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Developmental Biology

The study of all aspects of development, from the genes and molecular events that control development to the structural changes that an organism undergoes as it develops. This study is facilitated by a vast array of new technologies, adopted from molecular biology, genetics and cell biology.

Historical roots of developmental biology

- Embryology (the descriptive study of development)
- Embryologists (W. Roux, 1888; H. Driesch, 1892) conducting experimental manipulations of embryos showed that genetic information is inherited equally by all cells during development.
- Early cytologists (E.B. Wilson, 1896) recognized that embryological changes were caused by cellular changes, which must be directed by genetic information
- Early geneticists (T. Boveri, 1902; T.H. Morgan, 1927) showed that a full complement of genes was necessary for normal development.
- Embryologists (H. Spemann and H. Mangold, 1924) conducting embryo transplant experiments showed that certain areas of an embryo were responsible for inducing the development of other areas.
- The discovery of growth factors (R. Levi-Montalcini and V. Hamburger, 1950's) laid the foundation for the modern study of developmental biology.

- Late nineteenth century:
- embryologists were trying to figure out how genetic information directs development
- Weismann's hypothesis that chromosomal material is divided unequally between daughter cells

- Wilhelm Roux 1888:

- experimented with frog embryos (first embryonic manipulations ever done)
- poked hot needle into 1 cell of 2-cell embryo, to kill that one cell
- observed that treated embryos developed abnormally
- concluded that the 2 cells must carry different information for development

- Hans Driesch 1892:
- experimented with sea urchin embryos (Fig. 3.15)
- separated the cells of 2-cell and 4-cell embryos
- even cells from 4-cell embryos could develop into normal embryos
- concluded that each cell retains all the genetic information necessary for normal development

- Theodor Boveri 1902:
- experimented with sea urchin eggs
- dispermic fertilization (2 sperm per egg)
- causes formation of 2 mitotic spindles (tetrafoil)
- egg divides into 4 cells with abnormal combinations of chromosomes
- cleavage continues but the embryos die
- concluded that normal development depends on the normal combination of chromosomes

- Thomas Hunt Morgan, 1927:
- did experiments on Drosophila
- used ionizing radiation to induce mutations in DNA
- created a huge library of Drosophila mutants
- many of the mutants have serious developmental defects
- correlated certain developmental defects with mutations in certain chromosomes (eg. the X chromosome)
- demonstrated that specific genes are located on specific chromosomes
- demonstrated that normal genes are required for normal development

- Hans Spemann and Hilde Mangold, 1924:
- did experiments on the amphibian Triturus
- transplanted tissue from the dorsal side (dorsal lip of blastopore) to other regions of early embryo
- found that transplanted tissue induced formation of a second neural axis
- laid the foundation for the concept of embryonic induction

- Rita Levi-Montalcini and Viktor Hamburger, 1956
- grafted mouse tumors into chick embryos and observed innervation of the tumors
- reasoned that the tumors must produce a factor that induces nerve growth
- purified the factor and called it Nerve Growth Factor (NGF)
- discovered the first "growth factor" and laid the foundation for modern developmental biology

- **Scope of developmental biology**
- Agricultural benefits: Genetic testing, selective breeding, transgenics and cloning of animals can provide breeders with superior livestock.

Medical benefits:

- Hormonal contraceptives (eg. "the pill")
- Expectant mothers can be warned about environmental stresses that are found to negatively influence development (eg. folic acid helps prevents neural tube defects)
- Genetic defects can be identified in developing embryos, and may potentially be corrected or treated
- Improvements in cancer detection and treatment arise from increased understanding of genes involved in cell growth
- In vitro reproduction
- Stem cells can be used to treat certain degenerative conditions
- Human cloning

GAMETOGENESIS

The origin and development of gametes is called gametogenesis. This may be divided into spermatogenesis and oogenesis.

Spermatogenesis deals with the development of male sex-cells called sperms in the male gonad or testis.

Oogenesis is the development of female sex-cells called ova or eggs in the female gonad or ovary.

