NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 413

COURSE TITLE: DEVELOPMENTAL BIOLOGY

BIO 413: DEVELOPMENTAL BIOLOGY

Course Writers/Developers **Mr O.A. Adekolurejo**

Department of Biolog

Adeyemi Colledge of Education,

P.M.B. 520, Ondo,

Ondo State Nigeria.

Email: oloyedeisrael@yahoo.com;loyekolurejo@yahoo.com

Ms F.C. Olakolu

Nigerian Institute for Oceanography and

Marine Research,

No 3 Wilmot Point Road, Bar-beach Bus-stop,

Victoria Island,

Lagos, Nigeria.

the middle; the eyes are always in our heads, not in our toes or gut. This creation of ordered form is called **morphogenesis**. How can the cells form such ordered structures?

- **The question of growth**. How do our cells know when to stop dividing? If each cell in our face were to undergo just one more cell division, we would be considered horribly malformed. If each cell in our arms underwent just one more round of cell division, we could tie our shoelaces without bending over. Our arms are generally the same size on both sides of the body. How is cell division so tightly regulated?

- **The question of reproduction**. The sperm and egg are very specialized cells. Only they can transmit the instructions for making an organism from one generation to the next. How are these cells set apart to form the next generation, and what are the instructions in the nucleus and cytoplasm that allow them to function this way?

- **The question of evolution**. Evolution involves inherited changes in development. When we say that today's one-toed horse had a five-toed ancestor, we are saying that changes in the development of cartilage and muscles occurred over many generations in the embryos of the horse's ancestors. How do changes in development create new body forms? Which heritable changes are possible, given the constraints imposed by the necessity of the organism to survive as it develops?

- **The question of environmental integration**. The development of many organisms is influenced by cues from the environment. Certain butterflies, for instance, inherit the ability to produce different wing colors based on the temperature or the amount of daylight experienced by the caterpillar before it undergoes metamorphosis.

What you will learn in this course

In this course, you have the course units and a course guide. The course guide will tell you briefly what the course is all about. It is a general overview of the course materials you will be using and how to use those materials. It also helps you to allocate the appropriate time to each unit so that you can successfully complete the course within the stipulated time limit.

The course guide also helps you to know how to go about your Tutor-Marked-Assignment which will form part of your overall assessment at the end of the course. Also, there will be tutorial classes that are related to this course, where you can interact with your facilitators and other student. Please I encourage you to attend these tutorial classes.

This course exposes you to Developmental Biology, a very important and interesting field in Biology.

Course Aims

This course aims to enable you to appreciate and understand some of the universal molecular and cellular events and processes that occur as an animal develops from an egg and a sperm into an adult organism.

Course Objectives

To achieve the aim set above, there are objectives. Each unit has a set of objectives presented at the beginning of the unit. These objectives will give you what to concentrate focus on while studying the unit and during your study to check your progress.

The Comprehensive Objectives of the Course are given below. By the end of the course/after going through this course, you should be able to:

1 Explain the historical background of development Biology
2 Explain the significance of gametogenesis and fertilization with relevant examples to various animals at different level of organization.
3 Describe the steps in the cleavage formation; segmentation, gastrulation and invagination.
4 Explain the process involves in animal and mammalian embryonic development.
5 Name the stages at which the first and second meiotic divisions take place
6 Outline the stages of spermiogenesis and organogenesis
7 Name the functions of Sertoli cells and granulosa cells
8 Define the stages at which meiotic arrest in oocytes normally occurs
9 Identify the components of developing ovarian follicles and of the oocyte at the time of ovulation.

Working through the Course

To successfully complete this course. You are require to read each study unit, read the textbooks materials provided by the National Open University.

Reading the reference materials can also be of great assistance.

Each unit has self –assessment exercise which you are advised to doat certain periods during the course you will be required to submit your assignment for the purpose of assessment.

There will be a final examination at the end of the course. The course should take you about17 weeks to complete.

This course guide provide you with all the components of the course how to go about studying and how you should allocate your time to each unit so as to finish on time and successfully.

The Course Materials

The main components of the course are:

1 The Study Guide

2 Study Units

3 Reference/ Further Readings

4 Assignments

5 Presentation Schedule

STUDY UNITS

The study units in this course are given below:

CONTENTS

Module 1: Introduction to Developmental Biology

Unit 1: Principles of Development in Biology

Unit 2: Anatomical Approach to Development

Module 2: Gametogenesis

Unit 1: Gametes Interaction during fertilization

Unit 2: The Determination of germ cell in different group of animals
Unit 3: Mitosis and Meiosis

Unit 4: Fertilization and gamete formation in major groups of organisms

Module 3: Fertilization and Cleavage Formation in Animals

Unit 1: Fertilization in Animals

Unit 2: Cleavage, morula and Blastocyst formation

Unit 3: Cleavage patterns in major groups of organisms

Module 4: Gastrulation, Invagination and Organogenesis Formation

Unit 1: Gastrulation and Invagination in major groups of organisms

Unit 2: Organogenesis

Module 5: General Embryology

Unit 1: General Embryology

Unit 2: Embryonic membrane and Placenta

In Module One unit one deals with the *early history, and current understanding of the beginning of development itself, the second unit focuses on* anatomical approach to development.

Module Two is concerned with the formation of gametes, egg and sperm formation, cell division and fertilization in major group of organism

Module Three, unit one, two and three deals with fertilization in animals ,cleavage blastocyst formation and major pattern cleavage.

In Module Four,two unit deal with gastrulation formation and invagination in some animals, unit three and four deals with the formatiom of germ layer and nervous system formation.

Module Five is concerned with general embryology as in unit one ,but unit two deal with early embryonic membrane ,placental function, infertility in male and female and birth control.

Each unit will take a week or two.Lectures will include an introduction, objectives reading materials, self assessmentquestion, conclusion, summary, tutor marked assignment (TMAs), references and other reading resources.

There are activities related to the lecture in each unit which will help your progress and comprehension of the unit. You are required to work on these exercises which together with the TMAs will enable you to achieve the objective of each unit.

Presentation Schedule

There is a time-table prepared for the early and timely completion and submissions of your TMAs as well as attending the tutorial classes. You are required to submit all your assignments by the stipulated date and time. Avoid falling behind the schedule time.

Assessment

There are three aspects to the assessment of this course.

The first one is the self-assessment exercises. The second is the tutor-marked assignments and the third is the written examination or the examination to be taken at the end of the course.

Do the exercises or activities in the unit applying the information and knowledge you acquired during the course. The tutor-marked assignments must be submitted to your facilitator for formal assessment in accordance with the deadlines stated in the presentation schedule and the assignment file.

The work submitted to your tutor for assessment will account for 30% of your total work.

At the end of this course you have to sit for a final or end of course examination of about a three hour duration which will account for 70% of your total course mark.

Tutor Marked Assignment

This is the continuous assessment component of this course and it accounts for 30% of the total score. You will be given four (4) TMAs by your facilitator to answer. Three of which must be answered before you are allowed to sit for the end of the course examination.

These answered assignments must be return to your facilitator.

You are expected to complete the assignments by using the information and material in your reading references and study units.

Reading and researching into the references will give you a wider via point and give you a deeper understanding of the subject.

1 Make sure that each assignment reaches your facilitator on or before the deadline given in the presentation schedule and assignment file. If for any reason you are not able to complete your assignment, make sure you contact your facilitator before the assignment is due to discuss the possibility of an extension. Request for extension will not be granted after the due date unless there is an exceptional circumstance.
2 Make sure you revise the whole course content before sitting for examination. The self-assessment activities and TMAs will be useful for this purposes and if you have any comments please do before the examination. The end of course examination covers information from all parts of the course.

Course Marking Scheme

Assignment	**Marks**
Assignment 1-4	Four assignments, best three marks of the four count at 10% each - 30% of course marks.
End of course examination	70% of overall course marks
Total	100% of course materials

Facilitators/ Tutors and Tutorials

Sixteen (16) hours are provided for tutorials for this course. You will be notified f the dates, times and location for these tutorial classes.

As soon as you are allocated a tutorial group, the name and phone number of your facilitator will be given to you.

These are the duties of your facilitator:

- He or she will mark and comment on your assignment
- He will monitor your progress and provide any necessary assistance you need.
- He or she will mark your TMAs and return to you as soon as possible.

(You are expected to mail your tutored assignment to your facilitators at least two days before the schedule date).

Do not delay to contact your facilitator by telephone or e-mail for necessary assistance if

- You do not understand any part of the study in the course material.
- You have difficulty with the self assessment activities.
- You have a problem or question with an assignment or with the grading of the assignment.

It is important and necessary you acted the tutorial classes because this is the only chance to have face to face contact with your facilitator and to ask questions which will be answered instantly. It is also a period where you can point out any problem encountered in the course of your study.

Summary

Developmental Biology (413) is a course that introduces you to the developmental processes that lead to the establishment of the body plan of a variety of metazoan organisms from their start at fertilization through the stages of their development and on to entire organismal and post-embryonic development and the corresponding cellular and genetic mechanisms.

On the completion of this course, you will have an understanding of basic knowledge of animal developmental Biology. Gametogenesis and gamete formation during fertilization in both animal and mammals, how fertilization and cleavage is formed in different organisms including mammals, also you will understand the formation of invagination and gastrulation in embryology. In addition you will be able to answer the following questions:

1 Explain the historical background of development Biology
2 Explain the significance of gametogenesis and fertilization with relevant examples to various animals at different level of organization.
3 Describe the steps in the cleavage formation; segmentation, gastrulation and invagination
4 Explain the process involves in animal and mammalian embryonic development.
5 Name the stages at which the first and second meiotic divisions take place
6 Outline the stages of spermiogenesis and organogenesis
7 Name the functions of Sertoli cells and granulosa cells
8 Define the stages at which meiotic arrest in oocytes normally occurs
9 Identify the components of developing ovarian follicles and of the oocyte at the time of ovulation

The list of questions you are expected to answer is not limited to the above list.

I believe you will agree with me that Developmental Biology is a very interesting field of biology.

I wish you success in this course.

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BIO 413 DEVELOPMENTAL BIOLOGY

CONTENTS **PAGE**

Module 1: BIO 413 DEVELOPMENTAL BIOLOGY

Unit 1: Principles of Development in Biology

CONTENTS

1.0 INTRODUCTION

According to Aristotle, the first embryologist known to history, science begins with wonder: "It is owing to wonder that people began to philosophize, and wonder remains the beginning of knowledge." The development of an animal from an egg has been a source of wonder throughout history. The simple procedure of cracking open a chick egg on each successive day of its 3-week incubation provides a remarkable experience as a thin band of cells is seen to give rise to an entire bird. Aristotle performed this procedure and noted the formation of the major organs. Anyone can wonder at this remarkable yet common place phenomenon, but the scientist seeks to discover how development actually occurs. And rather than dissipating wonder, new understanding increases it.

Multicellular organisms do not spring forth fully formed. Rather, they arise by a relatively slow process of progressive change that we call **development.** In nearly all cases, the development of a multicellular organism begins with a single cell the fertilized egg, or **zygote**, which divides mitotically to produce all the cells of the body.

The study of animal development has traditionally been called **embryology**, from that stage of an organism that exists between fertilization and birth. But development does not stop at birth, or even at adulthood. Most organisms never stop developing. Each day we replace more than a gram of skin cells (the older cells being sloughed off as we move), and our bone marrow sustains the development of millions of new red blood

cells every minute of our lives. In addition, some animals can regenerate severed parts, and many species undero metamorphosis (such as the transformation of a tadpole into a frog, or a caterpillar into a butterfly). Therefore, in recent years it has become customary to speak of **developmental biology** as the discipline that studies embryonic and other developmental processes.

2.0 OBJECTIVES

At the end of this unit, student should be able to:

Explain the historical background of development in biology, objectives accomplished and six general questions scrutinized by developmental biologists.

3.0 MAIN CONTENT

3.1 General Questions Scrutinized by Developmental Biologist

Development accomplishes two major objectives: it generates cellular diversity and order within each generation, and it ensures the continuity of life from one generation to the next. Thus, there are two fundamental questions in developmental biology: How does the fertilized egg give rise to the adult body, and how does that adult body produce yet another body? These two huge questions have been subdivided into six general questions scrutinized by developmental biologists:

- **3.1.1 The question of differentiation**. A single cell, the fertilized egg, gives rise to hundreds of different cell types muscle cells, epidermal cells, neurons, lens cells, lymphocytes, blood cells, fat cells, and so on. This generation of cellular diversity is called **differentiation**. Since each cell of the body (with very few exceptions) contains the same set of genes, we need to understand how this same set of genetic instructions can produce different types of cells. How can the fertilized egg generate so many different cell types?

- **3.1.2 The question of morphogenesis**. Our differentiated cells are not randomly distributed. Rather, they are organized into intricate tissues and organs. T hese organs are arranged in a given way: the fingers are always at the tips of our hands, never in the middle; the eyes are always in our heads, not in our toes or gut. This creation of ordered form is called **morphogenesis**. How can the cells form such ordered structures?

- **3.1.3. The question of growth**. How do our cells know when to stop dividing? If each cell in our face were to undergo just one more cell division, we would be considered horribly malformed. If each cell in our arms underwent just one more round of cell division, we could tie our shoelaces without bending over. Our arms are generally the same size on both sides of the body. How is cell division so tightly regulated?

- **3.1.4 The question of reproduction**. The sperm and egg are very specialized cells. Only they can transmit the instructions for making an organism from one generation to the next. How are these cells set apart to form the next generation, and what are the instructions in the nucleus and cytoplasm that allow them to function this way?

- **3.1.5 The question of evolution**. Evolution involves inherited changes in development. When we say that today's one-toed horse had a five-toed ancestor, we are saying that changes in the development of cartilage and muscles occurred over many generations in the embryos of the horse's ancestors. How do changes in development create new body forms? Which heritable changes are possible, given the constraints imposed by the necessity of the organism to survive as it develops?

- **3.1.6 The question of environmental integration**. The development of many organisms is influenced by cues from the environment. Certain butterflies, for instance, inherit the ability to produce different wing colors based on the temperature or the amount of daylight experienced by the caterpillar before it undergoes metamorphosis. How is the development of an organism integrated into the larger context of its habitat?

4.0 CONCLUSION

In this unit you learnt, the principles of developmental biology and the various questions that biologist scrutinized which summarise what developmental biology entails.

5.0 SUMMARY

You are introduce to the field of developmental biology, including some of its most fundamental; early history, and current understanding of the beginning of development itself. Developmental biology asks questions about how organisms come into being, how life forms, and how complex structures develop and are differentiated. These fundamental questions have been the subject of research for centuries; accordingly.

6.0 TUTOR-MARKED QUESTION

1 Explain the Aristotle first findings in development of bird
2 State the two objectives accomplished by the biologist
3 Explain the six general questions scrutinized by developmental biologists.

7.0 REFERENCES

Professor Scott Gilbert, Developmental biology 6th Edition

Unit 2 Anatomical Approach to Developmental Biology

CONTENTS

1.0 INTRODUCTION

A field of science is defined by the questions it seeks to answer, and most of the questions in developmental biology have been bequeathed to it through its embryological heritage. There are numerous strands of embryology, each predominating during a different era. Sometimes they are very distinct traditions, and sometimes they blend. We can identify three major ways of studying embryology:

- Anatomical approaches
- Experimental approaches
- Genetic approaches

While it is true that anatomical approaches gave rise to experimental approaches, and that genetic approaches built on the foundations of the earlier two approaches, all three traditions persist to this day and continue to play a major role in developmental biology. In recent years, each of these traditions has become joined with molecular genetics to produce a vigorous and multifaceted science of developmental biology.

But the basis of all research in developmental biology is the changing anatomy of the organism. What parts of the embryo form the heart? How do the cells that form the retina position themselves the proper distance from the cells that form the lens? How do the tissues that form the bird wing relate to the tissues that form the fish fin or the human hand?

There are several strands that weave together to form the anatomical approaches to development.

The first strand is **comparative embryology**, the study of how anatomy changes during the development of different organisms. For instance, a comparative embryologist may study which tissues form the nervous system in the fly or in the frog.

The second strand, based on the first, is **evolutionary embryology**, the study of how changes in development may cause evolutionary changes and of how an organism's

ancestry may constrain the types of changes that are possible.

