5TH SEM GENERAL

UNIT 3: DEVELOPMENTAL BIOLOGY

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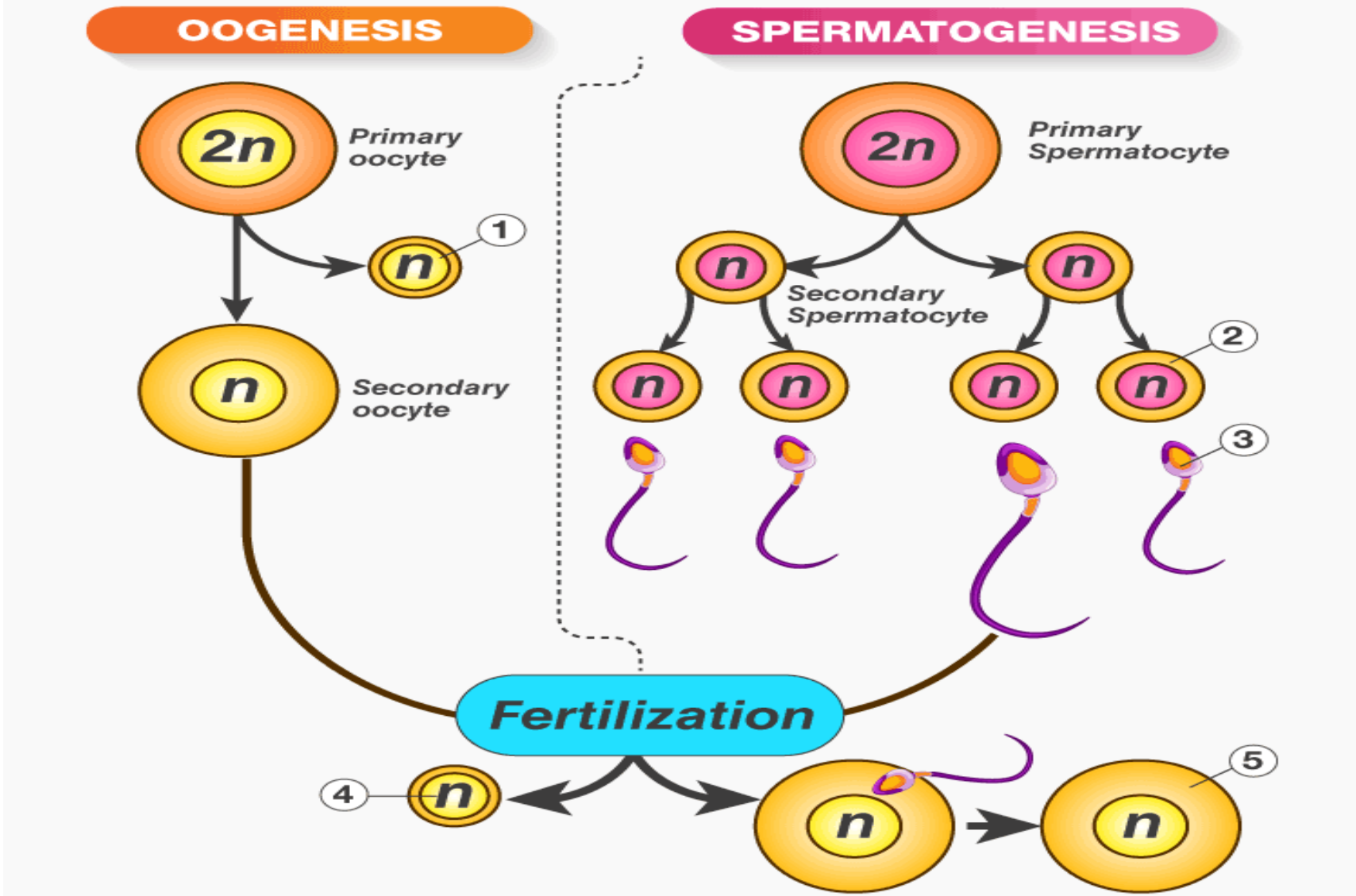
GAMETOGENESIS: SPERMATOGENESIS AND OOGENESIS

Gametogenesis occurs when a haploid cell (n) is formed from a diploid cell (2n) through meiosis. We call gametogenesis in the male spermatogenesis and it produces spermatozoa. In the female, we call it oogenesis. It results in the formation of ova.

SPERMATOGENSIS Vs OOGENSIS



An organism undergoes a series of changes throughout its life cycle. Gametogenesis (spermatogenesis and oogenesis), plays a crucial role in humans to support the continuance of generations.

Gametogenesis is the process of division of diploid cells to produce new haploid cells. In humans, two different types of gametes are present. Male gametes are called sperm and female gametes are called the ovum.

Spermatogenesis: Sperm formation

Oogenesis: Ovum formation

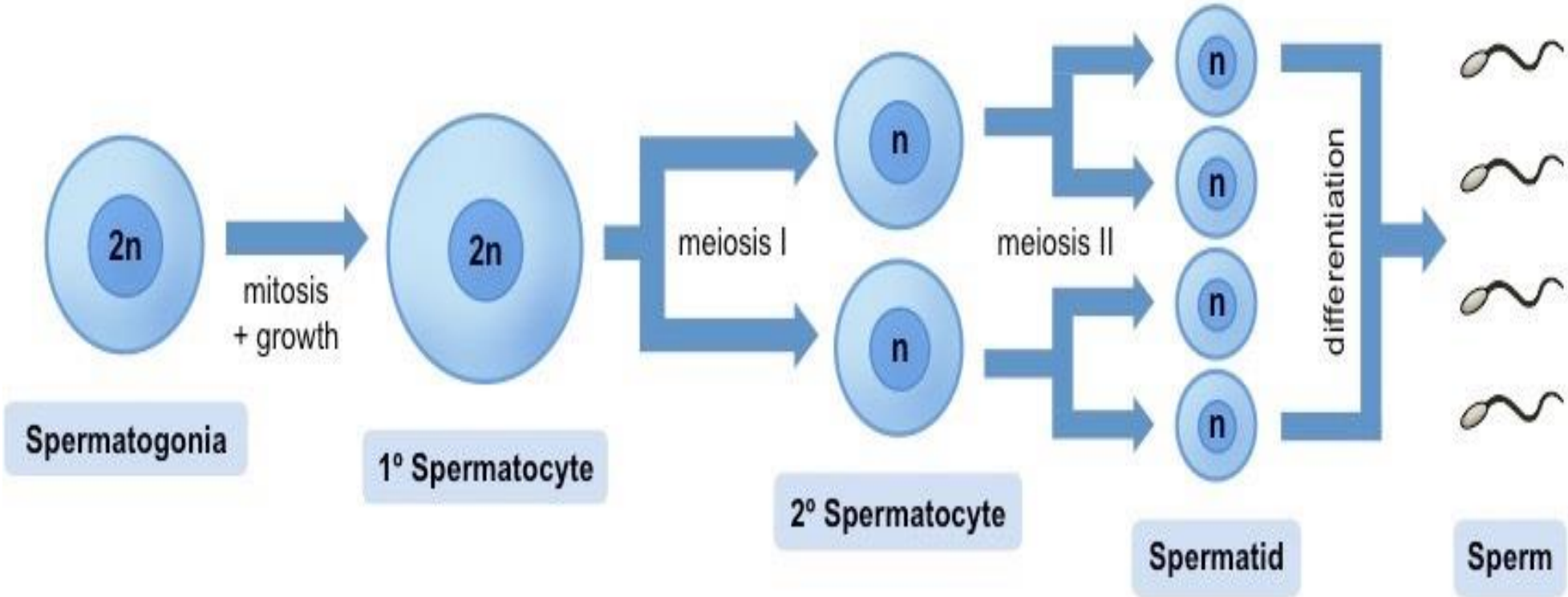
The process of gametogenesis occurs in the gonads and involves the following steps:
Multiple mitotic divisions and cell growth of precursor germ cells

- Two meiotic divisions (meiosis I and II) to produce haploid daughter cells

- Differentiation of the haploid daughter cells to produce functional gametes

Spermatogenesis

- Spermatogenesis describes the producton 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)

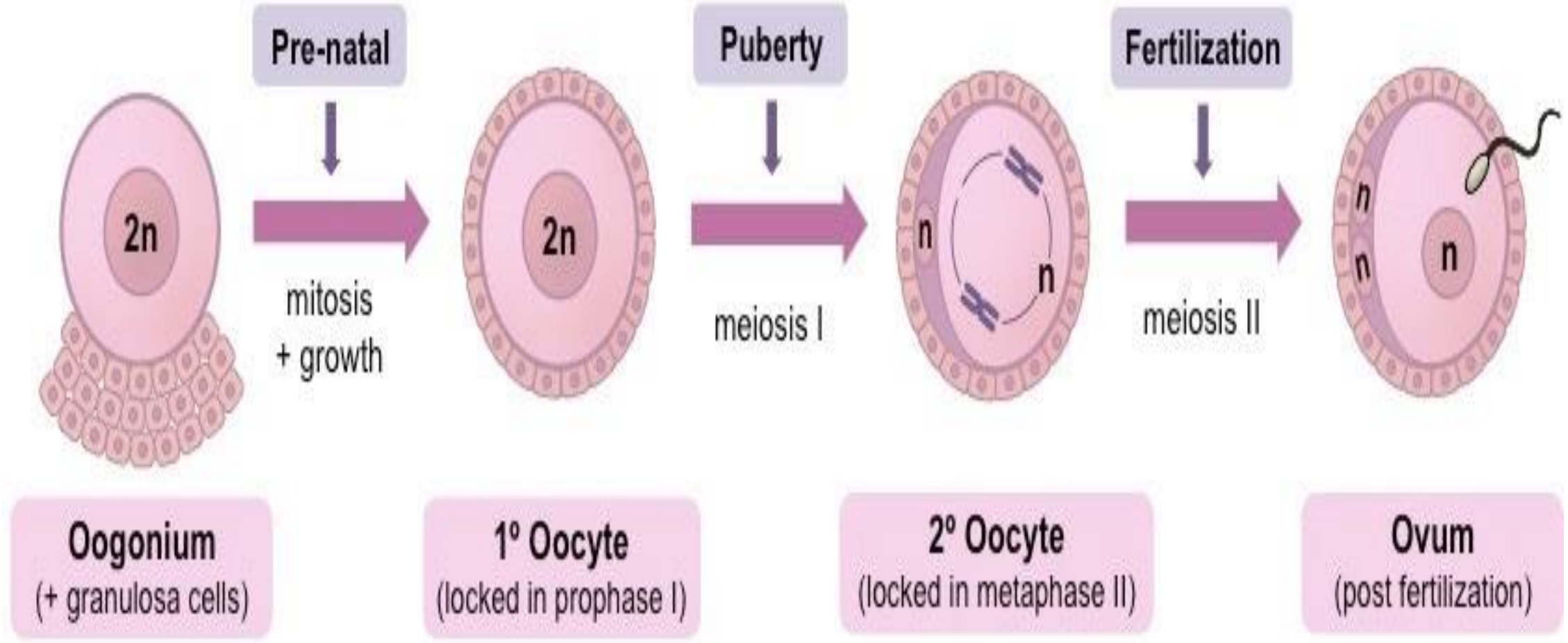

2n
mitosis
+ growth
2n
meiosis I
n
n
meiosis II
n
n
n
n
differentiation
Spermatogonia
1° Spermatocyte
2° Spermatocyte
Spermatid
Sperm

Oogenesis

- Oogenesis describes the production of female gametes (ova) within the ovaries (and, to a lesser extent, the oviduct)
- The process begins during foetal development, when a large number of primordial cells are formed by mitosis (~40,000)
- These 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, when a girl begins her menstrual cycle
- 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 fertilised by a sperm, chemical changes will trigger the completion of meiosis II and the formation of another polar body (the first polar body may also undergo a second division to form a third 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



Formation of Polar Bodie

1. Number of cells produced

In spermatogenesis, the cells divide equally during meiosis to produce four functional gametes
In oogenesis, the cells do not divide equally and as a result only one functional gamete is formed (plus 2 – 3 polar bodies)

2. Size of cells produced

In spermatogenesis, the cells that are formed following differentiation are all of equal size with equal amounts of cytoplasm
In oogenesis, one daughter cell (the ovum) retains all of the cytoplasm, while the other daughter cells form polar bodies
The polar bodies remain trapped within the surrounding layer of follicle cells until they eventually degenerate

Timing of the process

In spermatogenesis, the production of gametes is a continuous process that begins at puberty and continues until death

In oogenesis, the production of gametes is a staggered and finite process:

It begins before birth (prenatally) with the formation of a fixed number of primary oocytes (~40,000)

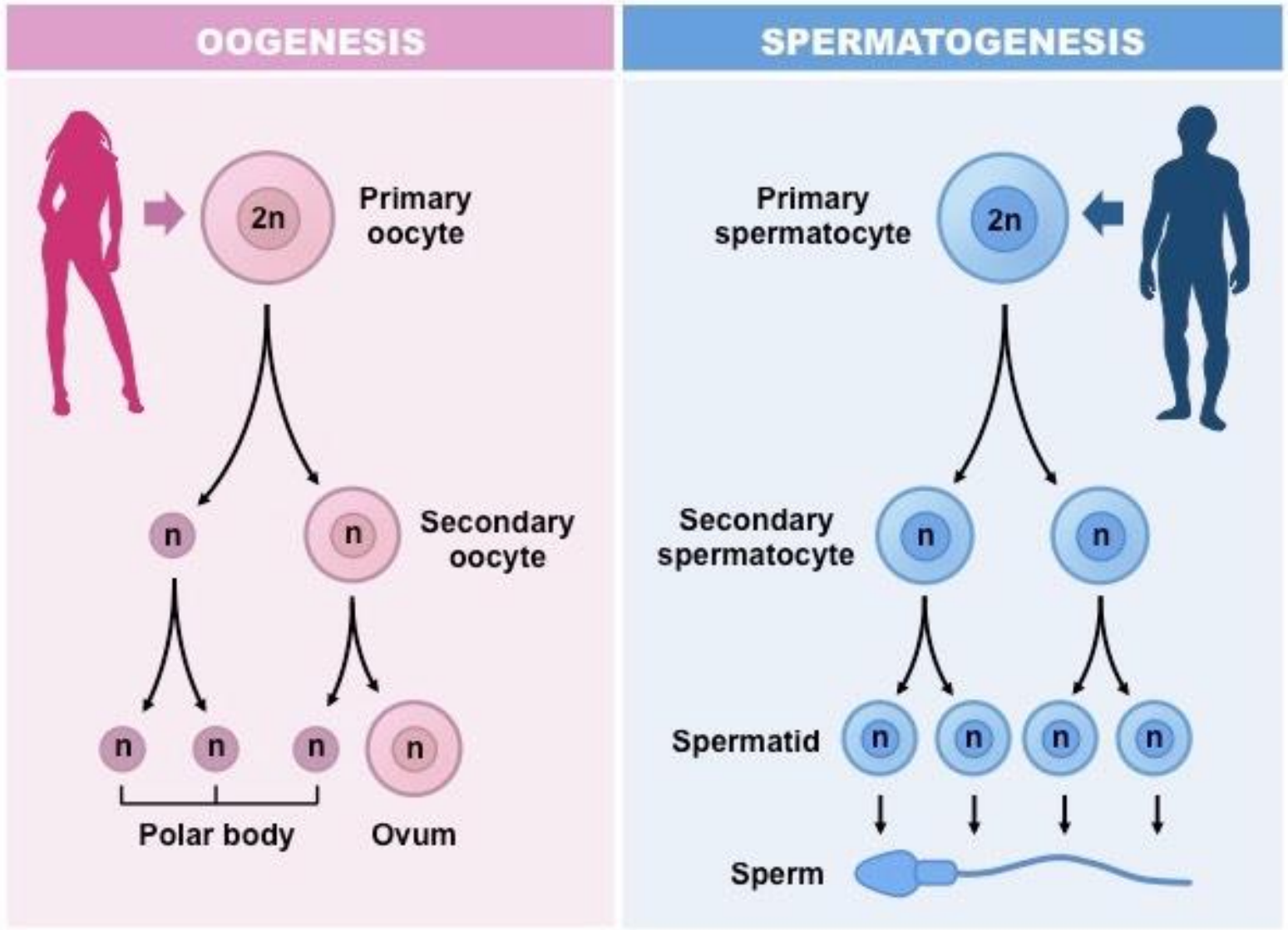
It continues with the onset of puberty according to a monthly menstrual cycle

It ends when hormonal changes prevent the further continuance of the menstrual cycle (menopause)

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

Gametogenesis Comparison



FERTILIZATION : SPERM EGG INTERACTION : ACTIVATION OF EGG: GAMETE FUSION IN SEA URCHIN

Fertilization is a multi-step process that involves the interaction of a mature, capacitated spermatozoon and ovulated egg. … Sperm and egg plasma membranes bind and fuse, resulting in incorporation of sperm contents into the egg cytoplasm. Fusion triggers egg activation and establishment of a block to polyspermy.

SUMMARY: Fertilization is a cell-cell recognition process that occurs between two distinct cells: a small asymmetric and motile sperm cell and a large and nonmotile egg. The stages of fertilization can be divided intofour processes: 1) sperm preparation, 2) sperm-egg recognition and binding, 3) sperm-egg fusion and 4) fusion of sperm and egg pronuclei and activation of the zygote.

. The specific structures of the sperm and egg that are important for fertilization will be discussed and experiments that led to the identification of the egg receptor for the sperm and the sperm receptor for the egg will be described. Membrane fusion of sperm and eggs is an incompletely understood process, but the discovery of proteins known as ADAM proteins on the sperm surface has suggested new mechanisms to explain sperm-egg fusion. Finally, we will consider how fertilized eggs prevent additional sperm from fusing (a condition known as polyspermy) and how the fertilized egg is activated to begin development

Discuss the sequential nature of fertilization in which ordered changes in the gametes "drive"
the process of fertilization toward completion.

2. Explain the role of specialized sperm and egg surface structures in fertilization.

3. Describe how egg and sperm receptors were identified.

4. Explain the current state of knowledge about sperm-egg membrane fusion and how sperm components are incorporated into the egg.

5. Describe how polyspermy is prevented and the fertilized egg is activated for development.

GLOSSARY:

Capacitation: The process by which the sperm becomes capable of fertilizing an egg.

Acrosome Reaction: A regulated exocytotic event in which an apical vesicle in the sperm head fuses with the sperm plasma membrane. The acrosome reaction is triggered in response to egg factors.

Acrosin: A serine protease released during the acrosome reaction.

Cortical Reaction: A regulated exocytosis in which apically localized vesicles (cortical granules) in the egg fuse with plasma membrane after fertilization.

Zona Pellucida: A coat surrounding the egg that contains three glycoproteins.

Galactosyl transferase: An oligosaccaride modifying enzyme that is usually found in the Golgi but in sperm is on the cell surface. Thought to be important as the sperm receptor for the egg.

Fertilin: An ADAM family protein on the sperm implicated in sperm-egg membrane fusion. Contains a fusion peptide resembling viral fusion peptides and a disintegrin domain involed in recognition

ADAM proteins: A family of proteins that contain A Disintegrin And Metalloprotease domain(s).

Pronuclei: The transitional male and female nuclei formed in the egg after fertilization. They fuse to form the diploid zygote nucleus.

Polyspermy: The condition in which more than one sperm fertilizes an egg. Polyspermy leads to defective development.

There are four stages to fertilization:

1. Preparation: Capacitation and acrosome reaction. Acrosomal vesicle fusion is the membrane fusion event of this stage.

2. Binding: Species-specific interaction of gametes.

3. Fusion: Merging of sperm and egg plasma membranes is the membrane fusion event of this stage.

4. Activation (of the zygote): Cortical reaction (fusion of cortical vesicles with the egg plasma membrane) and pronuclear fusion.

Eggs:

Eggs are large (~100 µm), symmetrical and nonmotile cells . Human eggs are arrested in metaphase
of the second meiotic division and complete meiosis only upon fertilization.
Their surface is covered by
microvilli .
Eggs are surrounded by a zona pellucida , which is a glycoprotein coatcomposed of three glycoproteins (ZPGP I-III). All three of the glycoproteins contain O- and N-linked oligosaccharides.
The zona pellucida is not an osmotic barrier (in fact, even virus are capable of penetrating it), however it is a barrier to the sperm.
The zona pellucida is the species specific barrier to fertilization as shown by the hamster experiment. Human sperm are incapable of fertilizing intact hamster eggs,
but can fertilize hamster eggs stripped of their zona pellucida.
This is used clinically to assess the fertilizing capacity of sperm

Sperm:

Sperm are small, asymmetrical and motile cells . They have three components:

1. Tail: Also referred to as the principal piece. The tail contains the flagellar apparatus, which is composed of "9 + 2" microtubules and accessory structures . The sliding of the microtubule is powered by the protein dynein. (Gibbons' movie of sliding microtubules)

2. Midpiece: at the proximal portion of the tail. Midpiece contains a sheath of mitochondria, which produce the ATP necessary for the beating of the tail.

3. Head: contains the spermatic haploid nucleus. Overlaying the head is a membrane bound vesicle, the acrosome. Sperm do not possess any organelles associated with protein synthesis (Golgi, RER or lysosomes).

The sperm plasma membrane is also highly differentiated and contains proteins localized in distinct regions . One of these, termed PH-30 or fertilin, is localized in the equatorial region of the sperm and is involved in sperm-egg plasma membrane fusion (see below)

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Acrosome:

The acrosome is a lysosomal-like compartment derived from the Golgi. It has a low pH and contains soluble hydrolases (serine protease acrosin). In cross-section through the head of a sperm, one would cross four membranes in traversing from the plasma membranes to the nuclear membrane. During the acrosome reaction, fusion of the outer acrosomal membrane with the plasma membrane releases the contents of the acrosome and exposes the inner acrosomal membrane as the functional outer boundary ofthe sperm head.

The Four Steps of Fertilization:

Step I. Preparation of the Sperm.

Ejaculated sperm are not ready to fertilize an egg when they enter the vagina. In response to the dilution of semen in the vagina, they undergo several changes, which are collectively known as capacitation.

1.Intracellular Ca++ levels increase.

2.Spermatic motility is activated and tails change beat frequency.

3.Sperm cell surface antigens are lost. The loss of these proteins renders the sperm more receptive to binding to the egg.

Step II. Sperm-Egg Binding

Because of the availability of gametes, the process of sperm-egg binding was first studied and understood in invertebrates . In sea urchins, the sperm head binds directly to the egg outer surface and this triggers the acrosome reaction. (Figs.1-9 and 1-10). The acrosomal contents are released and there is a balanced Na+ influx and H+ efflux, causing an increase in pH. The increased pHtriggers the dissociation of the profilactin complex (actin and profilin) and the released actin monomers polymerize to form a filament called the acrosomal process. This acrosomal process penetrates the eggcoatings to allow fusion of the sperm and egg plasma membranes. In sea urchins then, the sperm literally skewers the egg.

Sperm receptor on egg.

Dr. Paul Wassarman used a competition assay to isolate and identify the factor in the zona pellucida that
was involved in sperm egg binding (Fig. 1-11). Dr. Wassarman incubated sperm with zona pellucida
glycoproteins (ZPGPs) he had isolated from unfertilized and fertilized eggs. He found that sperm
preincubated with ZPGPs from unfertilized eggs were not able to fertilize eggs. Yet, when hepreincubated sperm with ZPGPs isolated from fertilized eggs, which are known not to bind sperm, the
sperm could still fertilize eggs (Fig. 1-12 and 1-13). This showed that the isolated ZPGPs from
unfertilized eggs contain a receptor for the sperm and that this receptor is modified after fertilization.
In follow up experiments, Dr. Wassarman purified ZPGP I, ZPGP II and ZPGP III and showed that only

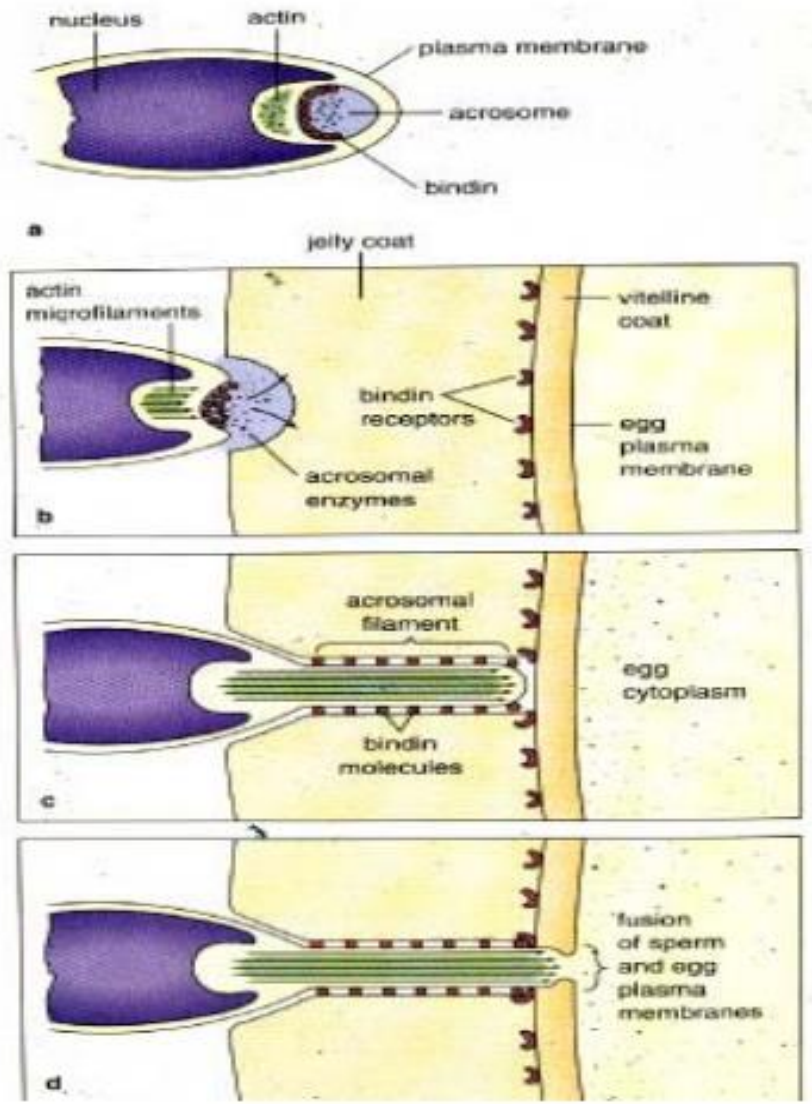


Fig. 1-10.

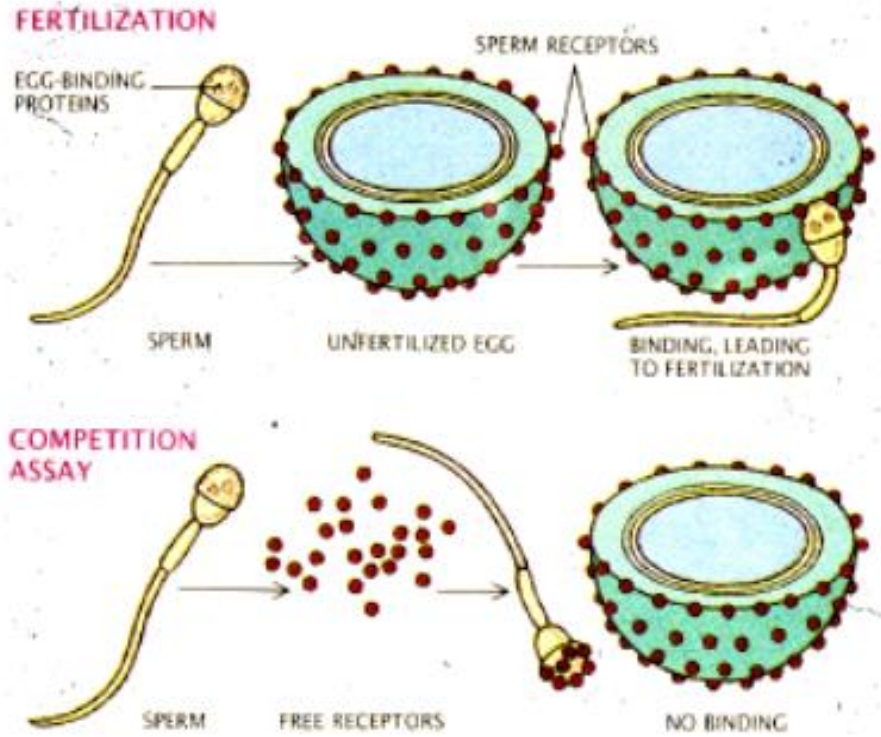


Fig. 1-11.

ZPGP III could prevent sperm binding to eggs showing that ZPGP III is the sperm receptor. By treating
ZPGP III with agents that selectively hydrolyzed protein (trypsin), N-linked glycoproteins (specific glycohydrolase) and O-linked glycoproteins (weak base), Dr. Wassarman showed that the part of ZPGP
III that was responsible for sperm binding was the O-linked oligosaccharide.

Egg receptor on sperm.
What sperm component is binding to the ZPGP III? Dr. Barry Shur was studying a Golgi enzyme
known as galactosyl transferase. This enzyme catalyzes the addition of galactosyl residues from a
donor nucleotide sugar, UDP-galactose, to the terminal end of O-linked oligosaccharides. As in all
enzymatic reactions, there are two stages in catalysis:
1.The enzyme binds the substrates (in this case UDP-gal and O-linked oligosaccharide).
2.The enzyme catalyzes the reaction and releases the products (in this case, UDP and the modified Olinked oligosaccharide with galacosyl residues on its ends)

It is important to understand that if one of the substrates is not present, the enzyme may be
able to bind the available substrate, but will not be able to catalyze the reaction. This is important in sperm binding. Dr. Shur found that sperm, which have no Golgi apparatus, have galactosyl transferase on the surface of their plasma membrane. When sperm are ejaculated, they have oligosaccharides bound to the galactosyl transferase. During capacitation, these coating glycoproteins are removed, allowing the galactosyl transferase, to bind to other carbohydrates it may encounter,such as those attached to ZPGP III. The sperm that do encounter the egg and its zona pellucida, bind ZPGP III through their galactosyl transferases (Fig. 1-14). At this point, UDP-gal

would normally bind to its site on galactosyl transferase, galactose residue would be transferred to the oligosaccharide and the modified oligosaccharide would be released. However, there is no high energy UDP-galactose in the extracellular fluid surrounding the egg so catalysis does not occur and the sperm remains tightly bound to the egg zona pellucida.