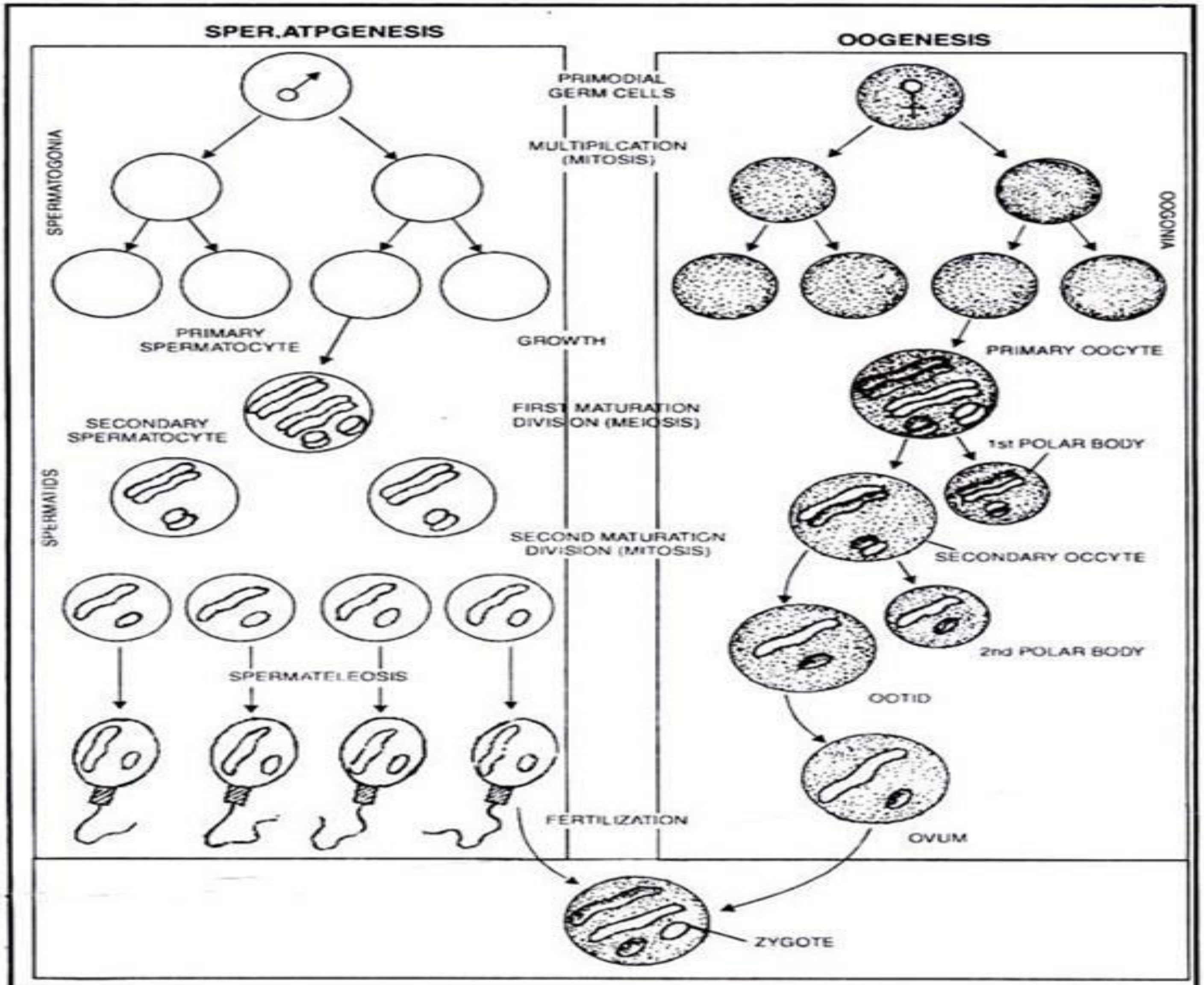

Fig. 3(B).1 Stages of gametogenesis (spermatogenesis and oogenesis).

1. **Spermatogenesis:**

The entire process of spermatogenesis can be divided into following two phases:

(A) Formation of Spermatid:

The male gonad known as testis is the site of spermatogenesis. In each vertebrate a pair of testes remains attached to dorsal body wall by a connective tissue called mesorchium. Each testis is formed of thousands of minute elongated and coiled tubules called seminiferous tubules. The inner lining of seminiferous tubules is called as germinal epithelium and is made of primordial germ cells (Primary germ cells) as well as some supporting nutritive cells. The primordial germ cells give rise to spermatids through the following steps (Fig. 3(B).2).