The third anatomical approach to developmental biology is **teratology**, the study of birth defects. These anatomical abnormalities may be caused by mutant genes or by substances in the environment that interfere with development. The study of abnormalities is often used to discover how normal development occurs.

The fourth anatomical approach is **mathematical modeling**, which seeks to describe developmental phenomena in terms of equations. Certain patterns of growth and differentiation can be explained by interactions whose results are mathematically predictable. The revolution in graphics technology has enabled scientists to model certain types of development on the computer and to identify mathematical principles upon which those developmental processes are based.

2.0 OBJECTIVES

At the end of this unit student should be able to:

1 Explain the anatomical approach to study embryology in development
2 State the principles of development
3 Describe the life cycles and the evolution of developmental patterns

3.0 MAIN CONTENT

3.1 Principles of Development: Developmental Anatomy

1. Organisms must function as they form their organs. They have to use one set of structures while constructing others.

2. The main question of development is, how does the egg becomes an adult? This question can be broken down into the component problems of differentiation (How do cells become different from one another and from their precursors?), morphogenesis (How is ordered form is generated?), growth (How is size regulated?), reproduction (How does one generation create another generation?), and evolution (How do changes in developmental processes create new anatomical structures?).

3. Epigenesis happens. New organisms are created de novo each generation from the relatively disordered cytoplasm of the egg.

4. Preformation is not in the anatomical structures, but in the instructions to form them. The inheritance of the fertilized egg includes the genetic potentials of the organism.

5. The preformed nuclear instructions include the ability to respond to environmental stimuli in specific ways.

6. The ectoderm gives rise to the epidermis, nervous system, and pigment cells.

7. The mesoderm generates the kidneys, gonads, bones, heart, and blood cells.

8. The endoderm forms the lining of the digestive tube and the respiratory system.

9. Karl von Baer's principles state that the general features of a large group of animals appear earlier in the embryo than do the specialized features of a smaller group. As each

embryo of a given species develops, it diverges from the adult forms of other species. The early embryo of a "higher" animal species is not like the adult of a "lower" animal.

10. Labeling cells with dyes shows that some cells differentiate where they form, while others migrate from their original sites and differentiate in their new locations. Migratory cells include neural crest cells and the precursors of germ cells and blood cells.

11. "Community of embryonic structure reveals community of descent" (Charles Darwin).

12. Homologous structures in different species are those organs whose similarity is due to their sharing a common ancestral structure. Analogous structures are those organs whose similarity comes from their serving a similar function (but which are not derived from a common ancestral structure).

13. Congenital anomalies can be caused by genetic factors (mutations, aneuploidies, translocations) or by environmental agents (certain chemicals, certain viruses, radiation).

14. Syndromes consists of sets of developmental abnormalities that "run together."

15. Organs that are linked in developmental syndromes share either a common origin or a common mechanism of formation.

16. If growth is isometric, a twofold change in weight will cause a 1.26-fold expansion in length.

17. Allometric growth can create dramatic changes in the structure of organisms.

18. Complex patterns may be self-generated by reaction-diffusion events, wherein the activator of a local phenomenon stimulates the production of more of itself as well as the production of a
more diffusible inhibitor.

3.2. Life cycles and the evolution of developmental patterns

Traditional ways of classifying catalog animals according to their adult structure. But, as Bonner (1965) pointed out, this is a very artificial method, because what we consider an individual is usually just a brief slice of its life cycle. When we consider a dog, for instance, we usually picture an adult. But the dog is a "dog" from the moment of fertilization of a dog egg by a dog sperm. It remains a dog even as a senescent dying hound. Therefore, the dog is actually the entire life cycle of the animal, from fertilization through death.

The life cycle has to be adapted to its environment, which is composed of nonliving objects as well as other life cycles. Take, for example, the life cycle of *Clunio marinus*, a small fly that inhabits tidal waters along the coast of western Europe. Females of this species live only 23 hours as adults, and they must mate and lay their eggs within this short time. To make matters even more precarious, egg laying is confined to red algae mats that are exposed only during the lowest ebbing of the spring tide. Such

low tides occur on four successive days shortly after the new and full moons (i.e., at about 15-day intervals). Therefore, the life cycle of these insects must be coordinated with the tidal rhythms as well as the daily rhythms such that the insects emerge from their pupal cases during the few days of the spring tide and at the correct hour for its ebb.

One of the major triumphs of descriptive embryology was the idea of a generalizable life cycle. Each animal, whether an earthworm, an eagle, or a beagle, passes through similar stages of development. The major stages of animal development are illustrated in Figure 1. The life of a new individual is initiated by the fusion of genetic material from the two gametes the sperm and the egg. This fusion, called **fertilization**, stimulates the egg to begin development. The stages of development between fertilization and hatching are collectively called **embryogenesis**.

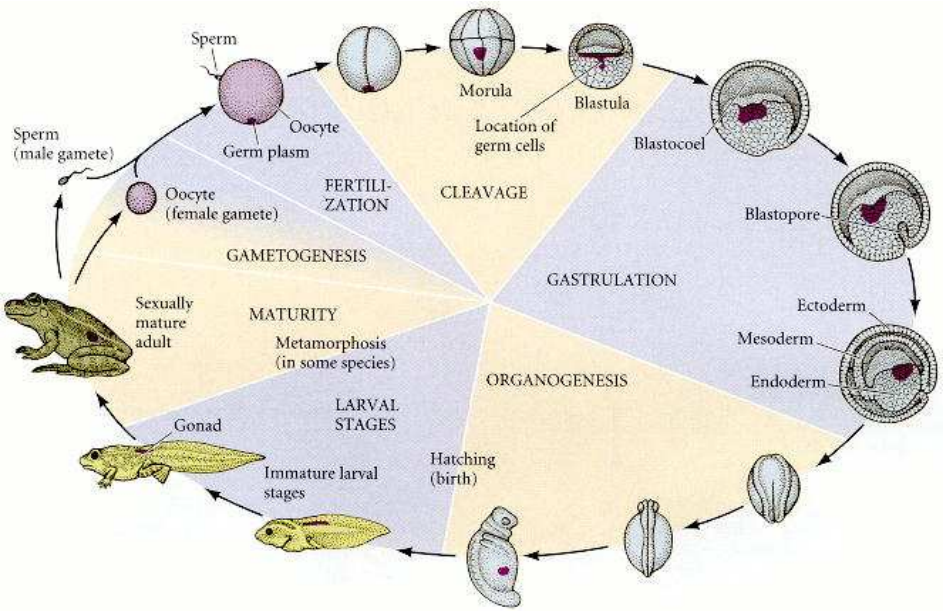

Figure 1: Circle of Life: The Stages of Animal Development

Throughout the animal kingdom, an incredible variety of embryonic types exist, but most patterns of embryogenesis are variations on five themes:

1. Immediately following fertilization, **cleavage** occurs. Cleavage is a series of extremely rapid mitotic divisions wherein the enormous volume of zygote cytoplasm is divided into numerous smaller cells. These cells are called **blastomeres**, and by the end of cleavage, they generally form a sphere known as a **blastula**.

2. After the rate of mitotic division has slowed down, the blastomeres undergo dramatic movements wherein they change their positions relative to one another. This series of extensive cell rearrangements is called **gastrulation,** and the embryo is said to be in the **gastrula** stage. As a result of gastrulation, the embryo contains three **germ layers**: the ectoderm, the endoderm, and the mesoderm.

3. Once the three germ layers are established, the cells interact with one another and rearrange themselves to produce tissues and organs. This process is called

organogenesis. Many organs contain cells from more than one germ layer, and it is not unusual for the outside of an organ to
be derived from one layer and the inside from another. For example, the outer layer of skin comes from the ectoderm, while the inner layer (the dermis) comes from the mesoderm. Also during organogenesis, certain cells undergo long migrations from their place of origin to their final location. These migrating cells include the precursors of blood cells, lymph cells, pigment cells, and gametes. Most of the bones of our face are derived from cells that have migrated ventrally from the dorsal region of the head.

4. Many species have specialized portion of egg cytoplasm which gives rise to cells that are the precursors of the **gametes** (the sperm and egg). The gametes and their precursor cells are collectively called **germ cells**, and they are set aside for reproductive function. All the other cells of the body are called **somatic cells**. This separation of somatic cells (which give rise to the individual body) and germ cells (which contribute to the formation of a new generation) is often one of the first differentiations to occur during animal development. The germ cells eventually migrate to the gonads, where they differentiate into gametes. The development of gametes, called **gametogenesis**, is usually not completed until the organism has become physically mature. At maturity, the gametes may be released and participate in fertilization to begin a new embryo. The adult organism eventually undergoes senescence and dies.

5. In many species, the organism that hatches from the egg or is born into the world is not sexually mature. Indeed, in most animals, the young organism is a **larva** that may look significantly different from the adult. Larvae often constitute the stage of life that is used for feeding or dispersal. In many species, the larval stage is the one that lasts the longest, and the adult is a brief stage solely for reproduction. In the silkworm moths, for instance, the adults do not have mouthparts and cannot feed. The larvae must eat enough for the adult to survive and mate. Indeed, most female moths mate as soon as they enclose from their pupa, and they fly only once to lay their eggs. Then they die.

4.0 CONCLUSION

In this unit you learnt, the anatomical approach to study embryology in development, the principles of development and life cycles and evolution of developmental patterns.

5.0 SUMMARY

The anatomical approach form the basis of research in developmental Biology. It involves changing anatomy of the organisms from cells to tissue to organ to system, for example what part of the embryo form the heart, how do the cells that form the retina position themselves the proper distance from the cells that form the lens?etc. The several strands weaved together to form anatomical approach to development are comparative embryology, evolutionary embryology, teratology embryology and mathematical modeling.

6.0 TUTOR-MARKED ASSIGNMENT

1 Explain the anatomical approach to study embryology in development
2 State the principles of development
3 Describe and explain the life cycles and the evolution of

developmental patterns.

7.0 REFERENCES

Professor Scott Gilbert, Developmental biology 6th Edition

MODULE 2: GAMETOGENESIS

Unit 1: Gametes Interaction during fertilization

CONTENTS

1.0 INTRODUCTION

The sperm and the ovum are highly specialized haploid cells, that are formed through a complex set of cell divisions, differentiation and maturation steps called gametogenesis. In mammals, the life history of germ cells begins during embryonic life with the extragonadal appearance of primordial germ cells and the colonization of the genital ridges, where germ cells associate with somatic cells; it continues with their multiplication, growth and maturation, and ends at fertilization. The oocyte undergoes a tremendous growth and stockpiles a large amount of macromolecules. In contrast, the spermatozoon is an extremely streamlined, highly polarized cell, containing only elements for essential functions such as motility and a few critical enzymes to ensure efficient transmission of the paternal genome to the oocyte at fertilization. The union of sperm and egg is an extraordinary cell fusion event that gives rise to an original individual and triggers a very sophisticated developmental program.

2.0 OBJECTIVES

1 Define Gametogenesis with relevant examples in animal

2 Explain the development of spem and egg in gamete formation

3 Explain the various steps involve in gametes interaction

3.0 MAIN CONTENT

3.1 Gametogenesis

Gametogenesis is the process of formation of gametes from the germ cells in the testes and ovaries. The diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes. Depending on the biological life cycle of the organism, gametogenesis occurs by meiotic division of diploid gametocytes into various gametes or by mitotic division of haploid gametogenous cells.

At the stage of spermatogonia and oogonia, germ cells multiply by mitosis, subsequently, they undergo meiosis to become the fully matured gametes. Meiosis involves two consecutive divisions with only one DNA replication cycle and results in the production of haploid gametes. The pairing of homologous chromosomes is unique to meiosis. The first meiotic division enhances genetic variability by independent assortment (random distribution) of the different maternal and paternal homologs and by crossing-over between homologous non sister chromatids. The second meiotic division resembles a normal mitosis without DNA replication. Meiosis is dominated by prophase of the first meiotic division, that occupies a long period and is divided into 5 sequential stages—leptotene, zygotene, pachytene, diplotene and diakinesis—defined by morphological criteria**.**

3.1.1 Development of the Sperm

Spermatogonia develop from primordial germ cells that migrate into the undifferentiated gonad early in embryogenesis. In the wall of the forming seminiferous tubules two different kinds of cells are already clearly distinguishable at this stage: the supporting Sertoli cells, thought to derive from the surface epithelium of the genital ridge, and the spermatogonia, derived from primordial germ cells. During the fetal period, spermatogonia enter a dormant or arrested phase of development, and the Sertoli cells constitute most of the seminiferous epithelium. At sexual maturity, spermatogonia begin to increase in number. It is at this time that spermatogenesis really starts since this term usually refers to the sequence of events by which spermatogonia are transformed into spermatozoa. Spermatogenesis includes three main phases: spermatogonial multiplication, meiosis, and spermiogenesis. The cells at these different stages are called spermatogonia, spermatocytes and spermatids, respectively. In men spermatogonial multiplication occurs through regular intervals of 16 days. Spermatogonia can be divided in two main types, the noncycling ones (Ao), and those that will differentiate into spermatocytes after six mitotic divisions. Type (Ao) spermatogonia are able to repopulate the seminiferous epithelium when cycling spermatogonia decrease in number. The cycling spermatogonia provide the stem cell population for meiosis, which begins when preleptotene spermatocytes start DNA replication. Each primary spermatocyte, actually the largest germ cell in the tubules, undergoes the first meiotic division, forming two secondary spermatocytes that are about half the size of the primary spermatocyte. Subsequently, these two secondary spermatocytes undergo the second meiotic division, forming four haploid spermatids that are about half the size of secondary spermatocytes. The spermatids are gradually transformed into mature sperm by an extensive process of differentiation known as spermiogenesis; finally the differentiated sperm is released from

the seminiferous epithelium and becomes a free spermatozoon, a process called spermiation. In human the process of spermatogenesis extends over a period of about 60 days.

An intriguing and unique feature of spermatogenesis is that the developing male germ cells fail to complete cytoplasmic division during mitosis and meiosis, so that all the daughter cells, except for the least differentiated spermatogonia remain connected by cytoplasmic bridges. These bridges persist until the very end of sperm differentiation. It has indeed been shown that sperm nuclei are haploid but sperm cell differentiation is directed by the diploid genome.

The sperm cell consists of two morphologically and functionally distinct regions. A head containing an unusually highly condensed haploid nucleus and a tail propelling the sperm to the egg helping to enter through the egg coat. The DNA in the nucleus is inactive and extremely tightly packed as a result of its association with highly positively charged proteins, the protamines, instead of histones, which have been displaced during spermiogenesis. The head also contains a membrane-limited organelle, the acrosome, whose contents are thought to have a function in the penetration of the spermatozoon into the ovum. A variety of enzymes, including proteinases, glycosidases, phosphatases, arylsulfatases and phospholipases are present in the acrosome and in the preacrosomal membrane.

Sperm released from the seminiferous epithelium are not capable of fertilization. The long series of changes that the spermatozoa endure between casting off from the Sertoli cells, and fusing with the egg, i. e. till the fully functional state of the spermatozoa, is referred to as sperm maturation. Throughout their journey from testis to the proximity of the ovum, sperm cells are suspended in transudations and secretions of the male and female genital tracts. The chemical and physical nature of this medium progressively changes and the spermatozoa also change structurally, chemically and behaviourally. Several proteins from testicular and epididymal environment have been shown to bind to specific regions of the sperm surface that are involved in sperm maturation and in part of the gamete recognition process. Biochemical modifications of some sperm surface components are also involved, as well as an increase in interchromatin disulfide bonds for chromatin condensation during this travel which lasts several days. Sperm cells develop gradual motility and ability to bind and penetrate eggs as they progress from the caput to the cauda epididymidis.

Ejaculates contain complex secretions from the accessory glands—the Cowper's gland, prostate, and seminal vesicles—which, contain a variety of energy substrates, hormones, nonenzymatic and enzymatic proteins and various ions. The last step of sperm cell maturation is called capacitation, which is a functional term used to indicate the changes in mammalian spermatozoa that must occur in the female genital tract, or during *in vitro* incubations, as preparation for the acrosome reaction (see below). Capacitation is a reversible reaction which does not involve morphological changes; it is accompanied by a hyperactivation of sperm motility: the flagellar beat pattern changes from a low amplitude favoring progressive motility to a high amplitude with little progression. Capacitation includes a lowering of the cholesterol/phospholipid ratio in the sperm membrane, a loss of sperm surface coating components (loss of the antifertility factor from human seminal plasma) probably involved with the acquisition of zona pellucida binding activity, and the phosphorylation of some plasma membrane proteins.