Many studies support a role for galactosyl transferase as the sperm protein involved in sperm-egg binding, however, other proteins may be involved.

A recent genetic knockout of galactosyl transferase in mice yielded mice that were completely fertile and showed normal sperm-egg binding.

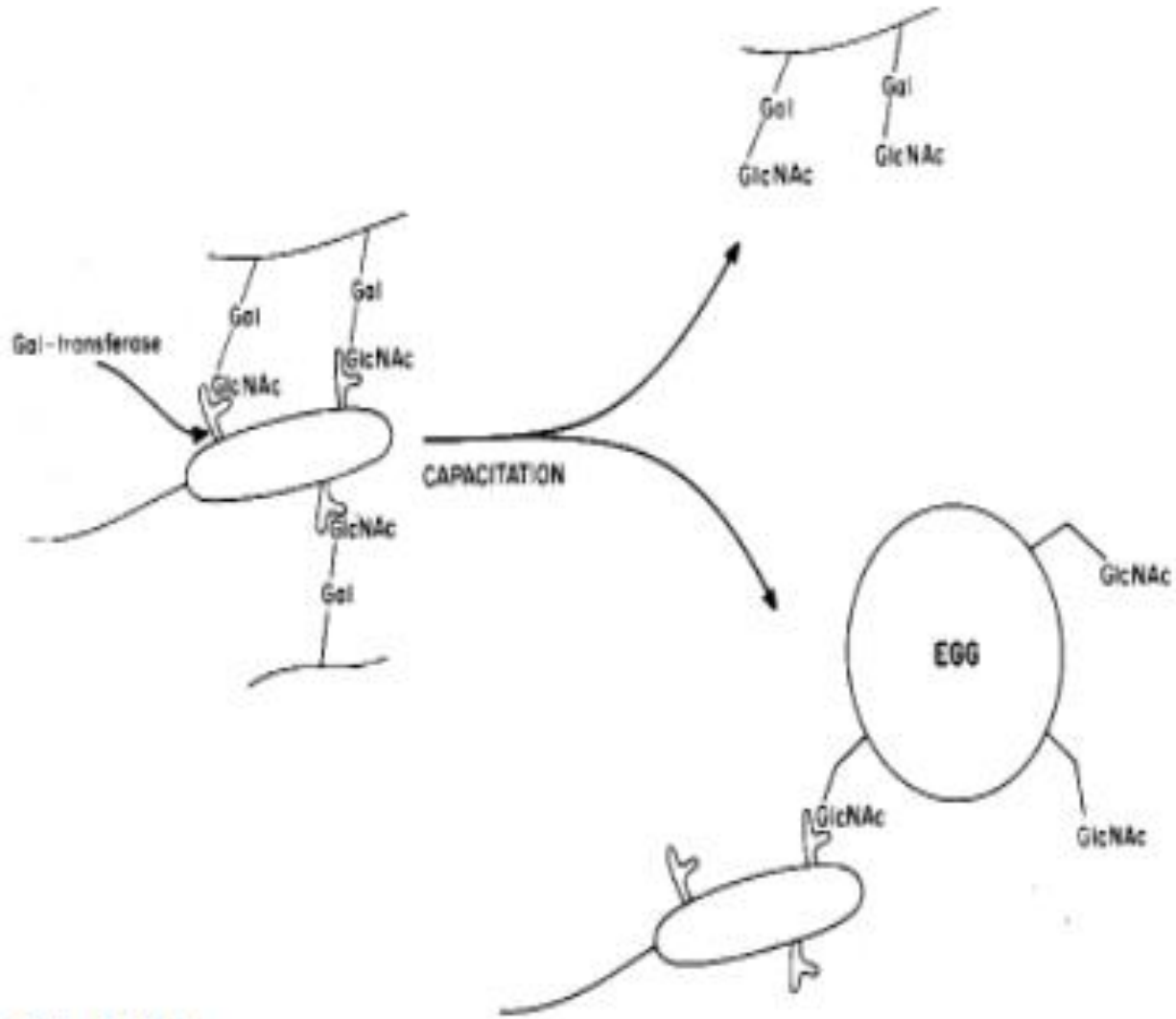

Gal
GlcNAc
Gal
GlcNAc
Gal-transferase
Gal
GlcNAc
GlcNAc
CAPACITATION
GlcNAc
Gal
EGG
GlcNAc
GlcNAc
GlcNAc

Fig.1-14

Acrosome reaction.

As a result of irreversible binding of the sperm to the egg, the zona pellucida triggers the acrosome reaction (Fig. 1-15). The outer plasma membrane of the acrosome fuses at multiple sites with the plasma membrane and the contents of the acrosome are released (Fig. 1-16). Two of the important components are acrosin, a serine protease, and N-acetylglucoaminidase. Acrosin bores a hole in the zona pellucida so that the sperm can reach the egg itself. N-acetylglucoaminidase hydrolyzes the O-linked oligosaccharides in ZPGP III to

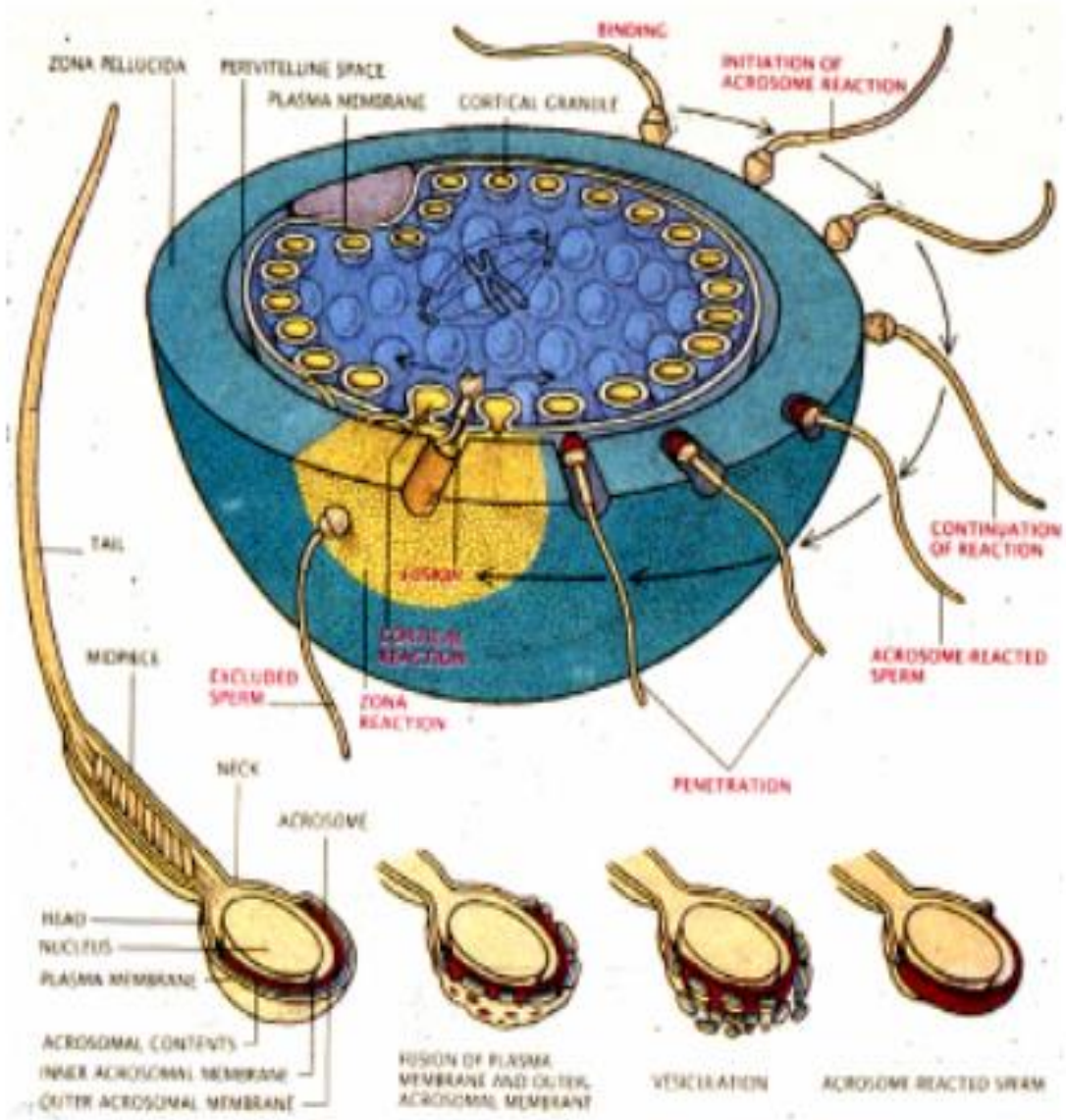


Fig. 1-15.

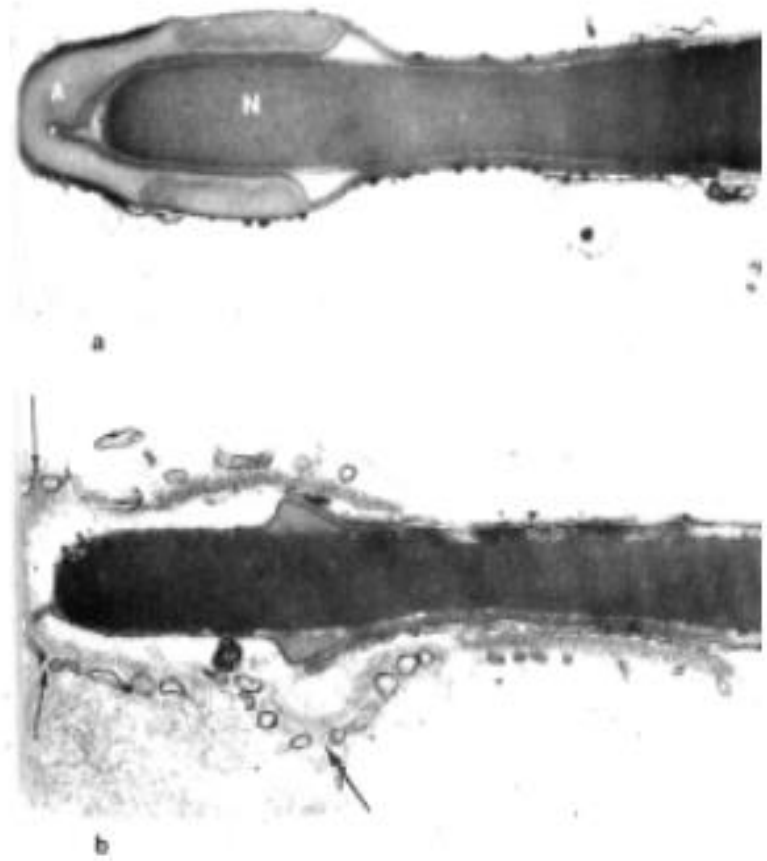


Fig. 1-16.

STEP III. Sperm-Egg Fusion.

For many years the process by which the plasma membrane of the sperm and egg fused was a black box. Recent studies by Drs. Judith White, Diana Miles, and Paul Primakoff and their colleagues, have now shed light on this process. Miles and Primakoff made an antibody to PH-30, a heterodimeric sperm membrane protein comprised of α and β subunits, and showed that this antibody blocked fertilization but did not block binding of sperm to eggs stripped of their zona pellucida. This suggested that PH-30 was involved in sperm and egg fusion and it was given the name fertilin.

Fertilin was one of the first proteins of a family of proteins known as ADAMs proteins (for A Disintegrin And Metalloprotease containing protein) that are involved in cell-cell recognition and cell fusion events.

Although the mechanism for how fertilin causes sperm-egg membrane fusion is not known, studies have supported its role in membrane fusion (Fig. 1-19).

For example, a peptide corresponding to the viral fusion peptide of

STEP IV. Activation - The Egg's Response.

The immediate events after fertilization include the egg's effort to prevent polyspermy.

Polyspermy refers to the fertilization of the egg by more than one sperm, resulting in zygotes with greater than a diploid amount of DNA. This causes early embryonic defects and arrest of development.

After sperm-egg fusion, the egg mounts the cortical reaction to prevent polyspermy. In all eggs, residing just under the plasma membrane there are membrane bound vesicles known as cortical granules.

When a single sperm penetrates the egg, the cortical granules adjacent to the site are triggered to fuse with the plasma membrane, exocytosing their contents into the perivitelline space (the space between the plasma membrane and the zona pellucida).

The cortical reaction is propagated over the surface of the egg by a wave of Ca++ . This was shown by the aequorin experiment in which the photoprotein aequorin phosphoresced in a wave from the site of sperm penetration of the egg (Fig. 1-25
and movie)

space:

1. Ovoperoxidase: In sea urchins, ovoperoxidase catalyzes the crosslinking of tyrosine residues in the extracellular matrix. This makes the extracellular matrix tough and insoluble (analogous to the tanning of leather) and a physical barrier is formed which prevents other sperm from fertilizing the egg. In mammals, ovoperoxidase does not catalyze tyrosine cross-linking to the point of insolubility. In mammals, its major effect is thought to be as a spermicial agent.

2. Hydrolase. Remember Wassarman's result showing that zona pellucida from fertilized eggs was incapable of blocking fertilization? Another cortical granule that is released is a specific hydrolase, which degrades O-linked oligosaccharides on ZPGP III. This renders the zona pellucida incapable of binding additional sperm, thus preventing polyspermy. Activation of the egg also includes the initiation of development of the new zygote. Protein synthesis and other metabolic processes are upregulated to provide for the developing embryo

GAMETE FUSION IN SEA URCHIN EGG

The union of the haploid sperm and the haploid egg in fertilization creates a single diploid cell, called a zygote, which will develop into an embryo. Fertilization does more, however, than just restore the full genetic complement of the animal. The processes associated with fertilization help the egg and sperm get together, prevent the union of the sperm and egg of different species, and guarantee that only one sperm will enter and activate the egg metabolically. In the accompanying animation, we examine fertilization using an invertebrate animal—a sea urchin—as an example.

The successful fusion of one sperm with one egg requires a sequence of cellular reactions in both of these haploid cells. During fertilization in a sea urchin, the sperm and egg undergo reactions that allow a sperm to recognize and fuse with the egg, followed by other reactions that prevent additional sperm from entering the egg. When more than one sperm cell fuse with one egg, this phenomenon is referred to as polyspermy.

The sperm performs its cellular reactions first, beginning with the release of acrosomal enzymes onto the egg's jelly layer. These enzymes digest through the jelly and allow the sperm's growing acrosomal process access to the egg. The sperm and egg recognize each other through a lock-and-key, species-specific binding between proteins on the surface of the acrosomal process and receptors on the egg. This recognition is especially important for organisms like the sea urchin, in which fertilization is external and the eggs and sperm are likely to encounter gametes from other species.

The species-specific binding allows the sperm to fuse with the egg. Immediately after this fusion, the egg undergoes two reactions that prevent additional sperm from gaining entry. The first, called the fast block to polyspermy, is a quick and short-lived response in which the egg's plasma membrane changes its electric polarity. The change in polarity inhibits other sperm from fusing.

In the mean time, the egg intiates a second, long-lived response, called the slow block to polyspermy. Associated with the slow block to polyspermy is a release of calcium ions into the egg cytoplasm from intracellular stores. The rise in cytoplasmic calcium activates the egg, thus initiating the first processes of development. The rise in calcium triggers cortical granules within the egg to release their contents and chemically alter the egg's outer layers. The vitelline envelope hardens to form the "fertilization membrane," which in turn rises away from the egg's plasma membrane. All of these responses make it more difficult for additional sperm to fertilize the egg.

The successful interaction of sea urchin sperm and egg usually exhibits a high degree of species specificity. In most in- terspecific inseminations sperm fail to adhere to or fuse with foreign eggs. Loeb (1916) probably was the first to suggest that species specificity of fertilization must re- side in the proteins carried on gamete sur- faces. During the past two years several gamete surface components have been iso- lated which appear to function in sperm- egg recognition and adhesion. The purpose of this mini-review is to dis- cuss some of these recent findings in rela- tion to the species specificity exhibited by invertebrate gametes. Special attention will be given to three interactions: The induc- tion of the sperm acrosome reaction by egg jelly, the attachment of spermatozoa to eggs and the fusion of sperm and egg plasma membranes. Comprehensive reviews of invertebrate and mammalian fertilization have recently appeared and their contents

will not be restated here (Epel, 1978; Epel and Vacquier, 1978; Gwatkin, 1978; Metz, 1978; Yanagimachi, 1977, 1978). Although most of the work to be mentioned concerns sea urchins, some exciting new data on ga- mete interactions in other animals will also be presented.

INTERACTION OF EGG JELLY WITH SPERM

The usually transparent egg jelly coat surrounding the sea urchin egg can be sol- ubilized by treatment of the eggs with sea- water at pH 5. After removal of the eggs, addition of sperm to the jelly solution may cause two visually observable and indepen- dent effects: the swarming of sperm into clusters (Loeb, 1916) and the acrosome reaction (both reviewed by Epel, 1978; Metz, 1978)

exocytosis of the acrosome granule located in the anterior apex of the cell and the ex- tension of the acrosome process (Epel, 1978; Metz, 1978). Under natural condi- tions the acrosome reaction can be induced by soluble egg jelly, by contact of sperm with the in situ jelly coat surrounding the egg or by contact of the sperm with the egg surface. Epel (1978) has review

Epel (1978) has reviewed the work on the dependence of the echinoid acrosome reac- tion on extracellular Ca^{2+}. Since then, Schackmann et al. (1978) have found the acrosome reaction of S. purpuratus sperm in response to eggjelly results in release of H^+ from the sperm coincident with the uptake or exchange of Ca^{2+}. Their studies with drugs and ionophores show the acrosome reaction to be absolutely dependent on en- trance of Ca^{2+} and Na^+ with the possible involvement of K^+ release

The chemotaxis of sperm to ovarian fluid of sessile marine hydroids and alcoholic ex- tracts of eggs of tunicates and chitons has been elegantly demonstrated using cine- matography (Miller, 1977; see review by Metz, 1978).

Ovarian or egg extracts in the bore of a pipette cause the immediate spe- cies specific swarming of sperm of manyhydromedusae to the pipette tip (Miller, 1979a, 19796). Miller (1979c) has most re- cently described the very interesting inter- actions of sperm and egg of the leptomedu- san Orthophxis caliculata. If sperm are added

to eggs before second polar body forma- tion they show no attraction for the egg, but swim randomly into the eggjelly where they become trapped and immobilized. But, if added after polar body II emission, the sperm move rapidly through the jellyand over the entire egg surface. The im- mobile sperm, trapped in the eggjelly, re- activate and also move to the egg surfacemembrane. The egg appears to release a species specific chemoattractant of about1000 mol wt 1 or 2 min after polar bodyII formation. Once at the egg surface the sperm undergo a head-to-head agglutina- tion only at the animal pole. This agglu- tination lasts 10 min after which the egg isno longer attractive to sperm. However, if the eggs are left unfertilized they continueto be attractive to sperm for at least 3 hours.

Is egg jelly essential for fertilization ?
The answer to this question is obviously no, since it has been known for several yearsthat blastomeres of 1, 2, 4 and 8 cell em- bryos can be refertilized after removal ofthe investing fertilization envelope and hyaline layer (Metz, 1978). One must con- sider sperm to be cells specialized for fusion with other cells, and it is known that induc- tion of the acrosome reaction directly against the naked egg or blastomere plasmamembrane may lead to gamete fusion and sperm incorporation

.However, although an indispensible role for egg jelly does not appear to exist, one can still ask whether egg jelly enhances the fertilizability of eggs. We have found (Vacquier et al., 1979) that L. pictus eggs treated 2 min with sea water at pH 4.7 and washed extensively with fresh sea water, show a great decrease in their sperm binding capacity and fertilizability that can be restored by addition of soluble jelly. We interpret this result by postulating the existence of an optimal amount of jelly

The interaction of egg jelly with sperm
The interaction of the fucose sulfate poly- saccharide with the sperm plasma mem- brane to induce the acrosome reaction, the swarming of sperm in response to jelly, and the chemotaxis of hydroid and tuni- cate sperm may all involve specific sperm membrane receptors. If so, do the recep- tors aggregate in the plane of the mem- brane to form or unmask ion channels? Or, do these egg jelly components act as deter- gents to remove proteins which block ion channels?

Can the fucose sulfate polymer be cleaved into a basic unit retaining biological func- tion?

In a species such as S. purpuratus, how is the jelly associated with the egg vitelline layer?

Is it only on the outer surface, or entirely throughout the vitelline, or possi- bly between the vitelline and the egg plasma membrane?

Many of these questions are now potentially answerable with the recent development of methods to radioiodinate and fluorescently label the surface of sea urchin and mouse sperm while keeping them viable and capable of fertilization (Gabel et al., 1978; Lopo and Vacquier, 1978

ADHESION OF SPERM TO THE EGG SURFACE

Many invertebrate eggs possess a glyco- protein coverlet closely adhering to the plasma membrane which is usually called the vitelline layer (VL). In sea urchins, the VL is the site of species specific sperm adhe- sion (reviewed by Metz, 1978). The bondbetween sperm and egg involves attach- ment of the acrosome process of the sperm on the outer VL surface. The VL can be isolated as an intact structure which retains its sperm binding quality (Glabe and Vac- quier, 1977a). The present idea is that species specific surface components of the sperm acrosome process bind to com- plementary "sperm receptors" on the egg VL and tightly cement the sperm to the VL.

Isolation of sperm surface components mediating gamete adhesion in sea urchins In a long series of papers over the past 12 years Aketa and his co-workers have iso- lated glycoproteins from sea urchin sperm and egg which appear to function in species specific gamete adhesion (reviewed by Metz, 1978). More recently, this group hasreported the isolation of a sperm factor which, when added to eggs, causes the eggs to lose their sperm binding capacity and fertilizability. This effect requires the pres- ence of Ca+2 and Mg*+ and is species specific (Aketa et al., 1978). When this factor is in- cubated with its putative egg surface recep- tor "sperm binding factor") it loses its fer- tilization inhibiting effect, another result which appears to be species specific. The factor is 5% protein and 95% carbohydrate.

FUSION OF GAMETE PLASMA MEMBRANES

The reasons for the induction of the ac- rosome reaction and the binding of sperm to the egg VL must surely be to enhance the probability of membrane fusion between the gametes. At this time, almost nothing is known about the relation between penetra- tion of the VL and membrane fusion. In sea urchin eggs the VL is very thin, being
only 100-300 A in diameter (Glabe and Vacquier, 1977a) and the acrosome process is short, being only about 1 mfi in length. The events of sperm attachment to VL, pen- etration of the VL and fusion of membranescell membrane

probably occur so rapidly in time that they would be impossible to separate from one another. One electron micrograph, how- ever, has been published showing a sea ur- chin sperm attached by its bindin to an egg
microvillus before extension of the acro- some process (Epel and Vacquier, 1978). In more favorable species, such as the horse- shoe crab Limulus polyphemus, the vitelline layer is roughly 40 fim in diameter and the
sperm acrosome process is at least that di- mension in length. In this species there are easily discernible phases of sperm attach- ment to the VL by the acrosome content, extension of the acrosome process through the thick VL and fusion of the tip of the process with the egg cell membrane (Brown,

3.TYPES OF EGG AND CLEAVAGE PATTERN

Fertilization …….Cleavage

• The transition from fertilization to cleavage is caused by the activation of mitosis promoting factor (MPF).

Cleavage, a series of mitotic divisions whereby the enormous volume of egg cytoplasm is divided into numerous smaller, nucleated cells.

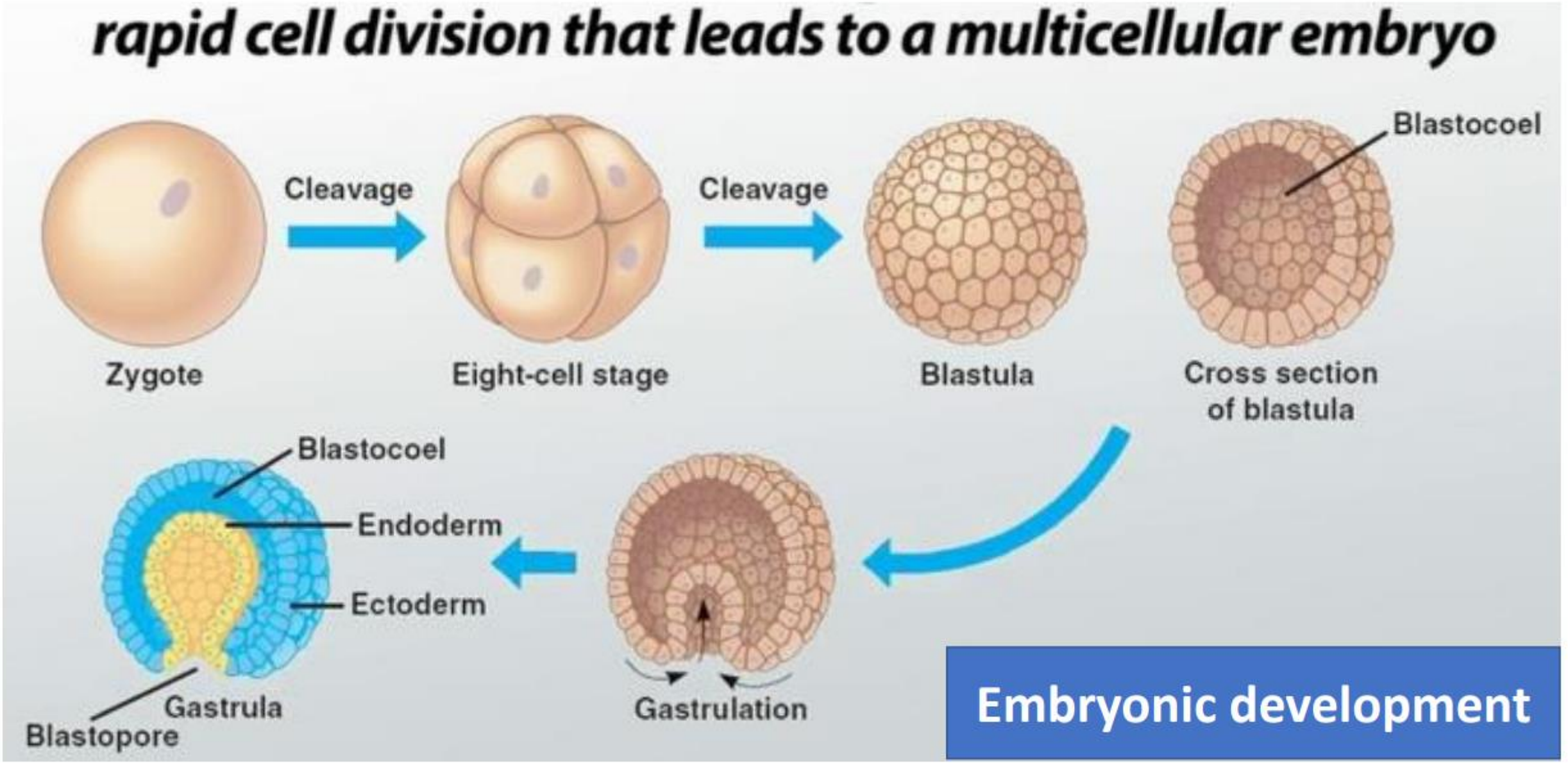
- These cleavage-stage cells are called blastomeres.
- In most species the rate of cell division and the placement of the blastomeres with respect to one another is completely under the control of the proteins and mRNAs stored in the oocyte by the mother.
- During cleavage, however, cytoplasmic volume does not increase. Rather, the enormous volume of zygote cytoplasm is divided into increasingly smaller cells.

One consequence of this rapid cell division is that the ratio of cytoplasmic to nuclear volume gets increasingly smaller as cleavage progresses.

- This decrease in the cytoplasmic to nuclear volume ratio is crucial in timing the activation of certain genes.
- For example, in the frog Xenopus laevis, transcription of new messages is not activated until after 12 divisions. At that time, the rate of cleavage decreases, the blastomeres become motile, and nuclear genes begin to be transcribed. This stage is called the midblastula transition.
- Thus, cleavage begins soon after fertilization and ends shortly after the stage when the embryo achieves a new balance between nucleus and cytoplasm.

