ZOO 501

DEVELOPMENTAL BIOLOGY

LABORATORY MANUAL

DEPARTEMENT OF BIOLOGY

VIRTUAL UNIVERSITY OF PAKISTAN

Contents:

Experiment No.1

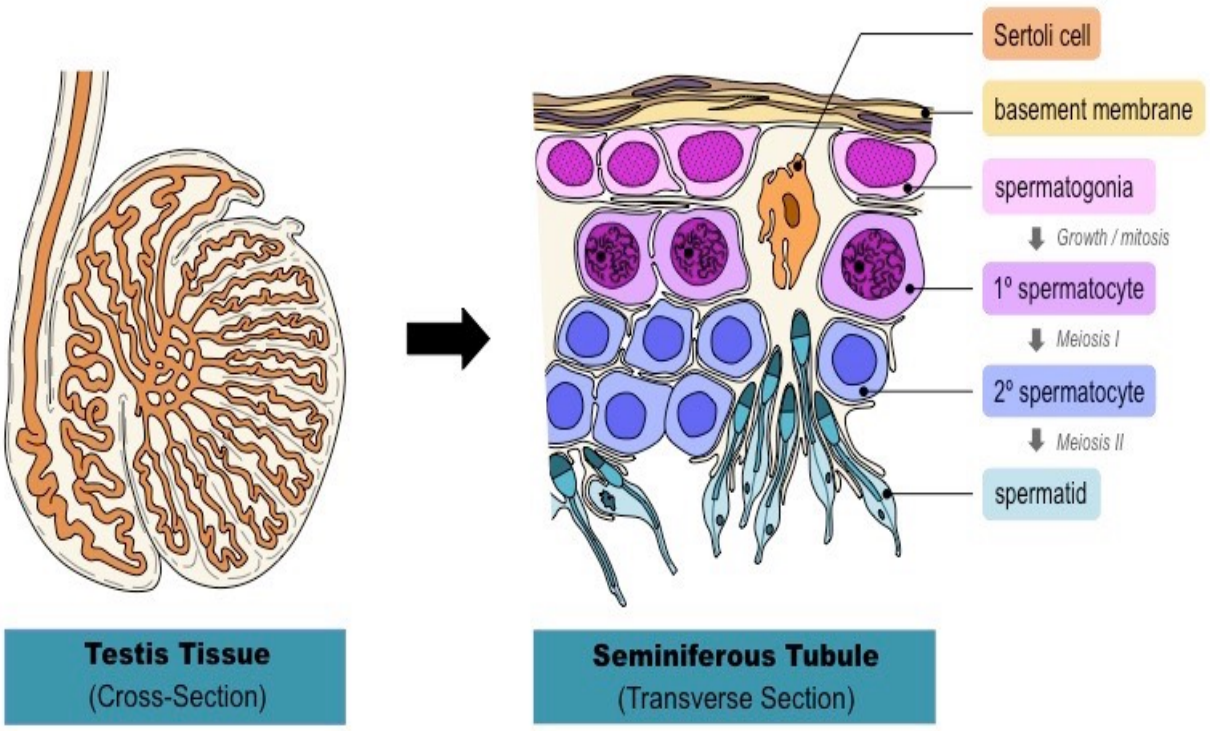
Study of structure of Testis

In males, the gametes are produced within the seminiferous tubules of the testes**.** The testis of mammals consist of following parts

- ❖ Vas deferens
- ❖ Epididymis
- ❖ Seminiferous tubules

Seminiferous Tubule:

- The testes are composed of seminiferous tubules which produce sperm
- Each tubule is surrounded by a basement membrane which is lined by germline epithelium
- The germline epithelium will divide by mitosis to make spermatogonia (which divide by meiosis to make spermatids)