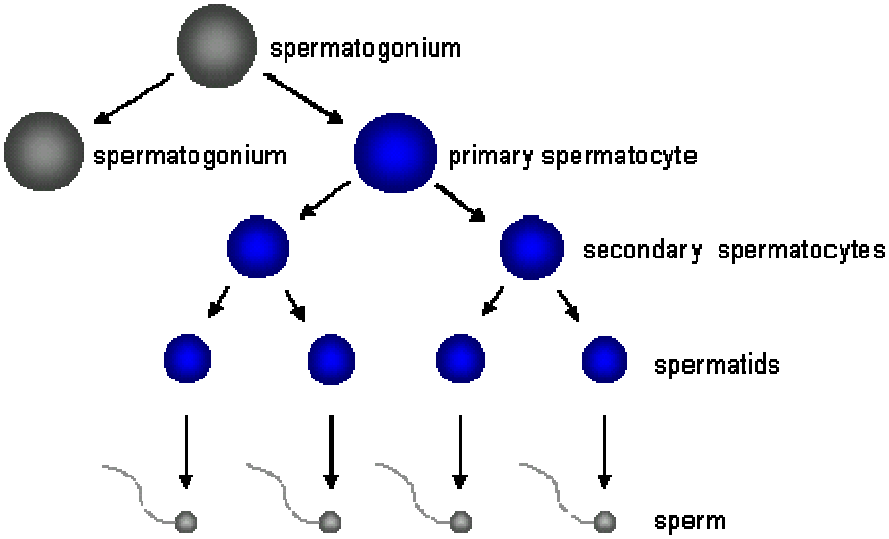

Figure 2: Sperm formation

3.1.2 Development of the egg

The unfertilized egg is the end product of a discontinuous course called oogenesis, that begins during fetal development and ends in the sexually mature adult. Oogonia develop from primordial germ cells in the ovary, and multiply by mitosis only during the fetal life. By the 5th month of gestation in women, all germ cells stop proliferation and enter meiosis but pause at the prophase of the first meiotic division; arrest may last from 12 to 50 years. The spherical dictyate oocytes become enclosed within a few squamous somatic cells to form what is called primordial follicles; the oocytes are then called primordial oocytes. It is in this period of life that the ovary contains the highest number of oocytes—about one to two millions—since many of them will degenerate before puberty and through the reproductive life of a woman. At puberty only about 300'000 primary oocytes remain. They represent a stockpile from which a few are selected at any given time for development towards preovulatory follicles containing fully grown oocytes. The oocyte and its surrounding follicle grow coordinately, rather than simultaneously. Indeed, the oocyte completes its growth before the formation of the follicular antrum, i.e., the major part of follicular growth occurs after the oocyte has stopped growing. The oocyte growth results in the formation of one of the largest cells in the body. During this period its volume increases more than 300-fold; from a diameter of about 20 µm at the primordial stage, the oocyte reaches a maximal diameter of about 120 µm. Completion of growth takes approximately 2.5-3 months. The nucleus of the growing oocyte, called the germinal vesicle, is particularly apparent and contains a very refractile nucleolus. During oocyte growth an extracellular coat develops around the plasma membrane. This acellular layer, called the zona pellucida (ZP), is constituted by three major glycoproteins (ZP1, ZP2 and ZP3) that are assembled into long, interconnected filaments to form a relatively porous coat about 5 µm thick.

From the time of puberty, one developing follicle is stimulated each month to mature to complete development and to ovulate. This means that during the approximately 40 years of a woman's reproductive life, only 400 to 500 eggs will have been released. All the rest will have degenerated. The LH surge released by the pituitary will, each month, activate one antral follicle to mature. Fully grown primary oocytes enclosed in Graafian follicles resume meiosis just prior to ovulation. This phase is called meiotic maturation. The first

macroscopically observable event of meiotic maturation is the dissolution of the nuclear membrane; this process is referred to as germinal vesicle breakdown or GVBD. The oocyte then progresses through metaphase, anaphase, and telophase of the first division, emits the first polar body, and, without stopping, enters the second division up to metaphase. It is around this time that ovulation occurs, by rupture of the follicle wall at the surface of the ovary. In the oviduct, the oocyte remains at metaphase II until it is triggered by fertilization to complete the second meiotic division.

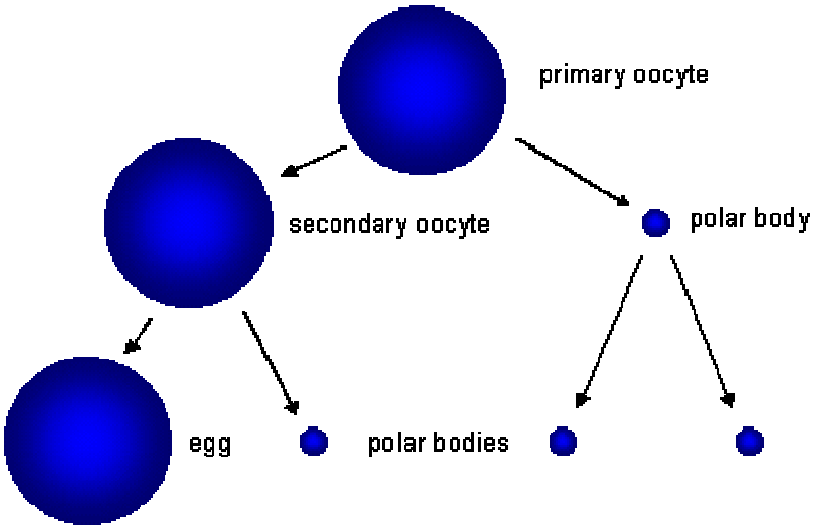

FIGURE 3: Formation of egg

4.0 Gamete Interaction

In comparison to the large number of spermatozoa laid down in the vagina at coitus, only very few sperm cells reach the ampulla and are found in the proximity of the egg. Although sperm attraction to follicular factor(s) has been claimed, sperm chemotaxis in mammalian fertilization has not been demonstrated. The leading role in the sperm-egg encounter is played by the molecular organization of their surfaces, and abundant evidence suggests that the species-specific gamete recognition and binding is mediated by receptor molecules at the gamete surface.

Initial contact between gametes occurs when the sperm attach to the unfertilized extracellular coat or zona pellucida. Capacitated, acrosome-intact sperm are capable of binding to the zona pellucida via the plasma membrane of the sperm head. Binding is an important prerequisite step for zona penetration because it initiates events that culminate in induction of the acrosome reaction.

One of the components of the zona pellucida (ZP3) representing the primary sperm receptor, is responsible for both the sperm-binding activity and the ability to induce a complete acrosome reaction. Acrosome-intact sperm bind to ZP3 in a relatively species specific manner, this gamete recognition and binding is mediated by carbohydrates and not by the polypeptide chain. Many sperm are released from the zona pellucida after undergoing the acrosome reaction, yet maintenance of sperm binding is achieved by interaction of acrosome-reacted sperm with ZP2; thus, ZP2 serves as a secondary receptor.

Putative ZP-binding-glycoproteins of spermatozoa have been recognized in various species. Several egg-binding proteins are envisaged on the sperm membrane that impart species specificity. The postulated candidates are the following: a 95kDa protein (p95SPERM) showing a tyrosine kinase activity that is stimulated on binding and whose activation requires aggregation, a 56kDa protein (p56) of unknown function, an antigen designated p200/220 (whose monoclonal antibody is named M42) necessary for zona-induced acrosomal reaction, another related antigen the SAA-1 antigen detected on all mammalian sperm acrosomes, a ß-1,4-galactosyl-transferase mediating fertilization by binding oligosaccharide residues on zona pellucida glycoprotein. Many evidence suggest also the possible involvement of protease inhibitor sites and mannosidase sites or of other molecules called spermadhesins showing features of serine proteases having lectin-like activity.

Proteolytic enzymes appear to participate in multiple phases of mammalian fertilization, including acrosome reaction, sperm binding to zona pellucida (ZP), ZP penetration and zona reaction, however, the enzymes involved have not been completely identified. A role for sperm proacrosin and acrosin, the best characterized sperm protease, in ZP binding and penetration has been postulated. Several observations suggest that the plasminogen activator/plasmin system might play a role in mammalian fertilization. First, both mouse gametes express plasminogen-dependent proteolytic activities: ovulated eggs contain and secrete tissue-type PA (t-PA) and ejaculated spermatozoa exhibit urokinase-type PA (uPA). Second, t-PA is significantly higher in follicular fluids and granulosa cells from follicles containing oocytes that can be fertilized *in vitro* compared to follicles containing oocytes that fail to fertilize. Third, the addition of plasminogen to the fertilization medium increases the frequency of eggs fertilized *in vitro*.

Sperm cells must undergo the acrosome reaction before they can penetrate the zona pellucida and fuse with the egg plasma membrane. Acrosome reaction progresses from multiple fusion-points between the plasma and outer acrosomal membranes, which expose the inner acrosomal membrane and the acrosomal contents (enzymes), to complete vesiculation and loss of the integrity of the acrosome. The acrosome reaction bears a strong resemblance to ligand-mediated exocytotic reactions in somatic cells proceeding through an intracellular signal transduction system, it involves the participation of a Gi protein, of phospholipase C and of protein kinase C. In addition, an increase in intracellular calcium is concomitant with the induction of acrosomal loss. Acrosome reaction can be induced by biological agents such as follicular fluid (progesterone), cumulus cells or zonae pellucidae or by physiochemical agents such as calcium ionophores, lysophosphatidylcholine and electropermeabilization or by the aggregation of zona binding sites on the sperm heads.

4.1 Sperm-egg fusion

After sperm entry into the perivitelline space, the final stages of sperm-egg interaction include the binding and fusion of the sperm and egg plasma membranes, and entry of the sperm into the egg. Sperm binding to the egg surface occurs on the lateral face of the head, with the firm point of attachment between the sperm and egg plasma membranes occurring at the equatorial segment. Little is known concerning the sperm and egg surface complementary molecules (binding sites) that participate in gamete plasma membranes fusion in mammals. It has been recently shown that a sperm surface protein (PH-30, a guinea-pig sperm antigen), known to be involved in sperm-egg fusion, shares biochemical characteristics with viral fusion proteins and contains an integrin ligand domain. These results suggest that an integrin-mediated adhesion event takes place and leads to fusion.

Fusion of a single sperm sets in motion a series of egg reactions to prevent additional sperm entry, thus avoiding the lethal consequences of polyspermy. Egg cortical reaction takes place soon after fusion, causing the zona pellucida to become " hardened " and refractory to both binding and penetration of supernumerary sperm. Zona binding is prevented by the inactivation of the sperm primary receptor (and acrosome inducer) ZP3 and zona penetration is stopped through modification of the sperm secondary receptor ZP2. The cortical reaction involves the exocytosis of cortical granules and the release of their enzymatic content into the perivitelline space. The oligosaccharides of ZP3 responsible for gamete recognition and adhesion are modified by cortical granule glycosidase(s) and the glycoprotein ZP2 undergoes limited proteolysis making the zona pellucida more insoluble and " hardened ", preventing the maintenance of binding of acrosome-reacted sperm to the zona pellucida. It has been suggested that the oocyte plasminogen activator may participate in this proteolytic process although the evidence is poor.

4.2 Egg activation and pronuclei formation

Gamete fusion triggers responses within the egg that culminate in the activation of the embryonic developmental program. Activation may also be induced partheno genetically under various physical or chemical stimuli, in all cases, calcium is an obligatory mediator. In mammals, sperm may cause both a persistent production of inositol trisphosphate (InsP3) and an increase in calcium permeability of the plasma membrane to maintain internal calcium oscillations. The early calcium increase induces cortical granule exocytosis (cortical reaction), which involves a signal transduction system that is similar to that of somatic cells, and that leads to the hardening of the zona pellucida. Activation leads to the resumption of the cell cycle: the second meiotic division is achieved, by the extrusion of the second polar body and the egg enters into interphase with formation of pronuclei. Pronuclear formation takes place a few hours after fertilization, and requires a calcium increase and a cytoplasmic alcalinization of the zygote. Following anaphase II, the egg chromosomes remaining in the cytoplasm disperse and the female pronucleus forms. Similarly, after cell fusion, the sperm nucleus is decondensed and transformed into a male pronucleus. The biochemical transitions responsible for the remodelling of the sperm nucleus consist of changes in the majority of sperm specific chromatin proteins and the acquisition of chromosomal proteins which induce a chromatin conformation compatible with fusion of male and female pronuclei. Maternal chromatin and sperm pronuclear development are regulated by common egg cytoplasmic factors involved in the regulation of the cell cycle and dependent on oocyte maturation. The pronuclear development in fertilized eggs is known to proceed through a series of transformations, which restore the transcriptional competence of the inactive gamete chromatin and re-establish the functional diploid genome of the embryo. Two stages of decondensation are observed: i) a very rapid chromatin expansion dependent on egg nucleoplasmin, and ii) a slow membrane-dependent decondensation involving protein migration into the nucleus reliant on nuclear envelope formation recruited from maternal pool.

The two pronuclei move towards the egg center, and spermaster increases in size during their migration. The end result of the migration of the pronuclei is their juxtaposition, following pronuclear envelope breakdown, giving rise to a group of chromosomes for the ensuing division. The spatial organization of microtubule arrays in a cell is largely dependent on organizing centers, the centrosomes. The proximal centriole of the sperm and its centrosomal material between apposed pronuclei are involved in the fertilization events. Human centrioles as those of other animals except the mouse are paternally derived.

Eventually, there is an intermixing of the maternally and paternally derived chromosomes to establish the genome of the embryo and hence the process of fertilization can be considered as concluded**.**

5.0 CONCLUSION

In this unit, you learnt about the formation of gametes from the developed sperm and egg. Gamete interraction through sperm-egg fusion and egg activation and pronuclei formation which lead to fertilization.This is a brief reviews of some aspects of gametogenesis and fertilization in mammals.

6.0 SUMMARY

Gametogenesis is the process of formation of gametes from the germ cells in the testes and ovaries. The diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes. Depending on the biological life cycle of the organism, gametogenesis occurs by meiotic division of diploid gametocytes into various gametes or by mitotic division of haploid gametogenous cells.

7.0 TUTOR-MARKED ASSIGNMENT

1 Define Gametogenesis with relevant examples

2 Explain the development of spem and egg in gamete formation

3 Explain the various steps involve in gametes interaction

8.0 REFERENCES/ FURTHER READING

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Unit 2 : The Determination of germ cell in different group of animals

CONTENT

1.0 INTRODUCTION

Gametogenesis, the processes by which the sperm and the egg are formed. Germ cells provide the continuity of life between generations, and the mitotic ancestors of our own germ cells once resided in the gonads of reptiles, amphibians, fishes, and invertebrates.

In many animals, such as insects, roundworms, and vertebrates, there is a clear and early separation of germ cells from somatic cell types. In several other animal phyla (and throughout the entire plant kingdom), this division is not as well established. In these species (which include cnidarians, flatworms, and tunicates), somatic cells can readily become germ cells even in adult organisms. The zooids, buds, and polyps of many invertebrate phyla testify to the ability of somatic cells to give rise to new individuals.