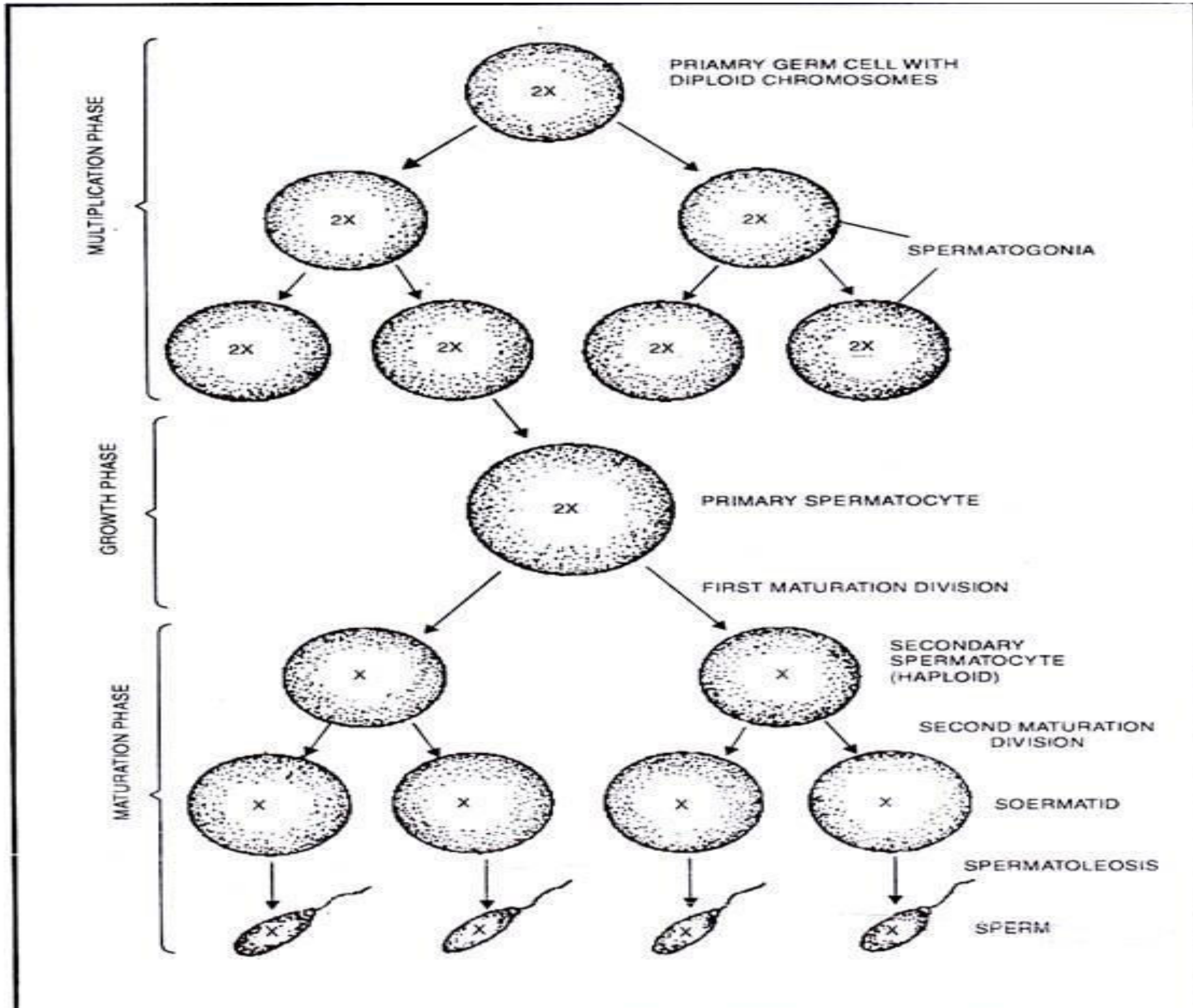

Fig. 3(B).2 Spermatogenesis (diagrammatic).