- The spermatids differentiate into functional spermatozoa, which are then released into the lumen of the tubule.
- These developing spermatozoa are nourished by Sertoli cells, which reside in the tubule lining.
- Outside of the tubules are blood capillaries and interstitial cells (Leydig cells) which produce testosterone.
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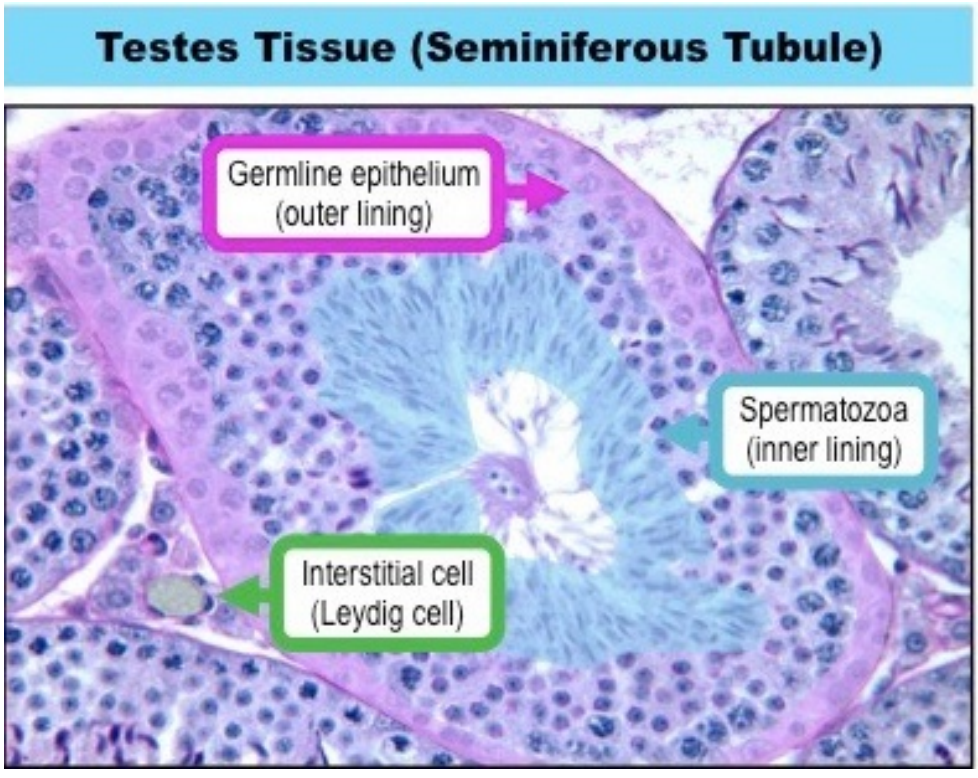
Cross-Sections of Male Reproductive Tissue:

Spermatogenesis in the seminiferous tubules starts at the outer lining of the tubule (*germline epithelium*)

- As the germ cells divide by meiosis they move towards the inner lining before being released into the lumen as *sperm.*
- Non-dividing cells within the tubule lining will represent the Sertoli cells, which nourish and support the spermatocytes
- Cells located externally to the tubule will likely represent interstitial cells (Leydig cells), which produce testosterone.



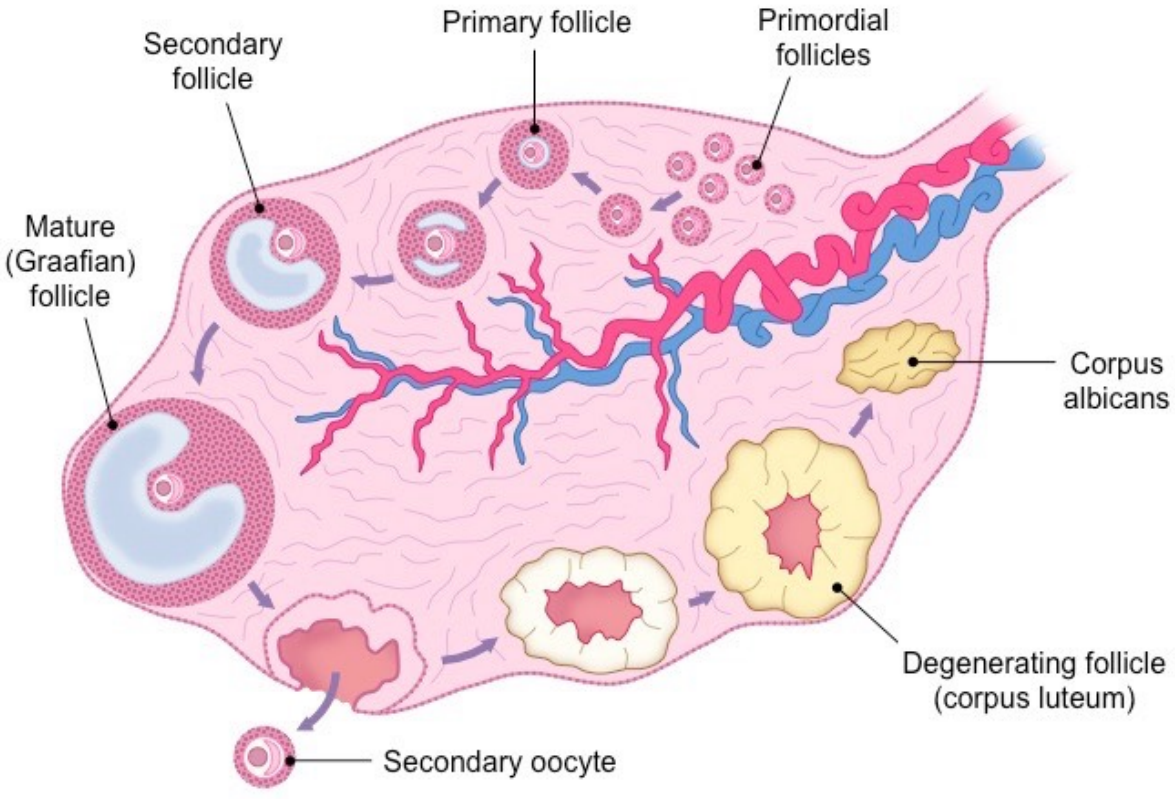
Experiment No.2

Study of structure of Ovary

In females, the gametes are produced by the ovaries.

