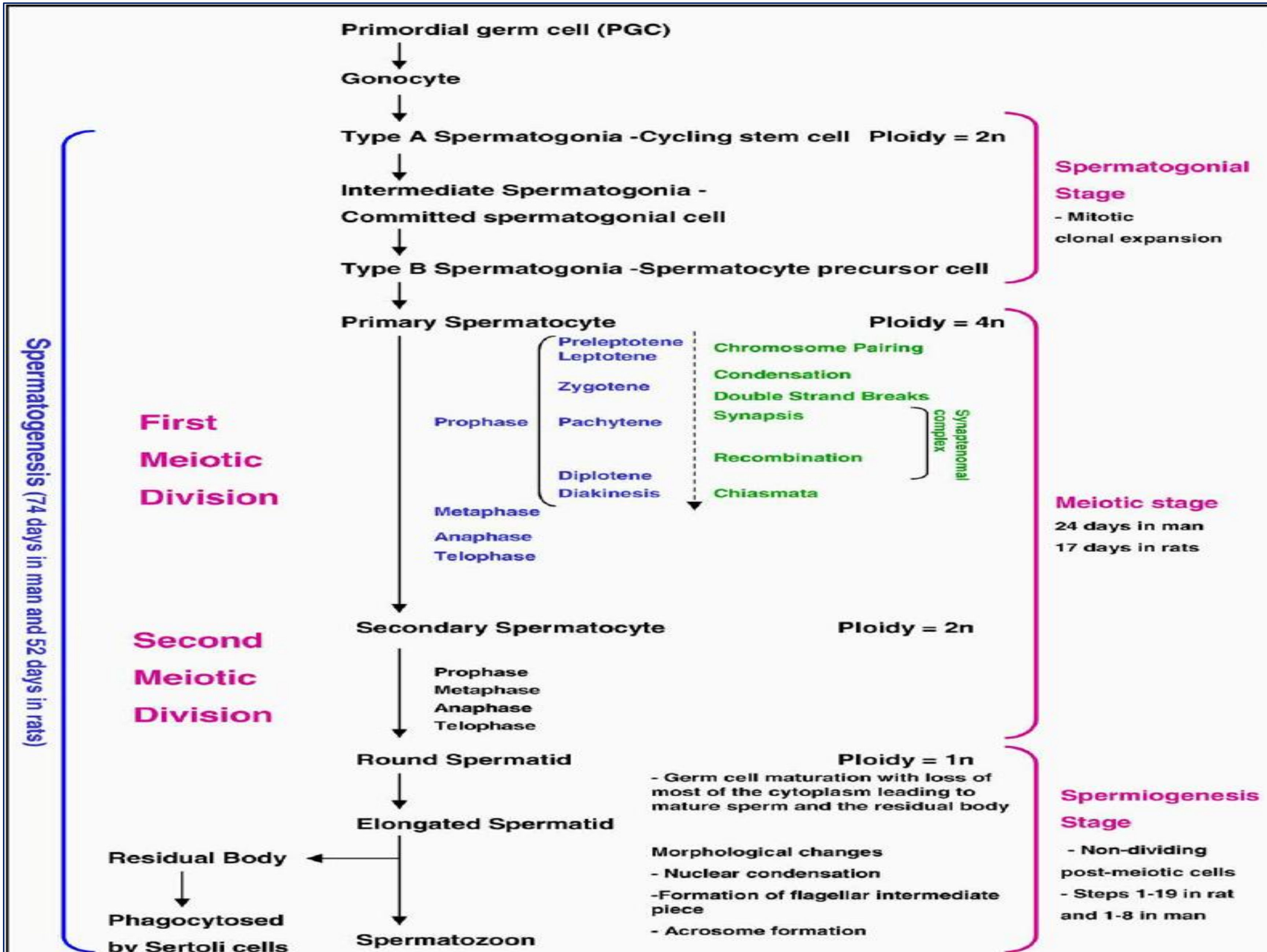
Spermatogenesis: (Formation Migration, Maturation and Ejaculation of Semen), Fine structure of Sperm, Semen and Its Composition

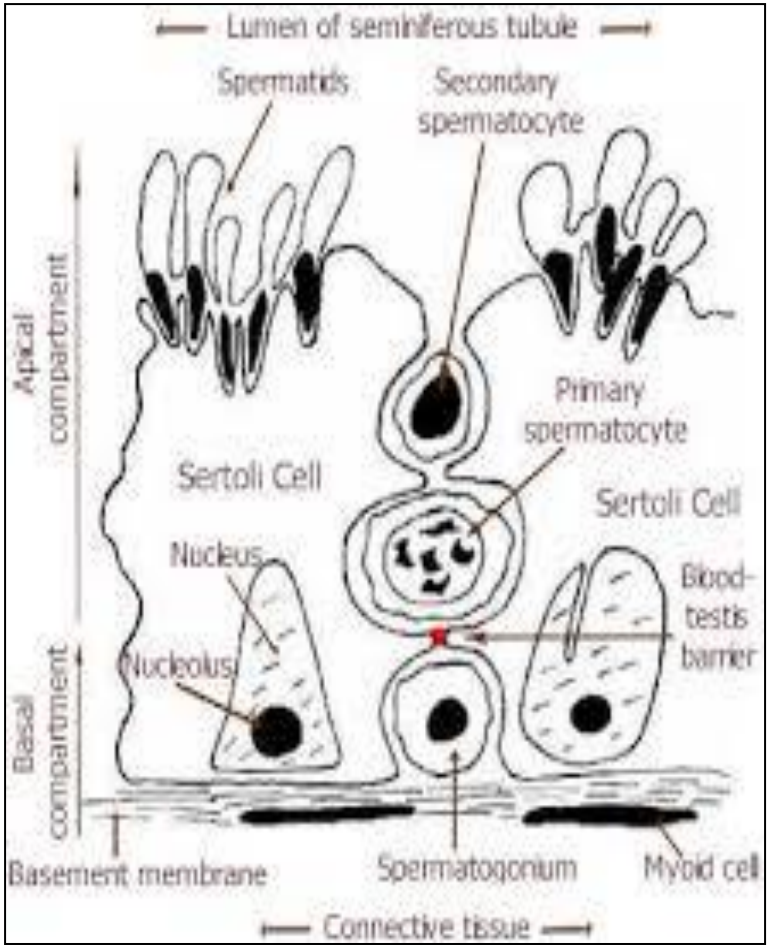
UNIT 3 VGO 604 | DR ANKESH KUMAR

Spermatogenesis-It is the different steps by which spermatogonia are transformed into spermatozoa in the testes it begin at puberty and continues into old age. The whole process take about 57-64 days

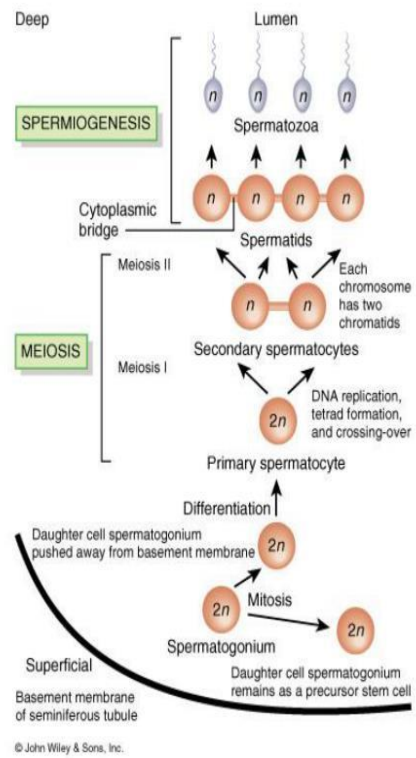
Time of Puberty in male Animals

Species	Puberty (Months)	Sexual maturity (Months)
Cow Bull	10-12	24-36
Buffalo Bull	24-36	36-48
Stallion	18-24	36-48
Ram	3-6	8-12
Buck	3-6	8-12
Boar	5-6	8-9
Dog	6-12	12