Cleavage

rapid cell division that leads to a multicellular embryo



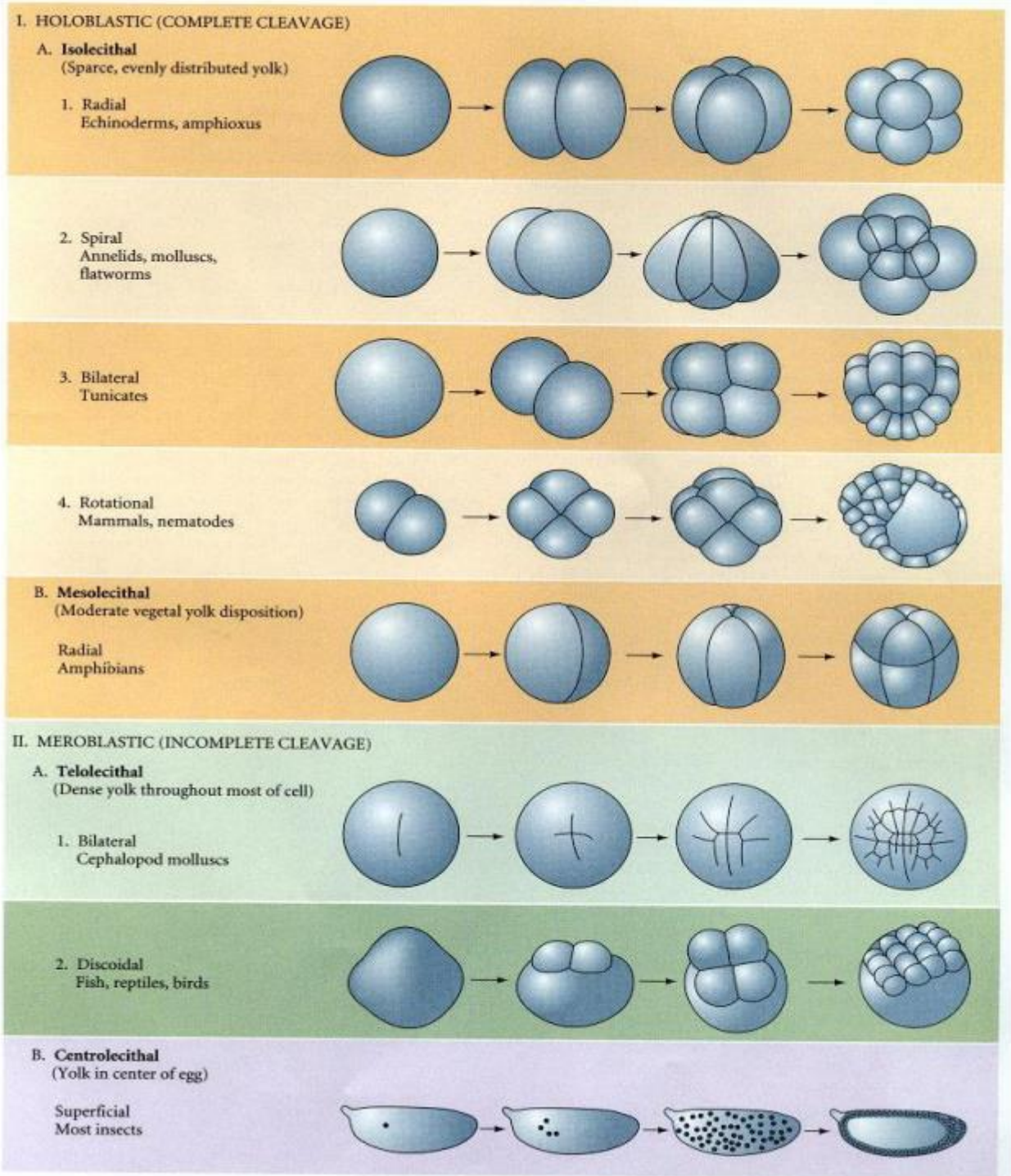
Embryonic development

CLEAVAGE 2

Division of first cell to many within ball of same volume (morula) is followed by hollowing of that ball to a blastula. Form of cleavage and blastulation depends on orientation of yolk and nucleus

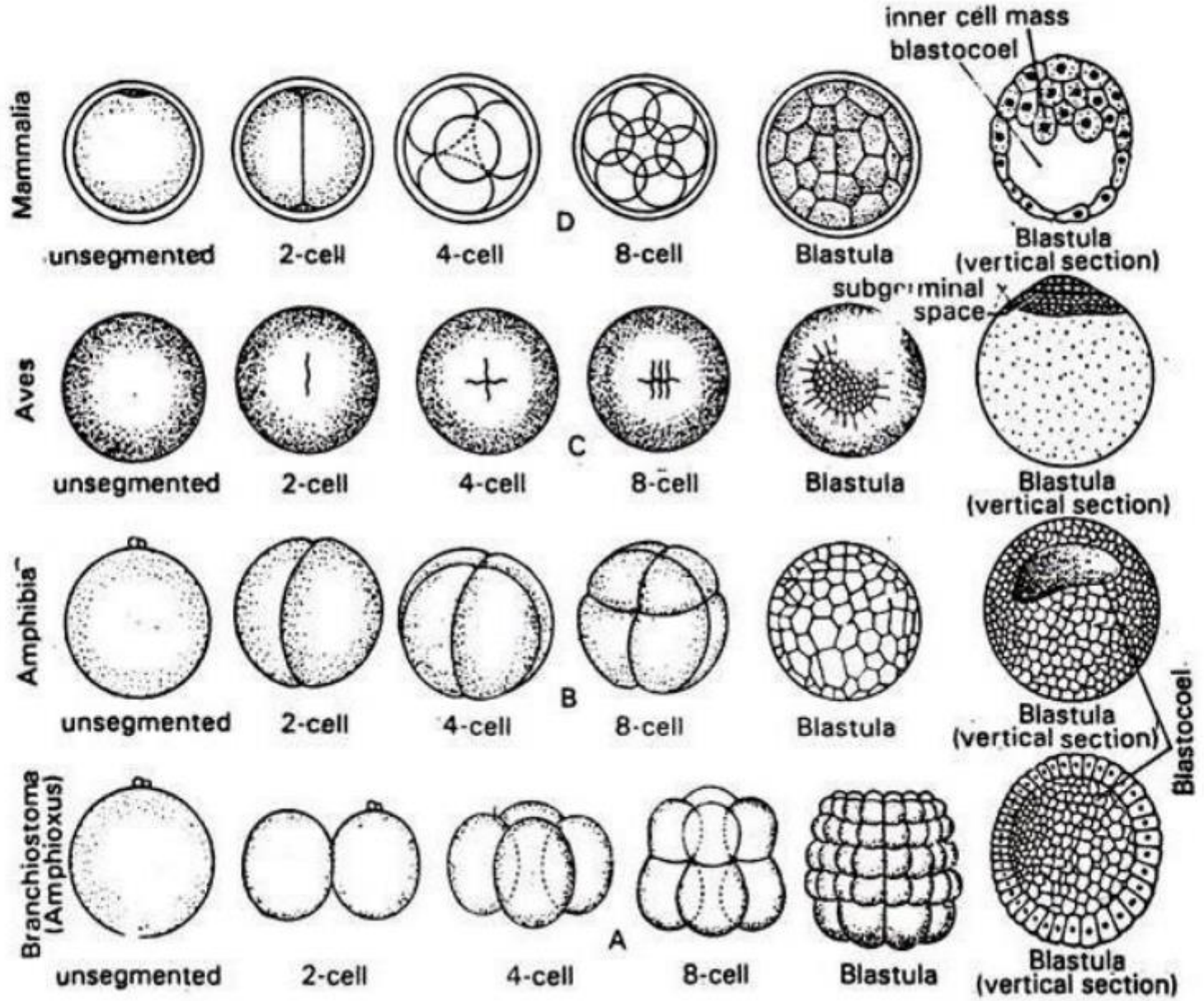
- In primitive chordates, division is even, towards a symmetrical blastula composed of cells of equal size
- In amphibians, holoblastic cleavage leads to asymmetrical blastula
- In reptiles and birds, meroblastic cleavage occurs, resulting in a cap of cells on top of the yolk
- In mammals, holoblastic cleavage occurs, creating a trophoblast containing a blastocoel, with inner disc of cells equivalent to a blastodisc

Patterns of embryonic cleavage



a. Holoblastic or total cleavage:
When the cleavage furrows divide the entire egg. It may be:
Equal: When the cleavage furrow cuts the egg into two equal cells. It may be radially symmetrical, bilaterally, symmetrical, spirally symmetrical or irregular.
Unequal:When the resultant blastomeres become unequal in size.
b. Meroblastic cleavage: When segmentation takes place only in a small portion of the egg resulting in the
formation of blastoderm, it is called meroblastic cleavage. Usually the blastoderm is present in the animal pole and the vegetal pole becomes laden with yolk which remains in an uncleaved state, i.e., the plane of division does not reach the periphery of blastoderm or blastodisc.
c. Transitional cleavage:
In many eggs, the cleavage is atypical which is neither typically holoblastic nor meroblastic, but assumes a transitional stage between the two.

Cleavage and blastula formation in chordate eggs



Types of Eggs

The types of eggs based on yolk characteristics are described as:

Isolecithal: sparse evenly distributed yolk, eg. sea urchin, mouse

Mesolecithal: moderate amount of yolk, often unevenly distributed,
eg. frog

Telolecithal: dense yolk concentrated at one end, eg. bird, reptile

Centrolecithal: yolk concentrated at the middle of the egg, eg. fly

Vegetal and Animal poles

Many eggs are polarized with a yolk rich pole, termed the vegetal pole and a yolk poor pole termed the animal pole, eg. frog.

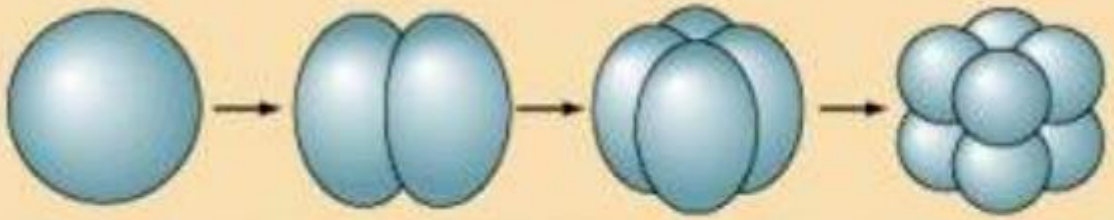
- The zygotic nucleus is generally displaced towards the animal pole.
- Zygotes with relatively little yolk (isolecithal and mesolecithal) cleave HOLOBLASTICALLY.
- The cleavage furrow extends all the way through the egg.
- While telolecithal and centrolecithal zygotes undergo MEROBLASTIC cleavage where the cleavage plane extends only to the accumulated yolk.
- In centrolecithal eggs (many insect eggs) cleavage is meroblastic and superficial, while in telolecithal eggs (birds and fish) cleavage is discoidal.

There are several types of cleavage symmetry seen in nature: radial(echinoderms, amphibians), spiral (mollusks, annelids), Bilateral (ascidians, tunicates), Rotational (mammals). The two figures below show examples of holoblastic and meroblasticcleavage symmetries.

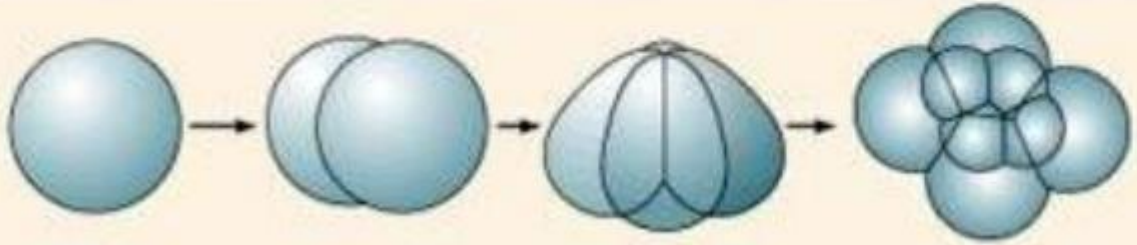
I. HOLOBLASTIC

A. **Isolecithal**

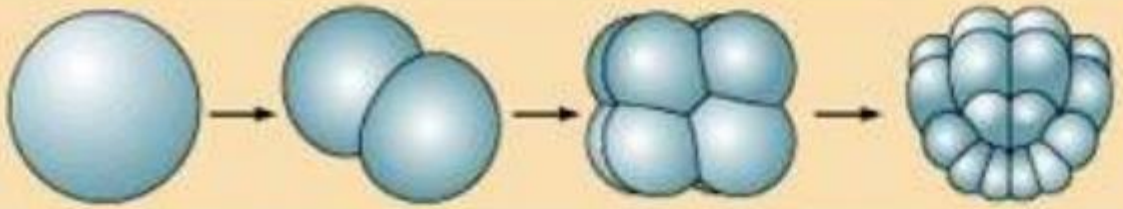
1. Radial
   Echinoderms, amphioxus



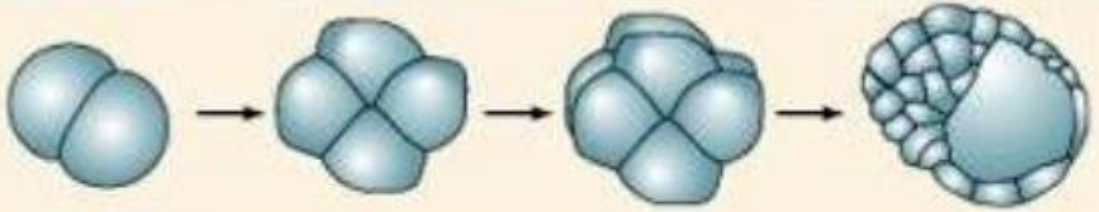
2. Spiral
   Annelids, molluscs, flatworms



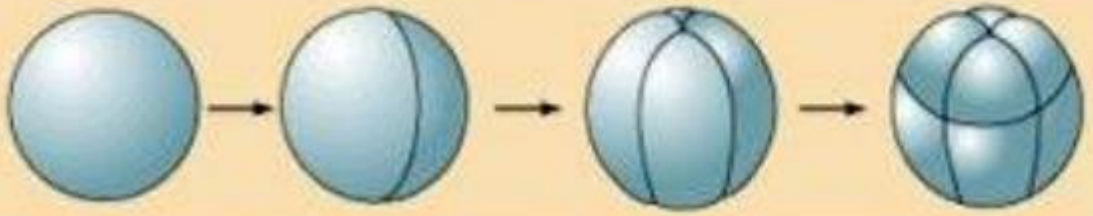
3. Bilateral
   Tunicates



4. Rotational
   Mammals, nematodes



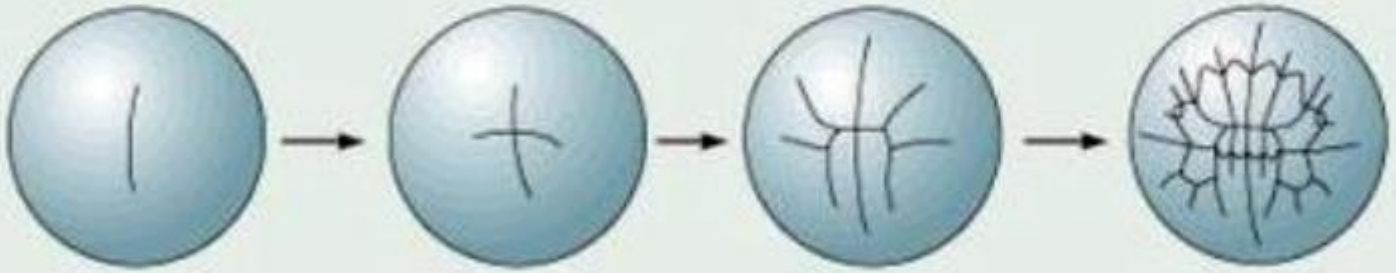
B. **Mesolecithal**
   Radial
   Amphibians



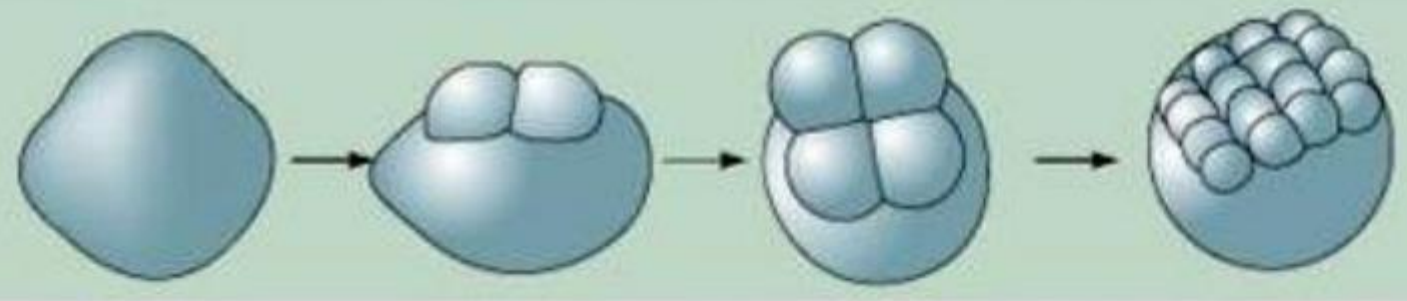
II. MEROBLASTIC

A. **Telolecithal**

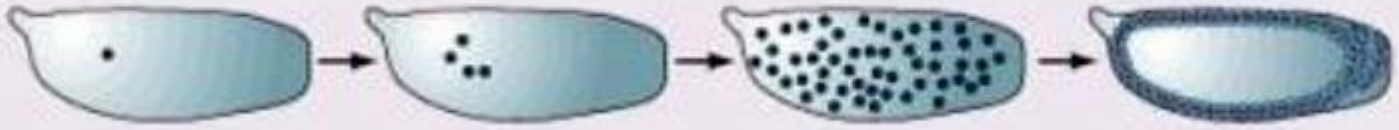
1. Bilateral
   Cephalopod molluscs



2. Discoidal
   Fish, reptiles, birds



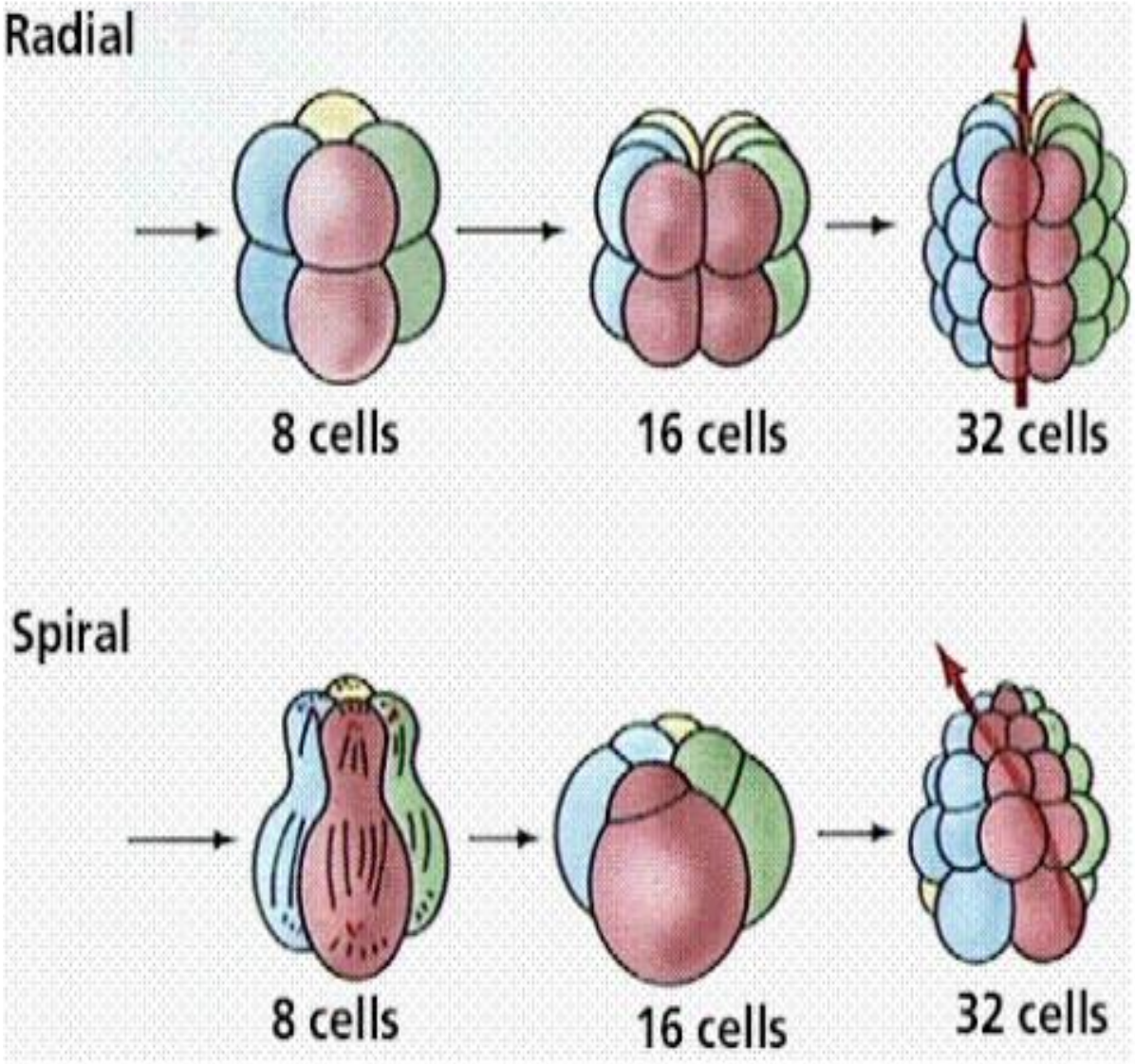
B. **Centrolecithal**
   Superficial
   Most insects



Radial & Spiral Cleavage

Radial – the cells divide such that each cell in thetop four cell plane isdirectly over one other cell in the bottom plane

- **Spiral - the cells divide at slight angles to one another, so that the none of the four cells in one plane of the eight-cell stage is directly over a cell in the other plane.**



Superficial cleavage-

This cleavage occurs in centrolecithal eggs. Here, the early divisions occur in the surface layer of the egg and cleavage furrows do not extend into the central yolk. In centrolecithal eggs , the zygote nucleus lies in the centre of the egg. It divides repeatdly without the div of the egg cytoplasm. As as result, a large number of nuclei are formed . These remain embedded in the undivided superficial layer of cytoplasm. The cytoplasm divides by furrow laid down from the surface towards inner side and separates into a large number of cells arranges around central yolk.

CONCEPT OF ORGANIZER AND INDUCTION

Organizers, which comprise groups of cells with the ability to instruct adjacent cells into specific states, represent a key principle in developmental biology. ... Similar experiments in chicken and rabbit embryos subsequently revealed groups of cells with similar instructive potential.

Definition. Embryonic induction describes the embryonic process in which one group of cells, the inducing tissue, directs the development of another group of cells, the responding tissue. Induction directs the development of various tissues and organs in most animal embryos; for example, the eye lens and the heart.

1. Introduction

The organizer is an embryonic tissue, which organizes the surrounding tissues to develop an embryo. The existence of the organizer was discovered by Spemann. He was given Nobel Prize in 1935 for his landmark discovery. Transplantation is a process in which small piece of a blastula, gastrula or even a embryo is cut off and inserted into suitably prepared incision of the same or another embryo. The embryo from which a part of tissue is taken is referred to as the donor, and the embryo to which the part is transplanted is called the host. The transplanted tissue is known as the graft.

2.Spemann's Experiment And The Embryonic Induction

Embryologist Hans Spemann succeeded in scratching out the cells of the dorsal lip of the blastopore of an early gastrula of a newt Triturus cristatus before their inward migration. Then he transplanted this small piece of tissue near the lateral lip of the blastpore of another Newt. He studied that the transplanted piece of dorsal lip invaginated and developed into a second notochord and the somites. It induced the host ectoderm above the location of the graft to form a neural groove. This neural groove was made up totally of host cells. It underwent normal development, forming a neural tube at first and then a central nervous system. At

last, a two-headed creature was formed. Thus, the dorsal blastopore lip of the blastula had the ability to induce theformation of the neural plate in the ectoderm of the host.This phenomenon is called neural induction.The presence of a notochord, kidney tubules and anadditional lumen in the endoderm corresponds to gut of thesecondary embryo. All these parts would not have developedif the transplant had not been present. Spemann concluded that the transplanted cells had changed the normal path of development of the host cells. The host cells above the graft were inspired to create a second central nervous system. This influence of one structure in the formation of another structure is called as embryonic induction. The structure, which induces the formation of another structure, is called inductor or organizer. The chemical substance that is emitted by an inductor is called an evocator. The tissue on which an inductor acts is called the responsive tissue

3. Primary, Secondary, Tertiary And Quaternary Organizers

PRIMARY ORGANIZER

When Spemann transplanted portions, only graft taken fromthe dorsal lip of the blastopore and the adjoining parts of the marginal zone were found to be able to induce. It is the capability of the dorsal lip of the blastopore (whentransplanted) to cause the production of a total embryo, that Spemann called Organizer. Spemann gave this dynamic region, the name of organizer because of its significance in

organizing the development. He imagined the organizer initiating the development of the morphogenesis and differentiation by inducing the structure of the neural tube.Thus, the dorsal lip of the blastopore is termed as the "Primary organizer".

SECONDARY, TERTIARY AND QUATERNARY ORGANIZERS As gastrulation continues,the different organ system of the embryo are laid down under the power of the primary organizer and they themselves then obtain the influence of inducing laterformed structures to develop. It is thus probable to know asequence of secondary, tertiary and quaternary organizers, which are set in a short of chain of command at whose summit is the primary organizer. These developed tissues then work together with another tissue in rotation and induce it to develop. In other words, one tissue gives the stimulus for the development of the other tissue subsequently.

4. Structure of Organizer & Competence

STRUCTURE OF ORGANIZER

The organizer has special region. Each region has the capacity to induce the development of a specific organ. There are two very important regions

(1) Head inductor and

(2) Trunk inductor

Head inductor: The head inductor is anterior part of the chordo-mesoderm, because it induces the development of head organs. The head inductors may be additionally divided

into the archencephalic inductor, which induces the prosencephalon, eyes and the nose rudiments and the duterencephalic inductor which induce the hind brain and the ear vesicles.Trunk inductor: The later part of the chordo-mesoderm functions as the trunk inductor. It induces the development of trunk organs and the tail bud.

The neural tube under the induction arises from the chordomesoderm. The endoderm and the mesoderm cannot develop into the neural tube under this stimulus. Therefore, the ectoderm has the competence to develop into the neural tube but the endoderm and the mesoderm do not have the competence to develop into the neural tube.

Competence

Waddington in 1932 introduced the concept of competence.It is always related to particular stimuli and particular corresponding responses. Competence is a term, which sums up the ability of the enzyme complement of the embryonic cell to adopt to a particular ratio of metabolites. When the ectoderm of amphibian embryo is transplanted from various developmental stages of blastula to early neurula, it gradually loses neural competence. With aging, the ectoderm gradually loses its capacity to respond to the inductive stimulus of chordo-mesoderm. Therefore, an isolated ectoderm unexposed to neural induction, and

ectoderm transplanted too late, differentiate into epidermis only. Late neurula epidermis no longer convertible into neural tissue becomes competent to respond to other inductors under the influence of eye vesicle, hindbrain and forebrain respectively. It differentiates into lens, ear vesicle and nasal pits during post-neurula stage of development. Thus, competence is a time-limited phenomenon with a beginning and an ending. As the age of the embryo advances, the competence of the various structures gets gradually reduced.

5. Induction Process In The Chordates The primary organizer was first noticed in Urodele Amphibians. It was found that dorsal lip of the blastopore and the roof of the archenteron of lower chordates and other vertebrates have the same function in development. In Cyclostomes, especially in lampreys, the dorsal lip of the blastopore, when transplanted into the blastocoel of another young gastrula, induces the development of a secondary embryo. In Bony fishes induction of secondary embryos were formed by grafting the posterior border of the blastodisc into the blastocoel of another embryo.

In frog, the formation of a secondary embryo can be induced by the transplantation of the dorsal lip of the blastopore into the blastocoel of a young gastrula. In reptiles, archenteron has the same inducing activity as in
other vertebrates. In the birds, the anterior half of the primitive streak was established to be the inducing part

6. Eye development

Eye development presents a typical pattern of series of inductive events:

1. The chordo-mesoderm, a primary organizer induces the demarcation of the fore-brain and the optic area within it. The development of eyes starts with the evagination of a pair of sac like optic vesicles from the lateral walls of the forebrain.
2. The external surface of the optic vesicle now compresses out and invaginates inside, so that the vesicle is changed into a dual walled cup like structure "The optic cup".
3. The optic cup serves as a secondary organizer inducing the structure of the lens. Since the optic vesicle is transforming into an optic cup, the somatic ectoderm overlying the cup undergoes a thickening to form a structure known as "Lens placode". The placode then

invaginates inside and finally pinches away from the parent ectoderm to form a lens vesicle lying in the pupil.

4. The lens substituting together with the retina work as a tertiary organizer of the cornea. The layer of mesenchyme left at the front of the anterior chamber of eye joins with the overlying somatic ectoderm to produce the cornea. Thus, it becomes evident from the foregoing account in the induction sequence, that one tissue supply the stimulus for the development of the other tissue. Since each structure is induced, it can further induce additional structures. The waves of induction could further lead to arranged, accurately timed, total, and planned embryo of the animal.

7. Mechanisms of neural induction Development of the ectoderm overlying the roof of the archenteron into neural tissue suggests a direct action upon the ectodermal cells, either by surface interaction or bychemical mediation.

1. One of the broad possibilities is surface interaction of the cells at the inductive interface. The contact of the two cellular layers may provide a device whereby the structural pattern or geometry or behaviour of the ectodermal cell membranes is altered directly by the underlying chorda-mesodermal cells. Thus, the spatial configuration of the latter membranes might induce a change in the spatial configuration of the ectodermal cell membranes, this in turn producing in the interior of the cell, changes that determine its development into neural plate.

2. Another broad possibility is a chemical mediation of the inductive effect. Therefore, a chemical substance
produced and released by inducing chorda-mesoderm cells at the archenteron-ectoderm interface may act
upon or enter the ectodermal cells to initiate cellular activities leading to neural development. From the experiments done later on, it was suggested that both of these possibilities are very much involved for neural

Chemical basis of neural induction:

It was found that many different tissues, embryonic or adult, live or dead from a great variety of species, were capable of inducing nervous tissue in amphibian embryos. Moreover, some foreign tissues were found to be much more potent inductors after they had been killed by heat, alcohol or petrol-ether treatment or by freezing or desiccation.

The organizer was then implanted into a living embryo in an appropriate stage of development and it was found that a killed organizer can still induce. Few inorganic agents such as iodine and kaolin, local injury, exposure to saline solutions of excessively high or low pH causes neural differentiation in ectoderm.

Artificial inducers such as solvents, acids and chemical dyes are employed for induction studies to understand the chemical nature of the inductors

Different chemical substances of dorsal lip or chordamesoderm are separated by different biochemical methods to find out the molecule which causes the neural induction and then the inductive capacity of each molecule was tested separately. Few experiments show that inducing substance or evocator is a protein.Exhaustive attempts were made by different embryologists to understand the real mechanism of neural induction. Some theories have been put forward to understand the mechanism of neural induction, out of which the most important are discussed here:

1. Protein denaturation theory of neural induction:

According to Ranzi, neural induction and notochord formation are related to protein denaturation. Site of notochord formation is amphibian gray crescent, which is a center of high metabolic activity. Such centers of greater metabolic activity correspond to sites of protein denaturation.

2. Gradient theory of neural induction:

Toivonen and Yamada stated that two chemically distinct factors are involved in the action of primary inductor. Out of these two factors, one is neutralizing agent and the other is mesodermalizing agent. Regional specifity of the embryonic axis arises from the interaction between two gradients: neuralizing principle has its highest concentration in the dorsal side of the embryo and diminishes laterally, while the mesodermalizing principle is present as an anteroposterior gradient with its peak in the posterior region.