Ovary:

- The ovary contains follicles in various stages of development
- These follicles will develop over the course of a menstrual cycle and hence will not always be apparent upon inspection
- Primordial follicles contain egg cells that have been arrested in prophase I (primary oocytes)
- Some of these follicles will develop each month into primary follicles and then secondary follicles

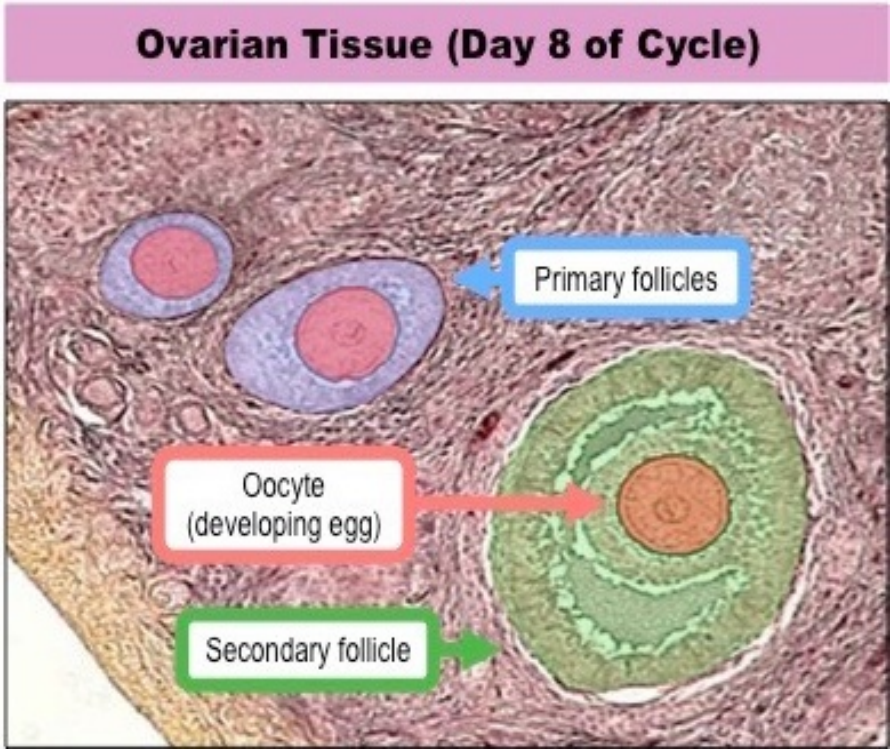


- Each cycle, one follicle will become a dominant Graafian follicle and rupture to release the secondary oocyte.
- The ruptured follicle will then develop into a short-lived corpus luteum, which secretes key ovarian hormones.
- Eventually the corpus luteum will degenerate to form a corpus albicans.

Cross-Sections of Female Reproductive Tissue:

Oogenesis in the ovaries is not a continuous process and proceeds according to a monthly menstrual cycle

- Consequently, certain developing follicles will only be visible at particular stages in the cycle
- As a follicle matures it will grow larger in size, allowing for comparisons to be made between primary and secondary follicles
- Unlike in spermatogenesis, the outer germline epithelium of the ovaries does not directly contribute to gamete formation.



Experiment No.3

Study of structure of Sperm

The male gamete (sperm) is small and motile and only contributes the male's haploid nucleus to the zygote.

Sperm

A typical human spermatozoon can be divided into three sections,

1. Head,
2. Mid-piece
3. Tail

Head region:

The head region contains three structures,

- A haploid nucleus,
- An acrosome cap
- Paired centrioles.