1. **Multiplication Phase:**

The primary germ cells multiply by repeated mitotic division. The cells produced after the final mitotic divisions are known as spermatogonia or sperm mother cells.

2. **Growth Phase:**

The spermatogonia do not divide for sometime but increase in size by accumulating nutritive materials from the supporting cells. In mammals such supporting cells are called cells of Sertoli. The enlarged spermatogonia are now called primary spermatocytes.

3. **Maturation Phase:**

During the phase of maturation, the primary spermatocytes divide by meiosis consisting of two successive divisions. The first division is reductional or dysjunctional reducing the chromosome number from ‘2n’ to ‘n’. These cells are celled secondary spermatocytes. Second division is equational resulting in formation of four daughter cells called spermatids.

(B) Spermiogenesis (Spermatoleosis):

This is the second phase of spermatogenesis during which the spermatids produced at the end of first phase are metamorphosed into sperm cells. The spermatid is a typical cell containing a nucleus and cytoplasmic organelles such as mitochondria, golgi bodies, centriole etc, but the nucleus only contains haploid number of chromosomes.

During spermiogenesis or spermatoleosis the following transformations occur in the spermatids:

1. The large spherical nucleus becomes smaller by losing water and usually changes its shape into elongated structure

2. The Golgi bodies condense into a cap called acrosome in front of the nucleus.

3. Nucleus and the acrosome combinedly form the head of the developing sperm while the cytoplasm with mitochondria and centrioles move downwards and form the cylindrical middle piece behind the head (Fig. 3(B).3).

4. The two centrioles of middle piece develop axial filaments which are bunched into a single thread and extend behind in the form of a long vibratile tail. Thus, spermatid is transformed into a motile sperm divisible into head, middle piece and tail.

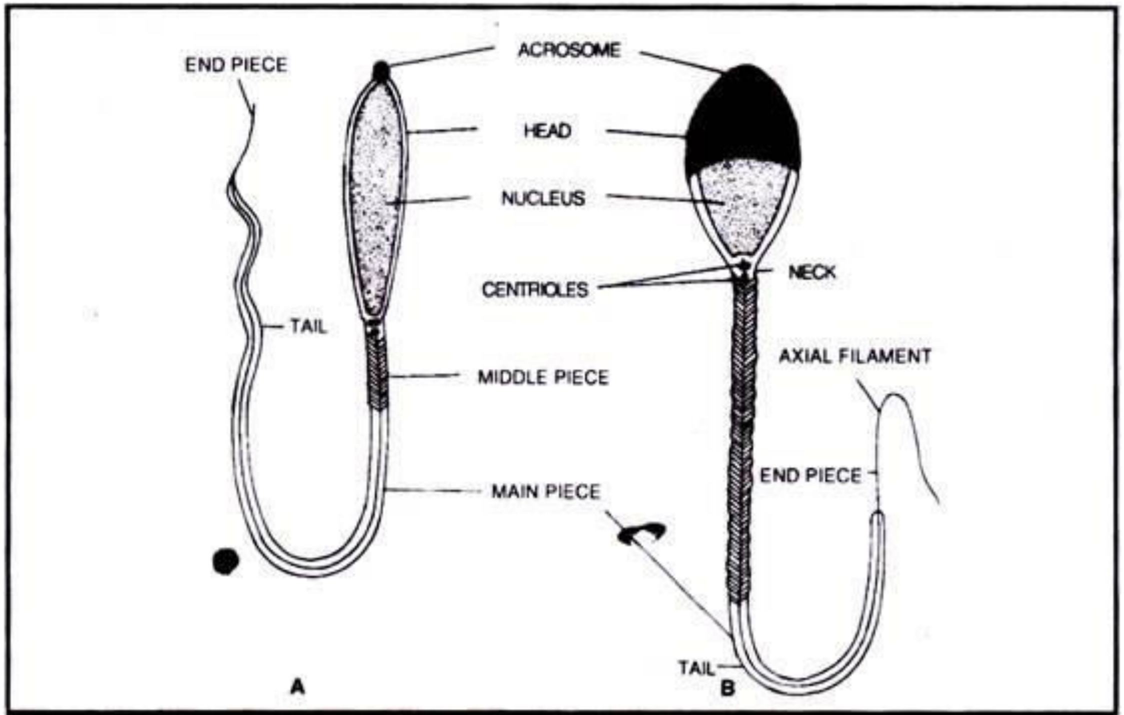

Fig. 3(B).3 Sperms of frog (A) and rabbit (B).