Primordial germ cell (PGC)
Gonocyte
Type A Spermatogonia -Cycling stem cell Ploidy = 2n
Intermediate Spermatogonia -
Committed spermatogonial cell
Type B Spermatogonia -Spermatocyte precursor cell
Spermatogonial Stage
- Mitotic clonal expansion
Primary Spermatocyte
Ploidy = 4n
Preleptotene
Leptotene
Zygotene
Prophase
Pachytene
Diplotene
Diakinesis
Metaphase
Anaphase
Telophase
Chromosome Pairing
Condensation
Double Strand Breaks
Synapsis
Recombination
Chiasmata
Synaptenomal complex
First Meiotic Division
Meiotic stage
24 days in man
17 days in rats
Secondary Spermatocyte
Ploidy = 2n
Second Meiotic Division
Prophase
Metaphase
Anaphase
Telophase
Round Spermatid
Ploidy = 1n
- Germ cell maturation with loss of most of the cytoplasm leading to mature sperm and the residual body
Elongated Spermatid
Residual Body
Phagocytosed by Sertoli cells
Spermatozoon
Morphological changes
- Nuclear condensation
-Formation of flagellar intermediate piece
- Acrosome formation
Spermiogenesis Stage
- Non-dividing post-meiotic cells
- Steps 1-19 in rat and 1-8 in man
Spermatogenesis (74 days in man and 52 days in rats)



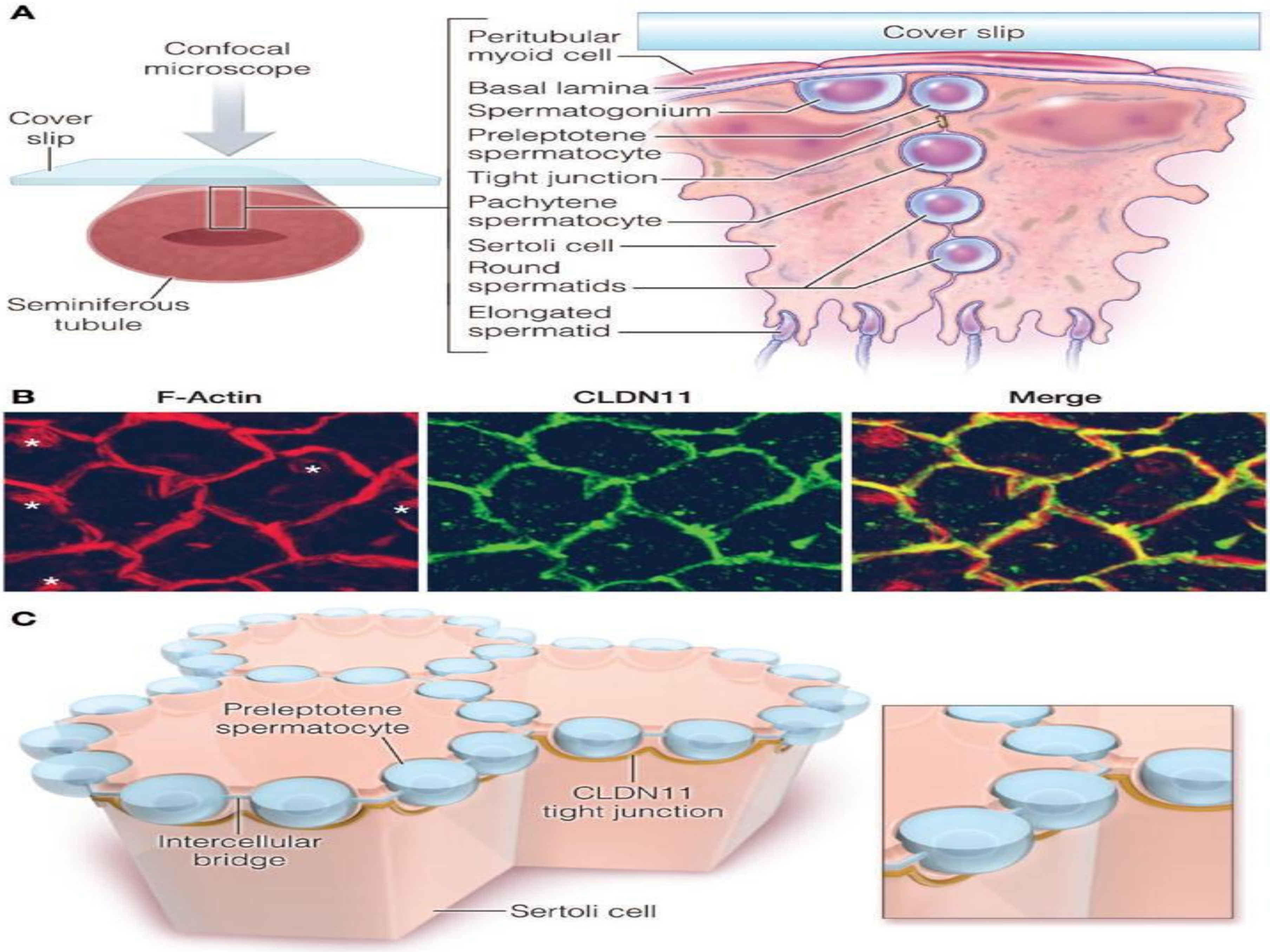
Spermatogenesis



- Secondary spermatocytes are formed
  - 23 chromosomes of which each is 2 chromatids joined by centromere
  - goes through meiosis II
- 4 spermatids are formed
  - each is haploid & unique
  - all 4 remain in contact with cytoplasmic bridge
  - accounts for synchronized release of sperm that are 50% X chromosome & 50% Y chromosome