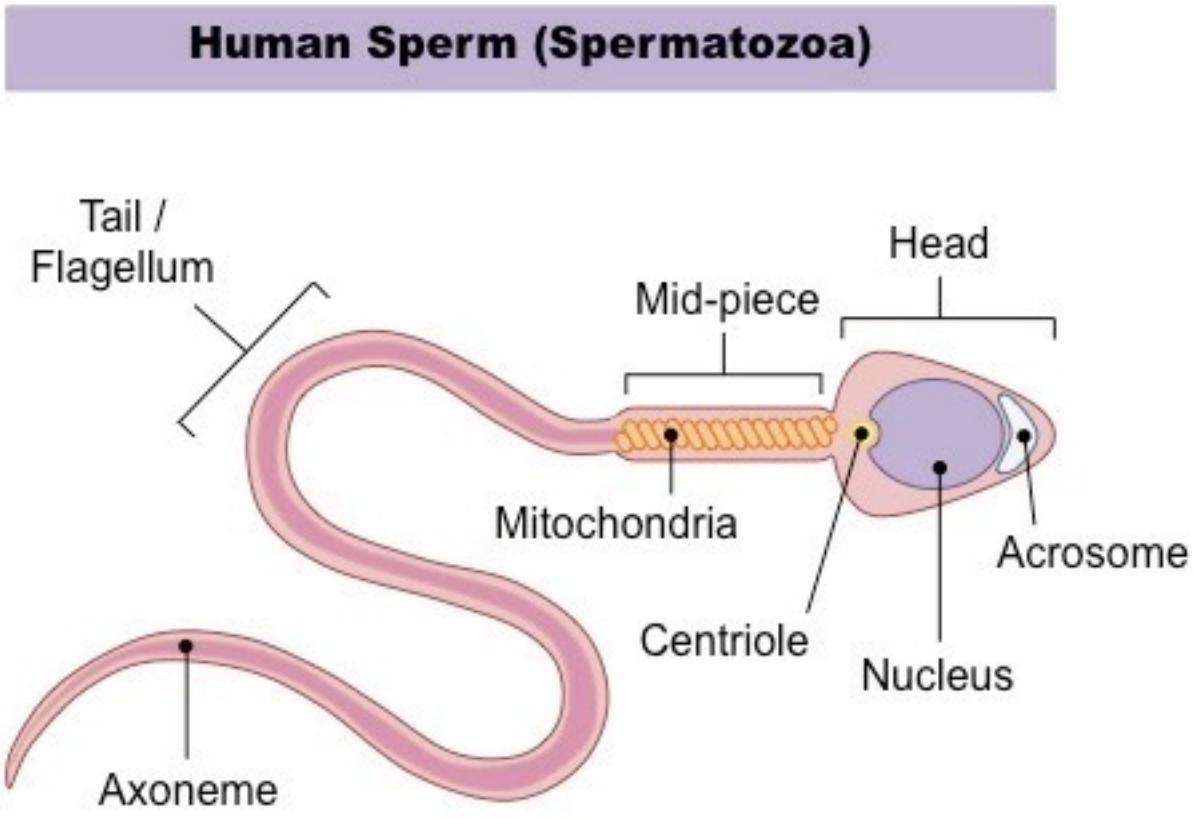
- The haploid nucleus contains the paternal DNA (this will combine with maternal DNA if fertilization is successful)
- The acrosome cap contains hydrolytic enzymes which help the sperm to penetrate the jelly coat of the egg
- The centrioles are needed by a zygote in order to divide (egg cells expel their centrioles within their polar bodies).

Mid-piece:

The mid-piece contains high numbers of mitochondria which provide the energy (ATP) needed for the tail to move

Tail:

The tail (flagellum) is composed of a microtubule structure called the axoneme, which bends to facilitate movement.



Experiment No.4

Study of structure of Egg

A typical egg cell is surrounded by two distinct layers

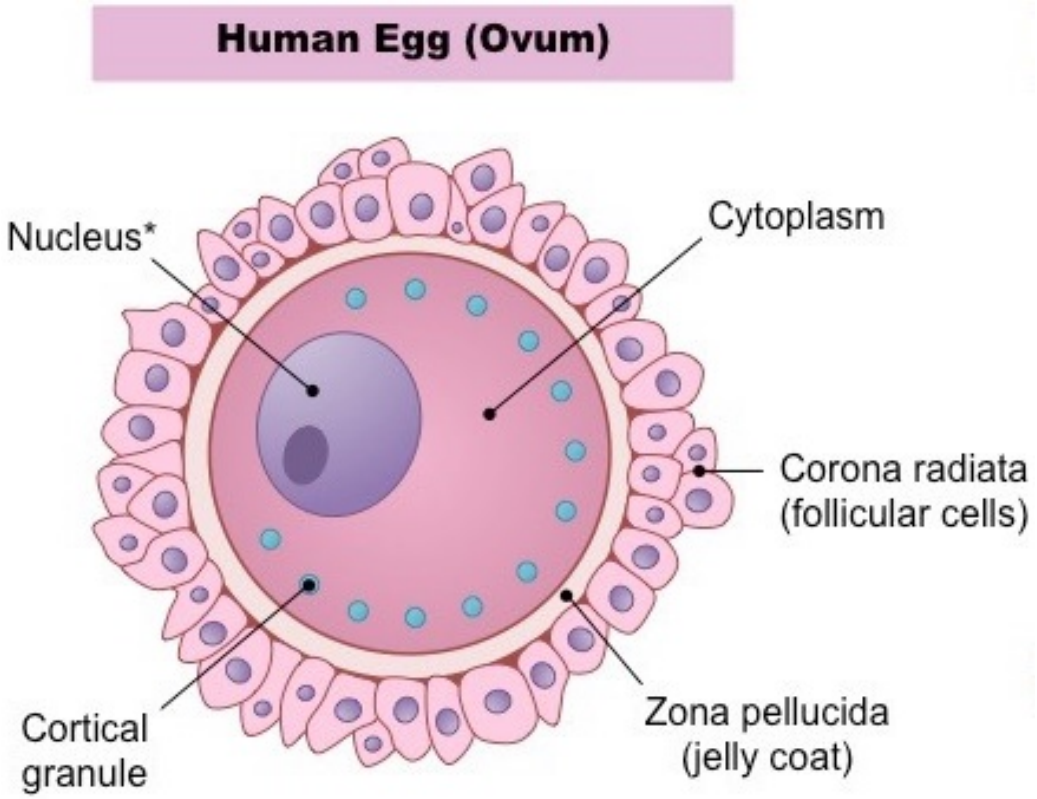
1. The zone pellucida (jelly coat)
2. Corona radiate

Zone or Zona pellucida:

The zona pellucida is a glycoprotein matrix which acts as a barrier to sperm entry.