In those organisms in which there is an established **germ line** that separates from the somatic cells early in development, the germ cells do not arise within the gonad itself. Rather, their precursors the **primordial germ cells** (**PGCs**) arise elsewhere and migrate into the developing gonads. The first step in gametogenesis, then, involves forming the PGCs and getting them into the genital ridge as the gonad is forming. Our discussion of the germ line will include

1. The formation of the germ plasm and the determination of the PGCs

2. The migration of the PGCs into the developing gonads

3. The process of meiosis and the modifications of meiosis for forming sperm and eggs

4. The differentiation of the sperm and egg

5. The hormonal control of gamete maturation and ovulation

2.0 OBJECTIVE

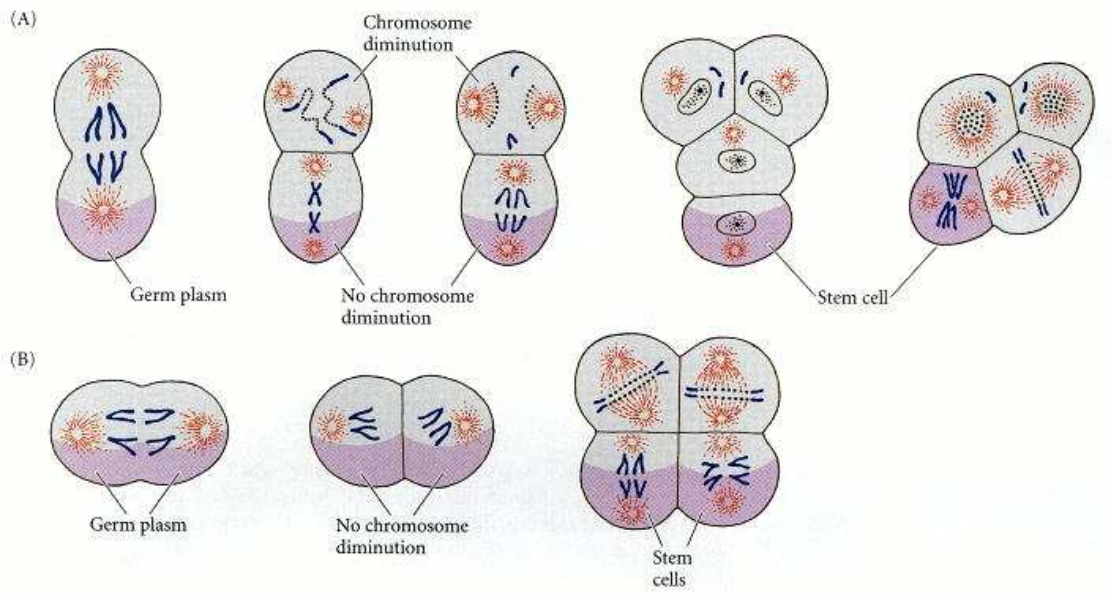
3.0 MAIN CONTENT

3.1 Germ Plasm and the Determination of the Primordial Germ Cells

All sexually reproducing organisms arise from the fusion of gametes sperm and eggs. All gametes arise from the primordial germ cells. In most animal species, the determination of the primordial germ cells is brought about by the cytoplasmic localization of specific proteins and mRNAs in certain cells of the early embryo (mammals being a major exception to this general rule). These cytoplasmic components are referred to as the **germ plasm**.

3.2.1 Germ cell determination in nematodes

This nematode has only two chromosomes per haploid cell, allowing for detailed observations of the individual chromosomes. The cleavage plane of the first embryonic division is unusual in that it is equatorial, separating the animal half from the vegetal half of the zygote. However, is the behavior of the chromosomes in the subsequent division of these first two blastomeres.

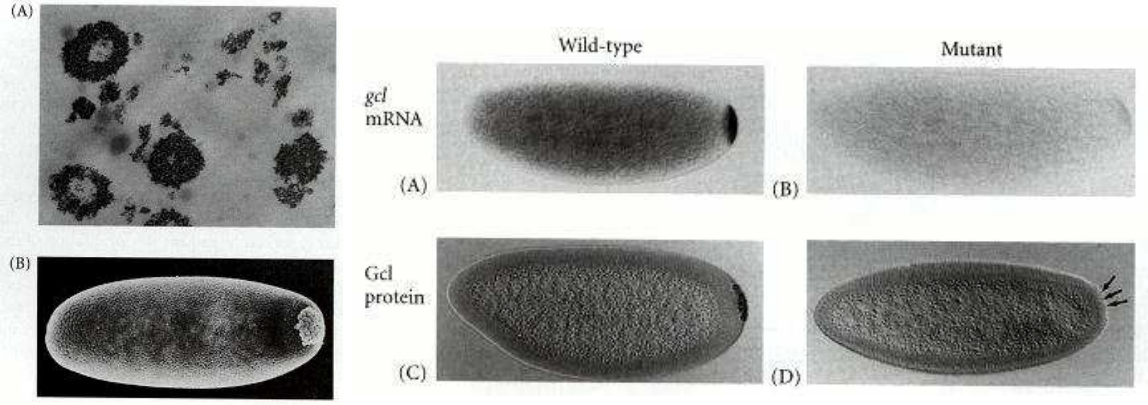


The ends of the chromosomes in the animal-derived blastomere fragment into dozens of pieces just before this cell divides. This phenomenon is called **chromosome diminution**, because only a portion of the original chromosome survives. Numerous genes are lost in these cells when the chromosomes fragment, and these genes are not included in the newly formed nucle. Meanwhile, in the vegetal blastomere, the chromosomes remain normal. During second cleavage, the animal cell splits meridionally while the vegetal cell again divides equatorially. Both vegetally derived cells have normal chromosomes. However, the chromosomes of the more animally located of these two vegetal blastomeres fragment before the third cleavage. Thus, at the 4-cell stage, only one cell the most vegetal contains a full set of genes. At successive cleavages, nuclei with diminished chromosomes are given off from this vegetalmost line, until the 16-cell stage, when there are only two cells with undiminished chromosomes. One of these two blastomeres gives rise to the germ cells; the other eventually undergoes chromosome diminution and forms more somatic cells. The chromosomes are kept intact only in those cells destined to form the germ line. If this were not the case, the genetic information would degenerate from one generation to the next. The cells that have undergone chromosome diminution generate the somatic cells.

In the nematode *C. elegans*, the germ line precursor cell is the P4 blastomere. The P-granules enter this cell, and they appear critical for instructing it to become the germ line precursor. The components of the P-granules remain largely uncharacterized, but they appear to contain several transcriptional inhibitors and RNA-binding proteins, including homologues of the *Drosophila* Vasa and Nanos proteins, whose function we will see below.

3.1.2.Germ cell determination in insects

In *Drosophila*, PGCs form as a group of cells (**pole cells**) at the posterior pole of the cellularizing blastoderm. These nuclei migrate into the posterior region at the ninth nuclear division, and they become surrounded by the **pole plasm**, a complex collection of mitochondria, fibrils, and **polar granules.** If the pole cell nuclei are prevented from reaching the pole plasm, no germ cells will be made. Nature has provided confirmation of the importance of both pole plasm and its polar granules. One of the components of the pole plasm is the mRNA of the ***germ cell-less*** (***gcl***) gene. The wild-type *gcl* gene is transcribed in the nurse cells of the fly's ovary, and its mRNA is transported into the egg. Once inside the egg, it is transported to the posteriormost portion and resides within what will become the pole plasm. This message gets translated into protein during the early stages of cleavage *gcl*-encoded protein appears to enter the nucleus, and it is essential for pole cell production. Flies with mutations of this gene lack germ cells.



Oskar appears to be the critical protein of this group, since injection of *oskar* mRNA into ectopic sites of the embryo will cause the nuclei in those areas to form germ cells. The genes that restrict Oskar to the posterior pole are also necessary for germ cell formation. Moreover, Oskar appears to be the limiting step of germ cell formation, since adding more *oskar* message to the oocyte causes the formation of more germ cells. Oskar functions by causing the localization of the proteins and RNAs necessary for germ cell formation. One of these RNAs is the *nanos* message, whose product is essential for posterior segment formation. **Nanos** is also essential for germ cell formation. Pole cells lacking Nanos do not migrate into the gonads and fail to become gametes.

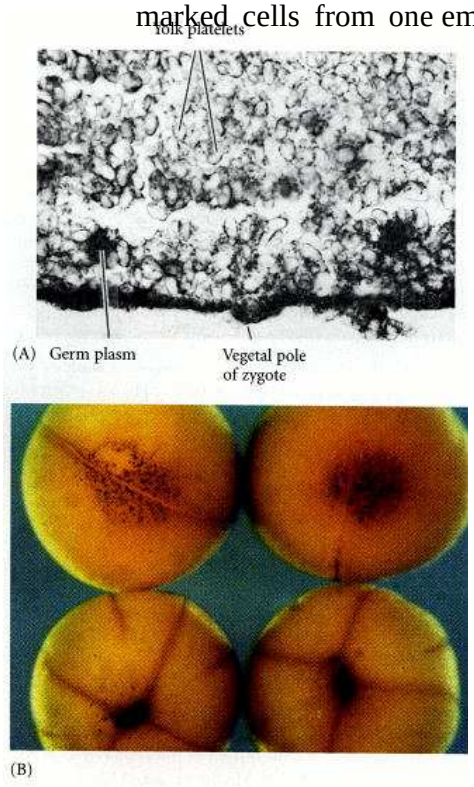
Nanos appears to be important in preventing mitosis and transcription during germ cell development. Another one of these RNAs encodes **Vasa**, an RNA-binding protein. The mRNAs for this protein are seen in the germ plasm of many species.

A third germ plasm component was a big surprise: **mitochondrial ribosomal RNA** (**mtrRNA**). Moreover, in normal fly eggs, the small and large mitochondrial rRNAs are located outside the mitochondria solely in the pole plasm of cleavage-stage embryos. Here, they appear as components of the polar granules. While mitochondrial RNA is involved in directing the formation of the pole cells, it does not enter them.

A fourth component of *Drosophila* pole plasm (and one that becomes localized in the polar granules) is a nontranslatable RNA called **polar granule component** (**Pgc**). While its exact function remains unknown, the pole cells of transgenic female flies making antisense RNA against Pgc fail to migrate to the gonads.

3.1.3 Germ cell Determination in amphibian

Cytoplasmic localization of germ cell determinants has also been observed in vertebrate embryos. Scientists showed that the vegetal region of fertilized frog eggs contains material with staining properties similar to those of *Drosophila* pole plasm . He was able to trace this cortical cytoplasm into the few cells in the presumptive endoderm that would normally migrate into the genital ridge. By transplanting genetically marked cells from one embryo into another of a differently marked strain, They showed that these cells are the primordial germ cell precursors.



They found that the germ plasm of unfertilized eggs consists of tiny "islands" that appear to be tethered to the yolk mass near the vegetal cortex. These germ plasm islands move with the vegetal yolk mass during the cortical rotation of fertilization. After the rotation, the islands are released from the yolk mass and begin fusing together and migrating to the vegetal pole. Their aggregation depends on microtubules, and the movement of these clusters to the vegetal pole is dependent on a kinesin- like protein that may act as the motor for germ plasm movement. Later, periodic contractions of the vegetal cell surface also appear to push this germ plasm along the cleavage furrows of the newly formed blastomeres, enabling it to enter the embryo.

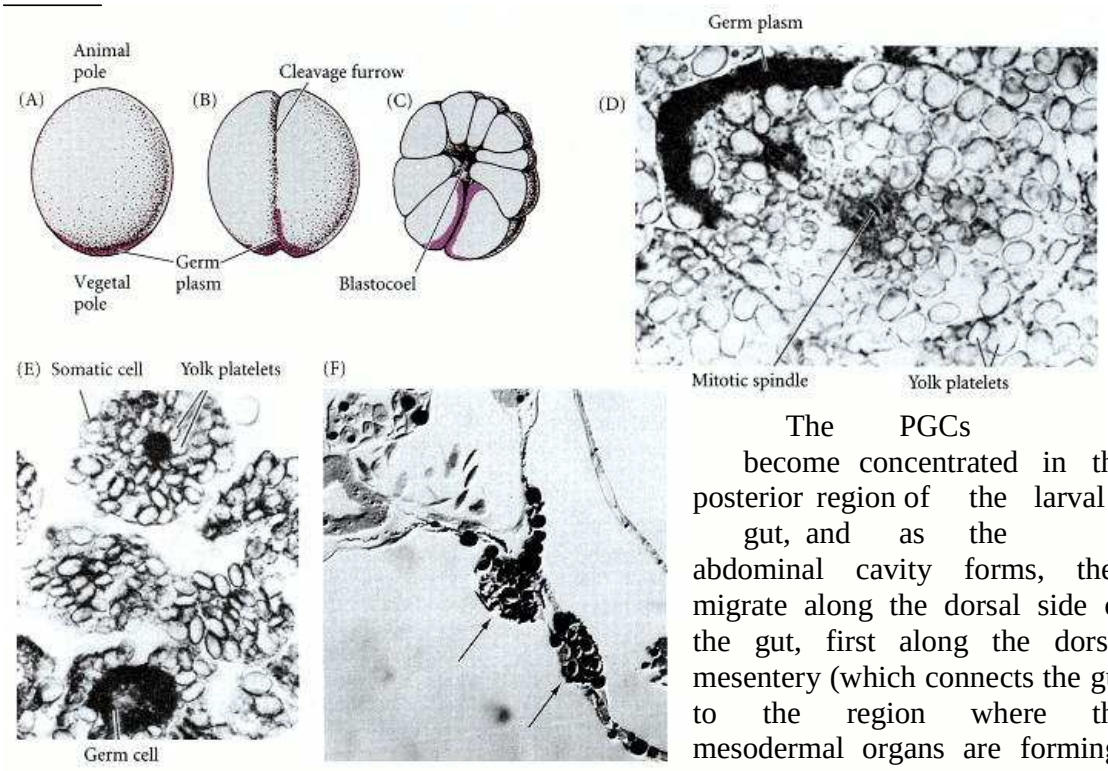
When ultraviolet light is applied to the vegetal surface (but nowhere else) of frog embryos, the resulting frogs are normal but lack germ cells in their gonads. Very few primordial germ cells reach the gonads; those few that do have about one- tenth the volume of normal PGCs and have aberrantly shaped nuclei. The *Xenopus* homologues of *nanos* and *vasa* are specifically localized to this region So, like the *Drosophila* pole plasm, the cytoplasm from the vegetal region of the frog zygote contains the determinants for germ cell formation. Moreover, several of the components are the same.

The components of the germ plasm have not all been catalogued. Indeed, in the birds and mammals, such a list has hardly even been started. Moreover, we still do not know the functions of the proteins and nontranslated RNAs found in the germ plasm. One hypothesis is that the components of the germ plasm inhibit both transcription and translation, thereby preventing the cells containing it from differentiating into anything else. According this hypothesis, the cells become germ cells because they are forbidden to become any other type of cell.

Germ Cell Migration

3.1.4 Germ cell migration in amphibians

The germ plasm of anuran amphibians (frogs and toads) collects around the vegetal pole in the zygote. During cleavage, this material is brought upward through the yolky cytoplasm, and eventually becomes associated with the endodermal cells lining the floor of the blastocoels.



The PGCs become concentrated in the posterior region of the larval gut, and as the abdominal cavity forms, they migrate along the dorsal side of the gut, first along the dorsal mesentery (which connects the gut to the region where the mesodermal organs are forming) and then along the abdominal wall and into the genital ridges.

They migrate up this tissue until they reach the developing gonads. *Xenopus* PGCs move by extruding a single filopodium and then streaming their yolky cytoplasm into that filopodium while retracting their "tail." Contact guidance in this migration seems likely, as both the PGCs and the extracellular matrix over which they migrate are oriented in the direction of migration. Furthermore, PGC adhesion and migration can be inhibited if the mesentery is treated with antibodies against *Xenopus* fibronectin . Thus, the pathway for germ cell migration in these frogs appears to be composed of an oriented fibronectin-containing extracellular matrix. The fibrils over which the PGCs travel lose this polarity soon after migration has ended.* As they migrate, *Xenopus* PGCs divide about three times, and approximately 30 PGCs colonize the gonads. These will divide to form the germ cells.