2. Oogenesis:

It occurs in the ovary of female animals. It is comparable to spermatogenesis so far as nuclear changes are concerned. But the cytoplasmic specialization in oogenesis is different from spermatogenesis.

It is divisible into following three phases:

1. Multiplication Phase:

The primary germinal cells of the ovary with diploid number of chromosomes (2n) divide several times mitotically so as to form a large number of daughter cells known as oogonia (Fig. 3(B) .4).

2. Growth Phase:

The oogonium does not divide but increases in size enormously to form a primary oocyte. The growth is associated with both nuclear and cytoplasmic growth. The nuclear growth is due to accumulation of large amount of nuclear sap and is termed as germinal vesicle. The cytoplasmic growth is associated with increase in number of mitochondria, endoplasmic reticulum and Golgi complex and accumulation of reserve food material called yolk or vitellin.

3. Maturation phase:

The primary oocyte undergoes two successive divisions by meiosis. The first division is meiosis-I and two unequal daughter cells are produced. The large cell is called secondary oocyte containing haploid (n) set of chromosomes (due to reductional or disjunctional division) and entire amount of cytoplasm. The smaller cell is called first polar body or polocyte containing ‘n’ number of chromosomes and practically no cytoplasm.

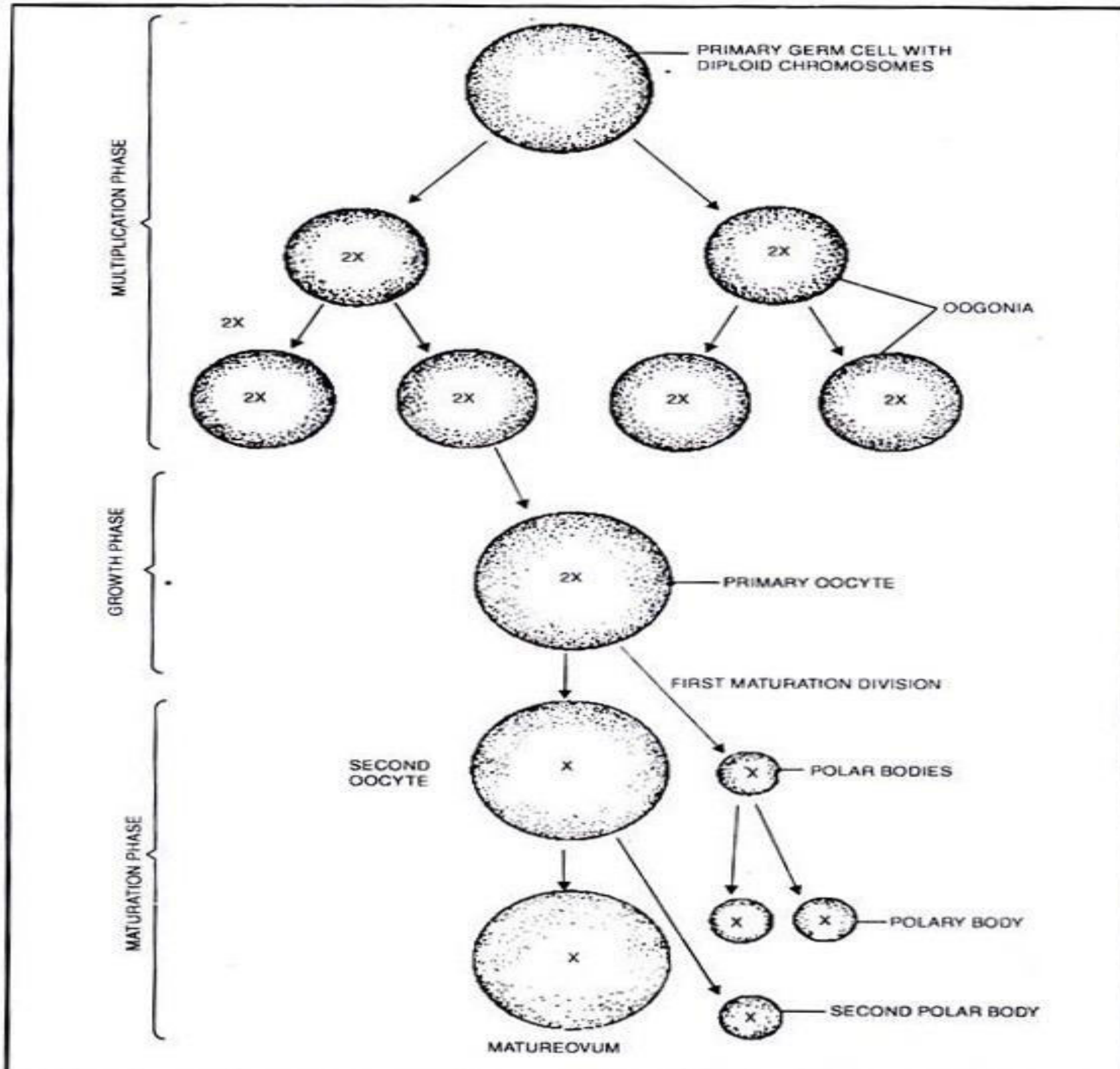

Fig. 3(B).4 Oogenesis (diagrammatic).

The secondary oocyte and first polar body then undergo second maturation division by meiosis-II which is an equational division. As a result of this division one large ovum is formed containing entire amount of cytoplasm and ‘n’ number of chromosomes and a second polar body like the first polar body.

Simultaneously, the first polar body may divide into two polar bodies or may not divide at all. Thus only one functional ovum is formed and the two or three polar bodies soon degenerate. In vertebrates the first polar body is formed after the primary oocyte is released from ovary and has entered into the oviduct. The second polar body is formed only when the sperm enters into ovum during fertilization.

(C) Ripening of Egg:

Oogenesis is followed by the formation of protective coverings called egg membranes. Primary membrane is formed surrounding the plasma membrane of ovum and is secreted by the ovum itself. It is called vitelline membrane in frog and zona pellucida in rabbit. The secondary membrane called chorion is formed from ovarian follicle cells. The tertiary membranes are secreted in oviduct when the ovum passes from ovary to outside. The egg white (albumin), calcareous shell etc. come under this category (Fig. 3(B).5).

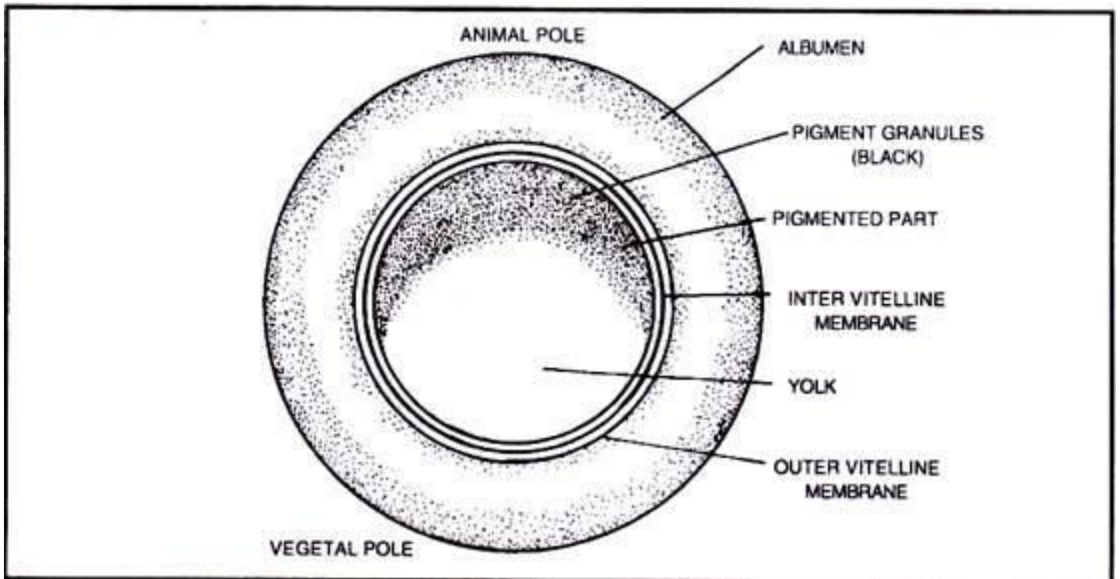

Fig. 3(B).5 Ovum of frog.