3. One factor hypothesis of neural induction:

Nieuwkoop using living notochord as the inductor, postulated that only one factor which first evokes ectoderm to form neural tissue and later causes ectoderm to transform into more posterior and mesodermal structures that are involved.

4. Ionic theory of neural induction:

According to Barth and Barth, the actual process of induction may be initiated by release of ions from bound
form, representing a change in the ratio between bound to free ions within the cell of early gastrula. Induction of
nerve and pigment cells in small aggregates of prospective epidermis of the frog gastrula were found to be dependent on the concentration of the sodium ions. Normal induction of nerve and pigment cells by mesoderm in small explants from the dorsal lip and lateral marginal zones of the early gastrula is dependent on the external concentration of sodium.

5. Genic basis of neural induction:

There are evidences that the component tissues of neural inductor become differentiated prior to ectodermal cells. During this process, the rate of transcription of mRNA and differential activation of genes becomes many fold, while the differentiation of ectodermal cells is set in only after mid-gastrulation. The mRNA by transcription from the DNA was required for translation of a specific protein for induction.

8. Summary

Organizers are recognized as tissues that influence and organize other tissues to differentiate and produce a tissue or structure that in normal course should not have been formed. Spemann's organizer is a dorsal lip of blastopore of Urodele Amphibians. If, it is transplanted autoplastically,

heteroplastically, homoplastically and xenoplastically in anyother location of the recipient embryo, a secondary embryo develops. It influences the other tissue, which again has power to induce the another. Thus, the first one is called primary organizer, the second one is called secondary organizer, the third one as tertiary organizer, etc.

The influence that comes from the organizer is known as embryonic induction. The chemical basis of this induction is analyzed and finally revealed that these are nucleic acids. In other animals, chorda mesoderm in mammals, blastodisc in fishes and the anterior parts of the primitive streak in bird act as primary organizer to induce neural cells as well as secondary embryos. The competence of the ectodermal cells reduces as age of the embryo advances.

EXTRA EMBRYONIC MEMBRANE OF BIRDS AND MAMMALS

Summary. The extraembryonic membranes consist of the chorion (the combination of trophoblast plus underlying extraembryonic mesoderm), amnion, yolk sac, and allantois. The amnion, a thin ectodermal membrane lined with mesoderm, grows to enclose the embryo like a balloon. There are four standard extraembryonic membranes in birds, reptiles, and mammals: the yolk sac which surrounds the yolk, the amnion which surrounds and cushions the embryo, the allantois which among avians stores embryonic waste and assists with the exchange of carbon dioxide with oxygen as well as the resorption of ...

Followings are the objectives of the lecture:

- What are extraembryonic membranes?
- Different types of extraembryonic membranes and their functions.

Extraembryonic membrane:

These are the membranes which do not form any part of the embryo proper but performs various functions which assist in the development of the embryo. These are discarded at the time of hatching. These membranes formed outside the embryo.

Amniotes: These are the vertebrates group whose eggs contain extraembryonic membranes for protecting the embryo.They lay eggs on the land.
Example: Reptiles, Birds and Mammals.

Anamniotes: These are the vertebrates group whose eggs do not contain extraembryonic membranes during embryonic development. They lay eggs in the water.
Example: Fish, Amphibia.

There are four types of extraembryonic membranes in birds:

1. Yolk sac.
2. Amnion.
3. Chorion.
4. Allantois.

1. Yolk sac:

- Formed from splanchnopleure (inner endoderm and outer mesoderm)
- Well developed in the animals with megalecithal egg as reptiles, birds and Prototheria.
- Formed completely on the 9 th day of incubation. In human it is vestigial.

Functions of Yolk sac:

- It surrounds the yolk. Its main function is in digestion. It serve as extraembryonic gut.
- It help in digestion of yolk and transfer the digested material to the developing embryo.
- First respiratory organ in the embryo.
- Form yolk sac placenta in the marsupials.

2. Amnion:

Formed of somatopleure (inner ectoderm and outer mesoderm).

It surrounds the embryo. It appears after 30 hours of incubation.

A amniotic cavity is present between the amnion membrane and the embryo, which filled with the amniotic fluid. In this fluid filled cavity embryo floats.

Functions of Amnion:

- Protection of the embryo from the mechanical injury and desiccation.
- Amniotic fluid acts as shock absorber.
- Protect from sudden temperature changes

3. Chorion:

Formed of somatopleure (outer ectoderm and inner mesoderm).

It forms the outermost boundary.

Space between amnion and chorion is called chorionic cavity which further provides protection to the embryo.

Functions of Chorion:

- In reptiles, birds and prototherians, chorion along with allantois acts as extra embryonic lung and helps in exchange of gases.
- Nutrition and protection.

4. Allantois:

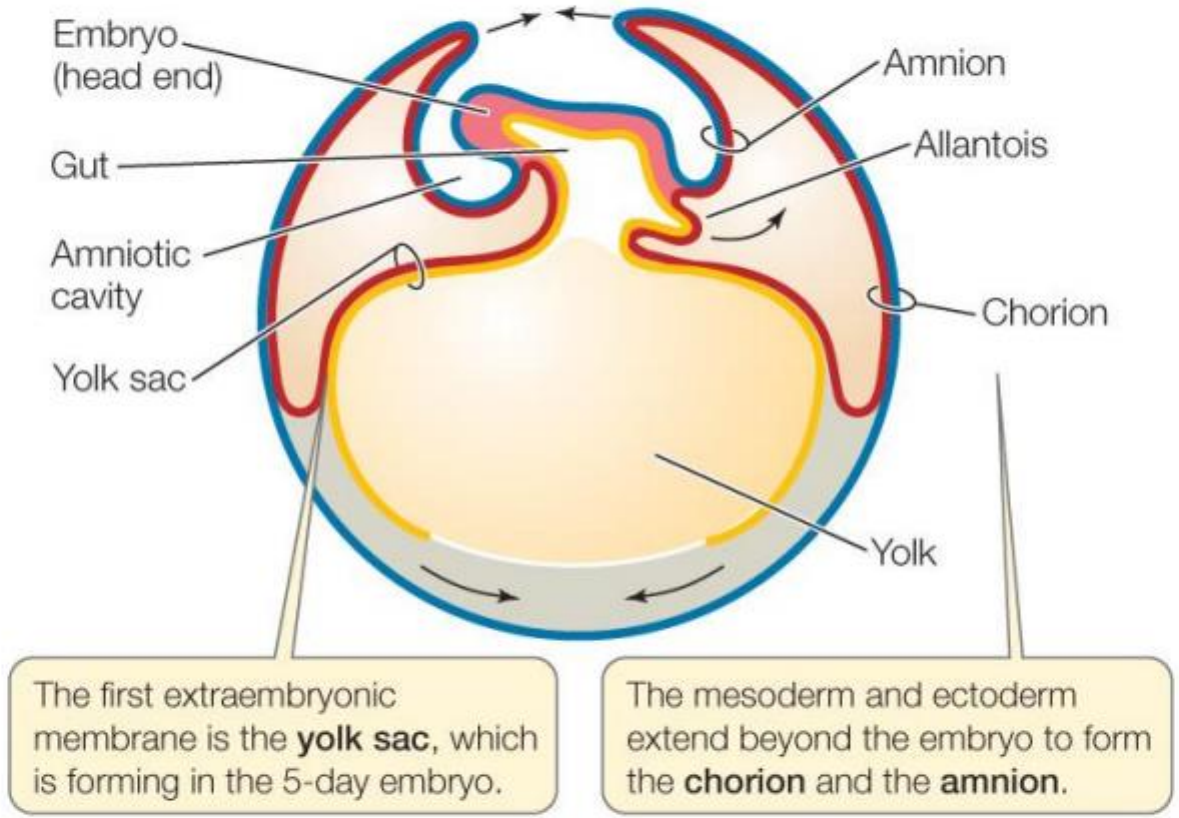
Formed of splanchnopleure (inner endoderm and outer splanchnic mesoderm).

Connected with the hindgut of the embryo.

Develops on the third day of incubation from the floor of the hind gut as a outgrowth.

Functions of Allantois:

- Act as reservoir for the storing the excretory wastes of the embryo. Considered as extra embryonic kidney.
- Also helps in digestion and nutrition from albumen and calcium of the shell.
- It grows in the chorionic cavity. Its outer membrane fuse with the inner membrane of the chorion and forms allantochorion which is highly vascular. Act as extraembryonic lung and provides surface for the gaseous exchange.



5 day chick embryo without shell

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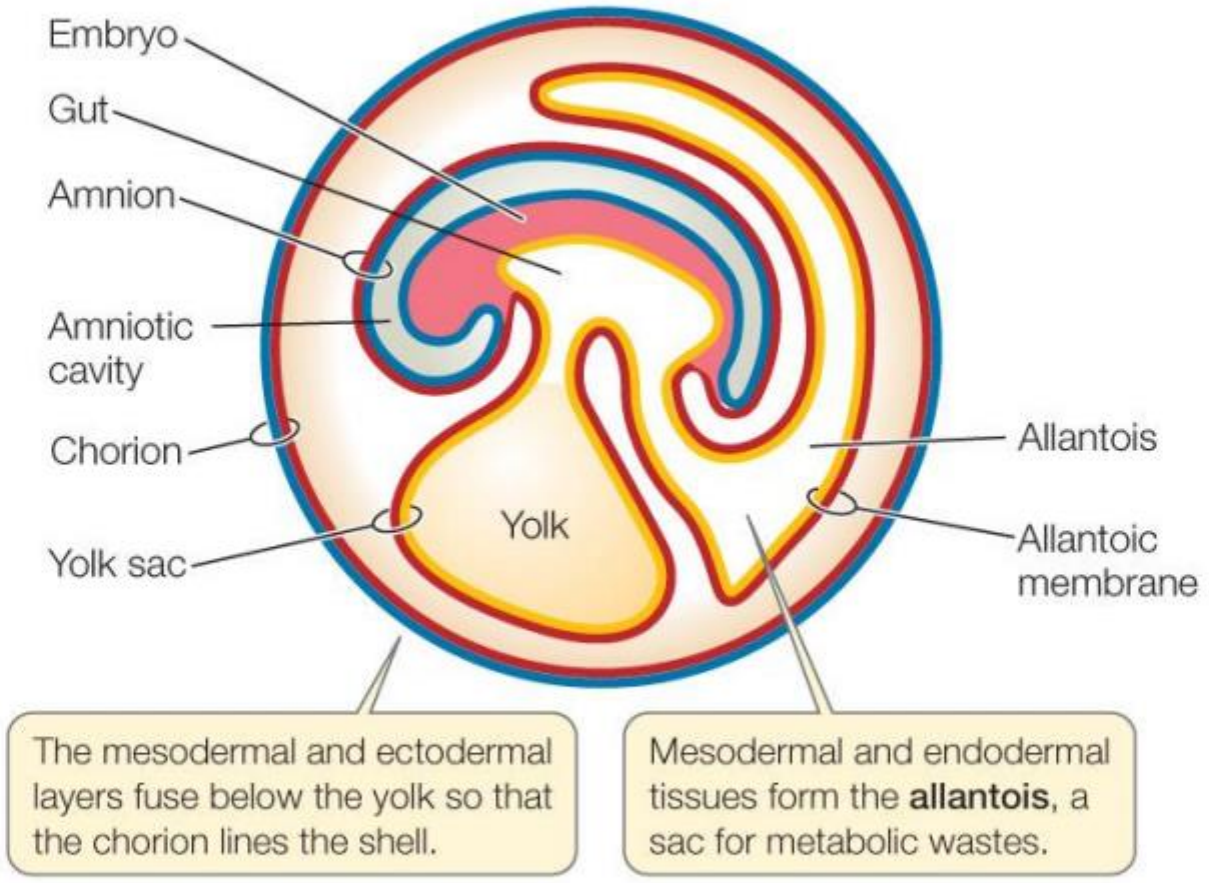

6 day chick embryo without shell

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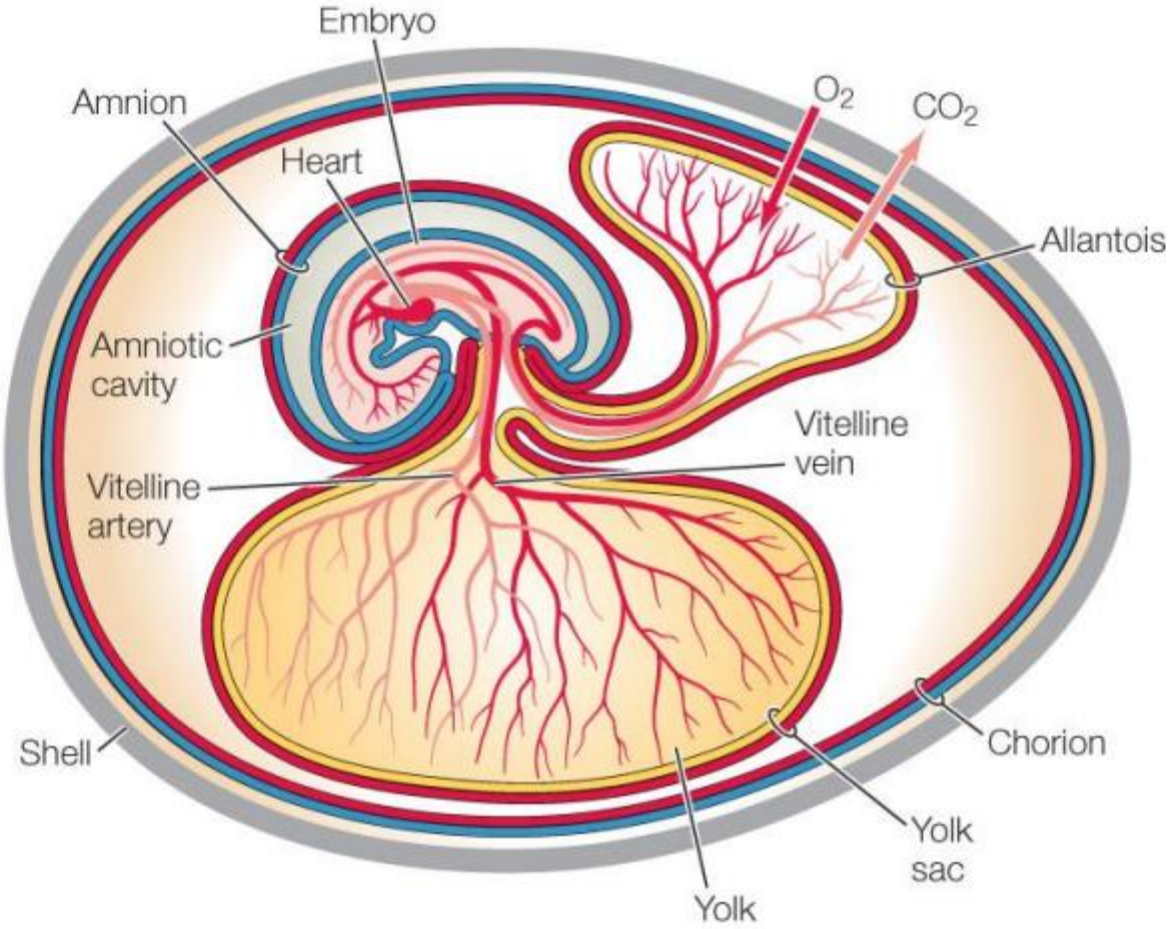

7 day chick embryo.

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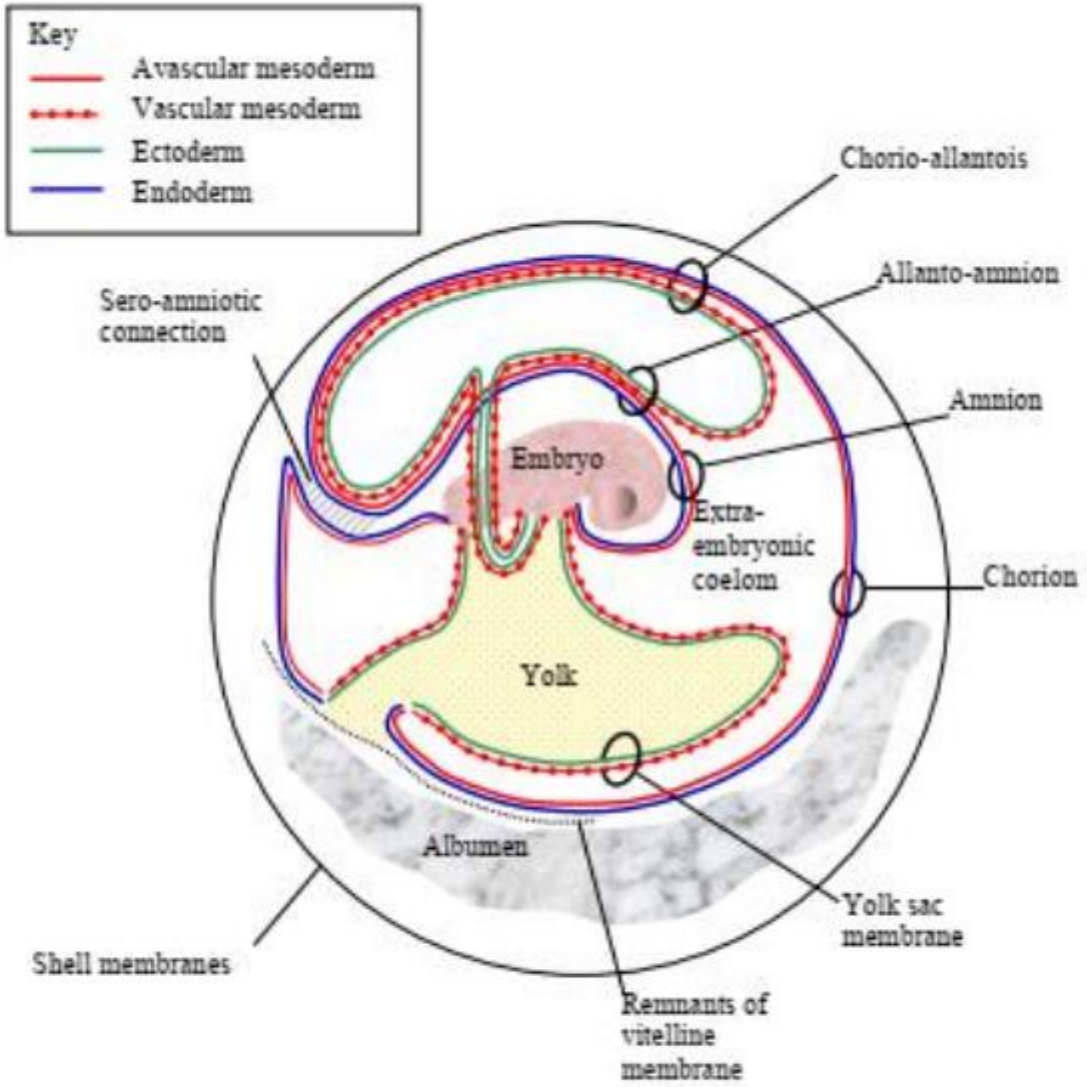
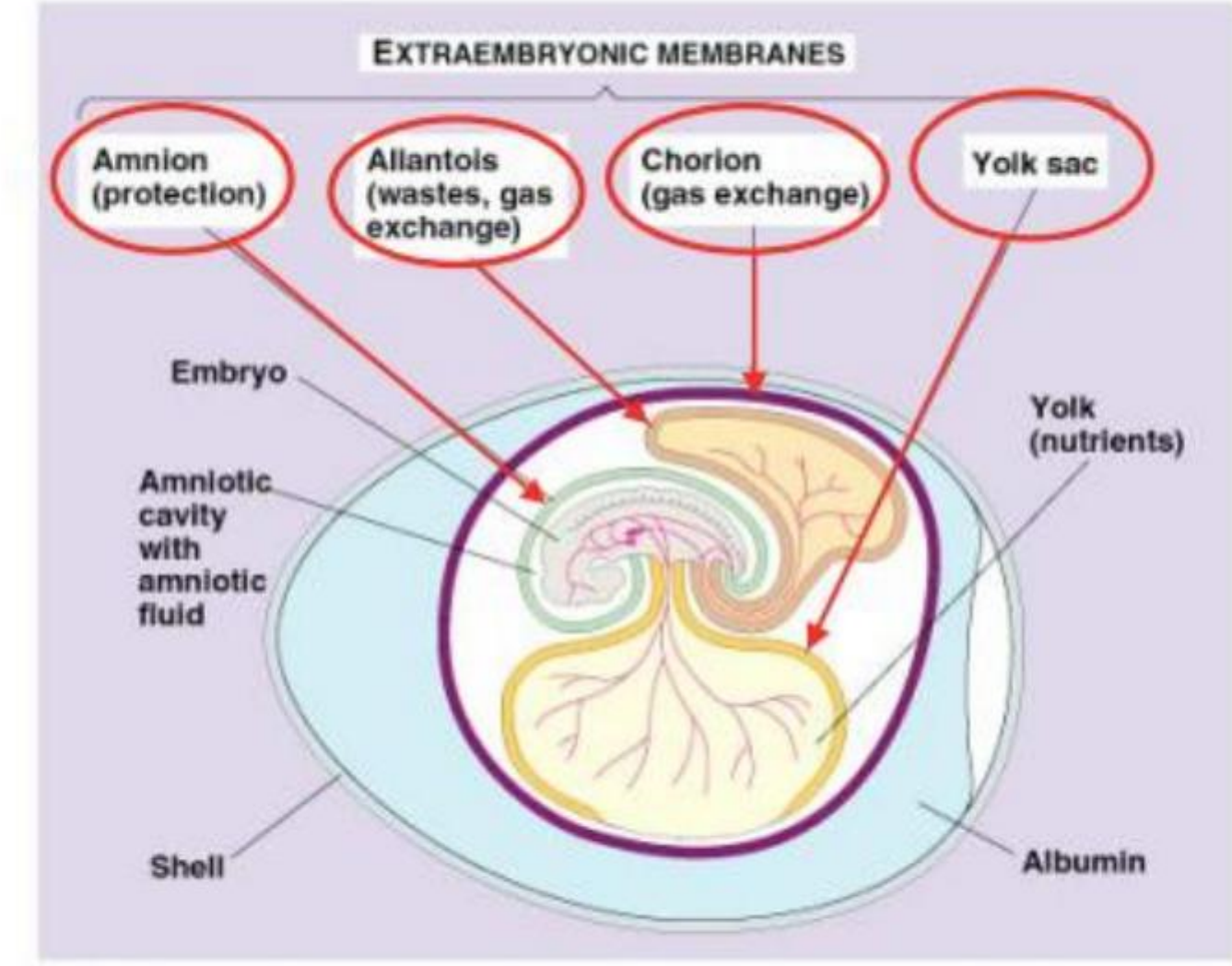


Extraembryonic membrane in Birds

Baggot,2009


EXTRAEMBRYONIC MEMBRANES
Amnion (protection)
Allantois (wastes, gas exchange)
Chorion (gas exchange)
Yolk sac
Embryo
Amniotic cavity with amniotic fluid
Yolk (nutrients)
Shell
Albumin
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The Extra-Embryonic Membranes of Mammals

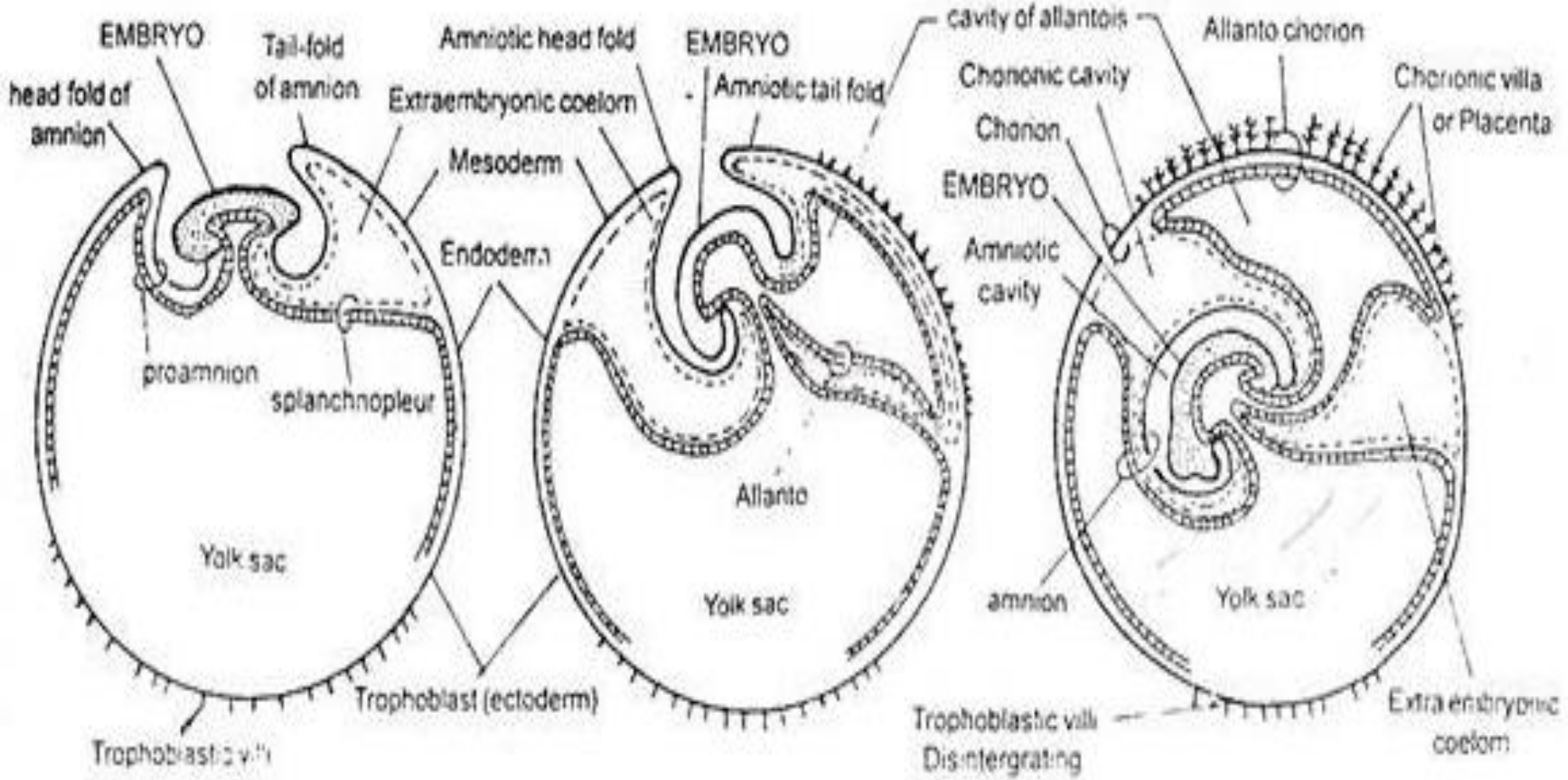
The extra-embryonic membranes of mammals also include the amnion, chorion, allantois and yolk sac.

Their origin and development is similar to that in chick except that the allantois in most mammals gives rise to a placenta.

1. Yolk sac:

The extra-embryonic endoderm spreading beneath the trophoblast (extra-embryonic) forms the yolk-sac. When mesoderm is formed, the somatic and splanchnic layers extend and penetrate between the yolk sac endoderm and trophoblastic ectoderm.

These establish the yolk sac and exocoel. Blood-vessels soon extend into the yolk sac establishing vitelline circulation. The yolk sac serves for the nutrition of embryo. In marsupials (e.g., Opossum) yolk sac wall is ultimately connected with uterine wall to form a yolk sac placenta.



Rabbit. Formation of foetal or extra-embryonic membranes. (A) Formation of head and tail folds of amnion. (B) Allantois formed. (C) Completed foetal membranes and placenta.

2. Amnion and Chorion:

The amnion is formed very early. The edges of the embryonic disc rise up as head and tail folds of the amnion; each consisting of trophoblastic ectoderm and somatic mesoderm. The tail-fold develops faster and receives the mesodermal layers earlier than the head-fold.

The fold meet over the embryo, for some time, a sero-amniotic connection indicates the point of meeting. Fusion of amniotic or somatopleural folds results in two membranes over the embryo: inner amnion and outer chorion. The fluid-filled amniotic cavity bounded by ectoderm between amnion and embryo serves to protect the embryo from mechanical shocks and prevents its dessication.

Chorion is made of outer ectoderm and inner somatic mesoderm. The space between chorion and amnion is the chorionic cavity or extra-embryonic coelom. Chorion develops larger secondary chorionic villi on the mesometrial side and establish connection with the uterine wall for absorbing the nutritive fluid.

3. Allantois:

In rabbit, the allantois grows out as a diverticulum from the hind-gut and soon becomes covered with the splanchnic layer of extra-embryonic mesoderm. Behind the embryo over a small disc-shaped area, the allantois comes into contact with chorion and their mesodermal layers fuse together and become highly vascular.

Thus a compound layer is formed called allanto-chorion or chorion – allantois. Its chorionic villi invade the maternal uterine wall forming an allantoic placenta for absorbing nutrients (in rabbit). Thus alanto-chorion of mammals is not only an efficient organ of respiration and excretion, but is also concerned with the supply of nourishment from the uterine wall.

In some mammals (man and apes), allantois remains rudimentary. It does not reach the chorion but remains buried as a small tube inside the body stalk (umbilical cord). Their chorion still forms a placenta known as chorionic placenta

REPRODUCTIVE CYCLE IN VERTEBRATES

reproductive cycle the cycle of physiologic changes in the reproductive organs, from the time of fertilization of the ovum through gestation and childbirth; see also reproduction.
sex cycle (sexual cycle) 1. the physiologic changes that recur regularly in the reproductive organs of nonpregnant female mammals.

Introduction

Some reproductive cycles of vertebrates are still little known or even unknown, and more particularly that of species which are difficult to study consequently to their geographic distribution or mode of life. This lack of knowledge can also be linked to the lack of economical interest. The works done in our laboratory and collaborating teams are devoted for a long time to the study of reproductive cycles in vertebrates. The first purpose of these works was to develop knowledge per se

Another reason is now related to the conservation of biodiversity. To protect a threatened species, it is indispensable to know its natural history and more especially its reproductive patterns. It is also useful to know reproductive features of a species which has been contrarily recognized as a pest, in order to control its proliferation. In both these cases, it is necessary to understand the consequences of environmental conditions on reproduction. Another reason, and not the least, is the knowledge of animal biology when a species becomesmodel used to study fundamental physiological and/or application to medical aspects. a

Essay # 1. Reproductive Cycles in Marine Fishes

Male Reproductive Organs:

A pair of testes is situated in the abdominal cavity, one on either side of the kidneys below the air bladder. These are enlarged and flattened structures suspended lengthwise by mesenteries (mesarchia). They may or may not be equal in size. Each testis is composed of follicles in which the spermatozoa formed make their way towards the exterior through the coiled tube, vasa efferentia.