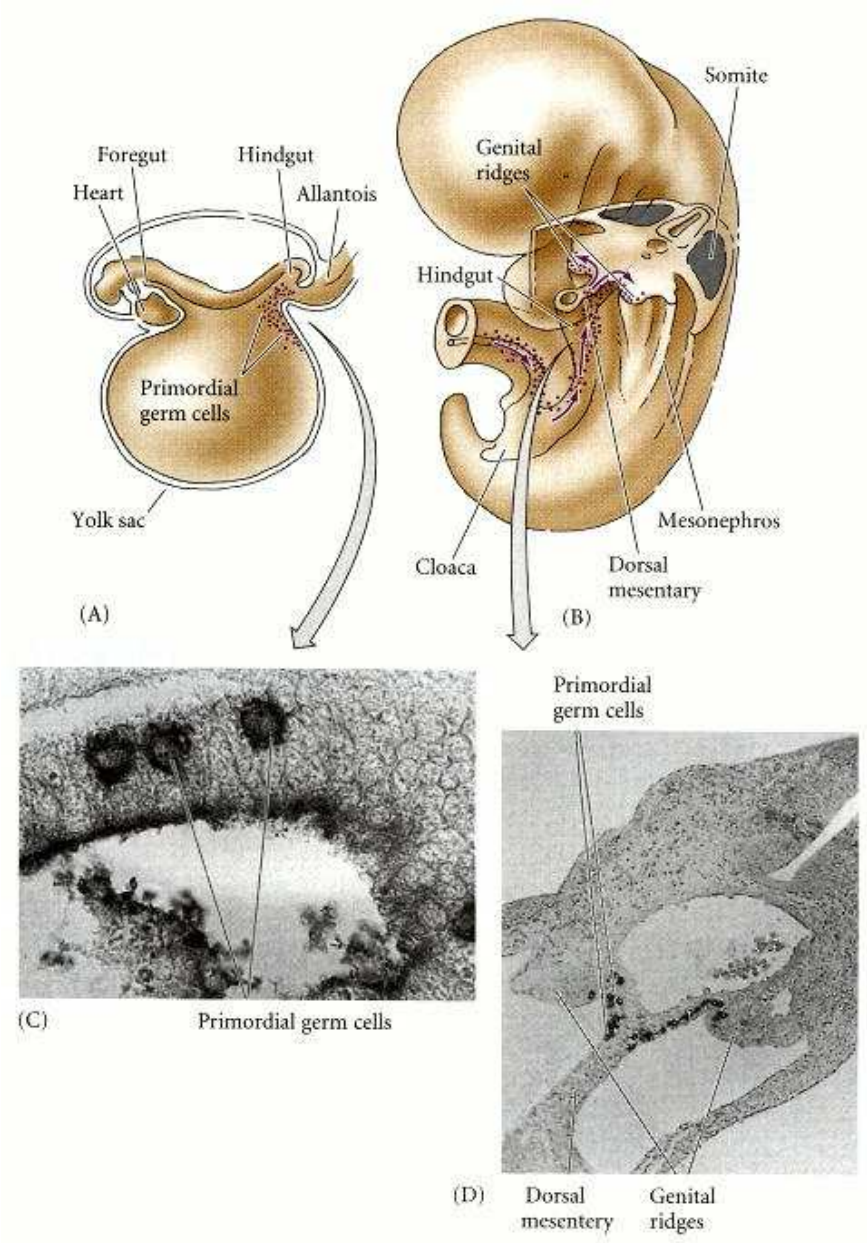
The primordial germ cells of urodele amphibians (salamanders) have an apparently different origin, which has been traced by reciprocal transplantation experiments to the regions of the mesoderm that involute through the ventrolateral lips of the blastopore. Moreover, there does not seem to be any particular localized "germ plasm" in salamander eggs. Rather, the interaction of the dorsal endoderm cells and animal hemisphere cells creates the conditions needed to form germ cells in the areas that involute through the ventrolateral lips. So in salamanders, the PGCs are formed by induction within the mesodermal region and presumably follow a different path into the gonads.

3.1.5 Germ cell migration in mammal

There is no obvious germ plasm in mammals, and mammalian germ cells are not morphologically distinct during early development. However, by using monoclonal antibodies that recognize cell surface differences between the PGCs and their surrounding cells, localized this region to the area that becomes extraembryonic mesoderm just posterior to the primitive streak of the 7-day mouse embryo. Here, about eight large, alkaline phosphatase-staining cells are seen. If this region is removed, the remaining embryo becomes devoid of germ cells, while the isolated region develops a large number of PGCs.

In normal mouse embryos, the germ cell precursors migrate from the extraembryonic mesoderm back into the embryo, by way of the allantois. The route of mammalian PGC migration from the allantois resembles that of anuran PGC migration. After collecting at the allantois (by day 7.5 in the mouse: The PGCs migrate to the adjacent yolk sac. By this time, they have already split into two populations that will migrate to either the right or the left genital ridge. The PGCs then move caudally from the yolk sac through the newly formed hindgut and up the dorsal mesentery into the genital ridge. Most of the PGCs have reached the developing mouse gonad by the eleventh day after fertilization. During this trek, they have proliferated from an initial population of 10 100 cells to the 2500 5000 PGCs present in the gonads by day 12.

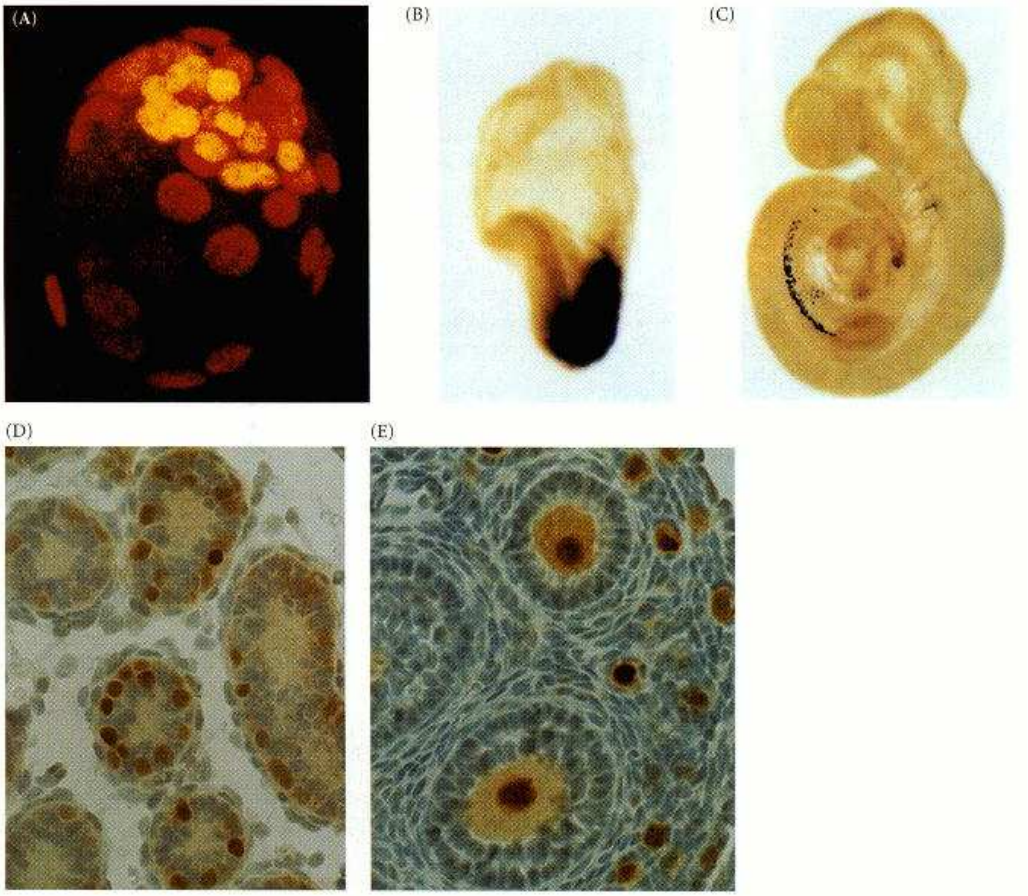
Like the PGCs of *Xenopus,* mammalian PGCs appear to be closely associated with the cells over which they migrate, and they move by extending filopodia over the underlying cell surfaces. These cells are also capable of penetrating cell monolayers and migrating through the cell sheets. The mechanism by which the PGCs know the route of this journey is still unknown.



Fibronectin is likely to be an important substrate for PGC migration, and germ cells that lack the integrin receptor for such extracellular matrix proteins cannot migrate to the gonads. Directionality may be provided by a gradient of soluble protein. In vitro evidence suggests that the genital ridges of 10.5-day mouse embryos secrete a diffusible TGF-β1-like protein that is capable of attracting mouse PGCs. Whether the genital ridge is able to provide such cues in vivo still must be tested.

Although no germ plasm has been found in mammals, the retention of totipotency has been correlated with the expression of a nuclear transcription factor, **Oct4**. This factor is expressed in all of the early-cleavage blastomere nuclei, but its expression becomes restricted to the inner cell mass.

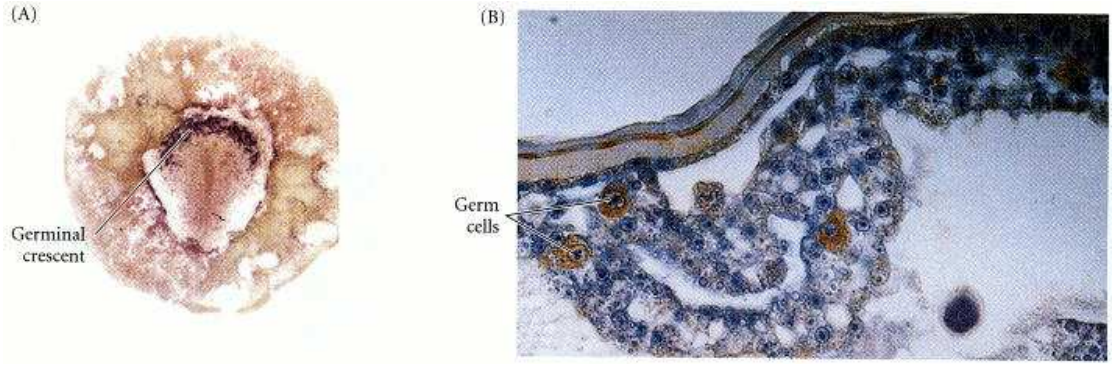
During gastrulation, it becomes expressed solely in those posterior epiblast cells thought to give rise to the primordial germ cells. After that, this protein is seen only in the primordial germ cells and oocytes.



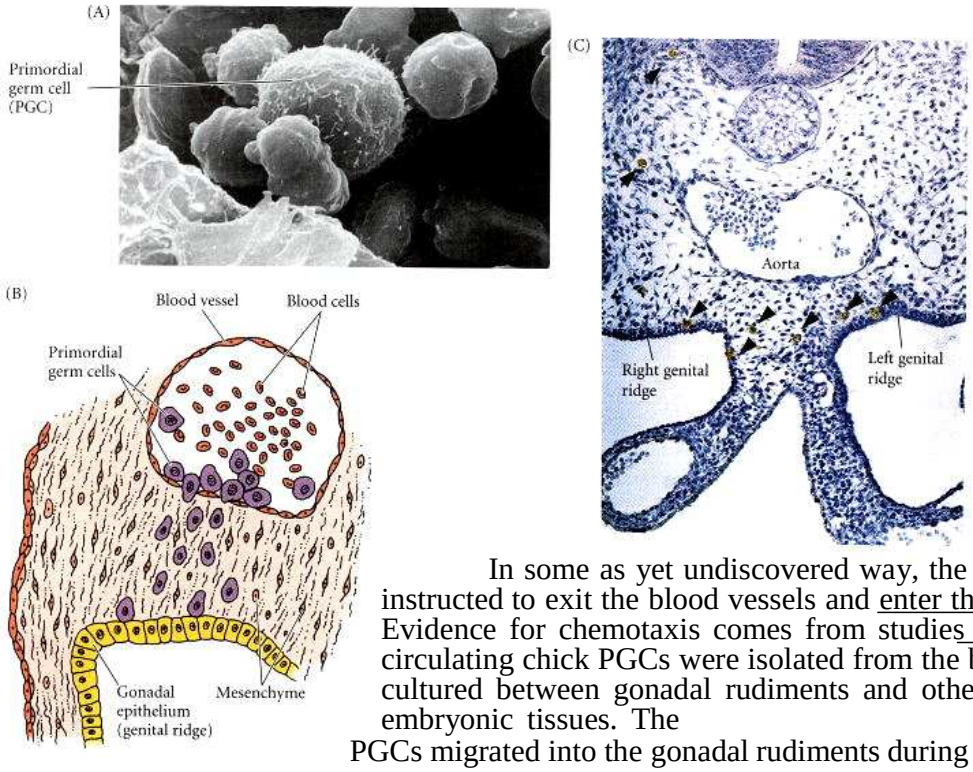
The proliferation of the PGCs appears to be promoted by stem cell factor, the same growth factor needed for the proliferation of neural crest-derived melanoblasts and hematopoietic stem cells. Stem cell factor is produced by the cells along the migration pathway and remains bound to their cell membranes. It appears that the presentation of this protein on cell membranes is important for its activity. Mice homozygous for the White mutation are deficient in germ cells (as well as melanocytes and blood cells) because their stem cells lack the receptor for stem cell factor. Mice homozygous for the Steel mutation have a similar phenotype, as they lack the ability to make this growth facto. The addition of stem cell factor to PGCs taken from 11-day mouse embryos will stimulate their proliferation for about 24 hours and appears to prevent programmed cell death that would otherwise occur

3.1.6 Germ cell migration in birds and reptiles

In birds and reptiles, the primordial germ cells are derived from epiblast cells that migrate from the central region of the area pellucida to a crescent-shaped zone in the hypoblast at the anterior border of the area pellucida. This extraembryonic region is called the **germinal crescent**, and the PGCs multiply there.

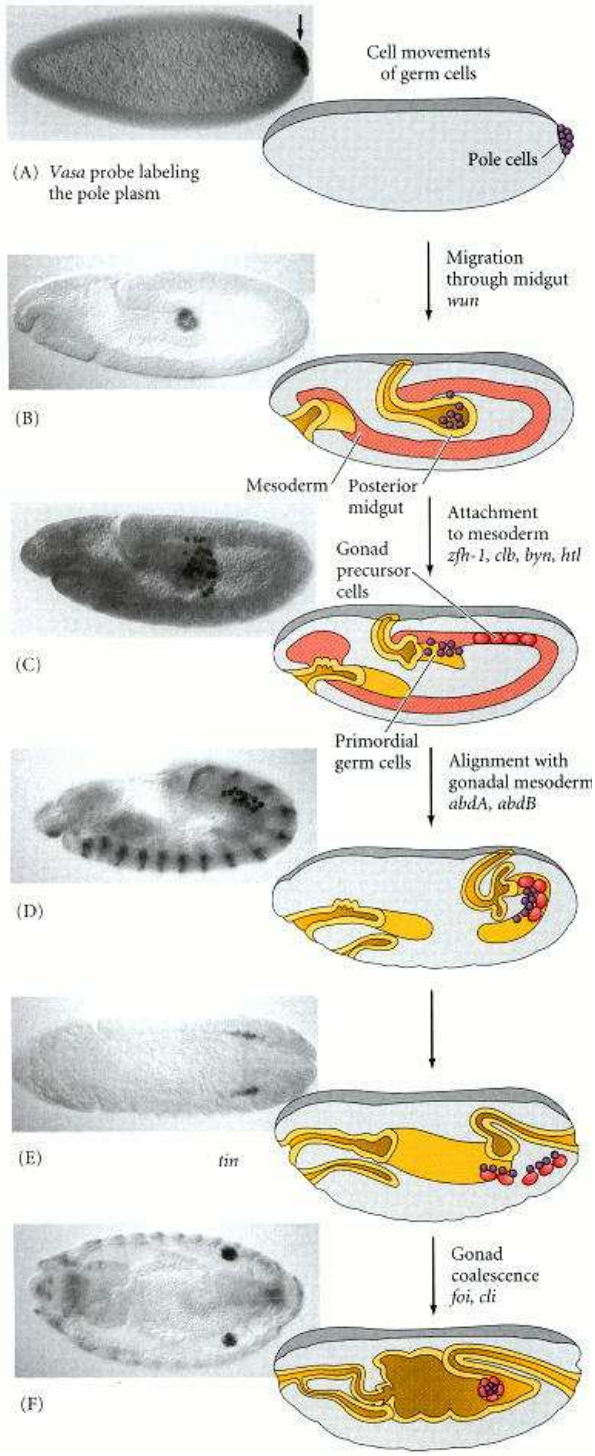


Unlike those of amphibians and mammals, the PGCs of birds and reptiles migrate to the gonads primarily by means of the bloodstream. When blood vessels form in the germinal crescent, the PGCs enter those vessels and are carried by the circulation to the region where the hindgut is forming. Here, they exit from the circulation, become associated with the mesentery, and migrate into the genital ridges. The PGCs of the germinal crescent appear to enter the blood vessels by **diapedesis**, a type of movement common to lymphocytes and macrophages that enables cells to squeeze between the endothelial cells of small blood vessels .