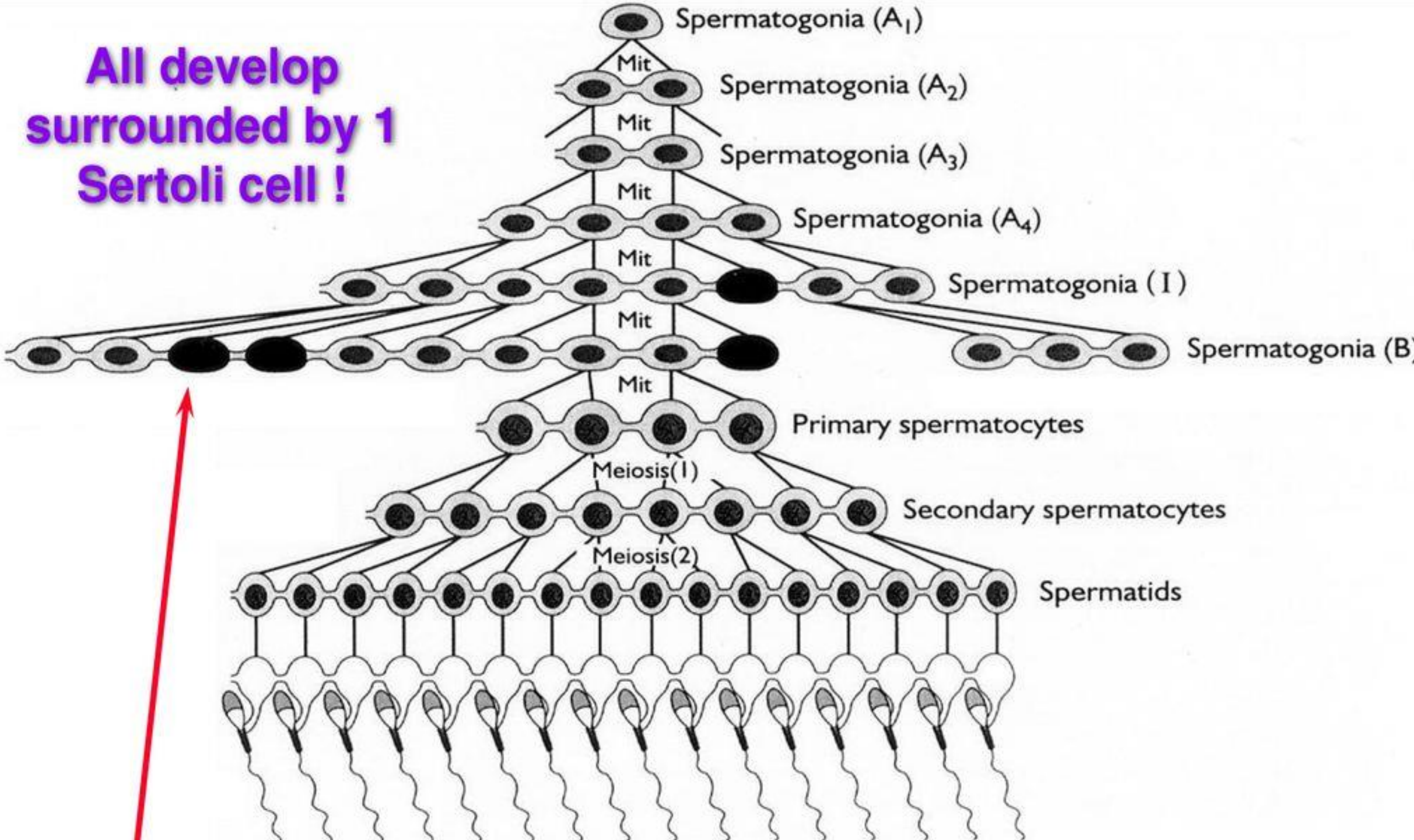
Tight junction and cytoplasmic bridge

Spermatogenesis takes place within seminiferous tubules of the testis. Extensive tight junctions between somatic Sertoli cells, referred to ultrastructurally as basal ectoplasmic specializations, create one of the body's tightest epithelial barriers and in doing so create separate functional compartments for diploid spermatogonia in the basal compartment, and differentiating spermatocytes and haploid spermatids in the adluminal compartment. To prevent autoimmunity and maintain a conducive microenvironment for differentiation, differentiating germ cells must cross this barrier without disrupting its functional integrity


A
Confocal microscope
Cover slip
Seminiferous tubule
Cover slip
Peritubular myoid cell
Basal lamina
Spermatogonium
Preleptotene spermatocyte
Tight junction
Pachytene spermatocyte
Sertoli cell
Round spermatids
Elongated spermatid
B
F-Actin
CLDN11
Merge
C
Preleptotene spermatocyte
CLDN11 tight junction
Intercellular bridge
Sertoli cell

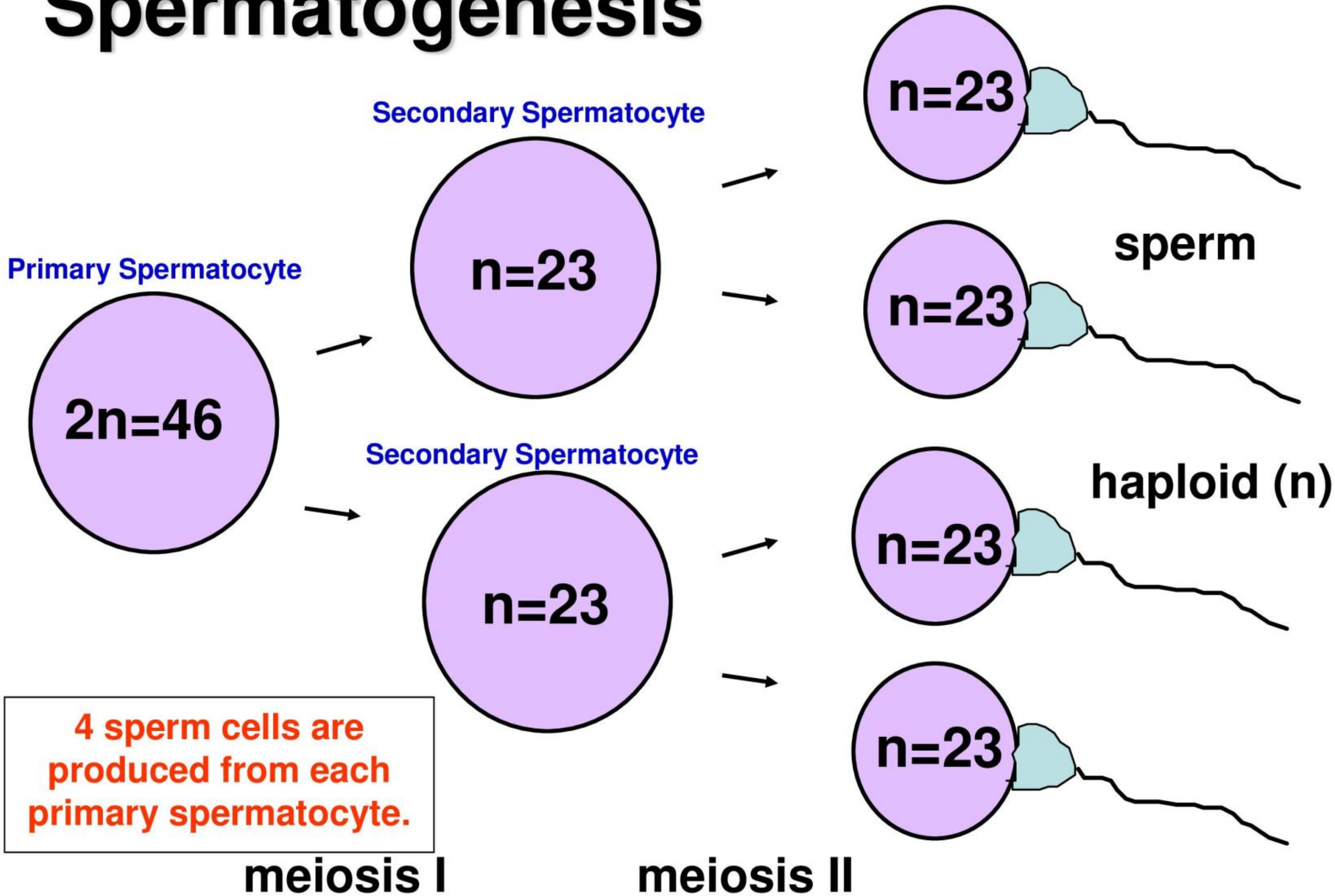
Cytoplasmic Bridges Present Among Daughter Cells