These open into the anterior end of the vas deferens. The vas deferens becomes enlarged to form the sac like seminal vesicles on each side before they open into a large triangular chamber, the urinogenital sinus, which finally opens into the cloaca on an elevated urinogenital papilla.

In bony fishes, each testis possesses a sperm duct in its posterior side and the two sperm ducts join posteriorly to open to the exterior through a common urinogenital pore placed behind the anus. Most often the testes are creamy white in color and smooth. The contents are granular.

In the earlier stages, testes are thread like, becoming thick and fleshy with age and maturity. In mature specimens, the testes occupy almost the entire length of the abdominal cavity and are rose red in color with ridges and furrows, which apparently divide them into a number of lobes.

The ovaries in majority of marine fishes are paired, elongated, sac-like structures extending lengthwise in the abdominal cavity, ventral to the kidneys. They are attached to the body wall by means of the mesovaria. In elesmobranchs, the oviduct with a funnel at its head end is situated anteriorly in the body cavity and conducts the eggs caudally to their cloacal exit.

In oviparous elasmobranchs, oviduct is modified anteriorly into a shell gland, while in ovoviviparous and viviparous fishes; it is enlarged to form uterus for retention of embryos during their development. In bony fishes, the anterior ends of the two ovaries are free but their caudal ends may become united into one.

The posterior end of each ovary continues into a short oviduct. The two oviducts open together to the exterior through a genital aperture. Generally both the ovaries are equal in size but occasionally they are unequal.

The paired ovaries vary greatly in their appearance, size and general structures during the growth. The size and extent of the ovaries in the body cavity vary with the stage of sexual maturity. The color varies from whitish in young through greenish when immature to golden yellow in ripe adults. Texture of the ovaries ranges from floccular to granular in adults.

Regarding maturity stages, the vast majority of fishes show cyclic or periodic reproductive behaviour. For the determination of maturity cycle, the most common method is to define the stage of maturity of the gonad. The International Council for the Exploration of the Seas (ICES) has recognized seven maturity stages, 1st and 2nd as immature, 3rd and 4th as maturing, 5th as mature, 6th as ripe and 7th as spent.

Earlier workers used different features of the gonads as the bases of classification of the maturity stages involving morphological and histological examination of the gonads of the fish under study. Morphological observations like color, shape and size of the gonads in relation to the body cavity were used for ascertaining the maturity stages.

Microscopic examination of gonadal products, covering various histological methods over a year can give an idea about the maturation cycle. In case of female fish, ova diameter frequency helps in predicting some of the events in the maturation cycle. In the first classification it is based on the size of the ova but later the diameter of the ova was taken into consideration.

Maturation and Spawnin

Development of Ova to Maturity:

The maturation cycle in a fish is largely dependent of the growth rate of the ova of different stages in the ovary and subsequently on their distribution in the mature ovaries. Maturation refers to cyclic morphological changes, which the male and female gonads undergo to attain full growth and ripeness. The definition does not include the complicated physiological changes involving endocrine control.

The following classification traces the development of ova from immature to ripe condition:

a) Immature Ova:

These include the minute transparent ova as they arise from the germ cells, from the time they could be distinctly recognized as possessing a nucleus and a protoplasmic layer.

(b) Maturing This:

These include all the small opaque ova in which the formation of yolk has just commenced, but which are not fully filled with yolk.

(c) Mature Ova:

This group includes all the ova that are opaque, full of yolk and with distinct yolk spherules, but still contained within the foll

(d) Ripe Ova:

Include all those fully mature large, free, fully or partly transparent eggs, which have burst from the follicles.

The common method followed by most workers is the ova diameter frequency distribution that gives an index of the progression of oocytes and their withdrawal from the ovaries.

Spawning Season:

Spawning season indicates the range and peak of maturity with respect to time. Spawning in teleost fishes occurs during a particular phase of reproductive cycle. Some breed once in a year (annual), some breed throughout the year at regular intervals and in some, such as Pacific salmon, death follows spawning. This implies that a sound Knowledge of the reproductive cycle of a species is essential in fisheries management and rational exploitation.

The spawning season can be determined by the following methods:

a. Occurrence of Mature Fishes:

In this method, observations carried out for at least a year are analyzed and their percentages calculated month-wise. Spawning season is determined on the basis of the distribution of the different maturity stages, particularly the dominance of advanced stages with respect to time.

b. Gonado-Somatic Index (GSI):

Gonado- somatic index is another method for studying the spawning season by following the seasonal changes in the gonad weight in relation to body weight.

c. Occurrence of Eggs and Larvae:

The variation in the peak spawning period is dependent on the availability of favorable environmental conditions. The spawning season extends from May to August with maximum activity during June and July. Spawning takes place at mid night or early morning. The breeding is in relatively shallow in shore waters and there is only one spawning season for the fish during its lifetime.

Spawning Periodicity:

In temperate as well as tropical waters, fishes exhibit different spawning periodicities, which are closely related with the development of eggs, thus establishing a close relationship between the structure of the ovary and the spawning behaviour. The length of the breeding period is extremely variable and some species may spawn only once, others twice, while still others several times in a year.

Environmental Factors Controlling Gonadal Cycles:

The process of maturation of gonads in fishes is controlled by internal as well as external factors so that the individuals spawn together during favorable environmental conditions. Internal factors comprise a series of hormone- controlled changes occurring both in testes and ovaries in such a way that peak maturation is attained during a stipulated time.

In many fishes coordination in spawning is achieved by shoaling behaviours. Very often, the spawning congregation involves migration over long distances and this is so adjusted that large number of individuals are available at the spawning ground. Both internal and environmental factors are responsible for the behaviours leading to congregation, while the fish is shoaling; the contents of both male and female gonads are released to the exterior where fertilization takes palace.

The process of fertilization is epidemic in nature as spawning by one individual induces the others to follow the same. Males reach the spawning grounds before the females and the presence of spermatozoa induces the females to shed their eggs. The internal physiological rhythm of gonadal maturation involving pituitary and gonadal interactions is adjusted to ensure that breeding occurs under most favorable environmental conditions for survival of the offspring.

Many environmental factors affect different phases of the breeding cycles. The proximate factors include light, temperature, and other physical factors while the ultimate factors are food availability and favorable growth conditions. Photoperiod also plays an important role.

Spawning period of tropical fishes is correlated with water characteristics such as temperature, salinity, wind and rain, which have a derisive influence on the beginning of the spawning season. These monsoon changes cause intermixing or upwelling of the hydrological factors. Temperature and salinity exert profound influence on the fish throughout its life cycle.

Temperature controls the metabolic and spawning activities while salinity not only exerts effect on spawning but also on osmotic balance of the body fluids of fishes. Optimum temperature for each species varies but generally lies between 17 to 24 °C. Favourable temperature and abundant food supply favor spawning in temperate countries but fail to trigger spawning in tropical waters

Gonado-Somatic Index:

Gonads undergo regular seasonal cyclic changes in weight, particularly in females. Such cyclical changes are indicative of the spawning seasons. Therefore, the gonado-somatic index is considered to be a method for studying the spawning season. Relative ovary weight indicates the state of maturity of the ovary.

Fecundity:

Fecundity is the number of ova formed in a season. In some fish like the herring, the number of yolky eggs in the ovary in a season can be accurately counted and this gives the fecundity of the fish. However, in Indian fish this is not possible because eggs are released from the ovary in batches.

Essay # 2. Reproductive Cycles in Freshwater Fishes:

Structure of Testes:

Testes of teleosts are covered over by tunica albuginea, a thin delicate membrane enveloping fibrous connective tissue. Internally each testis is lobulated and possesses somatic and germ cells. The tunica albuginea remains thin but the underlying stroma undergoes changes with maturity cycle, being thickest in spent testes.

The connective tissue fibres from the peripheral stroma penetrate and divide the lumen of testes into large number of seminiferous tubules or lobules of vaied shapes and sizes. The seminiferous lobules or tubules undergo changes in accordance with maturity cycle. The gonocoel, comprising of inter-and intra-lobular portions, is packed with both germ and somatic cells.

Although the germ cells are restricted to intralobular region, the somatic cells are distributed both within and outside the lobules. The architectural combination of these cells is highly variable during different seasons in relation to maturity stages.

Germ Cells:

The various types of germ cells that can be seen within a testis during its maturity cycle are sperm mother cells (resting or primary spermatogonia), secondary spermatogonia, spermatocytes, spermatids and spermatozoa .

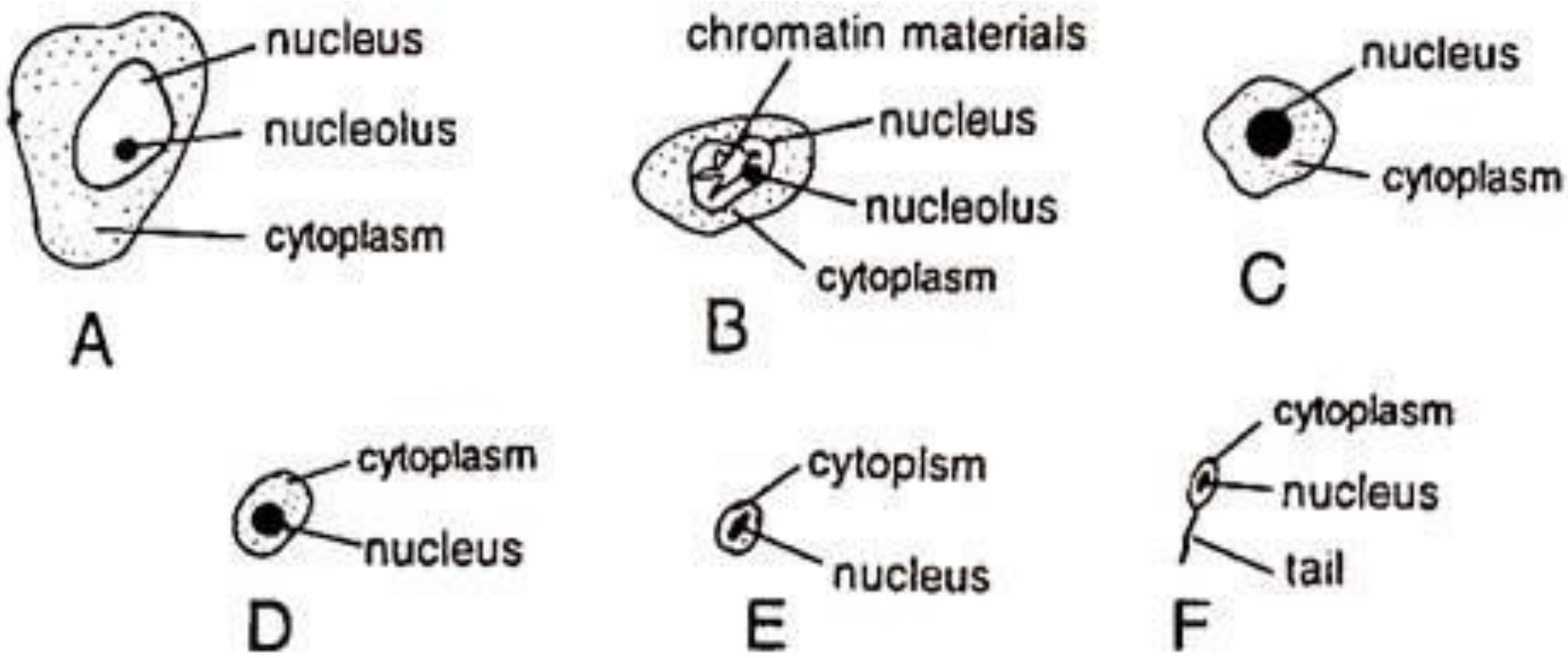


Fig 1 Male germ cells in the testis of a teleost fish : A–resting spermatogonium. B–secondary spermatogonium. C–primary spermatocyte. D–secondary spermatocyte. E–spermatid; F–spermatozoan.

Resting or Primary Spermatogonia or Sperm Mother Cells:

In size, these are the largest of germinal cells. They lie within the lobules close to the lobular wall. These cells are with clear eosinophilic cytoplasm and acentrally placed hyaline nuclei.

Secondary Spermatogonia:

These resemble primary spermatogonia except for being smaller in size and possession of chromatin threads in the nucleus, which radiate from nucleolus.

Spermatocytes:

These are of two types, primary and secondary and are more centripetally arranged in the lobules. Secondary spermatocytes are smaller in size but their cysts are comparatively larger. These possess hyaline cytoplasm and central chromatin material. Distinct nuclear membrane is often lacking.

Spermatids:

These are round, darkly stained cells usually filling the entire lobule. Each spermatid comprises of a prominent nucleus enclosed within a small layer of hyaline cytoplasm.

Spermatozoa:

These are derived from spermatids as a result of spermiogenesis and each sperm has an anterior darkly stained nucleus and a small posterior part drawn into a tail.

Somatic or Non-Germinal Elements:
Interstitial Tissue:

The testes of teleosts can be divided into two basic structural types; the first category includes fishes, which have testes with discrete interstitial cells between testicular lobules while the second category includes fishes with testes devoid of true interstitium, but characterized by possession of tubules with boundary cells.

Functionally, both these cell types are comparable with the Leydig cells of higher vertebrates. These cells secrete steroid hormones, which control development and maintenance of secondary sexual characters, and also the reproductive activity.

Phagocytes:

These are darkly stained eosinophilic cells with a well- defined nucleus and variable shapes. These make their appearance in the testes towards close of spawning season (in spent or partially spent testes) and grow in number and size; their size being maximum in a testis prior to initiation of its successive maturity cycle.

Migratory Cells:

Spindle shaped or oval migratory cells are present within the interlobular septa and tunica of the testes. These cells have been reported to play an important role in replenishment of new crop of germ cells. Based on the staining properties with Sudan black, and the changes in their shape and number, they are considered to be closely related to interstitial cells. In the majority of freshwater fishes ovaries are paired and elongated structures. They are placed along the ventro-lateral margins of the body cavity of the fish.

Structure of Ovary:

Ovaries in teleosts may be of gymnovarian or cystovarian type (Fig. 2A). Gymnovarian ovaries are naked since the investing cover, the tunic is absent and the ovulated eggs shed in coelom are carried by the oviducal funnels to the oviduct. In some fishes like salmon these ducts are absent.

The cystovarian ovaries, on the other hand, are closed by a fold of peritoneum the tynica, which continues posteriorlyl as oviducts. The tunic is made up of outer connective tissue and inner germinal epithelium. The germinal epithelium is projected into the ovarian lumen in the form of freely suspended folds.

Histologically ovary consists of developing oocytes in different stages (immature, maturing and mature) besides corpus luteum and corpus atreticum of ovulated and unvoulated follicles depending upon the stage of ovarian maturity. A mature oocyte is surrounded by three layers; the outer theca, middle follicular epithelium and innermost vitelline membrane.

The first two layers perform major functions in the transport of nourishment to the oocyte, resorption of corpus atreticum and formation of corpus luteum. Intestitial cells are the other histological elements observed in the ovaries in varying proportions at different times of the year. Theca, follicular epithelium, corpus atreticum, corpus luteum and interstitial cells are steroid hormone producing sites in the ovaries of different fishes.

Atretic Follicles:

The term corpora atretica refers to preovulating follicles turning atretic. The cells of egg membranes phagocytose the yolk in a developing ovum and ultimately degenerate. This process clears the ovary of debris of oocytes, which fail to mature.

The formation of corpus atreica occurs in four stages:

Stage I:

This stage is characterized by the breakdown of the ovum and hypertrophy of granulose cells.

Stage II:

Invasion of follicular space by granulose cells, fragmentation of vitelline membrance, and phagocytosis of yolk by granulose cells are the most conspicuous changes, which occur at this stage.

Stage III:

Complete occupation of oocytic space by hypertrophied cells and completion of phagocytosis of yolk takes place during this stage.

Stage IV:

All the above structures disintegrate and the vascular space is completely filled by cell masses. Yolk is completely absent in the atreticum.

Yellow body:

In most of the freshwater fishes the follicular envelope after the release of the ovum is retained in the ovary. Such discharged follicles consist of granulose and theca, which lie exterior to vitelline membrane. The follicles hypertrophy through developmental stages to form a solid corpus luteum.

The formation of corpus luteum occurs in the following stages:

Stage 1:

After ovulation, the postovulatory follicle shows a large cavity surrounded by the hypertrophied granulose and thecal layers. The granulose cells are columnar in shape with basal spherical nuclei. The theaca becomes thicker and consists of fibrous elements and connective tissue. Some of the thecal cells appear glandular. The theca is highly vascular.

Stage 2:

In this stage the size of postovulatory follicles is reduced. The granulose layer forms villus-like projections, which are irregularly placed in the lumen. These cells are further hypertrophied and their cytoplasm is eosinophilic. There is an increase in the thecal vascularity.

Stage 3:

In the third stage, the lumen of the postovulatory follicle is further reduced due to continued shrinkage. There is no vascularization of the granulose cells but they contain a few blood cells. The thecal gland cells become vacuolated.

Stage 4:

The hypertrophied granulose lutein cells form a mass and occupy most of the lumen, which is drastically reduced. The postovulatory follicle becomes a multilayered structure and contains several blood cells. There is an increase in the pycnosis of ganulosa cell nuclei.

Stage 5:

In this stage the postovulatory follicle is greatly reduced. The ganulosa cells are randomly arranged and separated from each other suggesting dissolution of intercellular cohesion between them. All the nuclei are pycnotic. The thecal vascularity is deceased and it becomes more fibrous.

Stage 6:

The postovulatory follicle in this stage is reduced to a very small structure. The number of granulose lutein cells is decreased due to degeneration. The thecal layer invades the residual luteal cells. The thecal vscularity is greatly reduced.

Ovarian Cycles:

In teleosts with cystovarian ovaries, the oviducts are not the modified Mullerian ducts, but they are the posterior continuation of mesovarium or ovarian tunic. The ovarian cycle in all the fishes follows a more or less identical pattern of maturation starting from a transparent infantile ovary comprising of oocytes in cytoplasmic differentiation stage.

Followed by a growing ovary in which vitellogenesis is initiated and completed with gonadosomatic index (GSI) recording highest values. A rapid decline in GSI and simultaneous appearance of corpora lutea indicates spawning activity.

On the basis of histo-morphology the ovarian cycle of freshwater fishes is divisible into the following four stages:

Stage I:

Ovaries during the first stage are translucent structures with no externally visible ova. Oocytes are enveloped by follicular epithelium. A darkly staining body, the yolk nucleus of Balbiani (YNB) is visible clearly in the cytoplasm of oocytes. Preparedness for nucleolar extrusion is a terminal point of this stage of ovary.

Stage II:

Ovaries increase in size and oocytes become visible externally. Appearance of vacuoles along periphery and initiation of yolk deposition in oocytes are the characteristics of this stage. Nucleolar extrusion may even extend to early stage II of maturation of oocytes.

Stage III:

Ovaries are filled with ova and occupy maximum space in the body cavity. A vitelline membrane appears around the oocyte. Yolk deposition is completed. A clear theca now appears around the follicular epithelium.

Stage IV:

Ovaries exhibit loose and flaccid appearance in this stage. In this stage large number of corpora lutea of discharge follicles in different stages of formation occur. Atretic follicles of different stages are also readily seen.

Three critical phases occur in the ovarian cycle at which the control mechanism operate, viz.:

(i) Initiation of oogenesis,

(ii) Transition into vitellogenic stage, and

(iii) Spawning.

Female Reproductive Cycle:
In majority of teleosts, the ovaries are paired and of cystovarian type. Within each ovary oocytes at different developmental stages are lodged. Interstitial cells lying within the ovarian tissue are also present. A mature oocyte is characterized by the presence of three investing membranes namely, outer theca, middle follicular epithelium and innermost vitelline membrane.

Interstitial cells, theca, follicular epithelium and corpus luteum are the sites of steroid hormone production. Presence of corpora atratica is a common feature of the ovary of fishes.

Male Reproductive Cycle:

Majority of teleost fishes have paired testes (Fig. 2B). Each testis has an outer covering, the tunica, and is internally divided into a number of lobules possessing both somatic and germ cells. Various types of germ cells present within the seminiferous tubules are the sperm mother cells (primary or resting spermatogonia), secondary spermatogonia, spermatocytes (primary and secondary), spermatids and spermatozoa.

The somatic cells of testis are interstitial cells, cells lying on the boundary of lobules and phagocytes. All these cells have been suggested to be the possible sites of androgen production. Sperm duct of the teleosts is the posterior continuation of mesarchium. Two types of sperm ducts occur in teleost fishes. The first is a conspicuous gland lying anterior to the duct and the second type is the seminal vesicle.

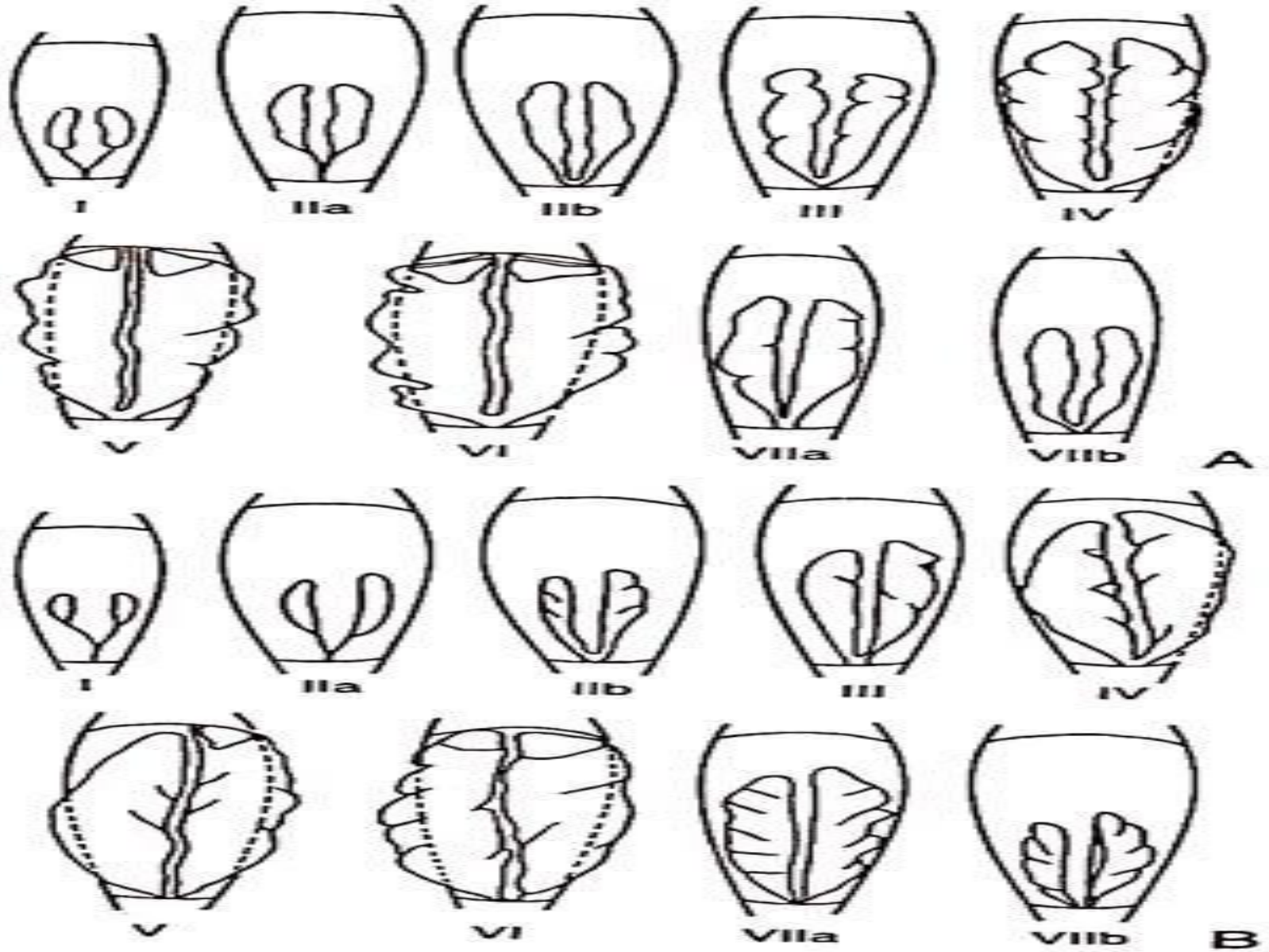


Fig. 2. Different maturity stages of a teleost fish. A—female. B—male.

Control of Gonadal Cycles:
External Control:

In the breeding season, two types of factors namely, the proximate and the ultimate factors exert influence. Proximate factors such as light, temperature and other physical factors regulate the development of reproductive organs and processes in breeding adult while ultimate factors like abundance of food and favorable growing conditions affect the survival of the young. Some of the more prominent environmental factors, which effect the reproductive cycle including the maturation and spawning, are discussed here.

Gonadal Maturation:

Light:

Day length is a major environmental variable synchronizing gonadal maturation with approximate season. Of all the environmental fluctuations, only length of day provides a reliable time marker for accuracy in animal breeding behaviour. In most of the fishes long photoperiod stimulates gonadal maturation.

Temperature:

Temperature is considered to be the most important exteroceptive factor controlling sexual cycles in temperate fishes. Winter dormancy is observed in the ovaries either in immature stage (stage I) or in completely mature (stage III) stage. This is an adaptation against acute cold winter conditions. This inhibitory stage is broken when the temperature starts to rise during the spring months of March and April.

Temperature and photoperiod acting together bring about maturation of the gonads. Both temperature and light influence reproduction. High temperature within the optimum range for a species is essential for efficient action of the photoperiod. Fish being poikilotherms, low temperature depresses metabolism when other environmental factors including light become ineffective.

Spawning:

Spawning, the most critical and sensitive phase in the gonadal cycle, is induced by sudden changes in climate like rise and fall in temperature or rain and floods. Temperature is an important variable effecting spawning activity of fishes in temperate areas. The role of light is indirect through the stimulation of photosynthetic activity of aquatic plants which results in a built up of dissolved oxygen content of water.

Increase in dissolved oxygen is considered to be an additional suitable habitat and stimulant for spawning activity in fishes. Day length and temperature fall during monsoon period offering suitable conditions for fish to spawn and survival of the offspring.

Rains and floods are responsible external factors favorable for triggering spawning in Indian major carps. Flood conditions created by premonsoon and monsoon rains act as stimuli for the onset of spawning. Stimulation of conditions of flooding by increasing pond water levels or by refilling Sun dried ponds induce spawning. It is not clearly known as to which specific factors created by rainfall and floods such as lowering of water temperature, dilution of electrolytes, increase in oxygen content and change of pH induce spawning.

Premonsoon rains in June cause the initiation of upstream movements of Indian carps towards their breeding grounds where they arrive and wait for flooding by monsoon rains. In the absence of such inundation, fishes evade spawning. It is observed that spawning does not occur in carps unless some rainwater is mixed with the pond water.

Other factors such as availability of food, DO and spawning grounds help in creating optimum conditions for spawners and juveniles. Spawning is sensitive to specific stimuli. Abrupt temperature changes in temperate fishes, and rainfall in tropical and subtropical species are the important triggers for spawning in Indian freshwater fishes.

In addition to established environmental factors, some social and ethological factors like visual contact, reaction to sounds of male and female, and the density of fishes in population also affect reproduction in fishes.

Endocrine Control:

The hormones secreted by the endocrine glands, chiefly the pituitary, control fundamental processes concerning development, reproduction and breeding cycles. Pituitary gland through the cyclic synthesis and release of gonadotrophic hormones regulates the gonadal cycles. Estrogens and androgens secreted by the gonads complete the cyclic chain of events through feedback systems.

Hypothalamic Control of Gonadal Cycle:

The hypothalamic neurosecretions at the time of breeding season release Gonadotrophic Releasing Hormones or factors (GnRH) and these factors stimulate the gonadotrophic cells in the anterior pituitary to secrete the gonadotrophic hormones. These hormones control the development and maturity of gonads (Fig. 3).