In some as yet undiscovered way, the PGCs are instructed to exit the blood vessels and enter the gonads. Evidence for chemotaxis comes from studies in which circulating chick PGCs were isolated from the blood and cultured between gonadal rudiments and other embryonic tissues. The PGCs migrated into the gonadal rudiments during a 3-hour incubation.

Germ cell migration in Drosophila



During *Drosophila* embryogenesis, the primordial germ cells move from the posterior pole to the gonads. The first step in this migration is a passive one, wherein the 30 40 pole cells are displaced into the posterior midgut by the movements of gastrulation. In the second step, the gut endoderm triggers active amoeboid movement in the PGCs, which travel through the blind end of the posterior midgut, migrating into the visceral mesoderm. In the third step, the PGCs split into two groups, each of which will becomes associated with a developing gonad primordium.

In the fourth step, the PGCs migrate to the gonads, which are derived from the lateral mesoderm of parasegments 10– 12. This step involves both attraction and repulsion. The product of the *wunen* gene appears to be responsible for directing the migration of the PGCs from the endoderm into the mesoderm. This protein is expressed in the endoderm immediately before PGC migration, and it repels the PGCs. In loss- of-function mutants of this gene, the PGCs wander randomly. Another gene needed for proper migration of the *Drosophila* PGCs is the product of the *columbus* gene. This protein is made in the mesodermal cells of the gonad, and it is necessary for the gonad to attract the PGCs. In loss-of-function mutants, the PGCs wanderrandomly from the endoderm, and if *columbus* is expressed in other tissues (such as the nerve cord), those tissues will attract the PGCs. In the last stage, the gonad coalesces around the germ cells, allowing the germ cells to divide and mature into gametes.

*This does not necessarily hold true for all anurans. In the frog *Rana pipiens*, the germ cells follow a similar route, but may be passive travelers along the mesentery rather than actively motile cells. The migration of fish PGCs follows a similar route, too, and there may be species differences as to whether the PGCs are active or passive travellers.

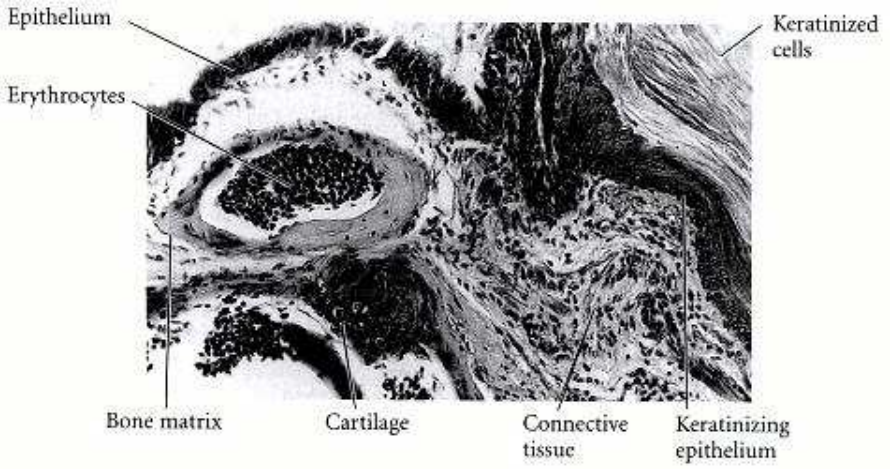
3.2 Embryonic germ (EG) cells

Stem cell factor increases the proliferation of migrating mouse primordial germ cells in culture, and this proliferation can be further increased by adding another growth factor, leukemia inhibition factor (LIF). However, the life span of these PGCs is short, and the cells soon die. But if an additional mitotic regulator basic fibroblast growth factor (FGF2) is added, a remarkable change takes place. The cells continue to proliferate, producing pluripotent embryonic stem cells with characteristics resembling the cells of the inner cell mass. These PGC-derived cells are called embryonic germ (EG) cells, and they have the potential to differentiate into all the cell types of the body. EG cells are often considered as embryonic stem (ES) cells and the distinction of their origin is ignored.

3.2.1Embryonic stem (ES) cells

Embryonic stem (ES) cells are the cells that are derived from the inner cell mass. ES cells and EG cells can be transfected with recombinant genes and inserted into the blastocyst to create transgenic mice. Such a mammalian germ cell or stem cell contains within it all the information needed for subsequent development. What would happen if such a cell became malignant? In one type of tumor, the germ cells become embryonic stem cells, like the FGF2-treated PGCs in the experiment above. This type of tumor is called a **teratocarcinoma**. Whether spontaneous or experimentally produced, a teratocarcinoma contains an undifferentiated stem cell population that has biochemical and developmental properties remarkably similar to those of the inner cell mass. Moreover, these stem cells not only divide, but can also differentiate into a wide variety of tissues, including gut and respiratory epithelia, muscle, nerve, cartilage, and bone. Once differentiated, these cells no longer divide, and are therefore no longer ma

lignant. Such tumors can give rise to most of the tissue types in the body.



Thus, the teratocarcinoma stem cells mimic early mammalian development, but the tumor they form is characterized by random, haphazard development.

4.0 Conclusion

In this unit you learnt about the determination of germ cell in various group of animals. Example nematode ,insect, amphibian , bird and reptiles and mammal

5.0 Summary

All gametes arise from the primordial germ cells. In most animal species, the determination of the primordial germ cells is brought about by the cytoplasmic localization of specific proteins and mRNAs in certain cells of the early embryo (mammals being a major exception to this general rule). These cytoplasmic components are referred to as the **germ plasm**.

The components of the germ plasm have not all been catalogued. Indeed, in the birds and mammals, such a list has hardly even been started.

The germ plasm of anuran amphibians (frogs and toads) collects around the vegetal pole in the zygote. During cleavage, this material is brought upward through the yolky cytoplasm, and eventually becomes associated with the endodermal cells lining the floor of the blastocoels.

6.0 Tutor-marked Assignment

1 Explain the term germ plasm and the position it takes during cleavage formation in various group of animals

2 Explain the migration of germ cell in insect,amphibian bird and reptiles and mammal

3 Explain the term embryonic germ (eg) cells and embryonic stem (es) cells

REFERENCES

Professor Scott Gilbert, Developmental biology 6th Edition

UNIT 3: MITOSIS AND MEIOSIS

CONTENT

1.0 INTRODUCTION

These describe the process by which the body prepares cells to participate in either asexual or sexual reproduction to make an entire organism. For either of these processes of reproduction we must first understand the basic Chromosome structure that the body uses in either Mitosis or Meiosis.

2.0 OBJECTIVES

At the end of this unit, student should be able to:

1 Explain the significance of meiosis sexual reproduction
2 Identify the different stages of gametogenesis in males and females in micrographs of testis and ovary
3 Name the stages at which the first and second meiotic divisions take place
4 Describe the stages of mitosis in cell division.

3.0 MAIN CONTENT

3.1 Mitosis

Mitosis is the reproduction of skin, heart, stomach, cheek, hair etc. cells. These cells are "Autosomal" cells. This is also a form of "Asexual" reproduction, where one organism or cell reproduces itself. Some organisms that reproduce asexually are hydra, bacteria, and single celled organisms.

Mitosis produces two daughter cells that are identical to the parent cell. If the parent cell is haploid (N), then the daughter cells will be haploid. If the parent cell is diploid, the daughter cells will also be diploid.

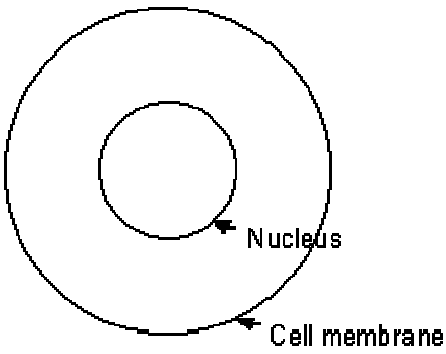
N □ N

2N □ 2N

3.1.1Function of Mitosis

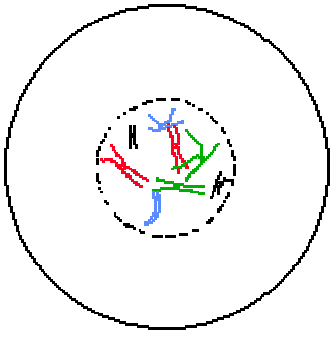
It allows multicellular organisms to grow and repair damaged tissue. The drawings below show chromosome movement and alignment in a cell from a species of animal that has a diploid number of 8. As you view the drawings, keep in mind that humans have a diploid number of 46.

3.1.2 Mitotic Division



Interphase

Chromosomes are not visible because they are uncoiled



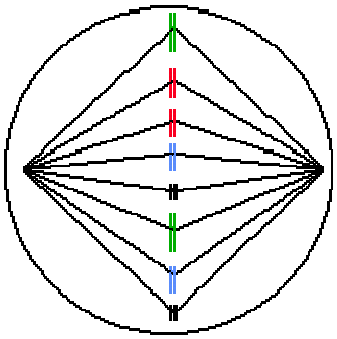
Prophase

The chromosomes coil.

The nuclear membrane disintegrates.

Spindle fibers (microtubles) form.

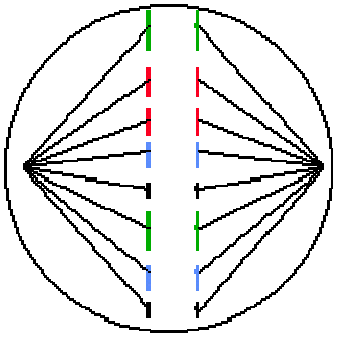
The drawing shows a cell with 8 chromosomes. Each chromosome has 2 chromatids for a total of 16 chromatids.



Metaphase

The chromosomes become aligned.

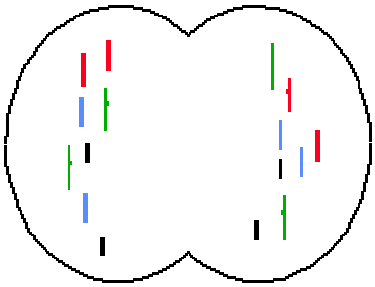
The drawing shows a cell with 8 chromosomes. Each chromosome has 2 chromatids for a total of 16 chromatids.



Anaphase

The chromatids separate; the number of chromosomes doubles.

The drawing shows a cell with 16 chromosomes. Each chromosome has 1 chromatid for a total of 16 chromatids.



Telophase

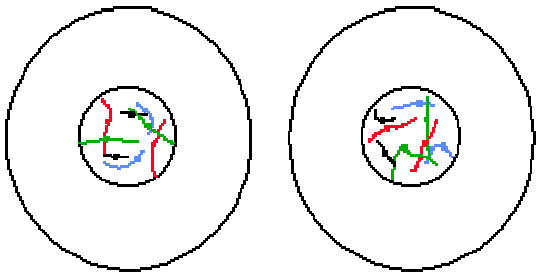
The cell divides into two.

The chromosomes uncoil.

The nucleus reforms.

The spindle apparataus disassembles.

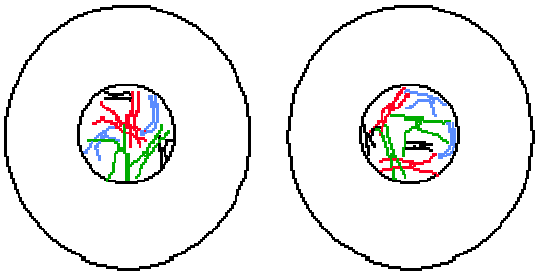
The drawing shows a cell with 16 chromosomes. Each chromosome has 1 chromatid for a total of 16 chromatids.



G_1 Interphase

The chromosomes have one chromatid.

The drawing shows two cells. Each cell has 8 chromosomes. Each chromosome has 1 chromatid for a total of 8 chromatids per cell.



G_2 Interphase

The chromosomes have two chromatids each.

The drawing shows two cells. Each cell has 8 chromosomes. Each chromosome has 2 chromatids for a total of 16 chromatids per cell

3.2. Meiosis

Meiosis is the sperm and egg production of cells. These cells are "Gamete" or "Sex" cells. Each cell has to go through the division process twice in order for the cell to end up with half the number of chromosomes. The cells pass on genetic information to the offspring. This is a form of "Sexual" reproduction, where one organism or cells reproduces by crossing with another organism or cell. Types of organisms that reproduce sexually are; plants, animals, and insects.

Meiosis produces daughter cells that have one half the number of chromosomes as the parent cell.

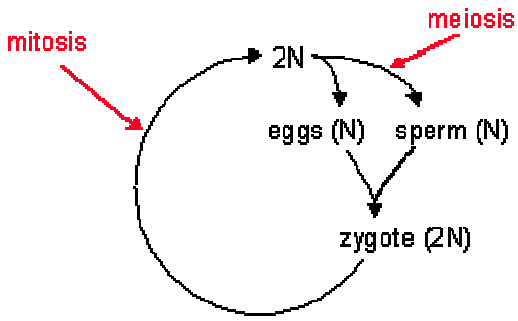
2N □ N

3.2.1 Function of Meiosis

1 Meiosis functions to reduce the number of chromosomes to one half. Each daughter cell that is produced will have one half as many chromosomes as the parent cell.

Meiosis is part of the sexual process because gametes (sperm, eggs) have one half the chromosomes as diploid (2N) individuals.

In animals, meiosis occurs only when gametes (sperm, eggs) are formed.



2 Meiosis enables organisms to reproduce sexually. Gametes (sperm and eggs) are haploid. It involves two divisions producing a total of four daughter cells.

A cell undergoing meiosis will divide two times; The first division is meiosis 1 and the second is meiosis 2. The phases have the same names as those of mitosis. A number indicates the division number (1st or 2nd).

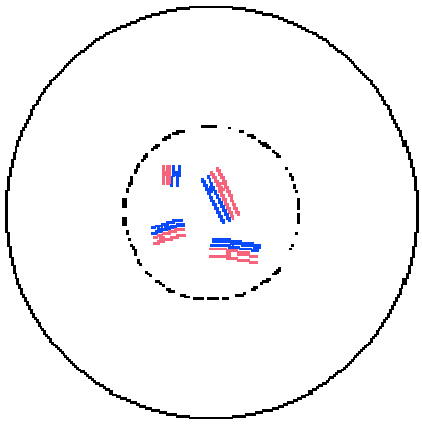
meiosis 1: prophase 1, metaphase 1, anaphase 1, and telophase 1

meiosis 2: prophase 2, metaphase 2, anaphase 2, and telophase 2

In the first meiotic division, the number of cells is doubled but the number of chromosomes is not. This results in 1/2 as many chromosomes per cell.

The second meiotic division is like mitosis; the number of chromosomes does not get reduced.

3.2.1 Meiotic Division

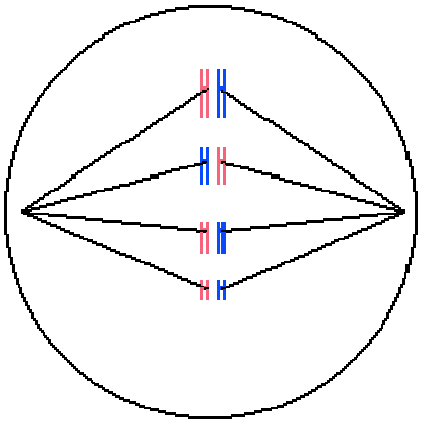


Prophase I

Homologous chromosomes become paired.

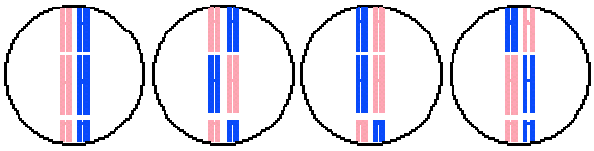
Crossing-over occurs between homologous chromosomes.