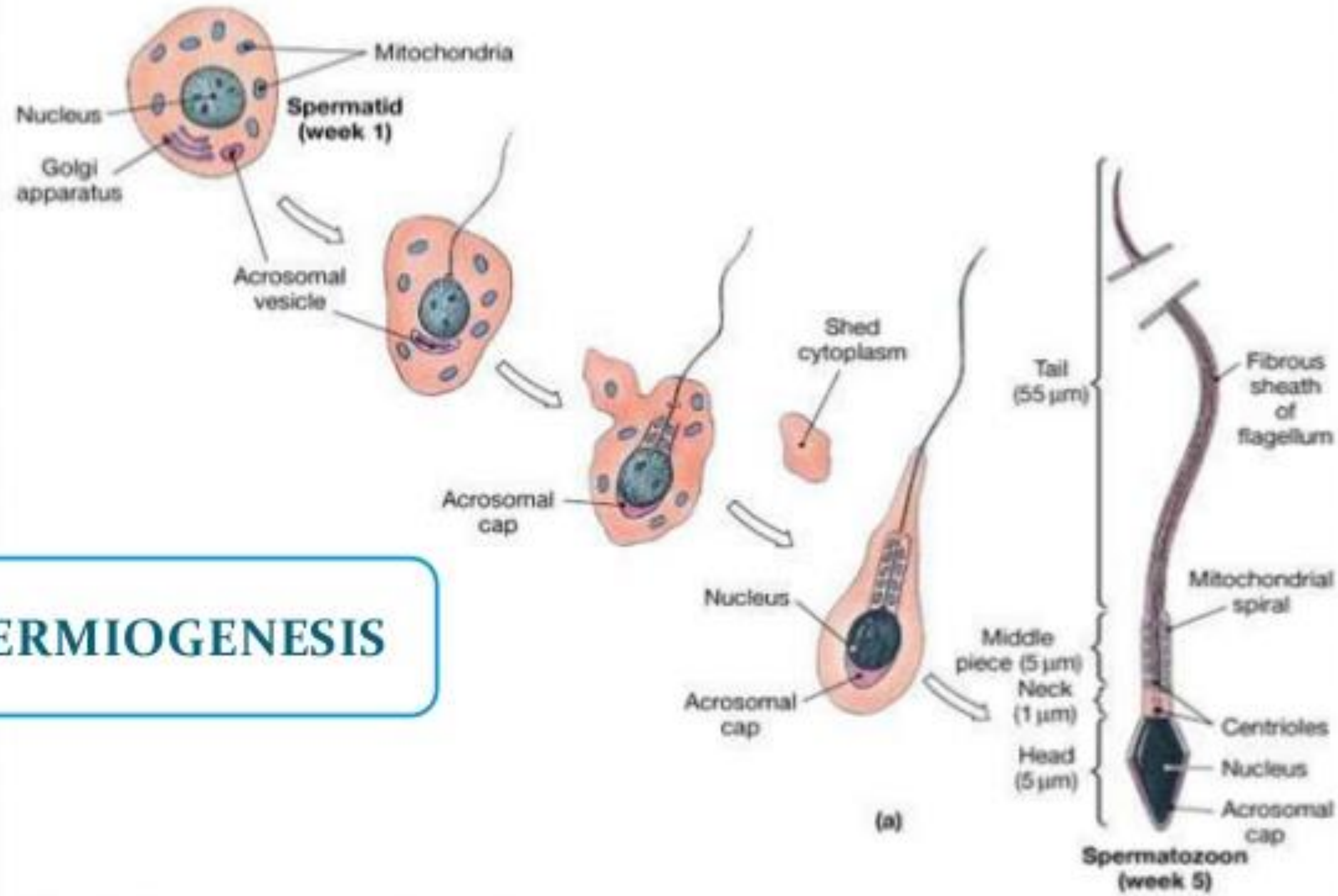
All develop surrounded by 1 Sertoli cell !



Degenerating Spermatogonia (Apoptosis, as high as 75%)

Spermatogenesis





SPERMIOGENESIS

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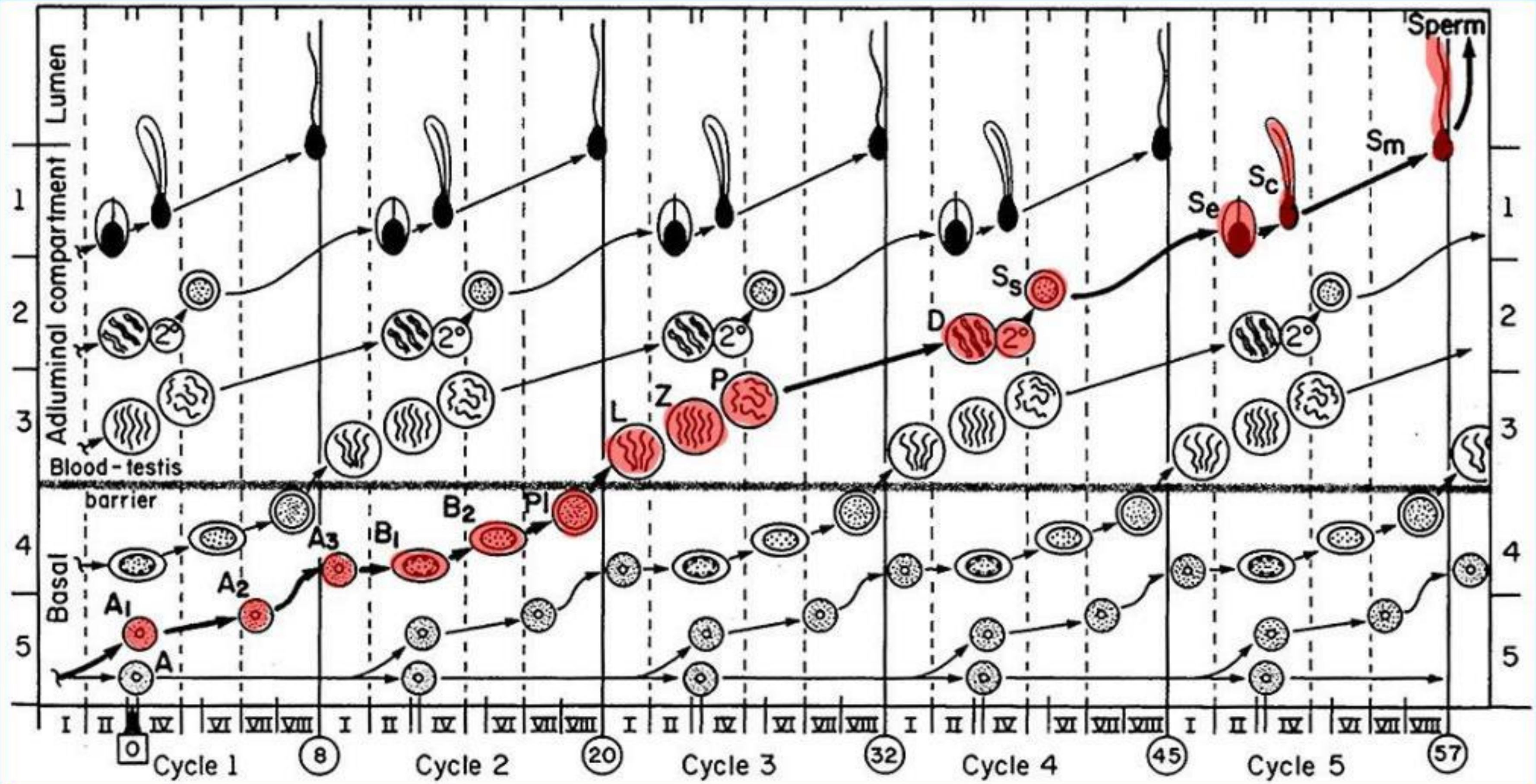
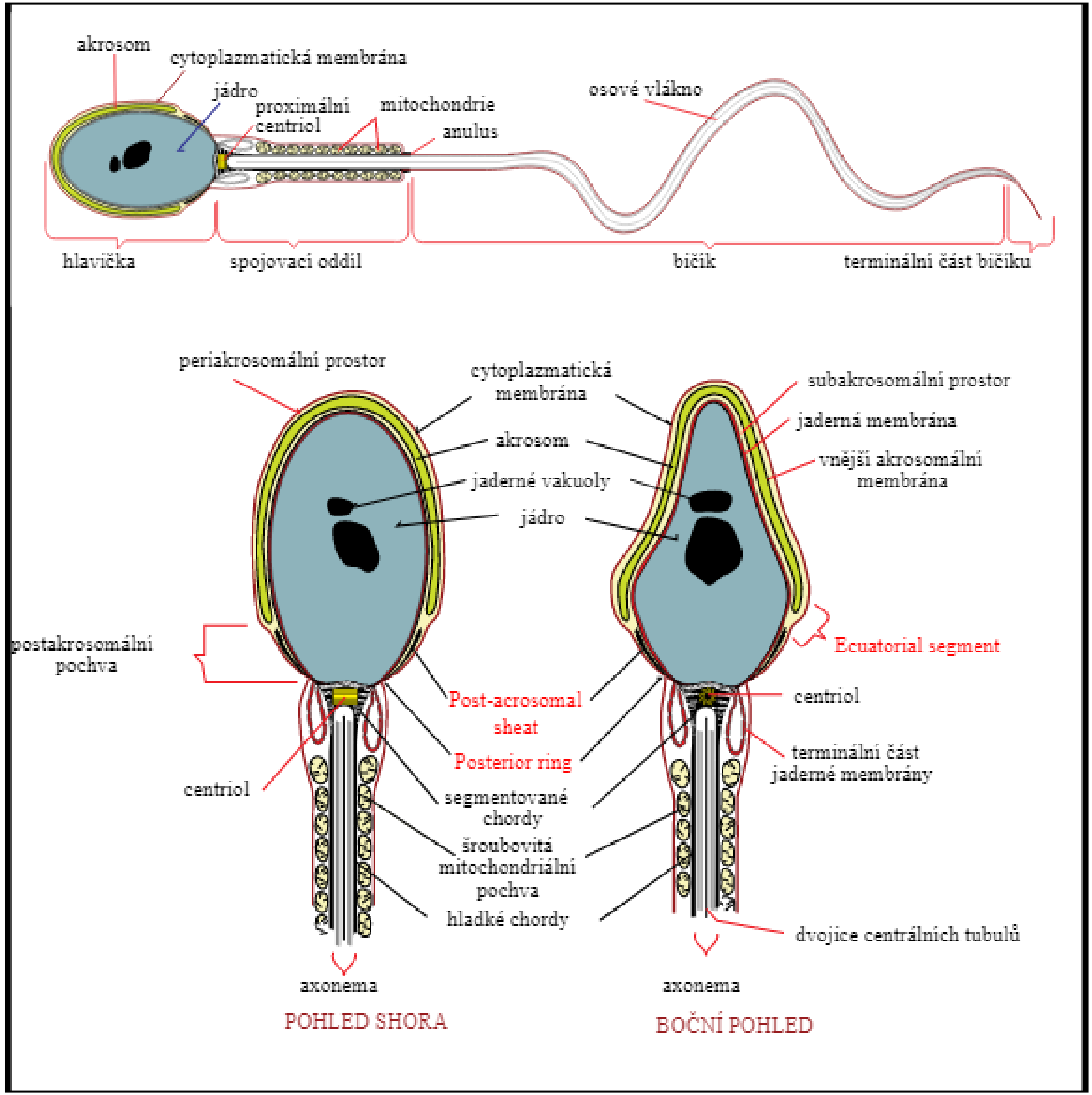
Spermatogenesis