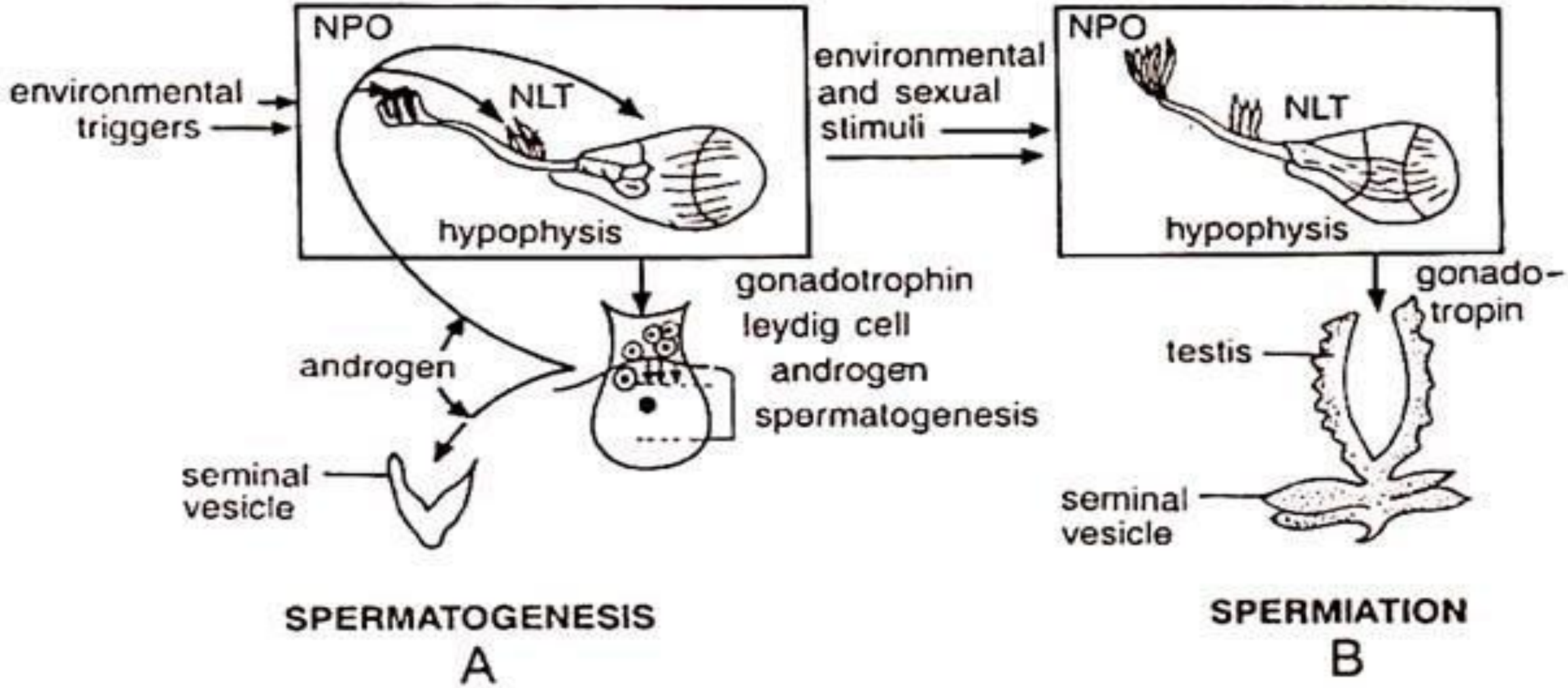


Fig 3 The hypothalamo-hypophyseal-gonadal axis and its relationship with spermatogenesis in a catfish

Hypophyseal Control of Gonadal Cycle:

Pituitary gland exerts control over reproduction. The oogonial stage of ovary is dependent upon gonadotropins. Gonadotrophic control comes into play during transition of gonads from cytoplasmic growth phase into vitellogenesis/ active spermatogenesis. Plasma vitellogenin levels and ovarian weight decrease after treatment with antiserum raised against a catfish gonadotropin fraction.

Spawning is also under the influence of gonadotropins. Heavy accumulation of secretory granules in gonadotrophs and their subsequent release shows that Leutenizing Hormone (LH) is involved during this phase. Human Chorionic Gonadotropin (HCG) also induces spawning in carps.

Gonadal Steroids:

Gonadal steroids by their action on gonads bring about the differentiation of gonoducts and gametogenesis. Estrogens exert a negative feedback control over pituitary gonadotropins during vitellogenesis resulting in an increased hepato-somatic index. Best known action of estrogens during vitellogenesis is to induce hepatic synthesis of vitellogenin, which is taken up by oocytes and the process is a gonadotropin dependent event.

Comparing the actions of estradiol, estrone and estriol in inducing vitellogenesis, estradiol is the most active steroid. In addition to estrogen, the androgen, progestin and corticosteroids are known to affect the female reproductive cycle. These steroids play a role in oocyte maturation and ovulation (Fig. 4).

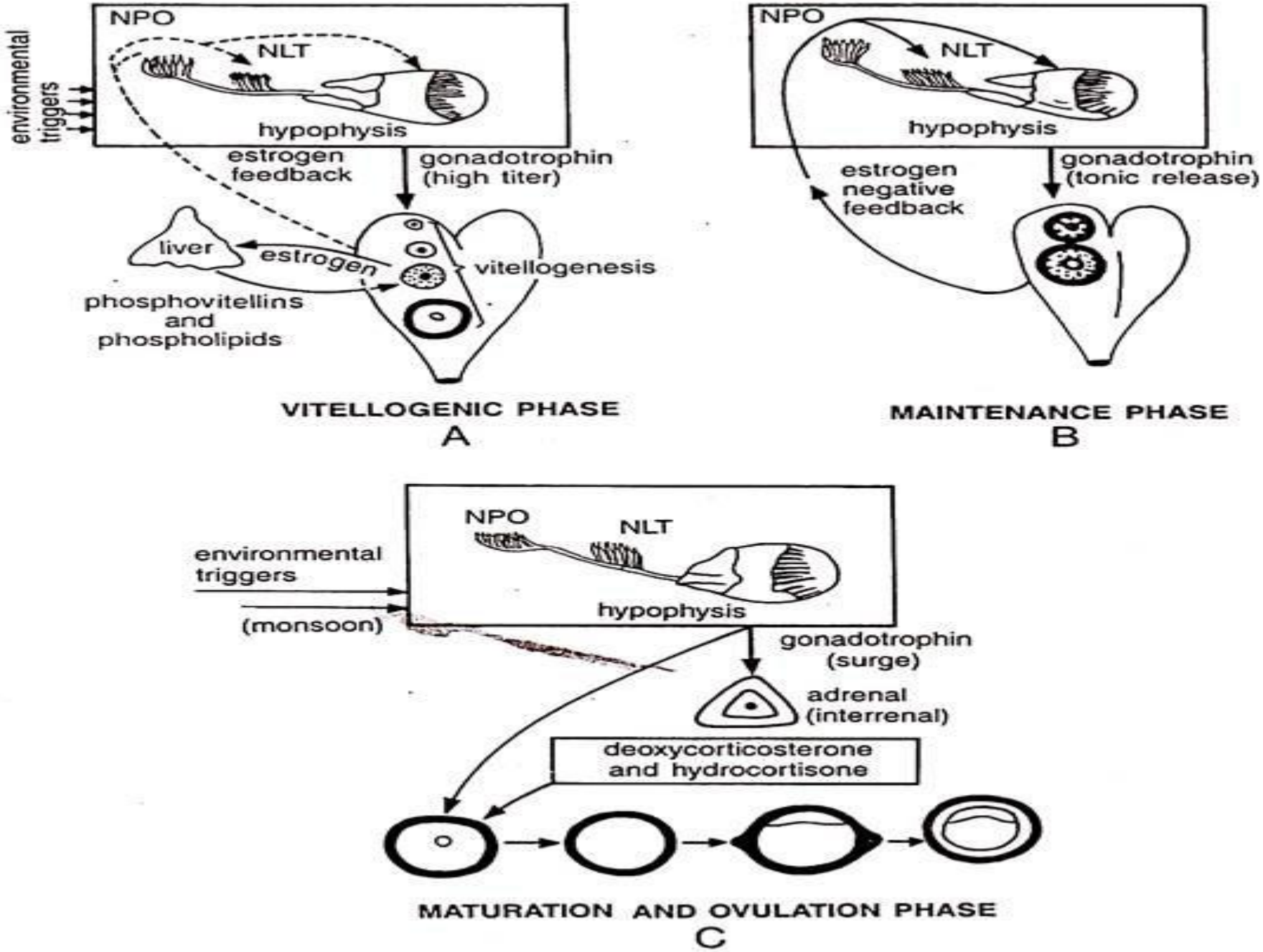


Fig. 4. The hypothalamo-hypophyseal-gonadal axis and its relationship with oogenesis in a catfish.

Prostaglandins are also involved in induction of ovulation in fishes probably by stimulating follicular contraction. The two compounds clomiphene (antiestrogen) and cyclofenil (non-steroidal compound with hormonal properties) have different modes of action.

Clomiphene acts through hypothalamo-hypophyseal-ovarian axis while cyclofenil has a bimodal action. It acts like clomiphene and exerts effect on ovary by increasing the sensitivity of ovaries to available gonadotropins.

Essay # 3. Reproductive Cycles in Birds:

Male Reproductive Cycles in Birds:

Birds have well developed reproductive organs. In male birds, a pair of testes is present, but the females possess only one functional ovary. The gonads of birds have a dual function, secretion of hormones and production of germ cells.

Testes:

Testes occupy a dorsal position in the abdominal cavity in very close proximity of the kidney (Fig. 11). Each testis is covered by a membrane called tunica albuginia. Tunica albuginia encloses a large number of fine and coiled seminiferous tubules. Each tubule is surrounded by a membrane called tunica propria.

The inner surface of the tunica propria is lined by primary spermatogonia, which divide mitotically to form secondary spermatogonia during active phases of breeding cycle. Primary spermatocytes are formed by mitotic divisions of secondary spermatogonia. The primary spermatocytes undergo meiotic divisions to form secondary spermatocytes and spermatids. Spermatids are transformed into spermatzoa by the process of spermiogenesis.

Mature spermatozoa are present in bunches in the lumen of the seminiferous tubules during the breeding phase. Interstitial spaces are concentrated in inter-tubular spaces of inactive testis. However, interstitial cells are scattered in inter tubular spaces during progressive and breeding phases. The number of interstitial cells increases with the activity of the testis. The left testis is bigger than the right testis.

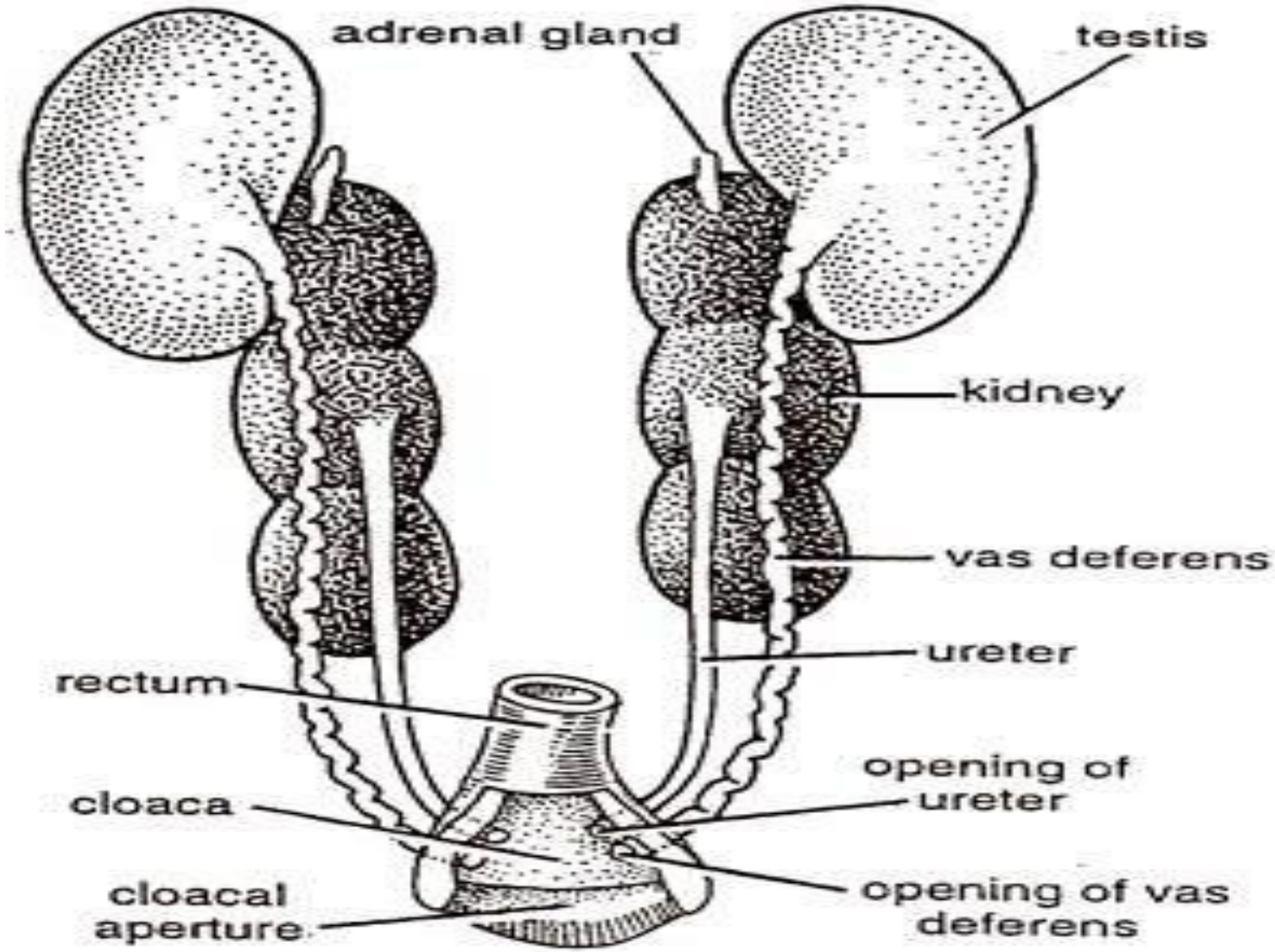


Fig. 11. Male urinogenital system of pigeon.

Female Reproductive Cycle in Birds:

Ovary:

In birds only the left ovary is functional. Ovary is whitish in color and irregular in shape. The size of the ovary varies from species to species (Fig. 12). Ovary is covered by a layer of germinal epithelium, which gives rise to primary and secondary follicles. The size and weight of the ovary change with the phase of the breeding cycle. The medullary part of the ovary is made up of highly vascularized connective tissue network.

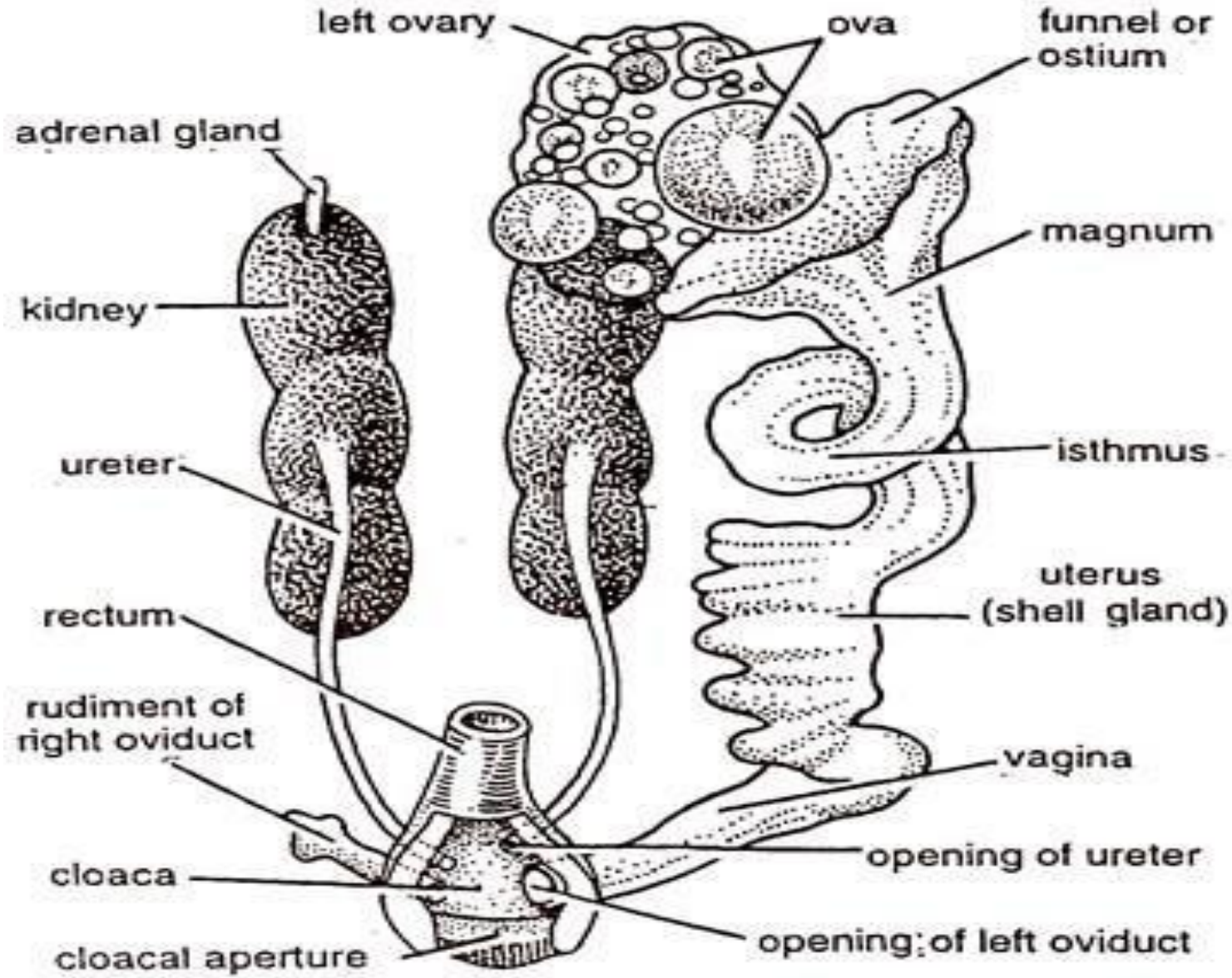


Fig. 12. Female urinogenital system of pigeon.

Gonadal Cycles:

Birds differ in their breeding seasons. Even those living together in a particular area and sharing a common habitat breed during different seasons. The cycle of gonads can be differentiated into four phases namely, quiescent, progressive, breeding and regressive phases. The duration of each phase, the pattern of development and regression of gonads differs in different species.

Weight of the testes and size are minimum during the quiscent phase when the seminiferous tubules are of minimum diameter and contain 1 – 3 layers of spermatogonia only. Interstitial cells are present in the inter tubular spaces in small groups and do not show any secretory activity. The quiscent phase is followed by progressive phase during which, the testicular activity increases gradually.

The volume and weight also increase. Seminiferous tubules become wider and spermatogonia start to divide mitotically. In the later stages of the progressive phase, spermatocytes and spermatids are formed from the divisions of spermatogonia. Initiation of spermiogenesis indicates the beginning of the breeding phase and marks the end of progressive phase.

During this phase, due to continuous cell divisions, the germinal cells increase in population. The seminiferous tubules become stretched and their lumen contains bunches of spermatozoa.

Patterns of Gonadal Cycles:

Due to seasonal variation in climatic conditions, birds show a wide spectrum of patterns of gonadal cycles. Weaverbirds, owls, crows, buntings, munia, etc. have a sharp and short breeding phase. Other birds like parakeets and spotted munia breed over an extended period of the year. Doves and pigeons have two breeding cycles in a year.

The rate at which gonads develop during the progressive phase shows variation between different species. Regression of the gonads may be slow or fast, depending upon the Species. Photosensitive and photoperiodic birds reveal very fast gonadal development and regression. Gonadal cycles are "mainly controlled by the genetic factors and secondarily by environmental factors. Climatic factors act through the neuroendocrine system.

Body Weight Cycles:

The body weight and gonadal cycles run parallel to each other. The relationship between body weight cycles and thyroid cycles vary from species to species. Changes in the body weight are mainly due to the fat content. Increase in lipid content helps the birds to meet the increased energy requirement for successful breeding and related activities in the breeding season.

Control of Gonadal Cycles:

Birds breed at a time of the year when the environmental conditions are the most favorable for both the parents and offspring. They select such time period when the biometeriological conditions ensure the maximum chances of survival of the young ones. Thus, the evolution of seasonal breeding due to annual gonad development cycle is of great adaptive significance.

Birds have developed mechanisms to synchronize their annual gonadal cycle and breeding with the periodicity of the most favorable months or season of the year. The mechanisms, which regulate the gonadal cycle are so efficient that, they enable a particular bird species to breed regularly and periodically during the particular month or season.

Environmental Factors:

The annual gonadal cycles are conditioned by internal rhythms of reproduction. Environmental factors also exert their influence. Environmental factors include day length and monsoon. Monsoon leads to drastic changes in humidity and rainfall, landscape, quantity and quality of food, day length and intensity of light. Indirectly it provides material for building nests.

Photoperiod:

Very sensitive mechanisms involving both internal and external factors control the reproductive cycles. Some birds dependent on photoperiod can detect small variations in day length and use them as cues for regulation of gonadal cycles. In general long photoperiods have a stimulatory effect on gonadal development while short day lengths inhibit or totally abolish cycles of the gonads.

The photoperiodic response of birds differs from species to species. Birds sensitive to photoperiods become refractive at the end of the breeding phase or when exposed to long photoperiods for longer periods of time. Decreasing day length induces gonadal regression in weaverbirds. Induction of gonadal regression due to photorefractoriness can be programmed by increasing the day lengths before or during the breeding phase, or by some hormones.

Termination of the photorefractory period is mediated by inborn circadian system of measurement of the duration of photoperiods. Changes in the activity of gonads during different months and under different photoperiods are due to the alterations in the levels of leutenizing hormone releasing hormone (LHRH) and leutenizing hormone (LH) in blood and pituitary gland.

Breeding cycles of birds not influenced by photoperiods (non-photoperiodic) are under the control of hormones secreted by the thyroid, adrenal, pineal and gonads. In finches, gonadal cycles are controlled by thyroid activity. The cycles of thyroid activity and that of gonads run parallel to each other or antiparallel in some birds.

In birds with inverse thyroid – gonadal relationship, removal of the thyroid leads to development of gonads in non-breeding season, their growth and maturity and breeding. In juveniles removal of the thyroid leads to precocious development of the gonads. On the other hand, injection of thyroid hormone leads to suppression of gonadal development and maturity, and abolition of gonadal cycles.

In birds where gonadal and thyroid cycles run parallel to each other, thyroid hormone is necessary for the normal development of gonads. In such birds, removal of the thyroid glands and administration of thyroid hormone produces effects depending upon the season or month of the year. Fluctuation in the level of the thyroid hormone also has a profound effect on birds sensitive to photoperiods.

Internal Factors:

Among the internal factors, thyroid, adrenal, pineal and gonadal hormones are involved in the regulation of gonadal cycles of birds. Hormones of the endocrine glands, acting alone or in combinations and depending on the species and phases of the reproductive cycles stimulate, inhibit or totally abolish the annual gonadal cycles. These hormones change the responses of birds to photoperiods. Hormones of gonads, thyroid and adrenal are involved in the initiation and termination of photorefractoriness in birds.

Thyroid Hormones:

Annual rhythm of thyroid activity runs inversely with the gonadal cycles in a number of birds. Gradual decrease in thyroid activity in finches is followed by the gonadal development, and the increasing levels of thyroid hormones induce regression of the fully developed gonads. An inverse relationship between thyroid and gonads is found in a number of birds like jungle bush quail, spotted munia, and female weaverbirds.

Gonadal Steroids:

Testosterone and estradiol secreted by the gonads also influence gonadal cycles. However, their effect is dependent upon the dose, time of administration, species and the nature of thyroid gonadal relationship. Low doses of testosterone have an inhibitory effect on the testicular cycle while high doses stimulate the testes.

In weaverbirds, testosterone irrespective of its dose, inhibits the gonadal cycle. Gonadal steroids stimulate or inhibit the gonads acting through the hypothalamo- hypophyseal axis. However, high doses of the hormones act directly on the gonads. In temperate birds gonadal steroids play a major role in the regulation of breeding cycles. Testosterone and estradiol regulate the photorefractoriness in male and female birds.

Adrenal Hormones:

Annual activity cycles of adrenal and gonads demonstrated two types of adrenocortical-gonadal relationship namely, parallel and anti parallel type. In parallel type of relationship, exhibited by weaverbird and common myna, increase and decrease in gonadal activity is associated with respective increase or decrease in the adrenocortical tissue activity. Corticosterone exerts positive or negative effects on gonads depending on the species and phase of administration. Adrenal medullary hormone inhibits gonadal activity.

Changes in the levels of thyroid and gonadal hormones and photoperiods alter the adrenocortical activity. However, the mechanism by which thyroid, gonadal hormones and photoperiod affect the adrenocortical activity is not understood.

Pineal gland hormone also influences gonadal activity. Removal of the pineal gland stimulates gonadal activity in weaverbird even under short day length condition. Injection of melatonin inhibits the testicular cycle in weaverbirds and common myna.

Removal of the pineal gland and administration of melatonin act on the gonads by changing the levels of leutenizing hormone releasing hormone from the hypothalamus and synthesis of leutenizing hormone and follicle stimulating hormone by the pituitary gland.

Gonadal development cycle in birds thus is determined by the cyclic release of gonadotropin releasing hormones from the hypothalamus and gonadotrophic hormones by the pituitary gland. External factors like rainfall, day length and temperature influence the cyclic release of hypothalamic releasing hormones and pituitary gonadotropins.

Role of Hypothalamo-Hypophyseal Axis:

Hypothalamo-hypophyseal complex plays an important role in the regulation of reproductive cycles in birds. LH and FSH are essential for the normal gonadal development cycles in birds. In juveniles as well as in adults, gonadal activity has been reported to increase much later than the regeneration of LH-dependent pigmental plumage in male lal munia and male weaverbird.

Secretion of LH starts in the beginning of the progressive phase, reaches its peak during the breeding phase and then declines rapidly during the regressive phase to become minimum during the quiscent phase. FSH is secreted mainly during the breeding phase and stimulates abrupt testicular and ovarian growth due to induction of spermatogenesis in male and follicular growth in females.

The hypothalamo-hypophyseal complex mediates effects of environmental factors and hormones on avian gonadal cycle (Fig. 13). This is the main seat of regulatory mechanisms, which regulate the circannual rhythm of gonad development in birds.

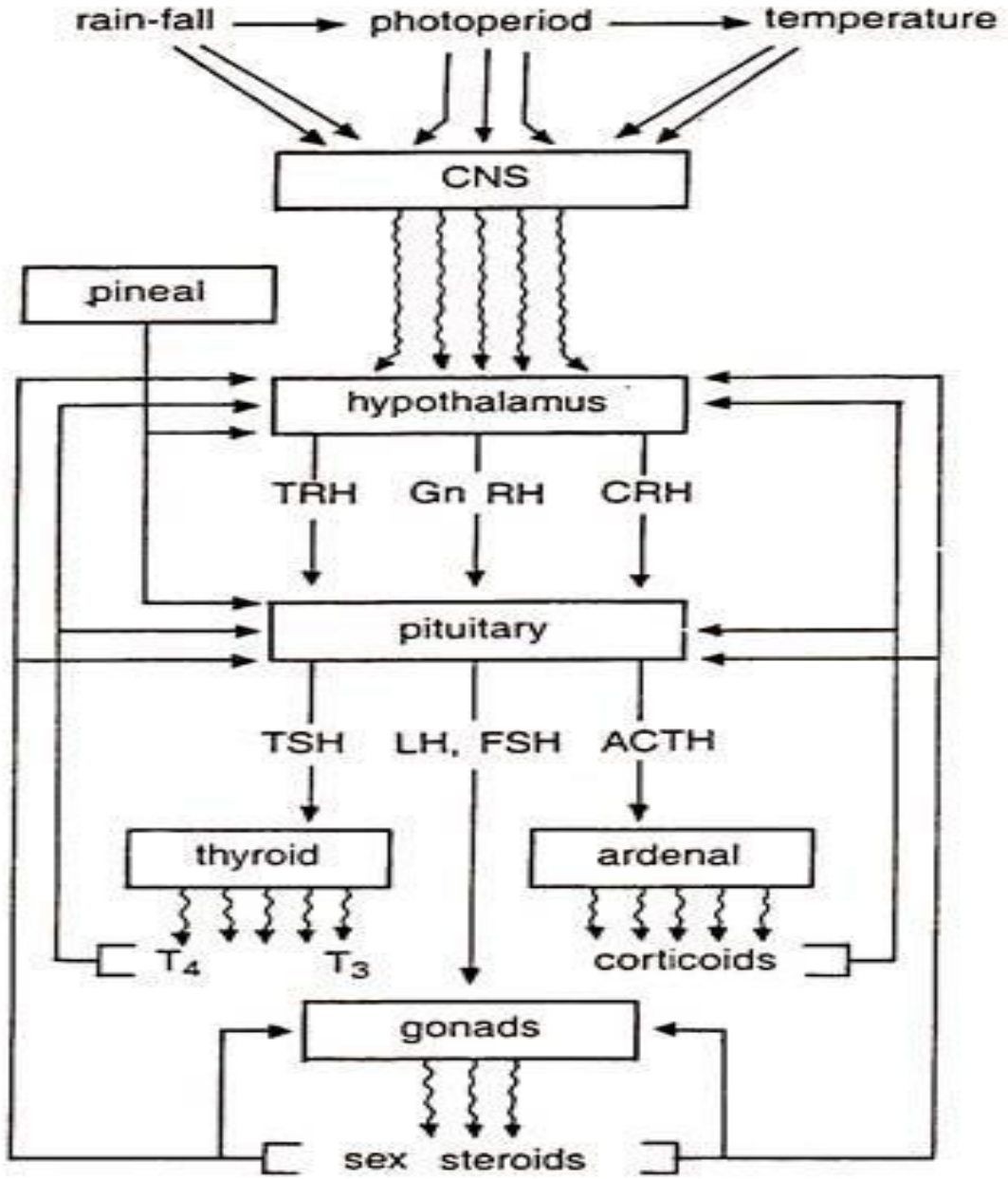


Fig. 13. Showing external and internal factors regulating gonadal cycles in birds.

Essay # 4. Reproductive Cycles of Lizards

Male Reproductive Cycle of Lizard:

Testes:

In lizards, the male reproductive system consists of a pair of oval to round testes and epididymis, which continue as vasa deferentia on both sides and open into the cloaca (Fig. 9). Development of the germ cells takes place in the seminiferous tubules.

In active testes, the seminiferous epithelium shows spermatogenesis from spermatogonia to spermatids and spermatozoa. Leydig cells along with blood vessels, stroma elements and connective tissue fibers occupy the interstitial space between the seminiferous tubules.