Crossing over

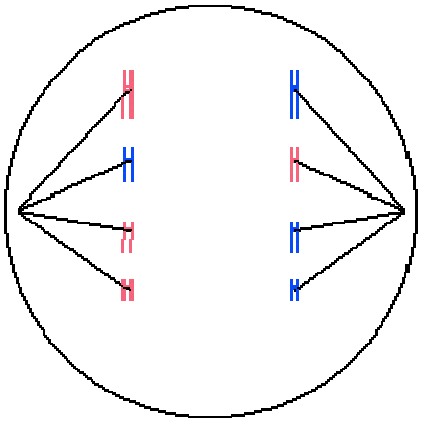


Metaphase I

Homologous pairs become aligned in the center of the cell.

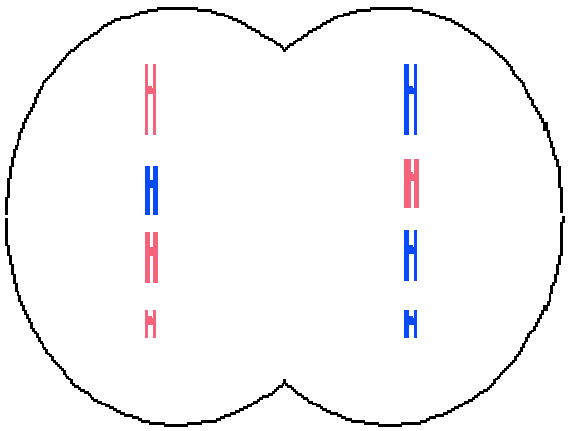


The random alignment pattern is called independent assortment. For example, a cell with 2N = 6 chromosomes could have any of the alignment patterns shown at the left..



Anaphase I

Homologous chromosomes separate.

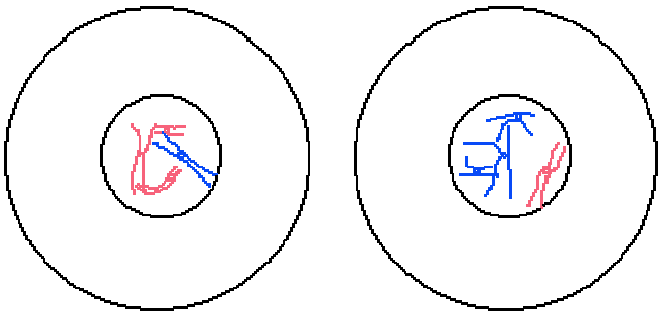


Telophase I

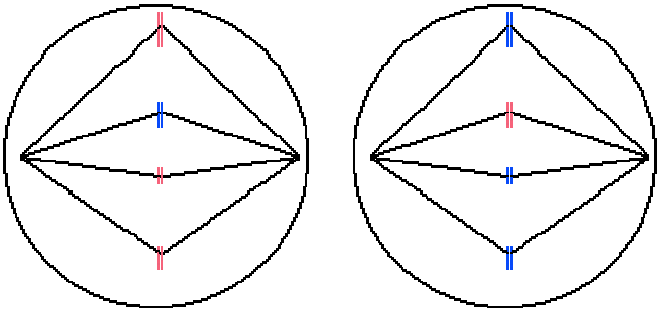
This stage is absent in some species

Interkinesis

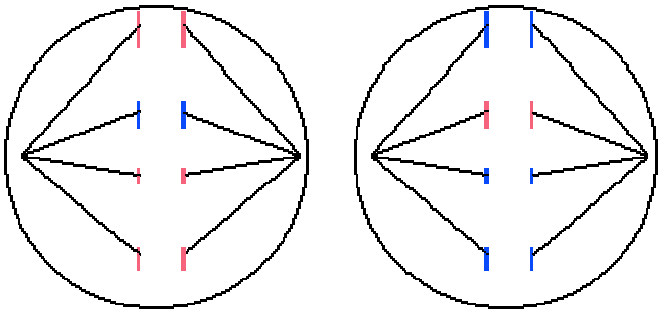
Interkinesis is similar to interphase except DNA synthesis does not occur.



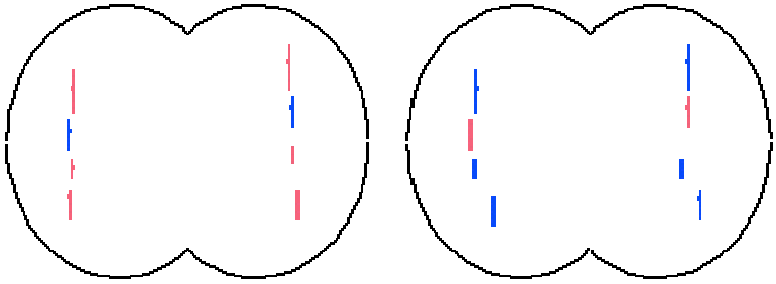
Prophase II



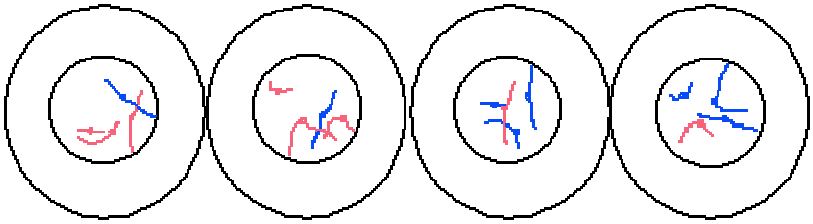
Metaphase II



Anaphase II

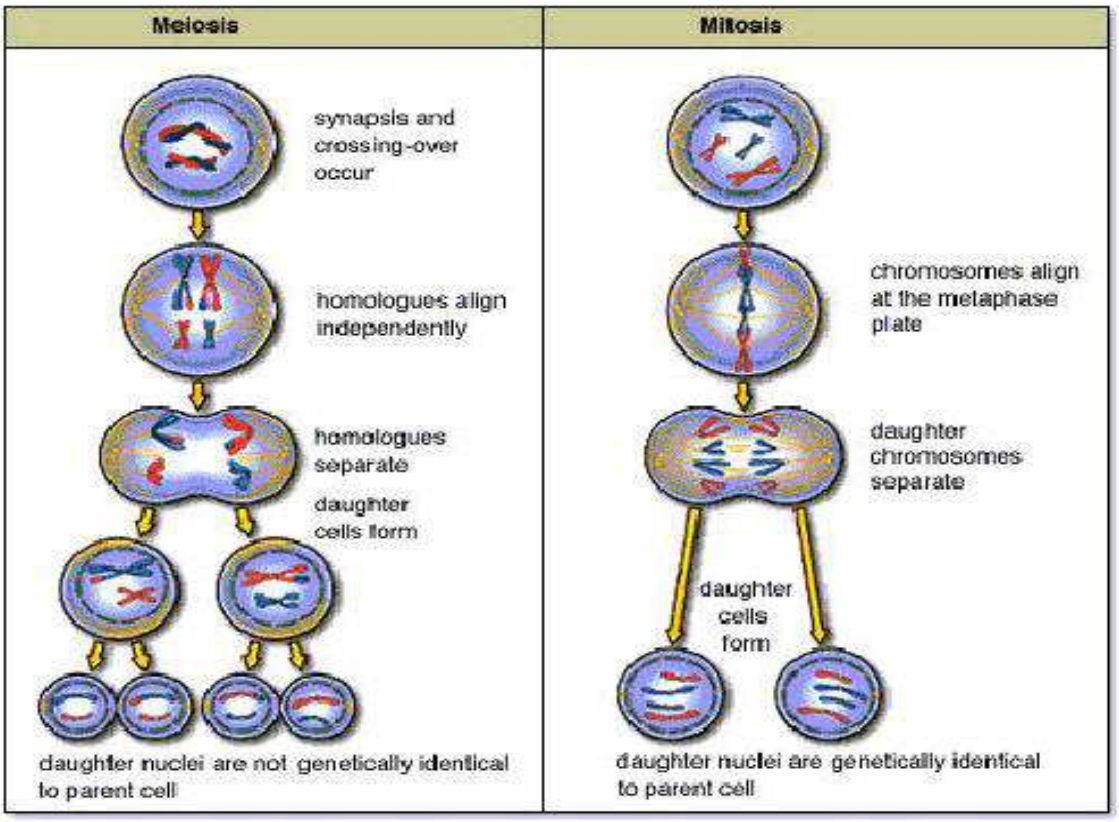


Telophase II



Daughter Cells

8 Major Differences between Meiosis and Mitosis



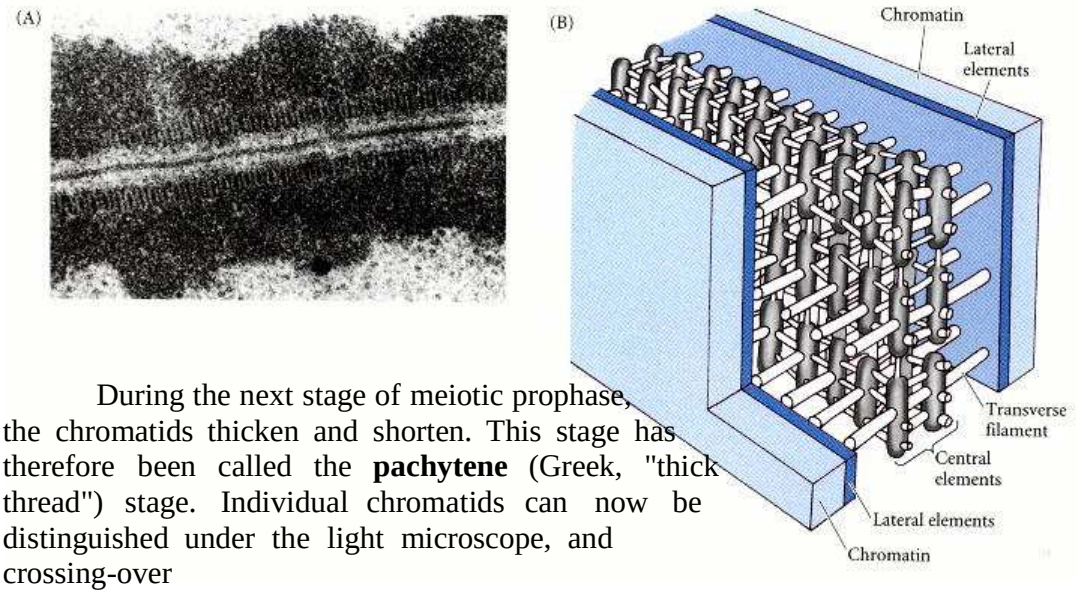
3.2 b Meiosis

Once in the gonad, the primordial germ cells continue to divide mitotically, producing millions of potential gamete precursors. The PGCs of both male and female gonads are then faced with the necessity of reducing their chromosomes from the diploid to the haploid condition. In the haploid condition, each chromosome is represented by only one copy, whereas diploid cells have two copies of each chromosome. To accomplish this reduction, the germ cells undergo meiosis.

After the germ cell's last mitotic division, a period of DNA synthesis occurs, so that the cell initiating meiosis doubles the amount of DNA in its nucleus. In this state, each chromosome consists of two sister **chromatids** attached at a common kinetochore (centromere). (In other words, the diploid nucleus contains four copies of each chromosome, but each chromosome is seen as two chromatids bound together. Meiosis entails two cell divisions. In the first division, homologous chromosomes (e.g., the chromosome 3 pair in the diploid cell) come together and are then separated into different cells. Hence, the first meiotic division separates homologous chromosomes into two daughter cells such that each cell has only one copy of each chromosome. But each of the chromosomes has already replicated (i.e., each has two chromatids). The second meiotic division then separates the two sister chromatids from each other. Consequently, each of the four cells produced by meiosis has a single (haploid) copy of each chromosome.

The first meiotic division begins with a long prophase, which is subdivided into

five stages. During the **leptotene** (Greek, "thin thread") stage, the chromatin of the chromatids is stretched out very thinly, and it is not possible to identify individual chromosomes. DNA replication has already occurred, however, and each chromosome consists of two parallel chromatids. At the **zygotene** (Greek, "yoked threads") stage, homologous chromosomes pair side by side. This pairing is called **synapsis**, and it is characteristic of meiosis. Such pairing does not occur during mitotic divisions. Although the mechanism whereby each chromosome recognizes its homologue is not known, synapsis seems to require the presence of the nuclear membrane and the formation of a proteinaceous ribbon called the **synaptonemal complex**. This complex is a ladderlike structure with a central element and two lateral bars. The chromatin becomes associated with the two lateral bars, and the chromosomes are thus joined together. Examinations of meiotic cell nuclei with the electron microscope suggest that paired chromosomes are bound to the nuclear membrane, and has suggested that the nuclear envelope helps bring together the homologous chromosomes. The configuration formed by the four chromatids and the synaptonemal complex is referred to as a **tetrad** or a **bivalent**.



During the next stage of meiotic prophase, the chromatids thicken and shorten. This stage has therefore been called the **pachytene** (Greek, "thick thread") stage. Individual chromatids can now be distinguished under the light microscope, and crossing-over may occur. Crossing-over represents exchanges of genetic material whereby genes from one chromatid are exchanged with homologous genes from another chromatid. Crossing-over may continue into the next stage, the **diplotene** (Greek, "double threads") stage. Here, the synaptonemal complex breaks down, and the two homologous chromosomes start to separate. Usually, however, they remain attached at various places called **chiasmata**, which are thought to represent regions where crossing-over is occurring. The diplotene stage is characterized by a high level of gene transcription. In some species, the chromosomes of both male and female germ cells take on the "lampbrush" appearance characteristic of chromosomes that are actively making

RNA. During the next stage, **diakinesis** (Greek, "moving apart"), the centromeres move away from each other, and the chromosomes remain joined only at the tips of the chromatids. This last stage of meiotic prophase ends with the breakdown of the nuclear membrane and the migration of the chromosomes to the **metaphase plate**.

During anaphase I, homologous chromosomes are separated from each other in an independent fashion. This stage leads to telophase I, during which two daughter cells are formed, each cell containing one partner of the homologous chromosome pair. After a brief **interkinesis**, the second division of meiosis takes place. During this division, the centromere of each chromosome divides during anaphase so that each of the new cells gets one of the two chromatids, the final result being the creation of four haploid cells.

Note that meiosis has also reassorted the chromosomes into new groupings. First, each of the four haploid cells has a different assortment of chromosomes. In humans, in which there are 23 different chromosome pairs, there can be 2^{23} (nearly 10 million) different types of haploid cells formed from the genome of a single person. In addition, the crossing-over that occurs during the pachytene and diplotene stages of prophase I further increases genetic diversity and makes the number of different gametes incalculable.

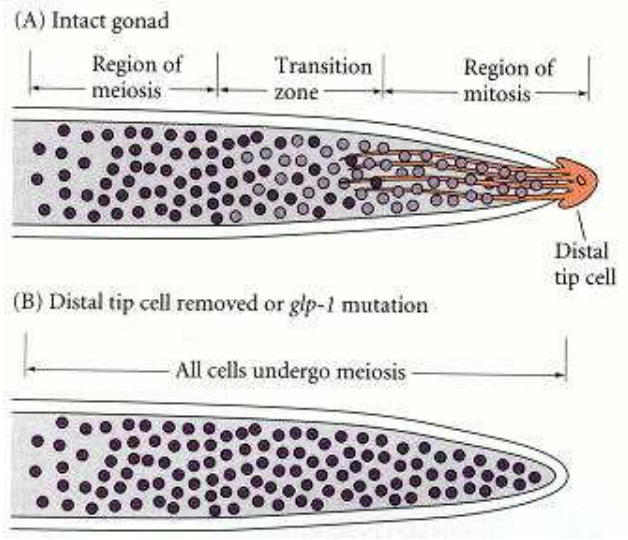
The events of meiosis appear to be coordinated through cytoplasmic connections between the dividing cells. Whereas the daughter cells formed by mitosis routinely separate from each other, the products of the meiotic cell divisions remain coupled to each other by **cytoplasmic bridges**. These bridges are seen during the formation of sperm and eggs throughout the animal kingdom.