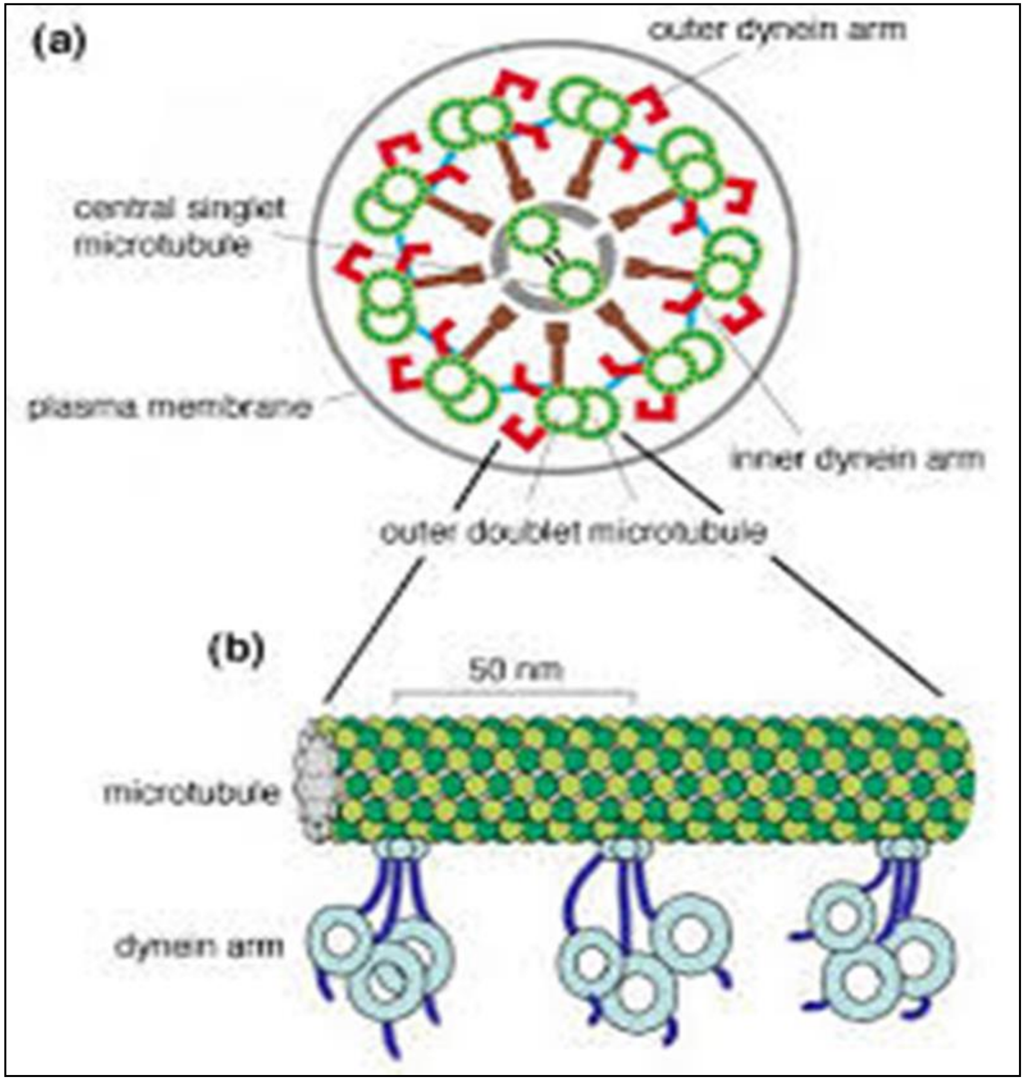


57 days

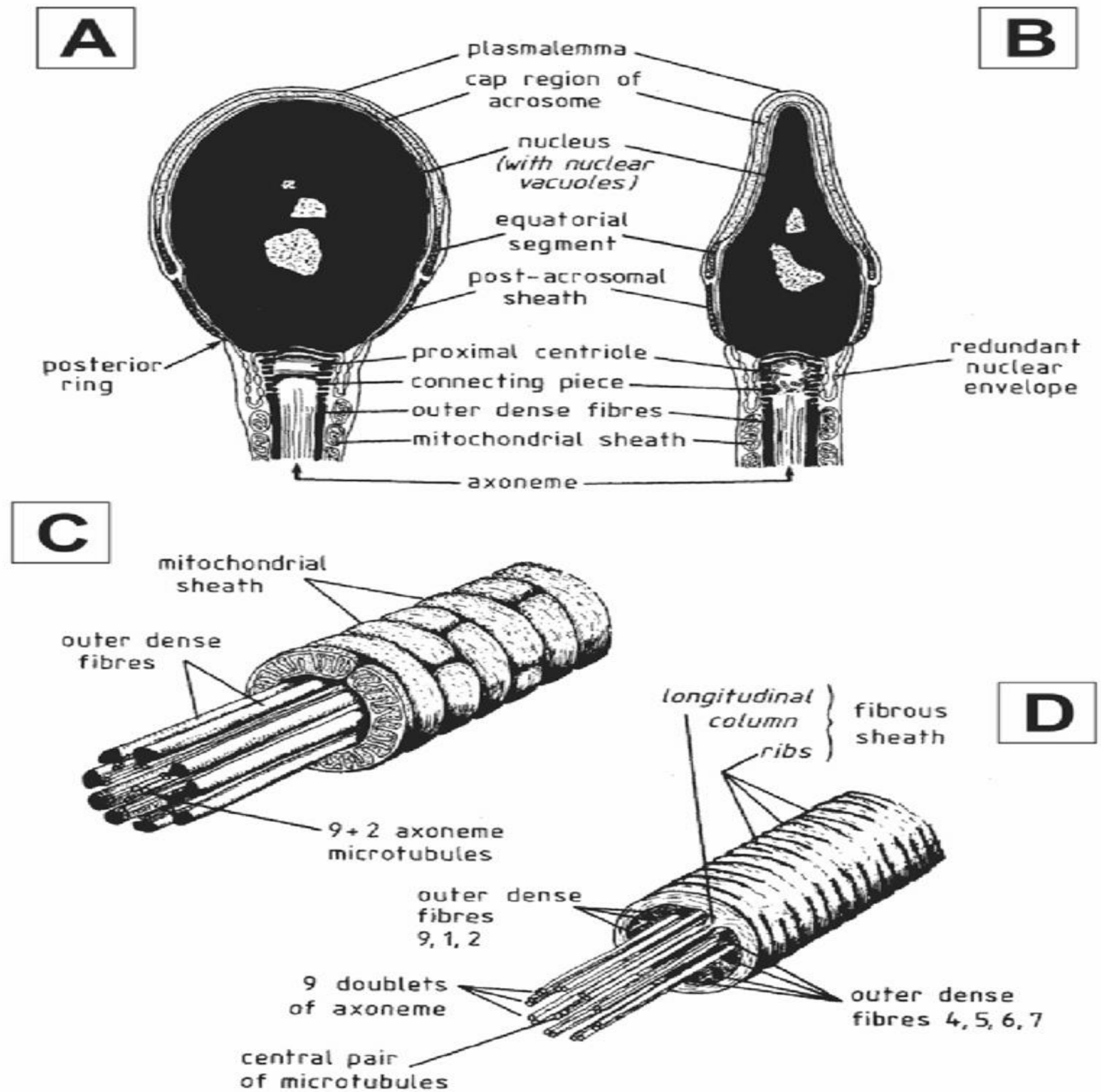
Stallion

Ultra structure of Sperm

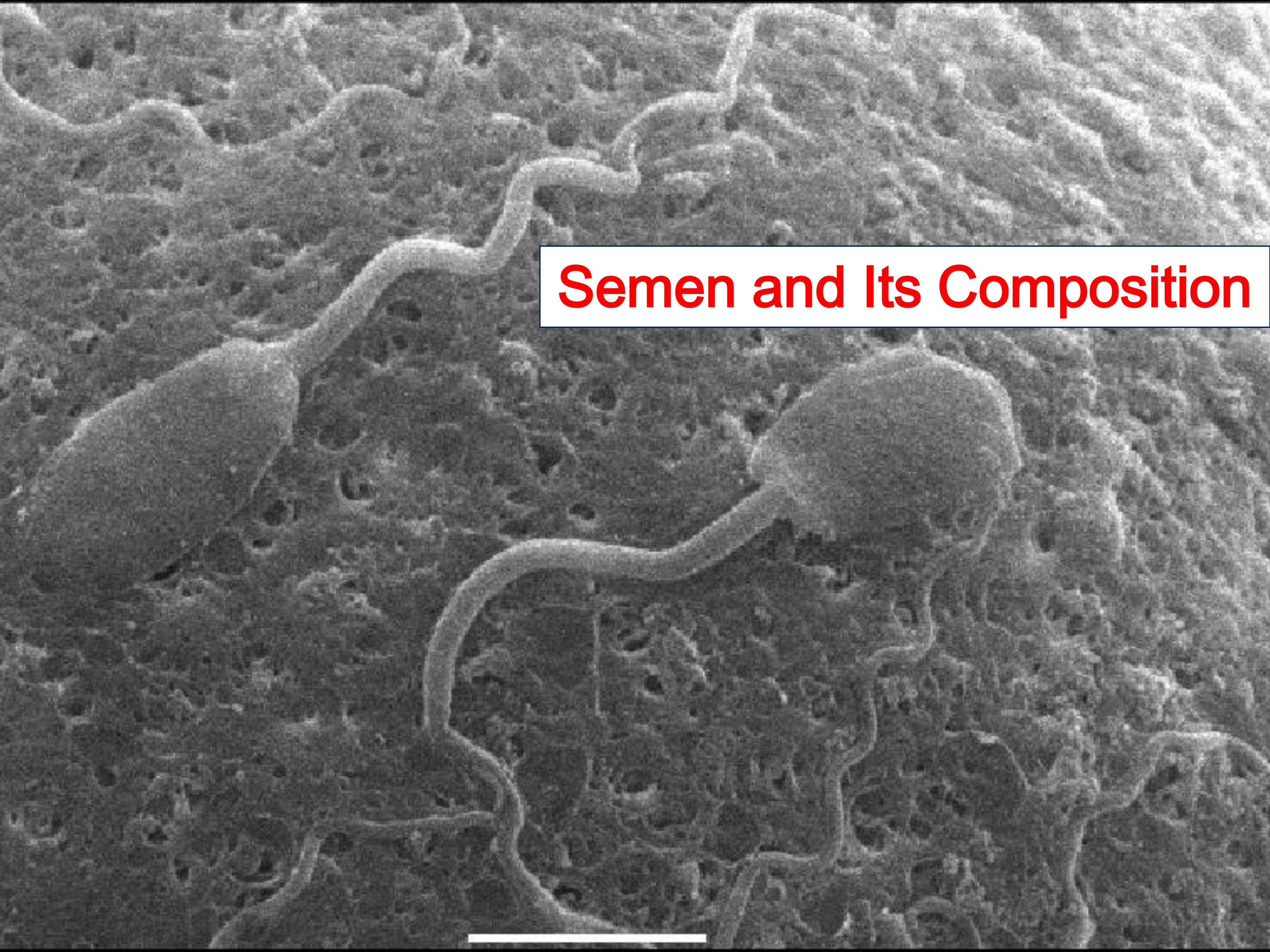


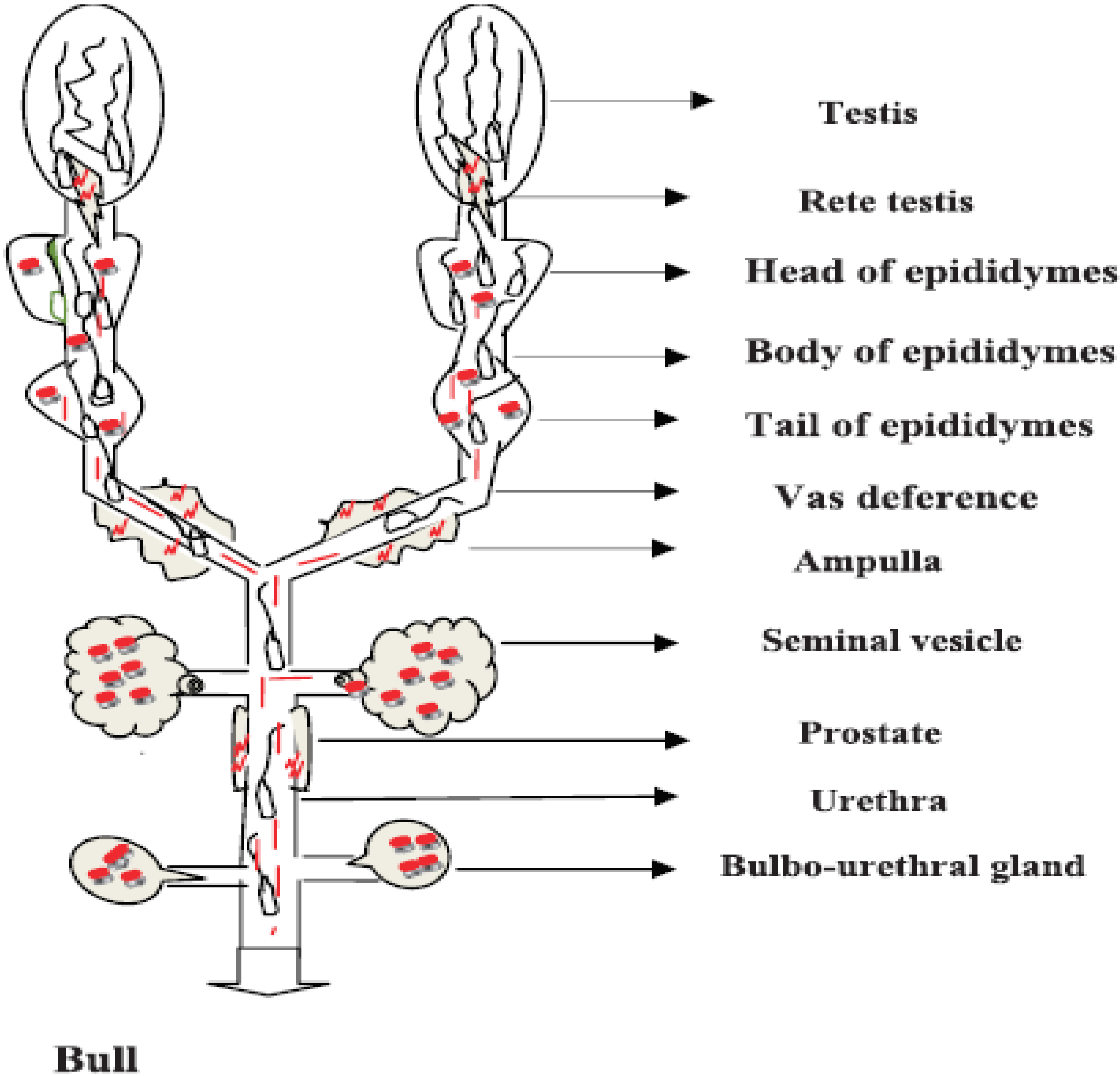


(a)
outer dynein arm
central singlet microtubule
plasma membrane
inner dynein arm
outer doublet microtubule
(b)
50 nm
microtubule
dynein arm


A
B
plasmalemma
cap region of acrosome
nucleus (with nuclear vacuoles)
equatorial segment
post-acrosomal sheath
posterior ring
proximal centriole
connecting piece
outer dense fibres
mitochondrial sheath
redundant nuclear envelope
axoneme
C
mitochondrial sheath
outer dense fibres
9+2 axoneme microtubules
D
longitudinal column
ribs
fibrous sheath
outer dense fibres 9, 1, 2
9 doublets of axoneme
central pair of microtubules
outer dense fibres 4, 5, 6, 7

Semen and Its Composition




Testis
Rete testis
Head of epididymes
Body of epididymes
Tail of epididymes
Vas deference
Ampulla
Seminal vesicle
Prostate
Urethra
Bulbo-urethral gland
Bull

Semen

- Semen consists of spermatozoa suspended in a fluid medium called seminal plasma.

- Seminal plasma is the fluid portion of semen, secreted by both the epididymis as well as the accessory glands before and during ejaculation.

- Seminal plasma is a complex fluid, which serves as a vehicle for transporting ejaculated spermatozoa towards their journey from the testes to their target, the oocyte

- Its pH varies with species—slightly acidic in bulls and rams and slightly alkaline in camelids

- Biochemical components of SP are secreted from rete testis, epididymis, and accessory sex glands (AG) of the male reproductive tract