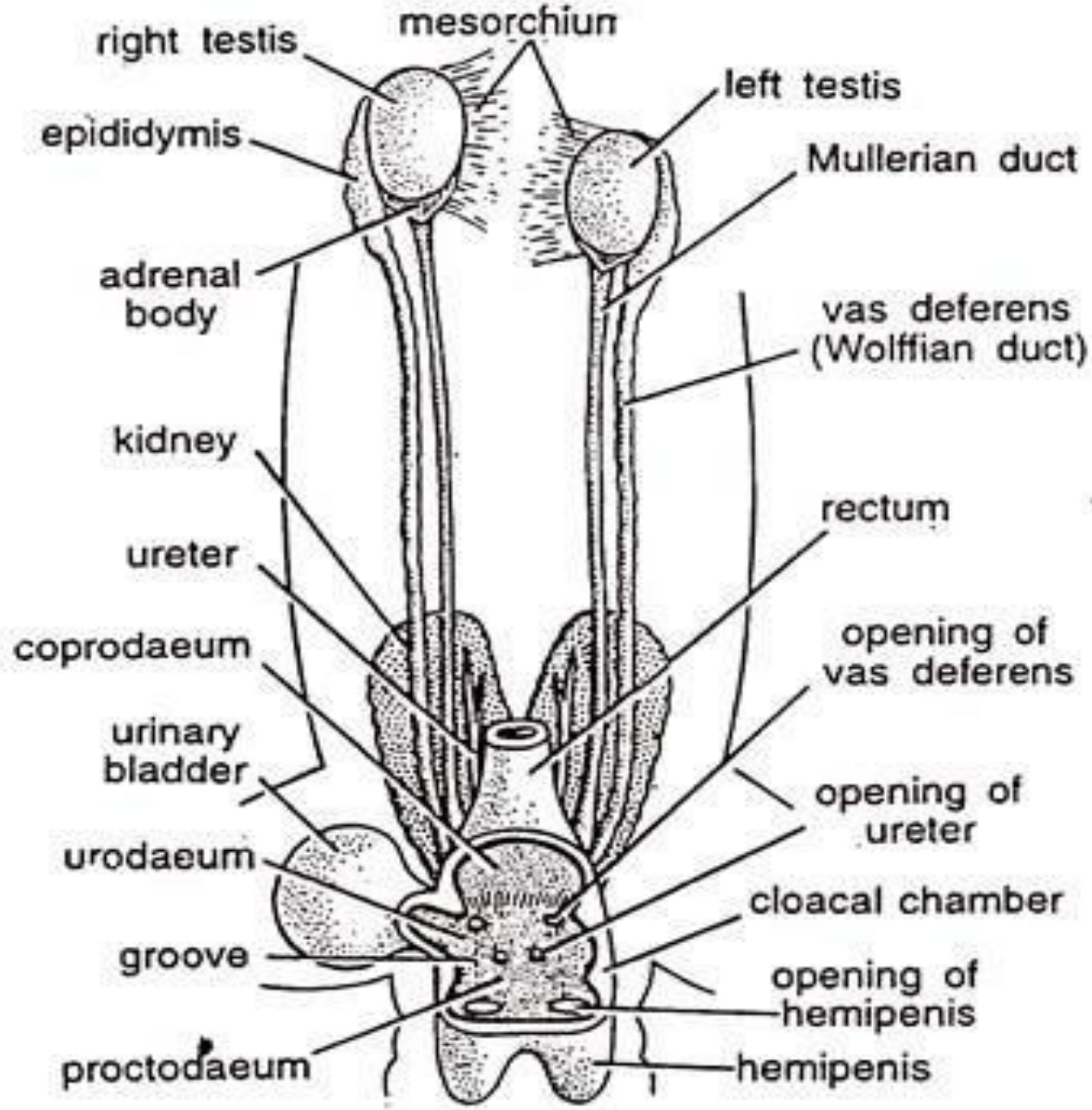


Fig. 9. Male reproductive system of a lizard.

Testicular Cycles:

The testes of reptiles show seasonal cycles both in the spermatogenetic activity and in Leydig cell function. Seasonal spermatogenetic cycle may or may not be synchronous with the activity of Leydig cells. Spermatogenesis in reptiles is of two types, pre-nuptial and post-nuptial. In the pre-nuptial type, sperms are produced immediately before or during mating season but in post-nuptial type, spermatogenesis takes place after the mating season.

Accordingly, in the pre-nuptial type mating occurs when the testes are spermatogenetically inactive but the sperms stored in the epididymis from the previous cycle are used during copulation. In species showing the postnuptial type, since viable sperms are stored for long periods in the epididymis during the regression of the testes, androgen production from the Leydig cells is temporarily separated from the spermatogenetic cycle. Snakes show both the types of cycles. Tropical lizards exhibiting seasonal reproductive activity are pre-nuptial and some species are reported to show continuous spermatogenesis.

The male reproductive cycle of lizards, on the basis of the size of testes, weight, histological appearance of spermatogenetic activity and the accessory reproductive organs, has been divided into three phases, the regenerative phase, reproductive phase and regressive phase. The phase of regeneration is marked by the onset of spermatogenetic activity when progressive cell divisions from spermatogonia upto spermatids occur.

This leads to the enlargement of the seminiferous tubules. The later phase shows increased size of testes and Leydig cells are few in number. Testes are biggest in size in the peak period of reproductive phase. The seminiferous tubules are maximally enlarged and the seminiferous epithelium is filled with various cell types, predominantly spermatids and spermatozoa lining the inner border of the epithelium and the lumen.

The regressive phase is marked by decrease in the size and weight of testes. The population of germ cells in the seminiferous tubules gets depleted as a consequence of which seminiferous tubules shrink. Due to tubular shrinkage, the interstitium and the Leydig cells become conspicuous. The duration of the regressive phase varies with the type of cycle. In lizards, the regressive period is generally longer extending upto six months after mating.

Cycle of Leydig Cells:

In testes, Leydig cells show well-defined seasonal lipid cycles. A slow postnuptial accumulation of cholesterol positive lipids in the Leydig cells takes place during the regressive phase. In the testes of snakes, the atrophic lipoidal Leydig cells are replaced by a new generation of Leydig cells

Cycle of Seminiferous Tubules:

In seasonal breeders, the seminiferous tubular epithelium shows conspicuous changes such as the postnuptial accumulation of lipids, which are cleared with the onset of testicular recrudescence. Sertoli cells show cyclic lipid accumulation and depletion along with the spermatogenetic cycle.

In the lizards accumulation of cholesterol positive lipid material follows the spermatogenetic cessation after the breeding season. The seasonal lipid cycles in the testes are related to the seasonal spermatogenesis and secretion of gonadotropins from pituitary gland.

Cycle of Lipid and Cholesterol:

In the annual reproductive cycles of reptiles, quantitative changes occur in the lipid, cholesterol and other steroid levels of the testes. In lizard testes, the lipid content is highest in the regressed phase and dramatic reduction occurs during the spermatogenetic recrudescence and lowest during the active phase. With the pos nuptial testicular regression, the lipid content again increases.

Cholesterol level also shows seasonal changes during the reproductive cycle. It is highest during the post nuptial lipid accumulation period and decreases with the initiation of spermatogenetic recrudescence suggesting active mobilization possibly by steroidogenesis. In some lizards cholesterol esters show sharp increase in the post breeding period and decrease during the recrudescence of the spermatogenesis

Female Reproductive Cycles of Lizards:

Ovary:

In lizards ovaries are bilobed, irregular and small structures lying in the body cavity attached to the dorsal body wall by means of mesenteries (Fig. 10). A limiting membrane consisting of a layer of squamous epithelium restricted to the dorsal surface surrounds each ovary.

The oogonia and oocytes are located in discrete areas known as germinal beds. The number and distribution of the germinal beds varies in different species. In some lizards and snakes there are two germinal beds in each ovary lying one on either side of the ovary.

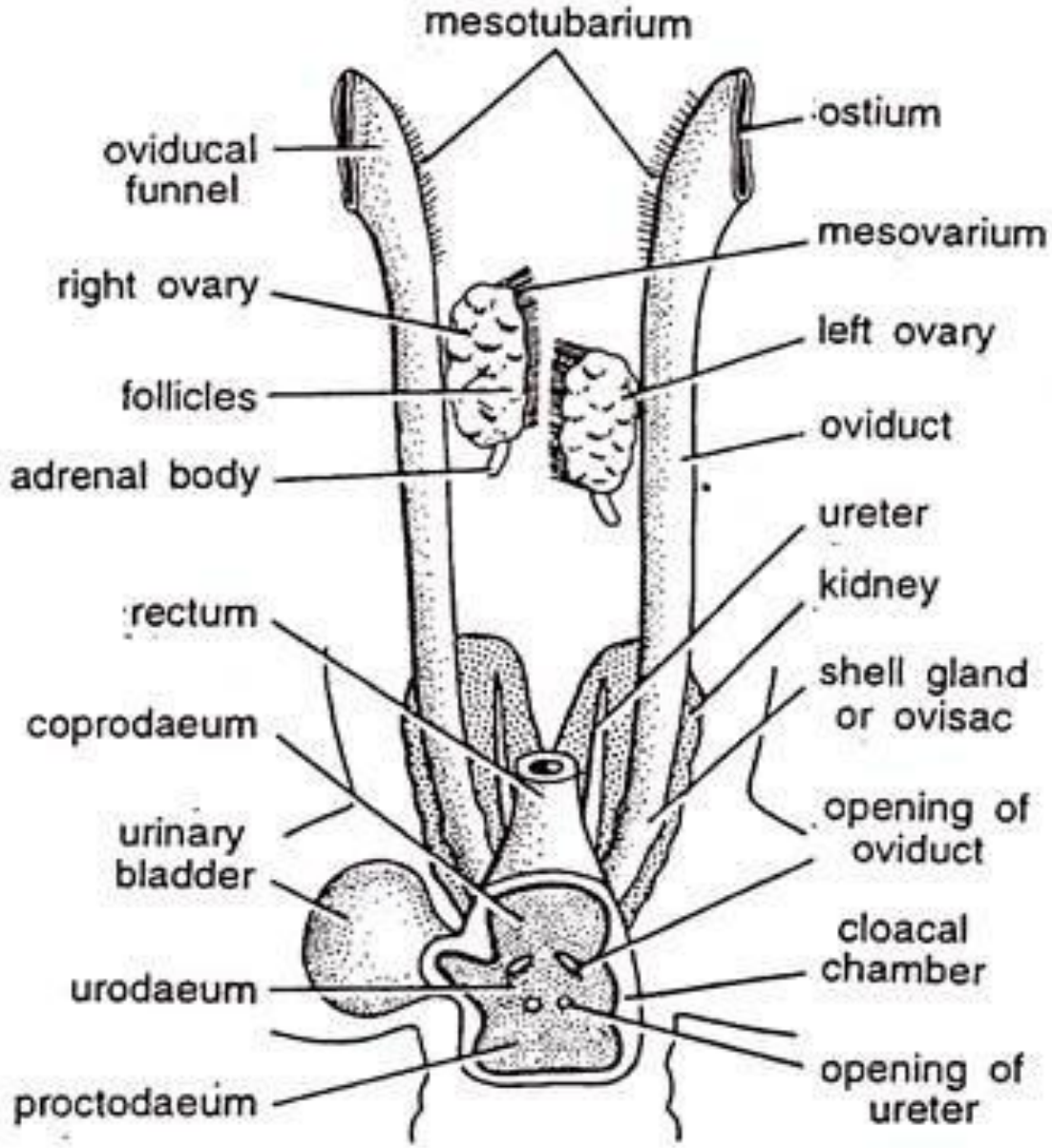


Fig. 10. Female reproductive system of a lizard.

Oogonia are smaller in size as compared to oocytes and contain little cytoplasm with a small centrally located nucleus. The primary oocytes are larger in size and contain more cytoplasm. Their nuclei are centrally placed, larger in size and vesicular in shape. The nucleolus stains deeply.

A single layer of granulosa cells surrounds the primary oocytes. As the primary oocytes grow in size, the granulosa cells are pushed out of the germinal bed. At this stage they are called as the developing follicles. The zona pellucida is surrounded by a 3-4-cell thick granulosa layer.

The cells in the granulosa layer are polymorphic consisting of three types of cells, small, intermediate and large. A layer of connective tissue called as theca surrounds the granulosa externally. The theca is further differentiated into an outer theca externa and an inner vascular theca interna.

Ovarian Cycles:

In lizards the ovarian cycle runs parallel to the testicular cycle. The seasonal changes in the development of the ovary are complex and can be differentiated into five stages. These include periods of regeneration, vitellogenesis, ovulation, gestation and regression.

During the period of regression, the ovaries are inactive and dormant showing no signs of growth. Decrease in weight follows the degeneration of ovarian components like corpora lutea and the follicles, which did not undergo vitellogenesis.

Towards the end of the regression period, some lizards like Mabuya carinata show slight increase in the weight of their ovaries. Ovarian follicles show signs of development and few follicles are in the stage of vitellogenesis.

In the regeneration phase there is a gradual increase in the weight of ovaries and conspicuous proliferation of the germinal beds with several oogonia and primary oocytes at different stages of development. A large number of developing follicles, a few persistent corpora lutea and atretic follicles of the previous cycles show various stages of degeneration.

Vitellogenesi stage is characterized by the process of yolk formation and deposition in the previtellogenesis follicles. Vitellogenesis continues for 30 – 40 days. In the beginning of the vitellogenesis stage, yolk accumulates at the periphery of the cortical ooplasm of the follicles.

In the period of ovulation the ovaries are heavy due to the presence of mature yolk follicles. Ovulation is preceded by a break in follicular epithelium. All the mature ova are expelled simultaneously from both the ovaries into the abdominal cavity from where they migrate to the oviducts. Transovarian migration of ovulated eggs is very common and this can be identified by the difference in the number of eggs in the oviduct and the number of corpora lutea in each ovary.

Mating occurs just before or at the time ovulation. Fertilization takes place in the infundibulum of the oviduct and the fertilized eggs are coated with albumen, shell membrane and shell in the oviduct. Eggs are retained in the uterus for different periods of time before oviposition. After ovulation, the follicles develop into corpora lutea and retain their activity for a part or full length of the period of gestation.

Control of the Gonadal Cycles:

Endocrine Control:

Pituitary gonadotrophs show cyclic changes in their secretory activity during seasonal reproductive cycles of reptiles. The role of pituitary hormones in controlling the testicular and ovarian cycles is an established phenomenon in reptiles.

Thyroid hormones exert both positive and negative effects on the gonads of reptiles. Removal of the thyroid glands results in regression of the gonads and this effect can be reversed by injection of thyroxin. Administration of thyroxin in the active phase produces antispermatogenetic effects.

Environmental Control:

In reptiles, reproductive cycles are influenced by rainfall, relative humidity, temperature and length of the day. Direct influence of rainfall on reproduction is not established in temperate reptiles. Indirectly rainfall can change the temperature of the microclimate and food availability. In tropical reptiles, rainfall influences

reproduction in females. Egg laying is dependent on moisture content.

Photoperiod initiates the seasonal recrudescence of the testes. Photo-receptiveness is restricted only to a short period in the annual cycle and at other times of the year lizards are not receptive to photoperiod. Effects of photoperiod are temperature dependent. Low temperature is inhibitory while high temperature is stimulatory.

Long photoperiods with high temperature stimulate testicular recrudescence in autumn. Temperature plays a primary regulatory role in the reproductive cycles of reptiles as compared to rainfall or photoperiod. In many lizards and snakes, increasing temperature stimulates ovarian development and testicular recrudesce

Essay # 5. Reproductive Cycles of Frogs: Male Reproductive Cycle of Frog: Testes:

Testes in anurans are paired; ovoid and compact structures lying near the kidneys. A short mesarchium connects them dorsally to the body wall. The vasa efferentia connecting the seminiferous tubules and the modified nephric elements running to the Wolffian ducts also travel through the mesarchium.

The germ cells are endodermal in origin. The two main elements of the testes are the seminiferous tubules and the interstitial tissue consisting of connective tissue, blood capillaries and closely packed small ovoid steroid secreting Leydig cells. The seminiferous tubules obtain their nutrients and other substances chiefly by diffusion from the vascular interstitial areas.

Spermatogenesis:

Spermatogenesis in amphibians is cystic type. Cells present in the cyst are derived from a single spermatogonium and are all in the same stage of development. However, a cross section of the seminiferous tubule shows cysts in different stages of spermatogenesis (Fig. 5).

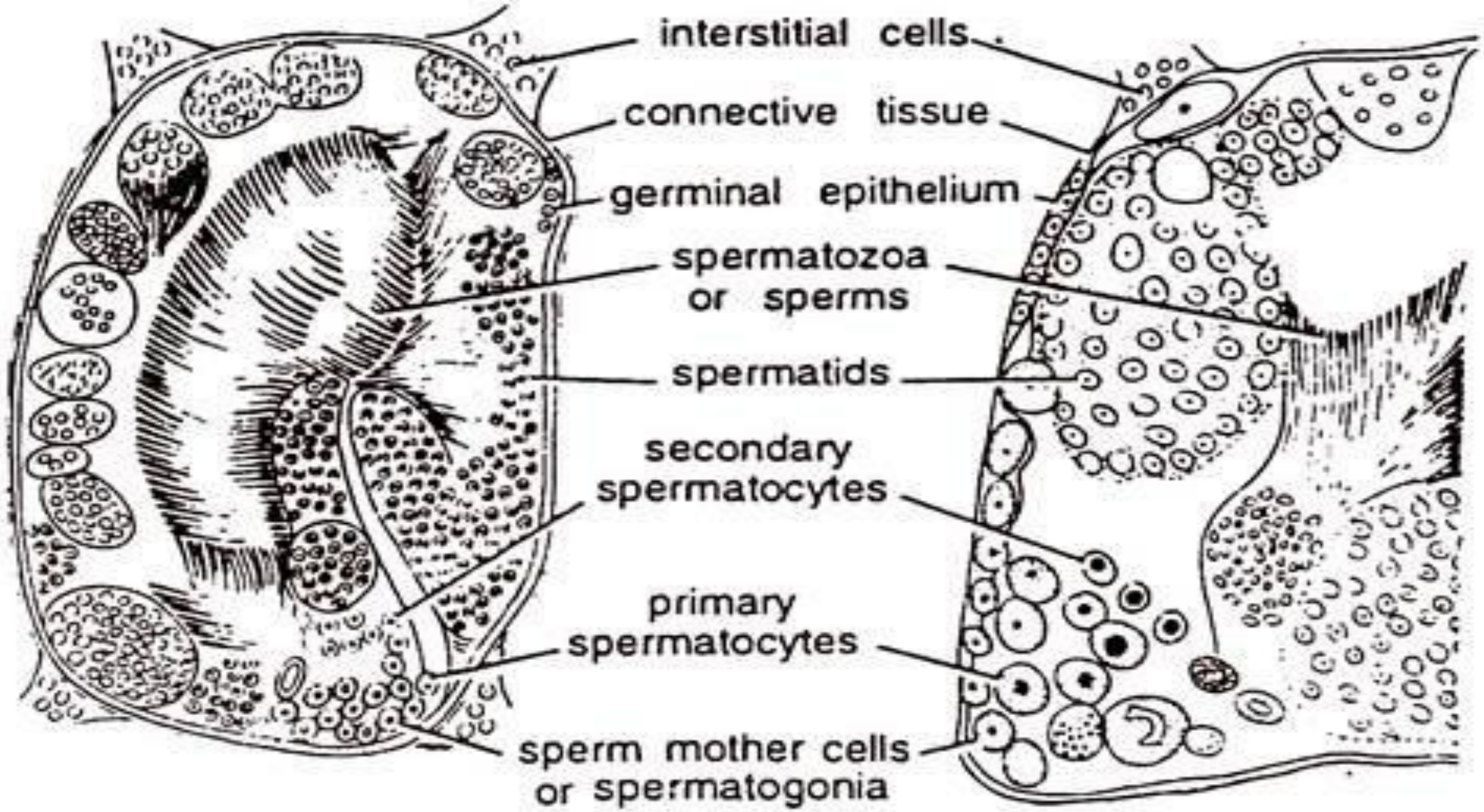


Fig. 5. Different stages in the spermatogenesis of frog.

The different stages of spermatogenesis are as follows:

Stage O:

Primary spermatogonia.

Stage I:

Secondary spermatogonia.

Stage II:

Primary spermatocytes.

Stage III:

Secondary spermatocytes.

Stage IV:

Spermatids.

Stage V:

Sperm bundles attached to the Sertoli cells.

The primary spermatogonia are the largest sperm cell type present in the adult testis. Each consists of large amount of eosinophilic cytoplasm and irregularly shaped nucleus. They lie singly adjacent to the basement membrane of the seminiferous tubule, each with one or two supporting cells associated with them.

Primary spermatogonia divide mitotically into two daughter primary spermatogonia or form a cyst through repeated mitotic divisions. The cyst is composed of the secondary spermatogonia, which divide repeatedly constituting the proliferation or multiplication phase.

About 8 divisions occur between the beginning and end of the multiplication phase. At the end of the last multiplication division, both cytoplasm and nucleus of the cells increase in size, and the cytoplasm becomes eosinophilic.

At the end of this growth period, the cells become somewhat larger in size, the cytoplasm entirely eosinophilic and the basophilic nuclei enter into the pre-reduction stage. These cells are now known as the primary spermatocytes. They lie either in the central part of the testis or attached to the wall of the seminiferous tubules.

The primary spermatocytic cysts show various stages of meiotic divisions. The first meiotic division produces secondary spermatocytes characterized by eosinophilic cytoplasm and strongly basophilic nuclear chromatin. The intercellular vacuoles increase in size and become more evenly distributed. The cell nests are located in the central parts of the testis tubules.

After the second meiotic division, spermatids are formed. The spermatids are smaller than the secondary spermatocytes and are globular cells, with eosinophilic cytoplasm and a spherical basophilic nucleus. With the starting of the spermatogenesis they become more and more elongated.

The intercellular vacuoles fuse into one big central vacuole and the cells are situated against the wall of the cyst. This type of cell nests is usually found attached to the wall of the seminiferous tubules. The heads of the maturing sperm cells are found embedded in the Sertoli cells. Each cell nest changes into about a dozen sperm bundles.

Sertoli Cells:

Sertoli cells are the only somatic elements found inside the seminiferous tubules. The Sertoli cells initially associated with the primary spermatogonia are flattened or triangular in shape with small, oval nuclei. The chromatin material is condensed. There may be 2-4 such cells surrounding a primary spermatogonium.

These are the follicular cells with the progress in spermatogenesis and formation of the cell nests of advanced stages; the Sertoli cells undergo progressive differentiation. The heads of several spermatozoa are found embedded in such fully differentiated cup-like Sertoli cells. They are generally attached to the basement membrane of the tubule wall and after spermiation, the cells burst and they are then visible freely floating in the lumen of the tubule.

Leydig Cells:

The interstitial cells or Leydig cells are small, compact and oval cells distributed between the seminiferous tubules. The size and shape of these cells as well as the morphological features of their nuclei show seasonal changes depending upon the species.

The Leydig cells, spherical in shape with large nuclei, conspicuous nucleoli and coarse chromatin granules are considered to be actively secreting androgens. Flat Leydig cells with reduced size of nuclei and possessing compact chromatin are inactive.

Testicular Cycles:

Spermatogenetic Cycles:

In frogs, production of primary and secondary spermatocytes, spermatids and sperm bundles occurs round the year showing that maturation of germ cells is not restricted to any specified part of the year. There is no appreciable seasonal variation in the rates of mitotic or meiotic activities.

The maximum weight attained by the testes during the breeding season is only 1.5 times of that found in other months. This is because proliferation and maturation of germ cells occur simultaneously and more or less at uniform rate through out the year. Secondary spermatogonia are produced through out the year but the mitotic activity in them is very prominent between April to June.

The primary and secondary spermatocytes, spermatids and sperm bundles are rapidly produced between April to June. It is interesting to note that even though secondary spermatogonia continue to divide and give rise to primary spermatocytes during the post-breeding period, very few secondary spermatocytes are formed in September – October 2nd these eventually undergo degeneration during November to February (Fig. 6).

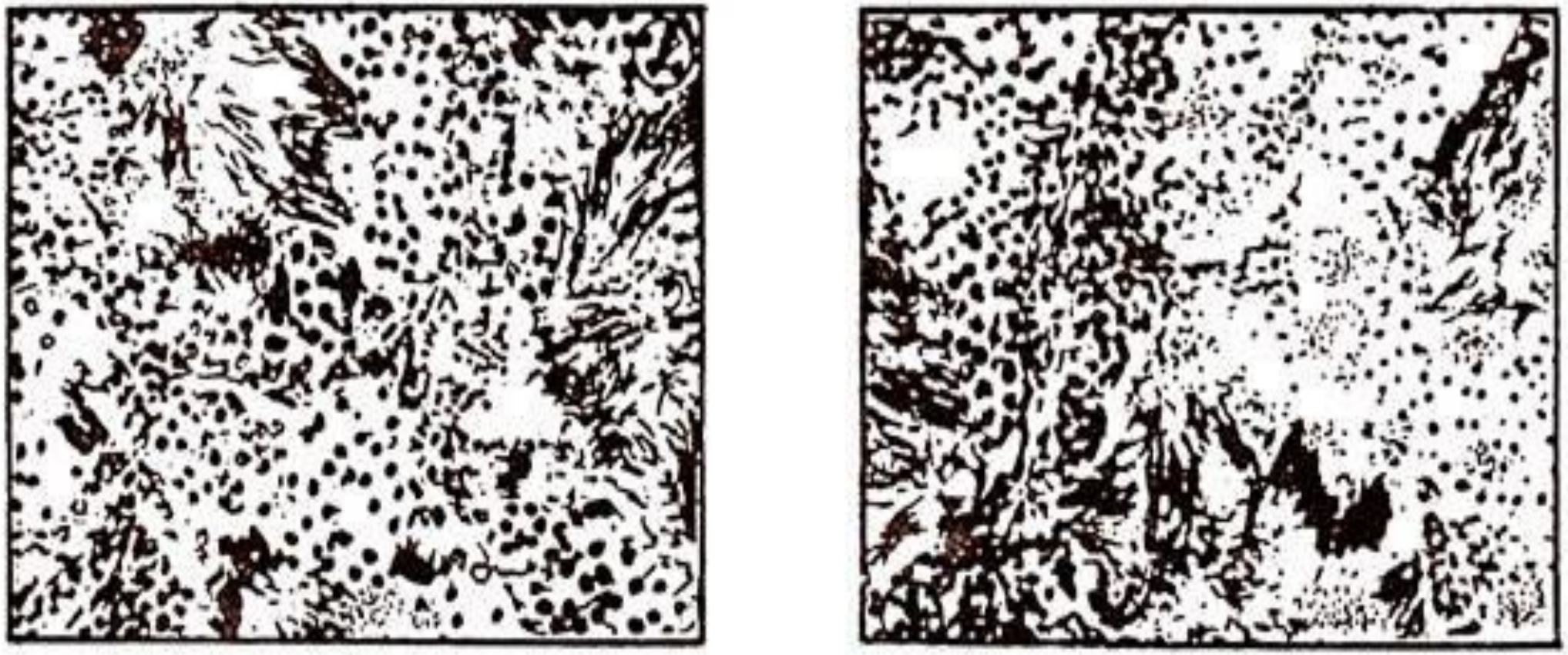


Fig. 6. Testis of frog in different seasons. A—December and B—September showing increased spermatogenesis.

In anurans living in the temperate zone, increase in the weight of testis —arks the proliferation of new germ cells and later with the onset differentiation or maturation of germ cells there is a decrease in the weight of testes. However, in the tropical anuran, both proliferation and maturation of germ cells occurs concurrently, the testis weight declines only after the evacuation of the spermatozoa during the breeding season.

Cycles of Leydig Cells and Steroidogenic Activity:

Leydig cells are round, abundant and exhibit maximum nuclear diameter during the breeding period. The nuclei of Leydig cells contain coarse chromatin granules in the of May and June. After breeding, the Leydig cells become flattened of shrunken and reduced in size and number.

They become less frequent but distinguishable from the connective tissue cells though with some difficultly. In this period they contain fine chromatin granules. Leydig cells continue to remain in this stage from August to March. In the months of April and May they rapidly increase in size and number, and become round.

The redistribution of chromatin material takes place due to which the coarse chromatin granules characteristic of secondary cells appear once again appear in their nuclei. In toads Leydig cells are round, abundant and show maximum nuclear diameter in April to August. This is followed by a marked decrease in nuclear diameter of Leydig cells during August – September.

However, from September to March, no further changes appear in the morphology of these cells. In April, Leydig cells number, size and chromatin granules content increase upto August. Thus, no drastic changes are noted in the Leydig cells of toads.

Control of Testicular Cycles:

The hormonal and environmental factors control and effect the annual changes in the testes. Temperate amphibians due to the lack of thermoregulatory mechanisms are greatly influenced by the changes in the temperature. Spermatogenesis does not take place during cold winter months and therefore, this constitutes the resting period.

Spermatogenetic activity is very high in summer months. Thus they show potentially continuous or discontinuous type of spermatogenetic cycles. Testicular cycles are controlled by both intrinsic and extrinsic factors.

Pituitary Control:
Gonadotrophic hormones produced by pituitary gland play important role in the regulation of spermatogenesis in all the vertebrates. In adult amphibians, mitotic proliferation of primary and secondary spermatogonia is dependent upon gonadotrophic hormones.

Annual changes in the testes are due to the influence of gonadotropins on the germinal epithelium. In green frog, the sensitivity of the germinal epithelium and the gonadotropin levels of the pituitary gland were higher in summer season. Even after the breeding season, testis of frog retains its sensitivity to gonadotropins.

In anuran, the activity of Leydig cells is also regulated by the hypophysial gonadotropins. Removal of the pituitary gland causes regression of these cells, which can be prevented by pituitary homogenates.

Role of Androgens:

The main sources of androgens in anuran are the Leydig cells. Androgens stimulate specific stages of spermatogenesis, development and maintenance of the secondary sexual characters such as the thumb pads and vocal sacs, and feedback regulation of pituitary gonadotropin secretion. The development and regression of thumb pads is due to the fluctuations in the leaves of androgens secreted by the Leydig cells.

In anurans of temperate zone, the annual cycles of Leydig cells activity is not the same as spermatogenetic cycle. Thumb pad development and Leydig cell activity are high when spermatogenetic activity is nil, but during the period of recovery of spermatogenetic activity, the thumb pads atrophy and the Leydig cells become inactive.