Significance:

1. The process leads to formation of germ cells or gametes.

2. The normal body cells known as somatic cells are diploid (2n) where as the germ cells are haploid (n).

3. During fertilization one haploid sperm unites with one haploid ovum to form a normal diploid somatic cell thus keeping the chromosome number constant generation after generation.

4. During first maturation division, the reshuffling of paternal and maternal genes take place resulting in variation.

FERTILIZATION

- The fusion of the sperm cell nucleus with the egg cell nucleus to produce a zygote (fertilized egg).

- Brings male and females gametes together – produces diploid zygote.

- It also activates the egg, triggering the beginning of embryonic development.

- Fertilization in mammals occurs in the oviduct.

- The ova is viable for approximately 24 hours after ovulation.

1. Encounter of spermatozoa and ova

2. Capacitation and contact

3. Acrosome reaction and penetration

4. Fusion of the sperm with the egg

5. Activation of ovum

Encounter of spermatozoa and ova

- During the fertile phase, millions of sperm travel from the vagina to the uterus and into the fallopian tubes.
- Chemotaxis – A chemical substance is found in the cortex of eggs.
- In general interaction is through special devices or particular forms of behavior.
- The primary need is a fluid medium for the act of fertilization and delivery of sperm to the eggs at the right time.
- 2 types of fertilization.

1.External

- Occurs outside of the body of the female.
- Increased number of eggs produced to insure the survival of the species
- E.g: fish and amphibians

2. **Internal**

- Occurs inside the body of the female
- Fewer number of eggs are produced
- Increased parental care insures species survival
- E.g: mammals, reptiles, birds

Several thousand sperm reach the egg and one will fertilize it.

When the sperm fuses with the egg it initiates a series of chemical changes that prevent any other sperm from entering.

1. **Capacitation**

- Sperm undergo capacitation (further maturation) within the female reproductive tract.
- occurs in the female's vagina.
- Vaginal secretions cause a molecular change in the sperm plasmalemma (removal of decapacitating factor - semen proteins, results in increased membrane fluidity,).
- Takes 4-5 hr in humans

- When the acrosome reaction occurs, a number of proteolytic enzymes are exposed or released.
- One or more of these enzymes is responsible for digesting the hole through the zona pellucida through which the sperm enters the perivitelline space.

1.Acrosome Reaction

- The male nucleus enters the egg cytoplasm and becomes the male pronucleus.
- As a result of the sperm fusing with the egg plasmalemma, the oocyte nucleus, which is at metaphase of the second meiotic division, completes that division giving rise to another polar body.
- Following the second meiotic division, what is now the nucleus of the ovum becomes the female pronucleus.
- The haploid male and female pronuclei move toward one and other, meet, and fuse to form the diploid nucleus of the zygote.
- The zygote will now proceed to undergo cleavage.

A series of morphological, physiological and molecular changes that occur in the egg in response to fusion of the sperm with the egg.

1. Release of Ca++ (calcium) stored in the egg endoplasmic reticulum - appears to be the critical step in the process.

2. Cortical reaction - rupture of cortical granules that occurs concurrently with the Ca++ release. Contents of granules are released into perivitelline space and cause “hardening” of the vitelline membrane or zona pellucida. Causes vitelline/fertilization membrane to rise away from surface of egg in some species.

3. In many species, an influx of Na+ (sodium) into the egg cytoplasm that causes a change in membrane potential - fast block to polyspermy.

4. In many species a reorganization of the egg cytoplasm.

5. In most cases, completion of meiosis by the egg.

6. An efflux of H+ (hydrogen) ions causing an increase in cytoplasmic pH - this activates previously inhibited synthetic pathways.