- AGs known as seminal vesicle, prostate, and bulbo-urethral glands contribute most of the volume of the ejaculate, and the seminal vesicle secretion constitutes the major portion of SP at ejaculation

- SP is made up of ions (Na^+, K^+, Zn^+ , Ca^{++}, Mg^{++}, Cl_2), energy substrates (fructose, sorbitol, glycerylphosphocholine), organic compounds (citric acid, amino acids, peptides, low– and high–molecular weight proteins, lipid, hormones, cytokines), and so on

- Nitrogenous components such as ammonia, urea, uric acid, and creatinine and reducing substances such as ascorbic acids, and hypotaurine also exist in the SP of ruminants

- The composition of SP is determined by the size, storage capacity, and secretory output of different organs of the male reproductive tract

- The chemical composition and function of AG secretions vary among species, among males within a species, and even among ejaculates of the same male

Role of SP

1. Activation and augmentation of the motility of spermatozoa
2. Buffering to provide the optimal osmotic and nutrient medium
3. Prevention of premature activation during physiological transport of spermatozoa and stabilization of the plasma membrane (PM) with capacitation inhibitors
4. Protection of spermatozoa from phagocytosis and destruction in an inflammatory environment in the horse
5. Regulation of sperm transport and elimination
6. Hastening of ovulation in cows (Marion, 1950) and induction of ovulation in pigs
7. Assistance in sperm-ovum interactions
8. Activation of the expression of embryotrophic cytokines and help toward preparing the maternal tract for the developing embryo, particularly by facilitating immune changes required to accommodate pregnancy