Corona radiate:

The corona radiata is an external layer of follicular cells which provide support and nourishment to the egg cell.



- Within the egg cell are numerous cortical granules, which release their contents upon fertilization to prevent polyspermy.

- Egg cells commonly include a haploid nucleus. The cytoplasm of the egg is called **ooplasm**. It contains a very little mount of yolk in human female and therefore it is **alecithal**. In animals where huge amount of yolk is present, the cytoplasm of egg consists of lipoproteins, pigment granules, water and along with other cytoplasmic organelles.

Experiment No.5

Study of process of Spermatogenesis

Gametogenesis is the process by which diploid precursor cells undergo meiotic division to become haploid gametes (sex cells).

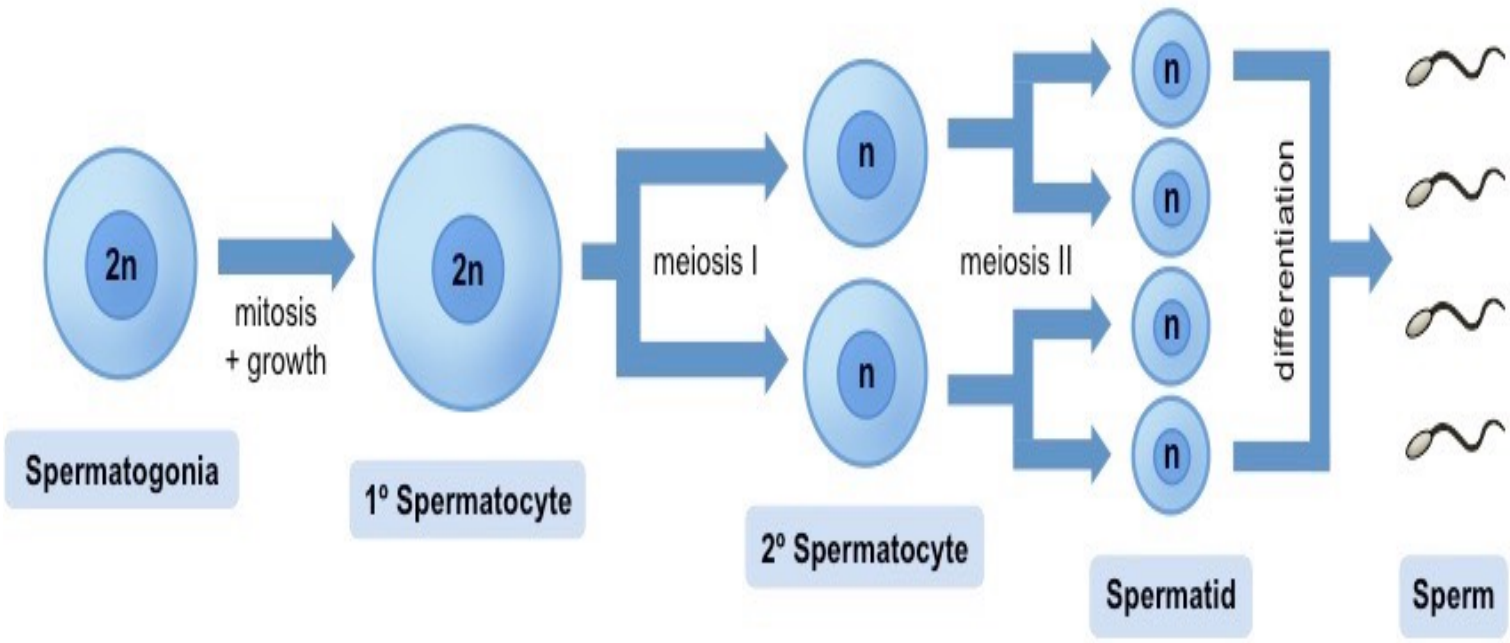
The process of gametogenesis occurs in the gonads and involves the following steps:

- Multiple *mitotic divisions* and *cell growth* of precursor germ cells.
- Two *meiotic divisions* (meiosis I and II) to produce haploid daughter cells.
- Differentiation of the haploid daughter cells to produce functional gametes.

Spermatogenesis:

In males, the process of gamete formation is called *spermatogenesis* and it produces spermatozoa (sperm).

- Spermatogenesis describes the production of spermatozoa (sperm) in the seminiferous tubules of the **testes.**



- The process begins at puberty when the germline epithelium of the seminiferous tubules divides by mitosis.
- These cells (*spermatogonia*) then undergo a period of cell growth, becoming *spermatocytes.*
- The spermatocytes undergo two meiotic divisions to form four haploid daughter cells (*spermatids*).
- The spermatids then undertake a process of differentiation in order to become functional sperm cells (*spermatozoa*).