7. Increase in metabolism - zygote gears up for development.

As early as 12 hours after fertilization you can see the two bundles of genetic material (two pronuclei), one from each parent.

By 18-20 hours after fertilization, these pronuclei fuse, and what starts out as two cells becomes one (called a zygote)

A multicellular organism in the early stages of development 2 four cell stage embryos.

Eight cell stage embryo

The beginning developmental processes are always the same in all animals:

1) cleavage

2) growth

3) differentiation

1. After fertilization the diploid ZYGOTE undergoes cleavage divisions in the oviduct

The first series of cell divisions by mitosis after fertilization Cell division is rapid, new cells do not take time for the growth phase G1 cell growth does not occur so cells decrease in size with each cleavage division.

Morula forms (solid ball of cells) Blastula forms (hollow ball of cells)

Cells begin to grow before dividing

Types of eggs

I. Structure The fully developed female gamete is called as ‘ovum’ or ‘egg’. It is non motile. The eggs are relatively larger than the male gametes. The egg performs different functions like, it provides haploid set of chromosome to the future embryo, and also it provides cytoplasm and food to the embryo. In egg, contains yolk and albumin. The yolk is yellowish colour and albumin is whitish colour in nature. Yolk is present inside the albumin.

Types of eggs On the basis of amount and distribution of yolk in the eggs, the eggs are classified by the embryologists as follows:

A. Types of eggs based on the amount of yolk:

On the basis of amount of yolk present in the eggs, the eggs are classified into fpur types: viz.,

1. Alecithal eggs
2. Microlecithal eggs
3. Mesolecithal eggs
4. Macrolecithal eggs

1. Alecithal Egg: When the egg contains no yolk, it is called alecithal egg. Eg. The eggs of eutherian mammals

2. Microlecithal eg gs: The eggs which contains small amount of yolk or reserved food, such eggs are called as ‘Microlecithal eggs’. The microlecithal eggs are found in Amphioxus, Cephalochordates, Tunicates and Eutherian mammals.

3. Mesolecithal eggs: The eggs which contains moderate amount of yolk, such eggs are called as ‘Mesolecithal eggs’. The mesolecithal eggs are found in Petromyzon, Dipnoi and Amphibia

4. Macrolecithal eggs: The eggs which contains enormous (large) amount of yolk, such eggs are called as ‘Macrolecithal eggs’. The macrolecithal eggs are found in cartilaginous and bony fishes, reptiles, birds, protherian mammals and insects.

B. Types of eggs based on the distribution of yolk: On the basis of distribution of yolk, the eggs are classified into three types. These are: viz.,

1. Homolecithal or isolecithal eggs
2. Telolecithal eggs
3. Centrolecithal eggs.

1. Homolecithal or isolecithal eggs: The eggs which contains small amount of yolk and distributed uniformly in the egg cytoplasm, such eggs are called as ‘Homolecithal or Isolecithal eggs’. The microlecithal eggs are homolecithal or isolecithal eggs.

2. Telolecithal eggs: The eggs in which the yolk is not distributed evenly. It is present at one side of the egg, may be at animal or vegetal pole. Such eggs are called as ‘Telolecithal eggs’. The mesolecithal and macrolecithal eggs are telolecithal eggs. Telolecithal eggs may further classified into three types:

(i) Slightly Telolecithal- This type of egg contains only a small quantity of yolk which is distributed unevenly. The vegetal pole has the highest concentration and the animal pole the lower (e.g. eggs of fishes).

(ii) Moderately Telolecithal -This type of egg contains a moderate quanilty of yolk which is distributed unevenly. Due to high concenteration of yolk in the vegetal hemisphere, the nucleus is shifted more towards the animal hemisphere (eg. amphibian egg).

(iii) Extremely Telolecithal -In this type of egg, due to the heavy deposition of yolk, the entire vegetal hemisphere and a major portion of the animal hemisphere are occupied by yolk. Due to this extremely uneven distribution of yolk, the ooplasm and nucleus are displaced towards the animal pole (eg. reptilian and avian eggs)

3. Centrolecithal eggs: The eggs in which the yolk is present in the centre of the egg cytoplasm, such eggs are known as ‘Centrolecithal eggs’. The eggs of insects are centrolecithal eggs.