9. SP plays an immunoregulatory role that is beneficial to survival of spermatozoa in the female reproductive tract. Several factors in SP, including proteins, cytokines, sex hormones, and prostaglandins, accompany the migration of spermatozoa to the female reproductivetract

10. This also possess potential biological capabilities to protect spermatozoa from different pathogens, both in the male and female reproductive tract

PROTEIN

- A study with bull semen revealed that the acidic proteins (13–16 kd) of SP could be used as a marker for high semen freezability, and a 25–26-kd SP protein could be a marker of low semen freezability

- Major epididymal proteins include lactoferrin, clusterin, procathepsin D, and cholesterol transfer protein, among others. It has been suggested that various epididymal proteins play different roles on spermatozoal function, either by modifying the sperm membrane surface or composition or by contributing to the preservation of sperm integrity

- Bovine seminal plasma proteins (BSP proteins) are emerging superfamily of proteins in mammals

- They are heparin-binding proteins, secreted by seminal vesicles Four proteins are identified as members of the BSP family, referred as BSP-A1, BSP-A2 , BSP-A3, and BSP- 30 kd, and they represent 70%–86% of the total protein content of bovine SP (Moura et al, 2007).

- BSP-A1 and BSP-A2 share the same primary structure, together called PDC-109(15–25 mg/mL in SP)

Protein found in SP: BSP, Spermadhesines , Calsemin (Ram also), osteopontin of 55 kd is also present in bull SP, Seminalplasmin