Environmental Factors:

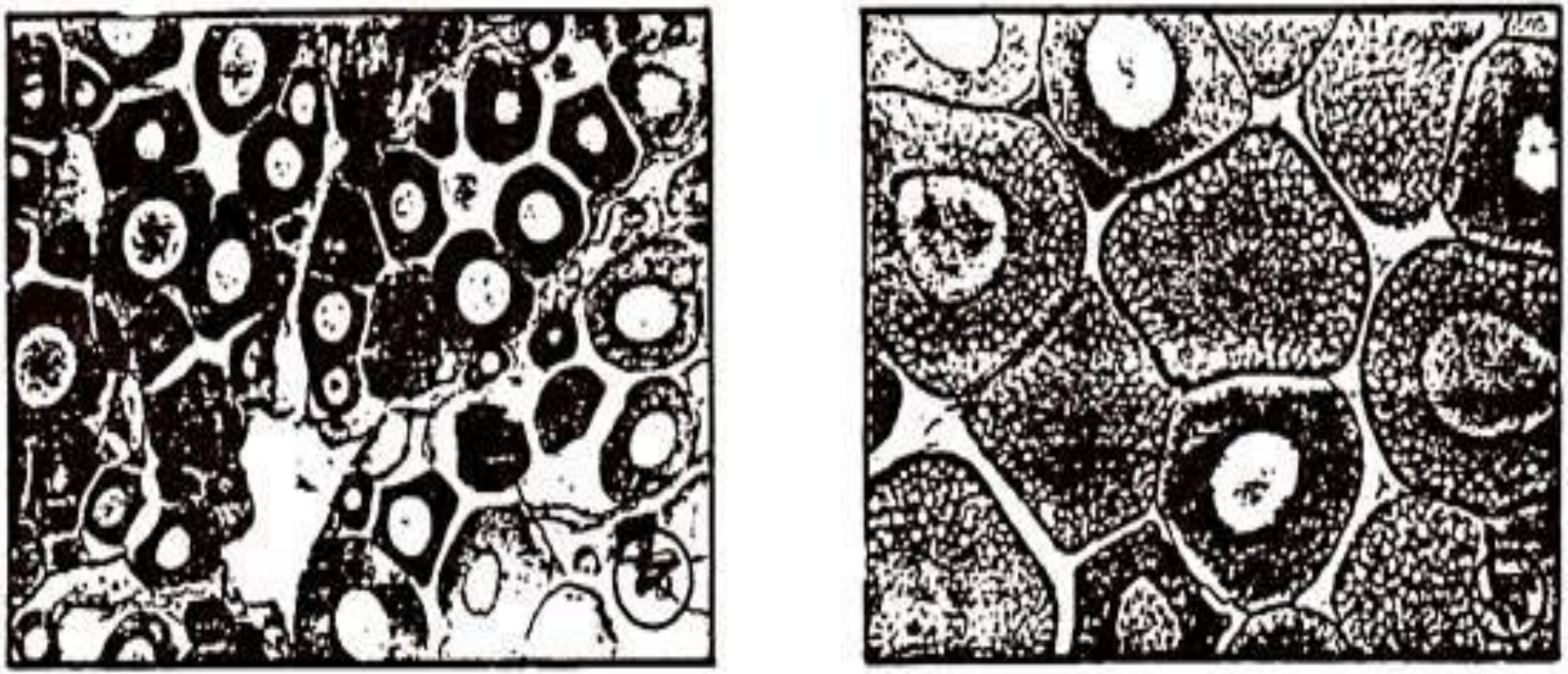
High temperatures between 20 to 25° C stimulate spermatogenesis but suppress the activity of Leydig cells in anurans. In winter low temperature has the opposite effect. In tropical countries rainfall is an important factor controlling the breeding activity as the frogs breed during the monsoon months.

Female Reproductive Cycle of Frog:

Ovary:

Ovaries of frogs are paired structures attached to the median surface of the kidneys by mesovarium. Each ovary is a hollow sac-like structure. Its wall is thrown into numerous folds and consists of a narrow cortical region covered by germinal epithelium.

Depending upon the phase of reproductive cycle, or the pattern of oogenetic activity, the ovary may contain oogonia, previtellogenic oocytes of different sizes, vitellogenic and fully-grown yolky oocytes. The corpora lutea are found in the ovaries during the immediate post-spawning period (Fig. 7).



A B

Fig. 7. Ovary of frog showing different stages : A–small primary oocytes; B–oocytes showing yolk vacuoles.

Oogenesis:

Oogenesis starts even in larvae before their metamorphosis. At the time of metamorphosis, the larva contains oogonia and primary oocytes that have entered their first growth phase of early diplotene stage. The oogonia possess large and highly lobed nuclei. Each nucleus contains a large, prominent nucleolus and many micronucleoli.

The oogonia are found scattered in the peripheral cortex of the ovary. The oogonia persist in the ovary of adults and are therefore found at all times in the germinal epithelium. The appearance of yolk vesicles marks the beginning of the vitellogen or the second growth phase of the oocytes.

Follicular Atresia:

Atretic follicles are commonly found in the ovary of amphibians and the histological changes that occur during atrophy of the follicles are divided into four stages:

Stage 1:

The characteristic features of the atretic follicle at this stage are hypertrophy of the follicular granulosa cells, appearance of vacuoles at the periphery of the oocyte, and coalescence of yolk platelets at the periphery of the oocyte.

Stage 2:

In this stage, the zona pellucida and follicular epithelium are not visible. The hypertrophied granulosa cells move into the ooplasm or the yolk material of the egg, forming conspicuous epithelial cells, and possess clear, vacuolated cytoplasm and a vesicular nucleus. The theca becomes

Stage 3:

The ooplasmic contents of the large, previtellogenic follicles are digested and removed by the phagocytic granulosa cells, leaving behind the hypertrophied thecal cells in the connective tissue.

Stage 4:

There is a gradual degeneration and disappearance of the phagocytic granulosa cells, leaving only the pigment and the original elements of the theca.

Yellow body:

The follicular membranes remain behind in the ovary after ovulation and give rise to post-ovulatory follicles or corpora lutea.

The histological changes occurring in the corpora lutea can be divided into the following four stages:

Stage 1:

This stage is characterized by the newly ruptured follicle, the follicular epithelium of which is almost similar to that of the preovulatory follicles. The thecal wall contains capillaries with blood and the tissue is vacuolated. The inner layer is formed of a dense continuous membrane.

Stage 2:

Both granulosa and thecal cells are relatively more hypertrophied. The follicle becomes collapsed and folded due to contraction.

Stage 3:

In the third stage the granulosa becomes multilayered due to further shrinkage or contraction of the follicle and the cavity of the post-ovulatory follicle is almost filled up by the hypertrophied granulosa cells. The hypertrophy of the theca is maximum at this stage.

Stage 4:

Further contraction of the follicle occurs at this stage and the granulosa cells are separated from each other and are irregularly distributed. There is a further reduction in size.

Stroma and Interstitial Cells:

The stroma of the ovary of frogs consists of cellular and fibrous connective tissue elements, which is distributed between the follicles, corpora lutea, interstitial gland cells, blood vessels, lymphatic spaces and nerve endings. The quantity and distribution of the ovarian stroma undergo marked seasonal variation in response to growth and recruitment of follicles. The interstitial gland cells are very scarce in the anuran ovary.

Ovarian Cycles:

The Morphology of the Ovary in Amphibians:

The morphology of the ovary of anurans varies with the phase of reproductive cycle and seasonal changes in the recruitment of oocytes, atresia and ovulation. During winter months due to the drastic decrease in temperature, development of the follicles is interrupted.

This inactive resting phase varies from species to species and ranges between 1 to 4 months. Oogenesis mainly occurs during spring and summer months. Vitellogenesis is completed either just prior to breeding or immediately after breeding.

In some frogs like Rana cyanopeltis oogenesis occurs throughout the year due to continuous oogenetic activity. About 25% of the total oocytes complete the vitellogenic growth and reach ovulatory sizes in May -June each year.

Breeding season spreads over 2-3 months from July to September and a mature female may shed as many as 300 eggs. Proliferation of oogonia and recruitment of oocytes start soon after the breeding months. Weight of the ovary is dependent upon the number of SGP oocytes.

Although atretic oocytes occur in all the months of the year, their number is higher during pre-breeding and breeding months. Toads breed during the monsoon months of June to August. In temperate anurans, the ovaries enter a period of quiscence before the initiation of the next ovarian cycle by recruiting a batch of small oocytes to the final growth phase.

Cycles of the Oviduct and Fat Bodies:

Seasonal changes also occur in the oviducts in correlation with the changes in ovaries. Seasonal variation in the weight of the oviducts is related to the number of SGP oocytes. Abdominal fat bodies also show seasonal changes in size and weight. Cycles of the fat bodies are inversely correlated to the ovarian cycles except during the immediate post-spawning period during which both the ovaries and fat bodies are reduced.

Control of Ovarian Cycles:

The reproductive cycles of amphibians are influenced by the changes in climatic factors such as temperature, rainfall, day length and relative humidity as they are poikilotherms and their breeding activities require water. Temperate amphibians show distinct seasonal changes in the ovarian follicular development, period of breeding and hibernation but anurans in tropical areas do not show such sharply defined seasonal changes in the gametogenetic activity.

Ovarian cycle in all amphibians is regulated by both intrinsic and extrinsic factors. The intrinsic factors mainly include the hypophyseal gonadotropins and ovarian estrogens. The extrinsic factors are temperature, light, rainfall and relative humidity. Sufficient food supply and water are essential.

Endocrine Control:

For normal functioning of the ovaries, hypophyseal gonadotropins are necessary. Removal of the pituitary gland or injection of antigonadotrophic chemicals causes degeneration of the ovary. However, the individual role of follicle stimulating hormone (FSH) and LH are less effective in amphibians because of their short half-life (Fig. 8)

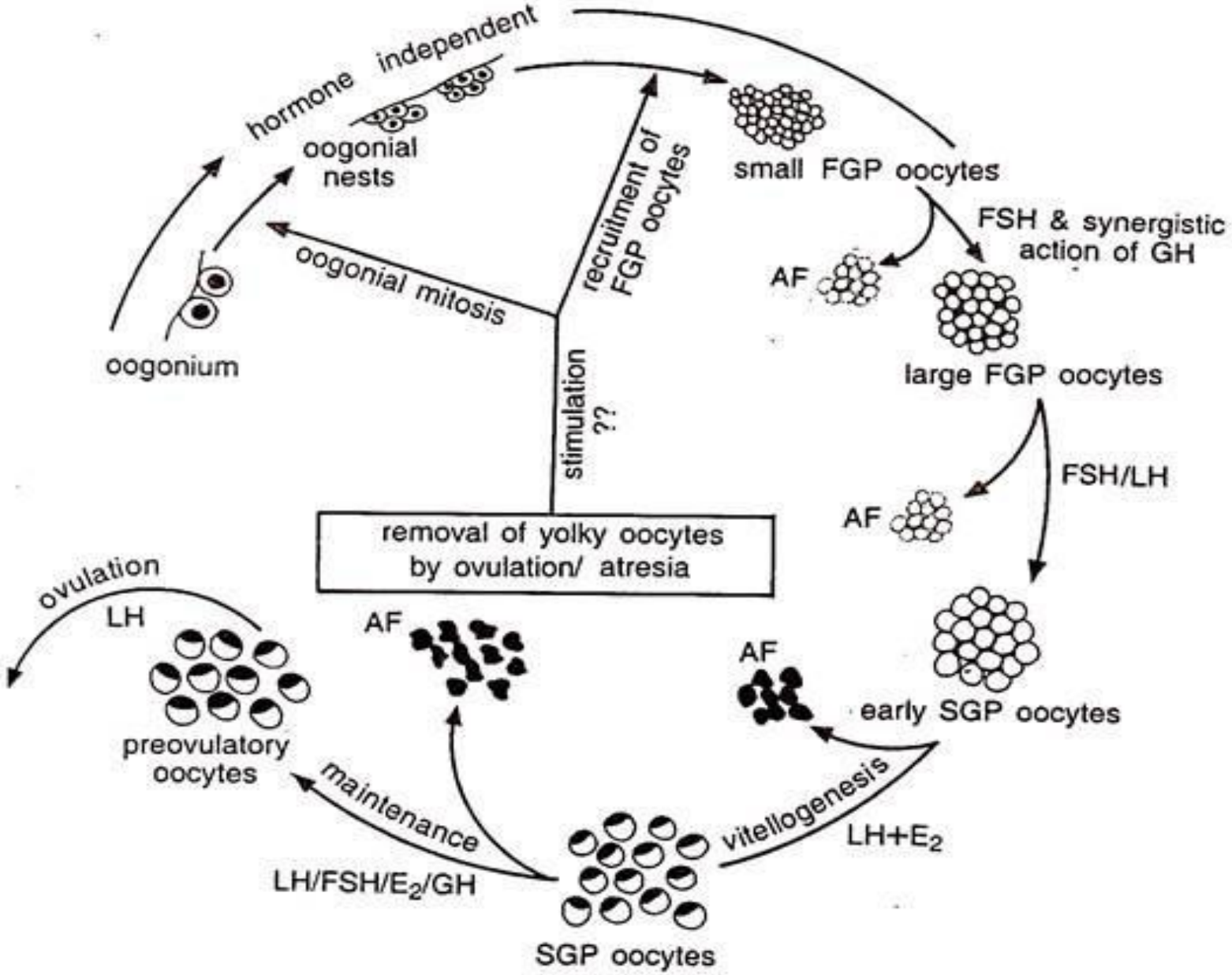


Fig. 8. Hormonal control of ovarian follicular development in frog.

REGENERATION IN VERTEBRATES AND INVERTEBRATES

In biology, regeneration is the process of renewal, restoration, and tissue growth that makes genomes, cells, organisms, and ecosystems resilient to natural fluctuations or events that cause disturbance or damage. Every species is capable of regeneration, from bacteria to humans. Regeneration can either be completewhere the new tissue is the same as the lost tissue, or incomplet where after the necrotic tissue comes fibrosis

At its most elementary level, regeneration is mediated by the molecular processes of gene regulation and involves the cellular processes of cell proliferation, morphogenesis and cell differentiation. Regeneration in biology, however, mainly refers to the morphogenic processes that characterize the phenotypic plasticity of traits allowing multi-cellular organisms to repair and maintain the integrity of their physiological and morphological states. Above the genetic level, regeneration is fundamentally regulated by asexual cellular processes Regeneration is different from reproduction. For example, hydra perform regeneration but reproduce by the method of budding.

Arthropods

Arthropods are known to regenerate appendages following loss or autotomy. Regeneration among arthropods is restricted by molting such that hemimetabolous insects are capable of regeneration only until their final molt whereas most crustaceans can regenerate throughout their lifetimes. Molting cycles are hormonally regulated in arthropods, although premature molting can be induced by autotomy. Mechanisms underlying appendage regeneration in hemimetabolous insects and crustaceans is highly conserved.

During limb regeneration species in both taxa form a blastema following autotomy with regeneration of the excised limb occurring during proecdysis. Limb regeneration is also present in insects that undergo metamorphosis, such as beetles, although the cost of said regeneration is a delayed pupal stage Arachnids, including scorpions, are known to regenerate their venom, although the content of the regenerated venom is different than the original venom during its regeneration, as the venom volume is replaced before the active proteins are all replenished

Annelids

Many annelids (segmented worms) are capable of regeneration. For example, Chaetopterus variopedatus and Branchiomma nigromaculata can regenerate both anterior and posterior body parts after latitudinal bisection. The relationship between somatic and germline stem cell regeneration has been studied at the molecular level in the annelid Capitella teleta. Leeches, however, appear incapable of segmental regeneration

Furthermore, their close relatives, the branchiobdellids, are also incapable of segmental regeneration. However, certain individuals, like the lumbriculids, can regenerate from only a few segments. Segmental regeneration in these animals is epimorphic and occurs through blastema formation. Segmental regeneration has been gained and lost during annelid evolution, as seen in oligochaetes, where head regeneration has been lost three separate times

Echinoderms

Tissue regeneration is widespread among echinoderms and has been well documented in starfish (Asteroidea), sea cucumbers (Holothuroidea), and sea urchins (Echinoidea). Appendage regeneration in echinoderms has been studied since at least the 19th century.In addition to appendages, some species can regenerate internal organs and parts of their central nervous system.In response to injury starfish can autotomize damaged appendages.

.Autotomy is the self-amputation of a body part, usually an appendage. Depending on severity, starfish will then go through a four-week process where the appendage will be regenerated. Some species must retain mouth cells to regenerate an appendage, due to the need for energy. The first organs to regenerate, in all species documented to date, are associated with the digestive tract. Thus, most knowledge about visceral regeneration in holothurians concerns this system

Planaria (Platyhelminthes)

Regeneration research using Planarians began in the late 1800s and was popularized by T.H. Morgan at the beginning of the 20th century. Alejandro Sanchez-Alvarado and Philip Newmark transformed planarians into a model genetic organism in the beginning of the 20th century to study the molecular mechanisms underlying regeneration in these animals.

Planarians exhibit an extraordinary ability to regenerate lost body parts. For example, a planarian split lengthwise or crosswise will regenerate into two separate individuals. In one experiment, T.H. Morgan found that a piece corresponding to 1/279th of a planarian or a fragment with as few as 10,000 cells can successfully regenerate into a new worm within one to two weeks.After amputation, stump cells form a blastema formed from neoblasts, pluripotent cells found throughout the planarian bodyNew tissue grows from neoblasts with neoblasts comprising between 20 and 30% of all planarian cells

Amphibians

Limb regeneration in the axolotl and newt has been extensively studied and researched. Urodele amphibians, such as salamanders and newts, display the highest regenerative ability among tetrapods. As such, they can fully regenerate their limbs, tail, jaws, and retina via epimorphic regeneration leading to functional replacement with new tissue. Salamander limb regeneration occurs in two main steps.

First, the local cells dedifferentiate at the wound site into progenitor to form a blastema Second, the blastemal cells will undergo cell proliferation, patterning, cell differentiation and tissue growth using similar genetic mechanisms that deployed during embryonic development. Ultimately, blastemal cells will generate all the cells for the new structure.

Hydra

Hydra is a genus of freshwater polyp in the phylum Cnidaria with highly proliferative stem cells that gives them the ability to regenerate their entire body. Any fragment larger than a few hundred epithelial cells that is isolated from the body has the ability to regenerate into a smaller version of itself. The high proportion of stem cells in the hydra supports its efficient regenerative ability.

Regeneration among hydra occurs as foot regeneration arising from the basal part of the body, and head regeneration, arising from the apical region. Regeneration tissues that are cut from the gastric region contain polarity, which allows them to distinguish between regenerating a head in the apical end and a foot in the basal end so that both regions are present in the newly regenerated organism

Aves (birds)

Owing to a limited literature on the subject, birds are believed to have very limited regenerative abilities as adults. Some studies on roosters have suggested that birds can adequately regenerate some parts of the limbs and depending on the conditions in which regeneration takes place, such as age of the animal, the inter-relationship of the injured tissue with other muscles, and the type of operation, can involve complete regeneration of some musculoskeletal structure.

. Werber and Goldschmidt (1909) found that the goose and duck were capable of regenerating their beaks after partial amputation and Sidorova (1962) observed liver regeneration via hypertrophy in roosters. Birds are also capable of regenerating the hair cells in their cochlea following noise damage or ototoxic drug damage. Despite this evidence, contemporary studies suggest reparative regeneration in avian species is limited to periods during embryonic development. An array of molecular biology techniques have been successful in manipulating cellular pathways known to contribute to spontaneous regeneration in chick embryos.

Mammals

Spiny mice (Acomys cahirinus pictured here) can regenerate skin, cartilage, nerves and muscle.
Mammals are capable of cellular and physiological regeneration, but have generally poor reparative regenerative ability across the group. Examples of physiological regeneration in mammals include epithelial renewal (e.g., skin and intestinal tract), red blood cell replacement, antler regeneration and hair cycling. Male deer lose their antlers annually during the months of January to April then through regeneration are able to regrow them as an example of physiological regeneration. A deer antler is the only appendage of a mammal that can be regrown every year.

Humans: Regeneration in humans

The regrowth of lost tissues or organs in the human body is being researched. Some tissues such as skin regrow quite readily; others have been thought to have little or no capacity for regeneration, but ongoing research suggests that there is some hope for a variety of tissues and organs.[1][88] Human organs that have been regenerated include the bladder, vagina and the penis

Reptiles

The ability and degree of regeneration in reptiles differs among the various species, but the most notable and well-studied occurrence is tail-regeneration in lizards. In addition to lizards, regeneration has been observed in the tails and maxillary bone of crocodiles and adult neurogenesis has also been noted. Tail regeneration has never been observed in snakes. Lizards possess the highest regenerative capacity as a group. Following autotomous tail loss, epimorphic regeneration of a new tail proceeds through a blastema-mediated process that results in a functionally and morphologically similar structure

Chondrichthyes

Studies have shown that some chondrichthyans can regenerate rhodopsin by cellular regeneration, micro RNA organ regeneration, teeth physiological teeth regeneration, and reparative skin regeneration. Rhodopsin regeneration has been studied in skates and rays. After complete photo-bleaching, rhodopsin can completely regenerate within 2 hours in the retina

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PARTHENOGENESISE

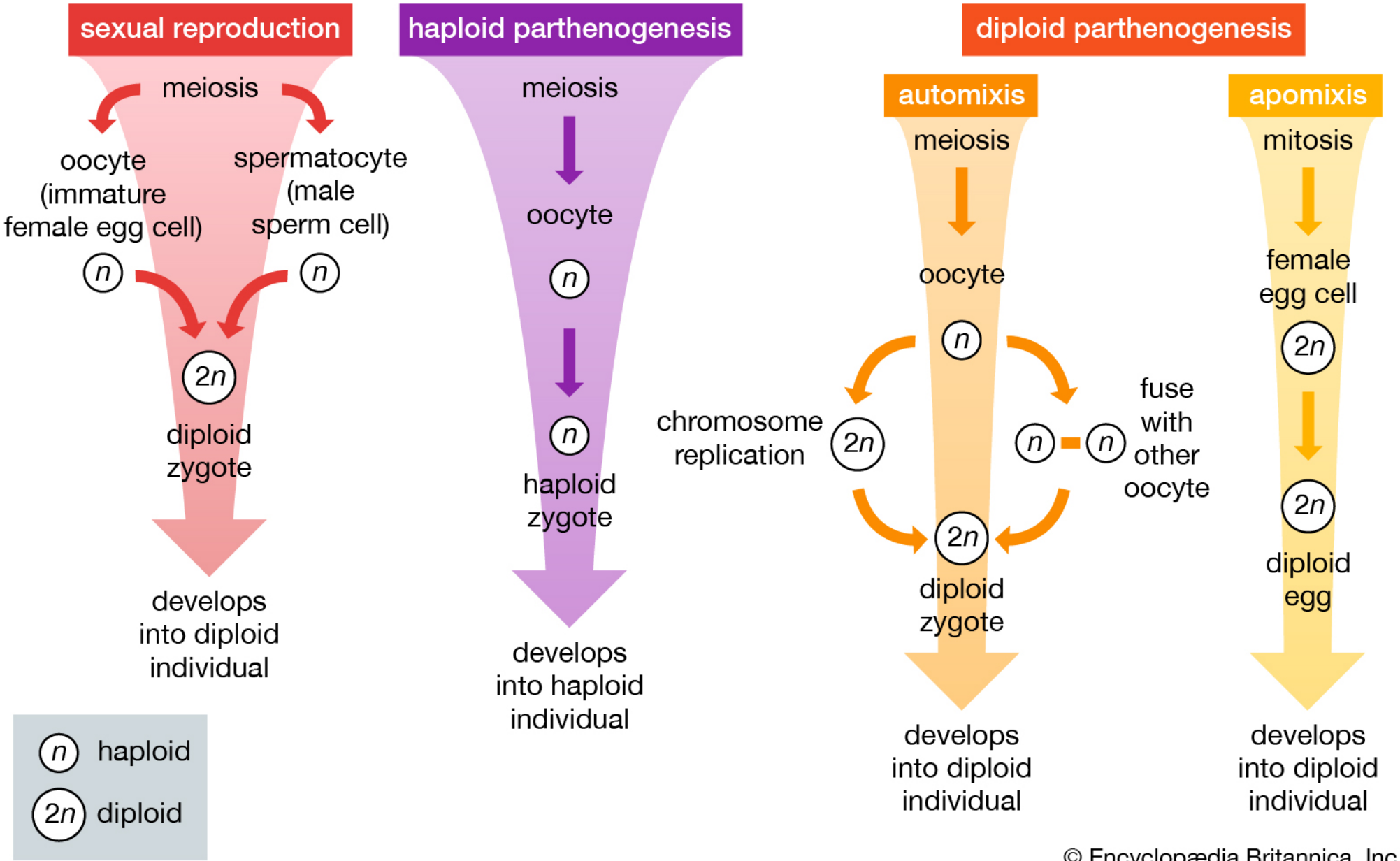
Parthenogenesis is a form of reproduction in which an egg can develop into an embryo without being fertilized by a sperm. Parthenogenesis is derived from the Greek words for “virgin birth,” and several insect species including aphids, bees, and ants are known to reproduce by parthenogenesis.

A form of asexual reproduction wherein the offspring develops from the egg or female gamete without the prior fertilization from the male gamete

Supplement

Parthenogenesis is regarded as a form of asexual reproduction since a zygote forms without the union between female and male gametes. It is a common means of reproduction in plants, invertebrates (such as water fleas, aphids, stick insects, some ants, bees and parasitic wasps), and vertebrates (such as some reptiles, amphibians, fish, and few birds).

The process of sexual reproduction versus several forms of parthenogenesis



Parthenogenesis, a reproductive strategy that involves development of a female (rarely a male) gamete (sex cell) without fertilization. It occurs commonly among lower plants and invertebrate animals (particularly rotifers, aphids, ants, wasps, and bees) and rarely among higher vertebrates. An egg produced parthenogenetically may be either haploid (i.e., with one set of dissimilar chromosomes) or diploid (i.e., with a paired set of chromosomes). Parthenogenic species may be obligate (that is, incapable of sexual reproduction) or facultative (that is, capable of switching between parthenogenesis and sexual reproduction depending upon environmental conditions). The term parthenogenesis is taken from the Greek words parthenos, meaning “virgin,” and genesis, meaning “origin.” More than 2,000 species are thought to reproduce parthenogenically.

Mechanisms

Parthenogenesis is sometimes considered to be an asexual form of reproduction; however, it may be more accurately described as an "incomplete form of sexual reproduction," since offspring of parthenogenic species develop from gametes. Gametes are reproductive cells that result from meiosis (or reduction division)—in which a specialized cell with a (diploid) double set of chromosomes undergoes two fissions of its nucleus. Meiosis gives rise to four gametes, or sex cells, which are haploid—in that each possesses half the number of chromosomes of the original cell (see meiosis).

Parthenogenesis may be apomictic or automictic. Apomictic parthenogenesis is one in which the mature egg cells produced through mitosis develop directly into embryos. The offspring are full clones of the mother. In automictic parthenogenesis, the gametes undergo meiosis and therefore are haploid.

Parthenogenesis may be facultative or obligate. A facultative parthenogenesis is one in which the female reproduce either sexually or asexually. Mayflies are capable of facultative parthenogenesis. They undergo parthenogenesis when viable males are absent from the habitat. Obligate parthenogenesis is one in which the organism reproduce only by asexual means. Certain species of reptiles (most of them are lizards) are capable of obligate parthenogenesis.

Pathenogenesis may also be arrhenotokous, thelytokous, or deuterotokous. The arrhenotolous parthenogenesis (arrhenotoky) is a form of parthenogenesis in which the unfertilized eggs develop into males. Thelytokous parthenogenesis (thelytoky) is a form of parthenogenesis in which unfertilized eggs develop into females. Deuterotokous parthenogenesis (deuterotoky) is one in which the unfertilized eggs may develop into males and females.

Word origin: from Ancient Greek parthénos ("virgin") + génesis ("origin, creation, generation")

Synonym(s):

Parthenogenesis can operate on either a haploid or a diploid cell. In haploid parthenogenesis, a rare form of parthenogenesis that occurs in a few species of bees, nematodes, and plants, offspring develop from haploid eggs to produce haploid adults. On the other hand, the process of diploid parthenogenesis, a more common and varied form of the phenomenon, may proceed along two pathways. Automixis (automictic parthenogenesis) is a postmeiotic process in which a haploid cell may either duplicate its chromosomes or join with another haploid cell.

In both cases, diploid zygotes develop and grow into diploid adults. Such organisms are not true clones of the mother, however, because the meiotic process separates and recombines the genetic material. A second form of diploid parthenogenesis, apomixis (apomicitic parthenogenesis), forgoes complete meiosis altogether. Instead, two genetically identical diploid egg cells are produced from a parent cell through mitosis (the process of cell duplication), and one or more of these daughter cells, which are both diploid and clones (that is, genetically identical) of the original parent cell, develop into a diploid offspring. Diploid parthenogenesis occurs in insects such as aphids as well as in some rotifers and flowering plants (see animal reproductive system and plant reprod

Parthenogenesis In Order Hymenoptera

In the insect order Hymenoptera (which includes bees, wasps, and ants), parthenogenesis can take one of three forms: arrhenotoky, thelytoky, and deuterotoky. In arrhenotoky, haploid males are produced from unfertilized eggs laid by mated (impregnated) females or by so-called secondary, or supplementary, queens, which have not been impregnated. In thelytoky, which occurs in many species of the suborder Symphyta (a group that includes the sawflies, the horntails, and the wood wasps), unmated females produce males. In deuterotoky, unmated females of some Symphyta produce females as well as males. The occurrence of these forms is not always mutually exclusive. For example, in Apis (bees), about 1 percent of the eggs laid by secondary queens may be female.

Sometimes associated with arrhenotoky, thelytoky, and deuterotoky is pseudoarrhenotoky (or paternal genome elimination). Pseudoarrhenotoky is a nonparthenogenic form of reproduction that occurs in the hymenopteran superfamily Chalcidoidea (a group of small parasitic wasps) and in some mites, Like arrhenotoky, pseudoarrhenotoky results in the production of haploid males. In this process, development begins as diploid organisms within fertilized eggs; however, as development progresses, males become haploid after the paternal contribution to the genome has been lost, eliminated, or deactivated.

Variations

A number of parthenogenic variations have been observed. Some aphids and water fleas undergo a type of parthenogenesis called heterogony or cyclic parthenogenesis. In these species, generations of offspring produced from fertilized eggs may alternate with those produced from unfertilized ones. Such an alternation of generations in both groups of insects is thought to result partly from seasonal temperature changes, with eggs produced through sexual reproduction having a greater ability to withstand the winter cold. They lie dormant until temperatures rise.

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Pseudogamy (gynogenesis, or sperm-dependent parthenogenesis) is another variation, which appears in the life cycle of a few insects, mites, and salamanders as well as the flatworm Schmidtea polychroa. S. polychroa is hermaphroditic and may be diploid (which can reproduce sexually) or polyploid (that is, with one or more additional sets of chromosomes). Whereas sexual reproduction requires sperm for fertilization, parthenogenic reproduction in this species involves sperm only to stimulate the initial development of the egg; the sperm's genetic material is not used.

THANK YOU