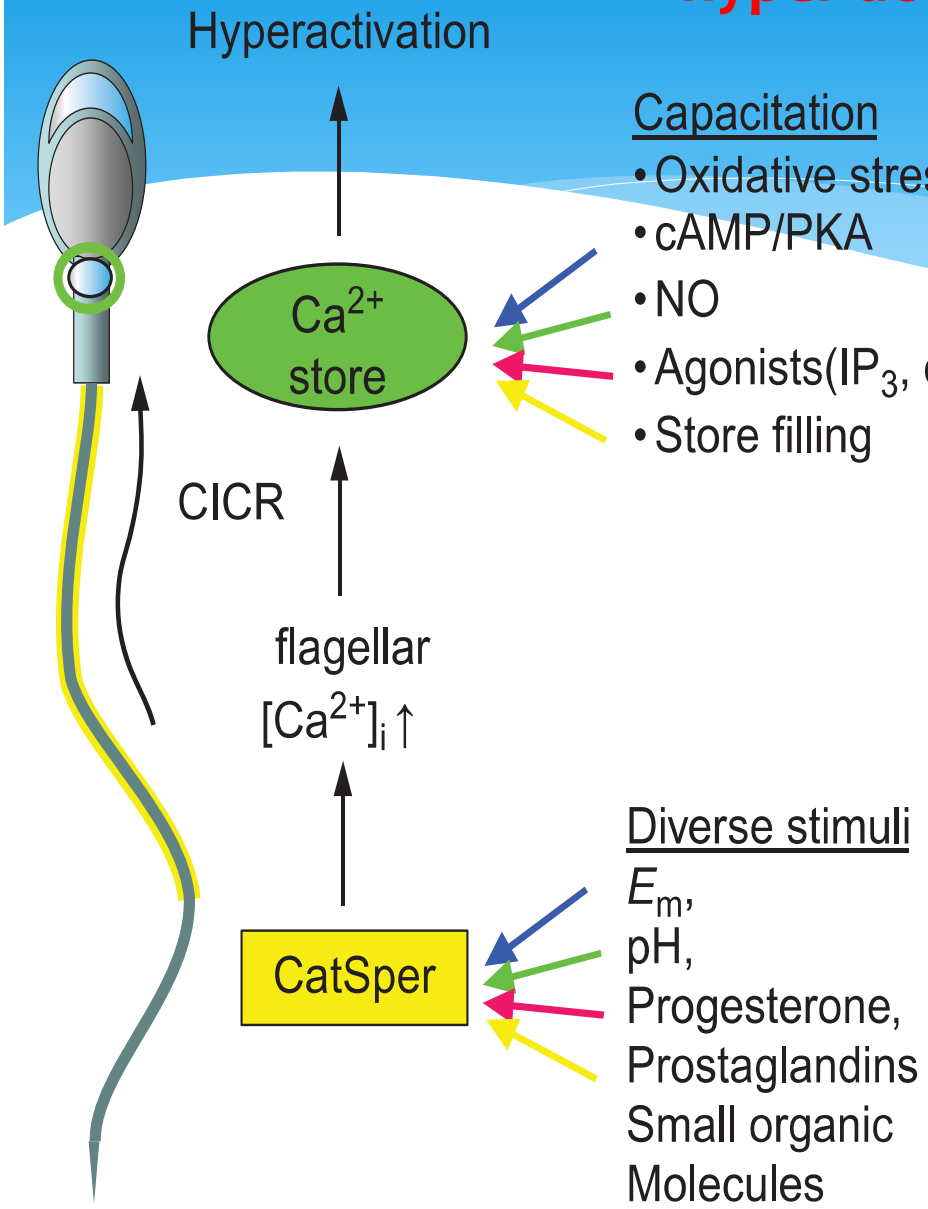
Sperm Ca^{2+} stores on the acrosome and at the sperm neck

* During the later stages of their development spermatozoa shed much of their cytoplasm including intracellular organelles. Thus **mammalian sperm contain no organised ER**. However, studies on the expression of Ca^{2+} store components and on the generation of $[Ca^{2+}]i$ signals suggest that **the remaining intracellular organelles function as Ca^{2+}-stores and play a significant role in the regulation of cellular function** (*Costello et al. 2009*).