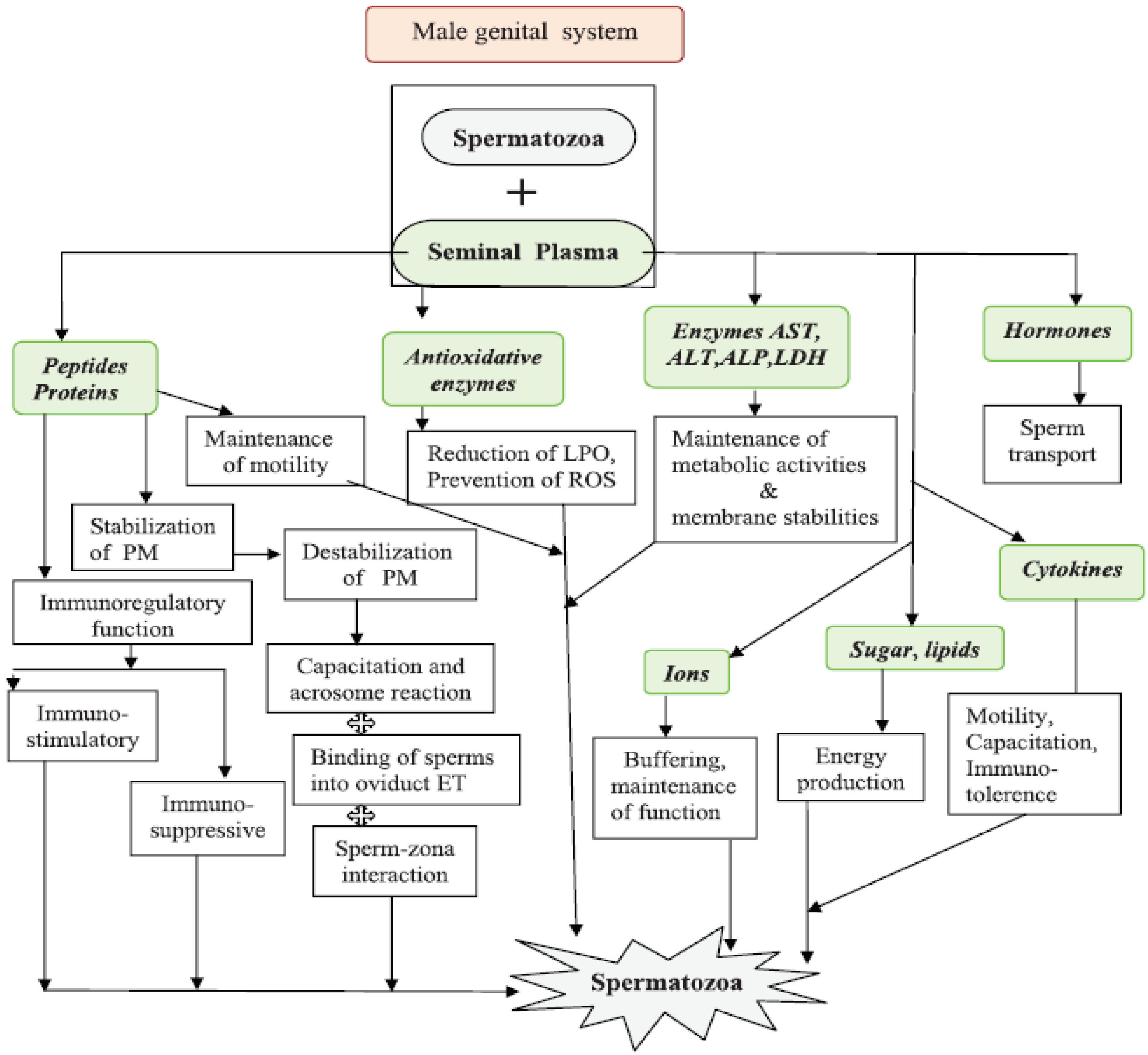


Figure 2. A model of seminal plasma (SP) structures and functions. The model focuses on the main components of SP and their functions on spermatozoa. PM indicates plasma membrane; AST, aspartate amino transferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; LPO, lipid peroxidation; ROS, reactive oxygen species; ET, epithelium.

Table 1. *Composition of seminal plasma in ruminants and camelids (values are mg/dL unless otherwise stated)*

Content	Bull[a]	Ram[b]	Goat[b]	Buffalo[c]	Old World Camelids[d]	New World Camelids[e]
Fructose	150–900	150–600	875	368–815	23.5	3–7
Glucose	300	0.9–1.6	4.8–8.8	13–52	29–42	4–8
Citric acid	340–1150	110–260	…	440–444	9.8	3.1–6.0
Total proteins, g/dL	3.8	2.30–2.50	0.77–1.48	…	1.6–2.6	3–4
Total lipids	29	254–396	…	150–175	87	51–115
Phospholipids	149.1	…	57	6.9–59.4	26–48	27–31
Cholesterol	312.16	…	…	117.83	15.3–25.9	0–8
Glutamic acid	1.0–8.0	4.5–5.2	…	4.28	…	…
Na	140–280	120–258	60–183	260–278	…	…
K	80–210	50–140	76–255	192–205	…	…
Ca	35–60	6–15	5–15	30	7.7–8.8	13–31
P	9	4.8–12.0	…	8–9	1.7–4.6	7–17
Cl	110–290	86	82–215	303–347	84–120	263–491
Mg	7–12	2–13	1–4	4.3–5.7	…	2.1–4.85
Zn	2.6–3.7	56–179	…	0.80–1.17	…	…
Testosterone, pg/mL	210–1310	25–375	…	970	…	…
Estrogen, pg/mL	20–166	…	…	43.67	…	…
Prostaglandins, ng/mL	5–10	500–20 000	…	…	…	…
ALP	246 BU/dL	14 895–40 818 mU/mL	…	315 BU/dL	…	50–3143 UI/L
AST	345–623 SFU/mL	190–256 mU/mL	…	166 units/mL	…	…
ALT	15.0–18.3 SFU/mL	39–148 mU/mL	…	34 units/mL	…	0–115 UI/L
LDH	1909 units/mL	968–1697 mU/mL	…	1621 BBU/mL	…	…

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate amino transferase; BBU, Berger-Broida units; BU, Bodansky units; LDH, lactate dehydrogenase; SFU, Sigma Frankel units; UI, international units.

Table 2. *Phospholipid composition (% of total phospholipids) of ruminant seminal plasma (Jain and Anand, 1976; Andrabi, 2009)*

Phospholipid	% of Total Phospholipids		
	Bull	Buffalo	Goat
Phosphatidyl choline (choline plasmogen)	17.6–32.9	17.3	15.7–22.4
Phosphatidyl choline	24.5–30.0	21.7–34.1	15.2–22.0
Phosphatidyl ethanolamine	5.4–10.5	10.8–11.7	1.1–15.6
Phosphatidyl ethanolamine	5.0–16.3	4.1–4.9	1.6–4.3
Sphingomylin	11.6–16.3	13.–13.8	10.6–19.8
Phosphatidyl serine	1.3	2.8	1.1–5.9
Phosphatidyl inositol	0.8	2.9	1.5–6.0
Lysophosphatidyl ethanolamine	1.2–2.2	5.6–6.6	4.3–14.2
Lysophosphatidyl choline	1.2–2.2	3.1–3.9	3.2–9.8
Diphosphatidyl glycerol	5.0–8.8	3.5–7.4	0.3–0.5
Phosphatidic acid	0.4	0.5	0.2–1.4

Amino acid

There is a broad range of amino acids in SP, and most of these originated in thetestes or epididymides. Their concentrations increase after ejaculation because of extensive proteolytic activity that takes place in semen (Mann, 1964). Amino acids serve as a readily oxidizable substrate for energy, yielding reactions in semen (Neumark and Schindler,1967). The amino acid found in greatest concentration in SP is glutamic acid, which is accompanied by a high level of glutamic oxaloacetic transaminase (GOT)activity (Flipse, 1960). L-Arginine is also present in ruminant SP and acts as a source of energy for normal sperm motility in the form of arginine phosphoric acid (Patel et al, 1998).

Enzymes

Other enzyme

I. AST
II. GPT
III. ALP
IV. LDH

These enzymes are good indictor of PM stability

Sources : testes , Epididymis

A positive correlation of AST/GOT activities in post thaw semen, with acrosome damage in ruminant spermatozoa and a negative correlation with fertility, were also reported by Zhao-Qi et al

Antioxidants—Spermatozoa are highly susceptible to lipid peroxidation by free radicals or reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), superoxide anion (O22), nitric oxide, and hydroxyl radical (2OH). In bovine and ram semen, ROS are generated primarily by dead spermatozoa via an aromatic amino acid oxidase–catalyzed reaction (Upretiet al, 1994).

Physiologically, ROS play an important role for sperm capacitation, acrosome reaction, and stabilization of the mitochondrial capsule in the mid-piece in bovine (Gonc¸alves et al, 2010), and their beneficial effects on sperm functions depend on the nature and concentration of the particular ROS involved (de Lamirande and Gagnon, 1992). Superoxide anion and nitric oxide participate in heparin-induced capacitation, whereas hydrogen peroxides induce capacitation in ejaculated bovine sperm (O'Flaherty et al, 1999). To maintain proper physiological activities a fine balance between ROS production and recycling around sperm cells is essential. Otherwise, any imbalance leads to the impairment of spermatozoal function through oxidative stress (Kim and Partha sarathy, 1998).


Thank You