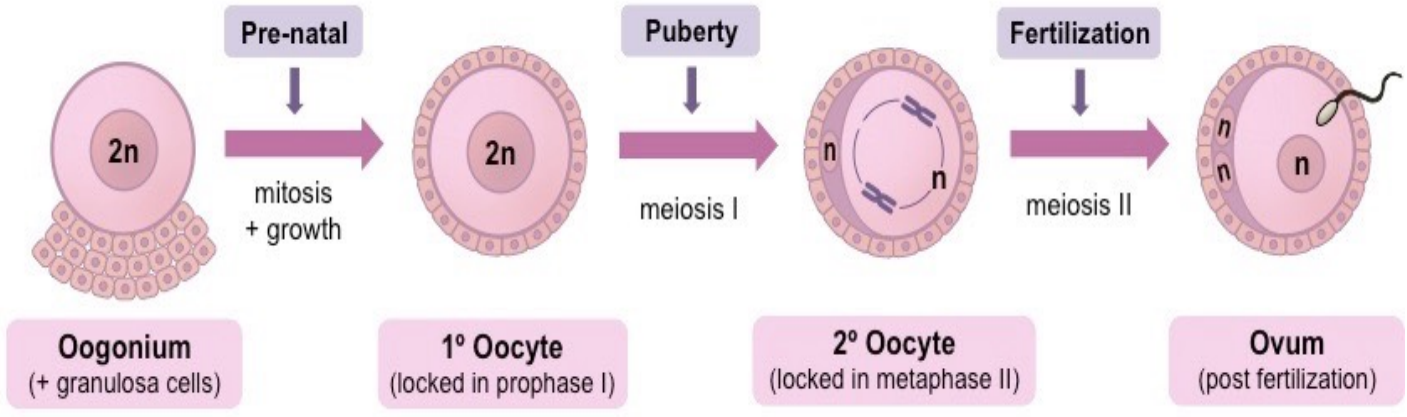
Experiment No.6

Study of process of Oogenesis

Oogenesis:

In females, the process of gamete formation is called *oogenesis* and it produces ova (eggs).

- Oogenesis describes the production of female gametes (ova) within the **ovaries**.
- The immature cells (*oogonia*) undergo cell growth until they are large enough to undergo meiosis (becoming *primary oocytes*)
- The primary oocytes begin meiosis but are arrested in prophase I when granulosa cells surround them to form follicles
- The primary oocytes remain arrested in prophase I until puberty.
- Each month, hormones (FSH) will trigger the continued division of some of the primary oocytes
- These cells will complete the first meiotic division to form two cells of unequal size
- One cell retains the entirety of the cytoplasm to form a *secondary oocyte*, while the other cell forms a polar body.



- The polar body remains trapped within the follicle until it eventually degenerates
- The secondary oocyte begins the second meiotic division but is arrested in metaphase II
- The secondary oocyte is released from the ovary (ovulation) and enters into the oviduct (or fallopian tube)
- The follicular cells surrounding the oocyte form a corona radiata and function to nourish the secondary oocyte
- If the oocyte is fertilized by a sperm, chemical changes will trigger the completion of meiosis II and the formation of another polar body.
- Once meiosis II is complete the mature egg forms a *ovum*, before fusing its nucleus with the sperm nucleus to form a zygote

Summary of the Differences between Spermatogenesis and Oogenesis

	Spermatogenesis	Oogenesis
Process		
Location	Occurs *entirely* in testes	Occurs *mostly* in ovaries
Meiotic divisions	Equal division of cells	Unequal division of cytoplasm
Germ line epithelium	Is involved in gamete production	Is not involved in gamete production
Gametes		
Number produced	Four	One (plus 2 – 3 polar bodies)
Size of gametes	Sperm smaller than spermatocytes	Ova larger than oocytes
Timing		
Duration	Uninterrupted process	In arrested stages
Onset	Begins at puberty	Begins in foetus (pre-natal)
Release	Continuous	Monthly from puberty (menstrual cycle)
End	Lifelong (but reduces with age)	Terminates with menopause

Experiment No.7

Study of Process of Human Fertilization

The process of fertilization in humans involves a number of key processes, including:

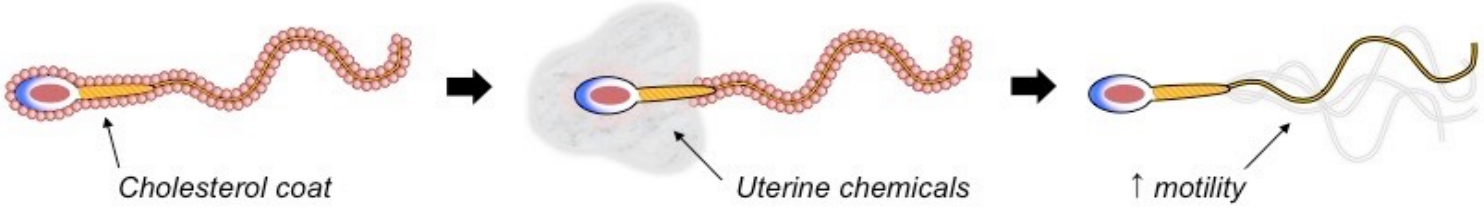
- ***Capacitation*** – biochemical changes which occur post ejaculation to improve sperm motility
- ***Acrosome reaction*** – the release of hydrolytic enzymes which softens the zona pellucida (jelly coat)
- ***Cortical reaction*** – hardening of the jelly coat post fertilization to prevent potential polyspermy