* In particular, **the acrosomal vesicle** at the apex of the head and **the collection of vesicular membranous structures (RNE/CRV) that occur at the sperm neck and anterior midpiece** (including the cytoplasmic droplet of human sperm) appear to be functionally **important Ca^{2+}-stores** (**shown in green**).



Model for triggering/regulation of CatSper-activated hyper-activation

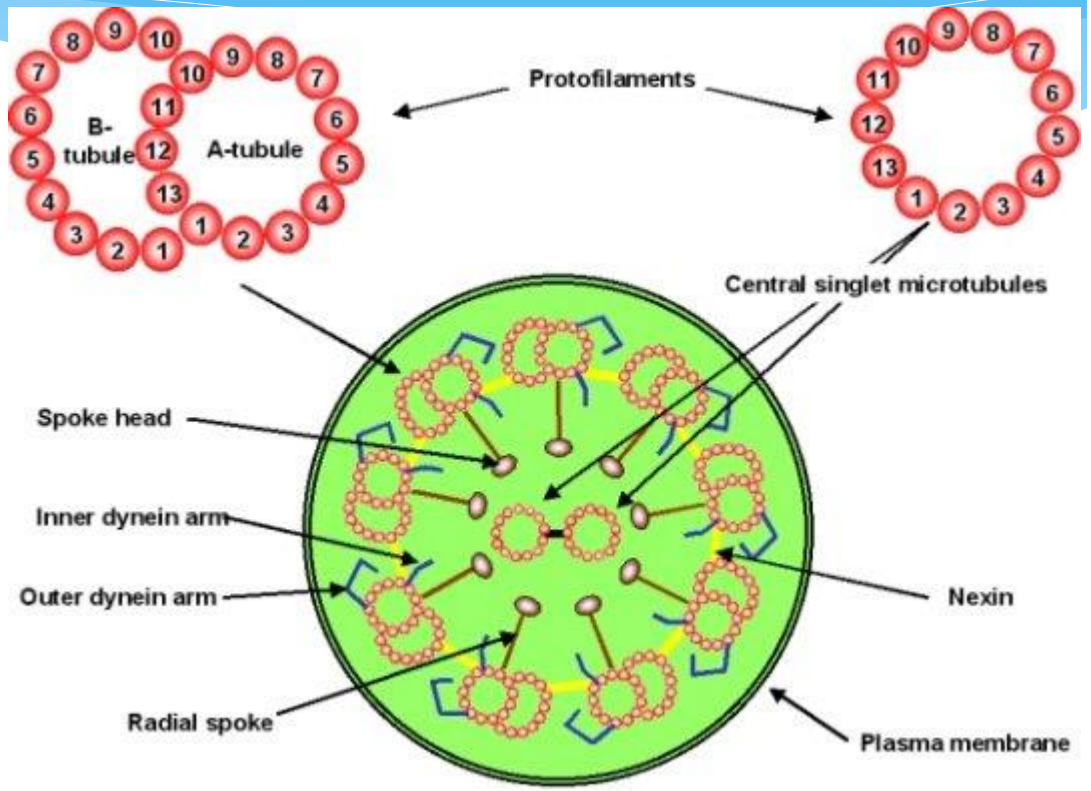


Susceptibility of the store to CICR is potentially regulated/sensitised by processes occurring during capacitation, as well as Ca^{2+} store filling and effects of agonists on Ca^{2+}-store release channels

Ca^{2+} from the flagellum diffuses forward, raising $[Ca^{2+}]i$ at the sperm neck and can mobilise stored Ca^{2+} by Ca^{2+}-induced Ca^{2+} release (CICR), triggering **hyperactivation**

CatSper channels in the flagellum (yellow box) **are activated by diverse stimuli** including intracellular pH (pHi), membrane potential (Em), progesterone, prostaglandins and other organic molecules

MOTILITY


Protofilaments
B-tubule
A-tubule
Central singlet microtubules
Spoke head
Inner dynein arm
Outer dynein arm
Radial spoke
Nexin
Plasma membrane

Early events at fertilization

Capacitated sperm has to cross several oocyte vestments prior to fertilize it

1. CCs
2. ZP
3. Oolemma