1. Capacitation:

Capacitation occurs after ejaculation, when chemicals released by the uterus dissolve the sperm's cholesterol coat

- This improves sperm motility (hyperactivity), meaning sperm is more likely to reach the egg (in the oviduct).
- It also destabilizes the acrosome cap, which is necessary for the acrosome reaction to occur upon egg and sperm contact.

Overview of Capacitation

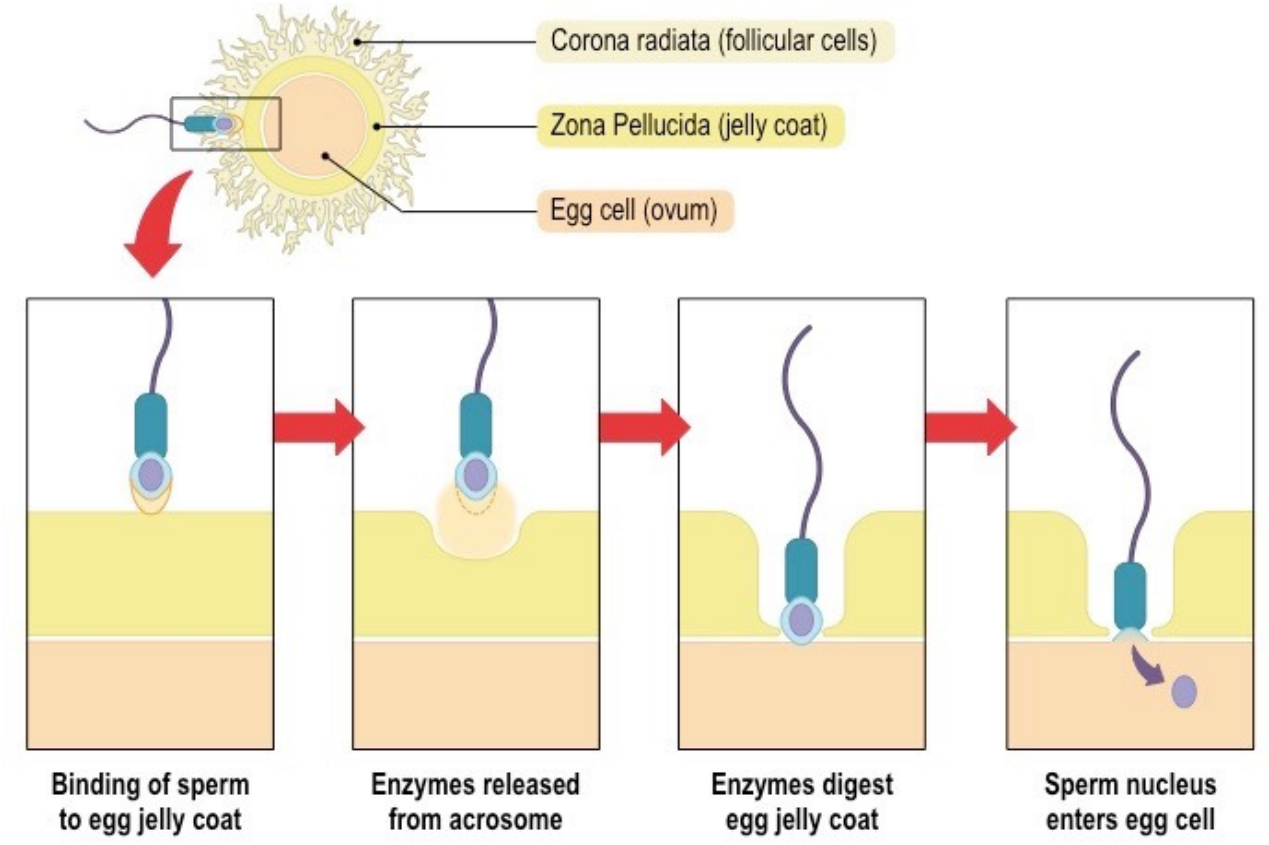


2. Acrosome Reaction:

When the sperm reaches an egg, the acrosome reaction allows the sperm to break through the surrounding jelly coat

- The sperm pushes through the follicular cells of the corona radiata and binds to the zona pellucida (jelly coat).
- The acrosome vesicle fuses with the jelly coat and releases digestive enzymes which soften the glycoprotein matrix.
- The sperm then pushes its way through the softened jelly coat and binds to exposed docking proteins on the egg membrane
- The membrane of the egg and sperm then fuse and the sperm nucleus (and centriole) enters the egg.

<u>Overview of the Acrosome Reaction</u>

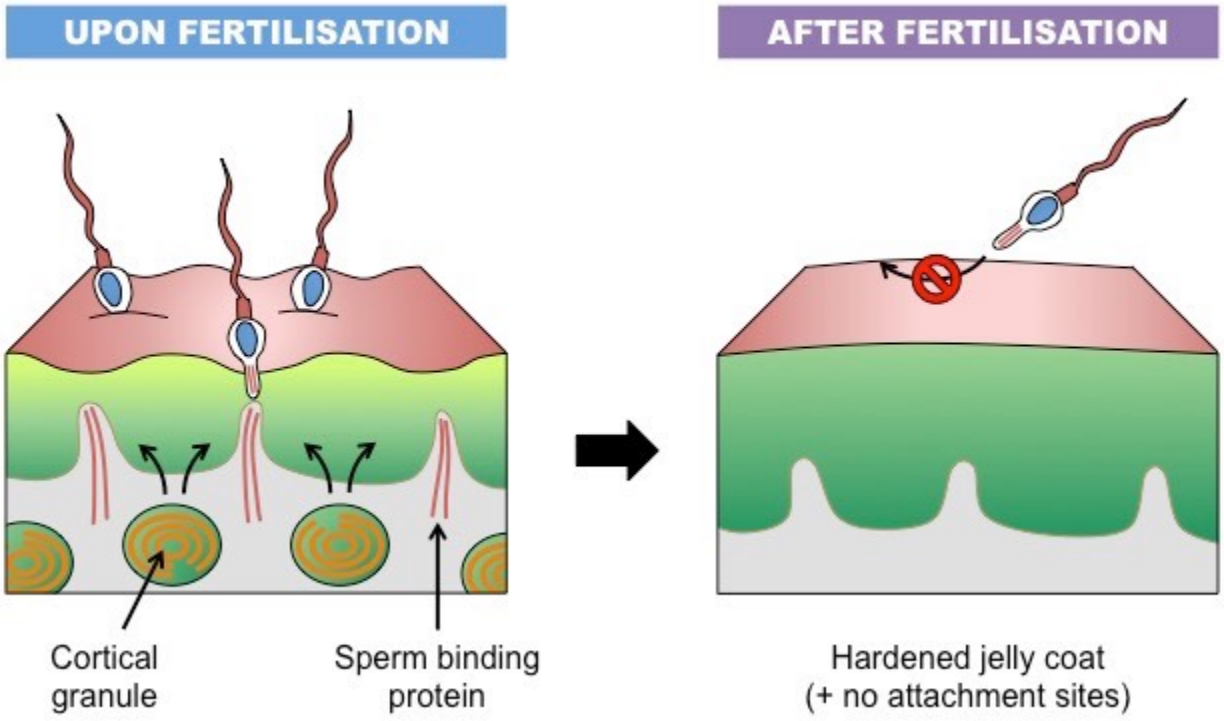


3. Cortical Reaction:

The cortical reaction occurs once a sperm has successfully penetrated an egg in order to prevent polyspermy

- Cortical granules within the egg's cytoplasm release enzymes (via exocytosis) into the zona pellucida (jelly coat).
- These enzymes destroy sperm binding sites and also thicken and harden the glycoprotein matrix of the jelly coat.
- This prevents other sperm from being able to penetrate the egg (polyspermy), ensuring the zygote formed is diploid.

Overview of the Cortical Reaction



Experiment No.8

Study of Process of Embryonic Development

Blastocyst Formation:

Following the fusion of an egg and sperm (fertilization), an influx of Ca^{2+} into the ova prompts the completion of meiosis II.

- ❖ The egg and sperm nuclei combine to form a diploid nuclei and the fertilized cell is now called a **Zygote.**

The zygote will undergo several mitotic divisions to form a solid ball of cells called a **Morula.**

- ❖ As the Morula continues to divide, it undergoes differentiation and cavitation (cavity formation) to form a **Blastocyst**.

A blastocyst is comprised of three distinct sections:

- An inner cell mass (that will develop into the embryo)
- A surrounding outer layer called the **trophoblast** (this will develop into the placenta)
- A fluid filled cavity called the **blastocoel**

Early Embryo Development

Implantation of Blastocyst:

The final stage of early embryo development is the implantation of the blastocyst into the endometrial lining of the uterus.

- The blastocyst breaches the jelly coat that was surrounding it and preventing its attachment to the endometrium.
- Digestive enzymes are released which degrade the endometrial lining, while autocrine hormones released from the blastocyst trigger its implantation into the uterine wall.

Only once the blastocyst is embedded within the uterine wall can the next stage of embryogenesis occur

- The growing embryo will gain oxygen and nutrients from the endometrial tissue fluid, ensuring its continued development.
- The entire process (from fertilization to implantation) takes roughly 6 – 8 days.