3.3 Mitosis or Meiosis? Sperm or Egg?

In many species, the germ cells migrating into the gonad are bipotential and can differentiate into either sperm or ova, depending on their gonadal environment. When the ovaries of salamanders are experimentally transformed into testes, the resident germ cells cease their oogenic differentiation and begin developing as sperm. Similarly, in the housefly and mouse, the gonad is able to direct the differentiation of the germ cell Thus, in most organisms, the sex of the gonad and of its germ cells is the same.

But what about hermaphroditic animals, in which the change from sperm production to egg production is a naturally occurring physiological event? How is the same animal capable of producing sperm during one part of its life and oocytes during another part? Using *Caenorhabditis elegans,* Kimble and her colleagues identified two "decisions" that presumptive germ cells have to make. The first is whether to enter meiosis or to remain a mitotically dividing stem cell. The second is whether to become an egg or a sperm.

Recent evidence shows that these decisions are intimately linked. The mitotic/meiotic decision is controlled by a single nondividing cell at the end of each gonad, the **distal tip cell**. The germ cell precursors near this cell divide mitotically, forming the pool of germ cells; but as these cells get farther away from the distal tip cell, they enter meiosis. If the distal tip cell is destroyed by a focused laser beam, all the germ cells enter meiosis, and if the distal tip cell is placed in a different location in the gonad, germ line stem cells are generated near its new position.

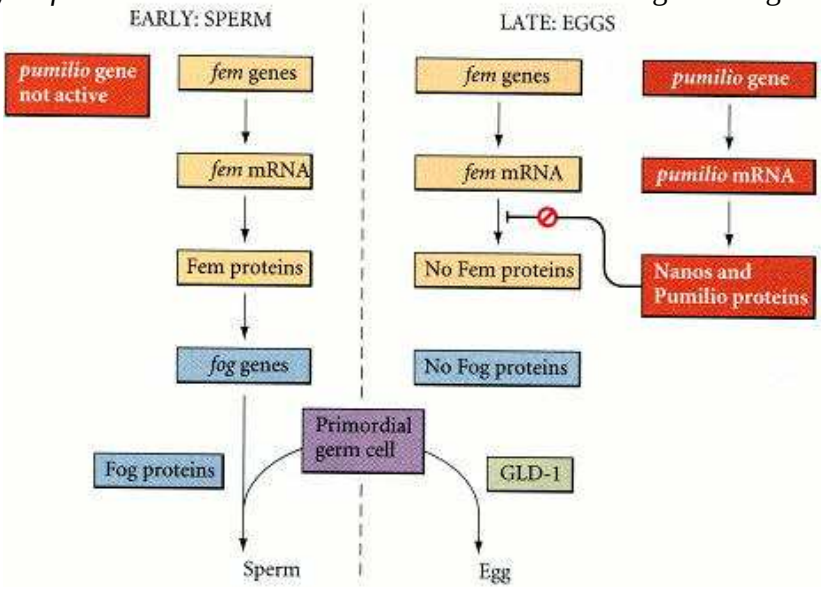


The distal tip cell extends long filaments that touch the distal germ cells. The extensions contain in their cell membranes the Lag-2 protein, a *C. elegans* homologue of *Delta.*

The Lag-2 protein maintains these germ cells in mitosis and inhibits their meiotic differentiation.

It is not surprising that this mutation encodes Glp-1, the *C. elegans* homologue of Notch the receptor for Delta. All the germ cell precursors of nematodes homozygous for the recessive mutation *glp-1* initiate meiosis, leaving no mitotic population. Instead of the 1500 germ cells usually found in the fourth larval stage of hermaphroditic development, these mutants produce only 5 to 8 sperm cells. When genetic chimeras are made in which wild-type germ cell precursors are found within a mutant larva, the wild-type cells are able to respond to the distal tip cells and undergo mitosis. However, when mutant germ cell precursors are found within wild-type larvae, they all enter meiosis. Thus, the *glp-1* gene appears to be responsible for enabling the germ cells to respond to the distal tip cell's signal.

After the germ cells begin their meiotic divisions, they still must become either sperm or ova. Generally, in each ovotestis, the most proximal germ cells produce sperm, while the most distal (near the tip) become eggs. This means that the germ cells entering meiosis early become sperm, while those entering meiosis later become eggs. The genetics of this switch are currently being analyzed. The laboratories of have isolated several genes needed for germ cell pathway selection, but the switch appears to involve the activity or inactivity of *fem-3* mRNA. Presents a scheme for how these genes might function.



During early development, the *fem* genes, especially *fem-3,* are critical for the specification of sperm cells. Loss-of-function mutations of these genes convert XX nematodes into females (i.e., spermless hermaphrodites). As long as the FEM proteins are made in the germ cells, sperm are produced. The active *fem* genes are thought to activate the *fog* genes (whose loss- of-function mutations cause the feminization of the germ line and eliminate spermatogenesis). The *fog* gene products activate the genes involved in transforming the germ cell into sperm and also inhibit those genes that would otherwise direct the germ cells to initiate oogenesis.

Oogenesis can begin only when *fem* activity is suppressed. This suppression appears to act at the level of RNA translation. The 3′ untranslated region (3′ UTR) of the *fem-3* mRNA contains a sequence that binds a repressor protein during normal development. If this region is mutated such that the repressor cannot bind, the *fem-3* mRNA remains translatable, and oogenesis never occurs. The result is a hermaphrodite body that produces only sperm. The *trans*-acting repressor of the *fem-3* message is a combination of the Nanos and Pumilio proteins (the same combination that represses *hunchback* message translation in *Drosophila*). The up-regulation of Pumilio expression may be critical in regulating the germ line switch from spermatogenesis to oogenesis, since Nanos is made constitutively. Nanos appears to be necessary in *C. elegans* (as it is in *Drosophila*) for the survival of all germ line cells.

5.0 CONCLUSION

In this unit, you learnt about different stages of cell division; mitosis and meiosis. Mitosis division for asexual reproduction, meiosis division for sexual reproduction, their functions and differences.

6.0 **SUMMARY**

Mitosis and Mitosis describe the process by which the body prepares cells to participate in either asexual or sexual reproduction to make an entire organism. For either of these processes of reproduction we must first understand the basic Chromosome structure that the body uses in either Mitosis or Meiosis. Mitosis produces two daughter cells that are identical to the parent cell while meiosis produces daughter cells that have one half the number of chromosomes as the parent cell. Mitosis allows multicellular organisms to grow and repair damaged tissue while meiosis is the sperm and egg production of cells, this cells form gamete of sex cells.

7.0 Tutor-Marked Assignment

The questions below refer to the following diagrams.

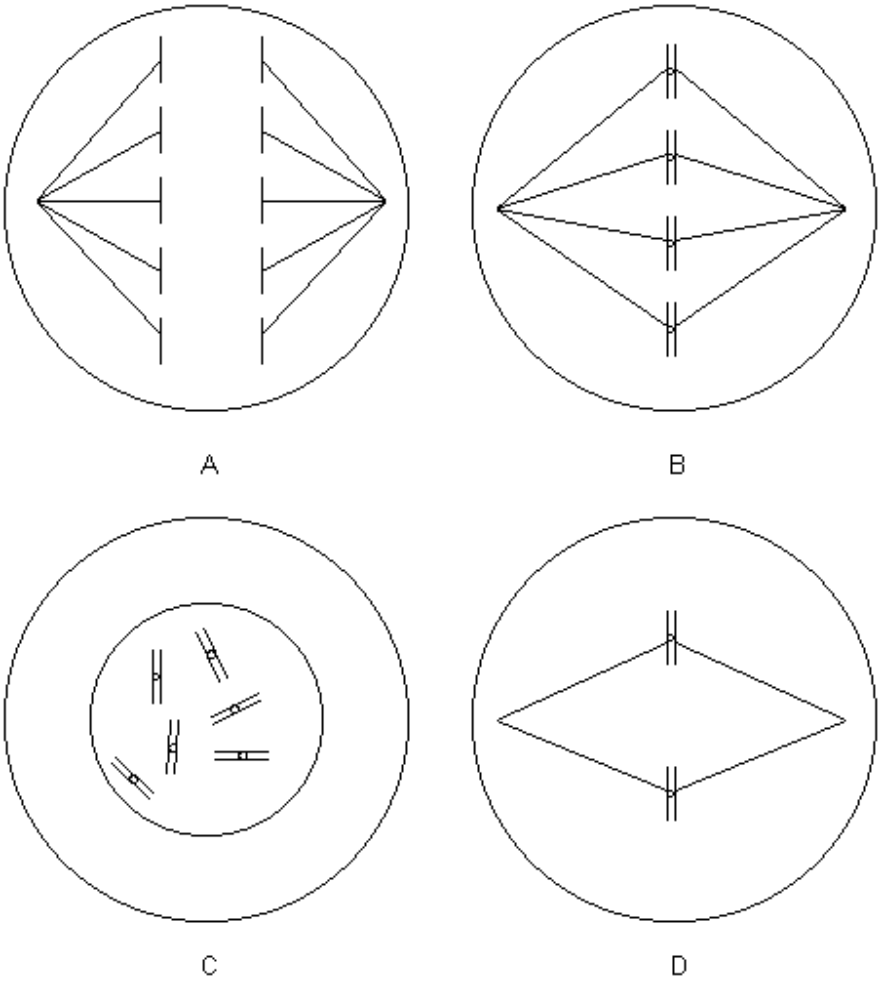


Diagram A represents a cell from an organism with a diploid chromosome number of 10.

1 Therefore, the diagram represents which one of the following stages?

A. interphase of mitosis
B. anaphase of mitosis
C. metaphase I of meiosis
D. prophase II of meiosis
E. anaphase II of meiosis

2. Diagram B represents a cell from an organism with a diploid chromosome number of 8. Therefore, the diagram represents which one of the following stages?

A. prophase of mitosis
B. metaphase of mitosis
C. metaphase I of meiosis
D. telophase I of meiosis
E. metaphase II of meiosis

3. Diagram B represents a cell from an organism with a diploid chromosome number of 4. Therefore, the diagram represents which one of the following stages?

A. prophase of mitosis
B. metaphase of mitosis
C. metaphase I of meiosis
D. telophase I of meiosis
E. metaphase II of meiosis

4. Diagram B represents a cell from an organism with a diploid chromosome number of 6. Therefore, the diagram represents which one of the following stages?

A.	Prophase		of	mitosis
B.	Metaphase		of	mitosis
C.	Metaphase	I	of	meiosis
D.	Telophase	I	of	meiosis
E.	Metaphase	II	of	meiosis

F. None of the above

5. Diagram C represents a cell from an organism with a diploid chromosome number of 6. Therefore, the diagram represents which one of the following stages?

A. prophase of mitosis
B. metaphase of mitosis
C. metaphase I of meiosis
D. prophase II of meiosis
E. metaphase II of meiosis

6. Diagram D represents a cell from an organism with a diploid chromosome number of 2. Therefore, the diagram represents which one of the following stages?

A. prophase of mitosis
B. prophase I of meiosis
C. prophase II of meiosis
D. metaphase of mitosis
E. metaphase I of meiosis
F. metaphase II of meiosis

8.0 REFERENCES/FURTHER

Regina Bailey, 1977. Sexual Reproduction: Gametogenesis. About.com Guide, 1997

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UNIT 4: FERTILIZATION AND GAMETE FORMATION IN MAJOR GROUPS OF ORGANISMS

CONTENTS

1.0 INTRODUCTION

Fertilization is the process whereby two sex cells (gametes) fuse together to create a new individual with genetic potentials derived from both parents. Fertilization accomplishes two separate ends: sex (the combining of genes derived from the two parents) and reproduction (the creation of new organisms). Thus, the first function of fertilization is to transmit genes from parent to offspring, and the second is to initiate in the egg cytoplasm those reactions that permit development to proceed.

Although the details of fertilization vary from species to species, conception generally consists of four major events:

1. Contact and recognition between sperm and egg. In most cases, this ensures that the sperm and egg are of the same species.

2. Regulation of sperm entry into the egg. Only one sperm can ultimately fertilize the egg. This is usually accomplished by allowing only one sperm to enter the egg and inhibiting any others from entering.

3. Fusion of the genetic material of sperm and egg.

4. Activation of egg metabolism to start development.

2.0 OBJECTIVES

At the end of this unit the student should be able to:

1 Explain Structure and function of sperm and egg in formation of Gametes

2 Explain acrosomal reaction in sea-urchin and mammal

3 Describe the fusion of genetic material in mammal and sea- urchin

3.0 MAIN CONTENT

3.1Structure of gametes

A complex dialogue exists between egg and sperm. The egg activates the sperm metabolism that is essential for fertilization, and the sperm reciprocates by activating the egg metabolism needed for the onset of development. But before we investigate these aspects of fertilization, we need to consider the structures of the sperm and egg the two cell types specialized for fertilization.

3.1.1Sperm

It is only within the past century that the sperm's role in fertilization has been known. Anton van Leeuwenhoek, the Dutch microscopist who co-discovered sperm in 1678, first believed them to be parasitic animals living within the semen (hence the term *spermatozoa,* meaning "sperm animals"). He originally assumed that they had nothing at all to do with reproducing the organism in which they were found, but he later came to believe that each sperm contained a preformed embryo. Leeuwenhoek (1685) wrote that sperm were seeds (both *sperma* and *semen* mean "seed") and that the female merely provided the nutrient soil in which the seeds were planted. In this, he was returning to a notion of procreation promulgated by Aristotle 2000 years earlier. Try as he might, Leeuwenhoek was continually disappointed in his attempts to find the

preformed embryo within the spermatozoa. Nicolas Hartsoeker, the other co-discoverer of sperm, drew a picture of what he hoped to find: a preformed human ("homunculus") within the human sperm. This belief that the sperm contained the entire embryonic organism never gained much acceptance, as it implied an enormous waste of potential life.

Most investigators regarded the sperm as unimportant.

The first evidence suggesting the importance of sperm in reproduction came from a series of experiments performed by Lazzaro Spallanzani in the late 1700s. Spallanzani demonstrated that filtered toad semen devoid of sperm would not fertilize eggs. He concluded, however, that the viscous fluid retained by the filter paper, and not the sperm, was the agent of fertilization. He, like many others, felt that the spermatic "animals" were parasites.

The combination of better microscopic lenses and the cell theory led to a new appreciation of spermatic function. In 1824, J. L. Prevost and J. B. Dumas claimed that sperm were not parasites, but rather the active agents of fertilization. They noted the universal existence of sperm in sexually mature males and their absence in immature and aged individuals. These observations, coupled with the known absence of spermatozoa in the sterile mule, convinced them that "there exists an intimate relation between their presence in the organs and the fecundating capacity of the animal." They proposed that the sperm entered the egg and contributed materially to the next generation.

These claims were largely disregarded until the 1840s, when A. von Kolliker described the formation of sperm from cells within the adult testes. He ridiculed the idea that the semen could be normal and yet support such an enormous number of parasites. Even so, von Kolliker denied that there was any physical contact between sperm and egg. He believed that the sperm excited the egg to develop, much as a magnet communicates its presence to iron. It was only in 1876 that Oscar Hertwig and Herman Fol independently demonstrated sperm entry into the egg and the union of the two cells' nuclei. Hertwig had sought an organism suitable for detailed microscopic observations, and he found that the Mediterranean sea urchin, *Toxopneustes lividus*, was perfect. Not only was it common throughout the region and sexually mature throughout most of the year, but its eggs were available in large numbers and were transparent even at high magnifications. After mixing sperm and egg suspensions together, Hertwig repeatedly observed a sperm entering an egg and saw the two nuclei unite. He also noted that only one sperm was seen to enter each egg, and that all the nuclei of the embryo were derived from the fused nucleus created at fertilization. Fol made similar observations and detailed the mechanism of sperm entry. Fertilization was at last recognized as the union of sperm and egg, and the union of sea urchin gametes remains one of the best-studied examples of fertilization.

Each sperm consists of a haploid nucleus, a propulsion system to move the nucleus, and a sac of enzymes that enable the nucleus to enter the egg. Most of the sperm's cytoplasm is eliminated during maturation, leaving only certain organelles that are modified for spermatic function. During the course of sperm maturation, the haploid nucleus becomes very streamlined, and its DNA becomes tightly compressed. In front of this compressed haploid nucleus lies the **acrosomal vesicle**, or **acrosome**, which is derived from the Golgi apparatus and contains enzymes that digest proteins and complex sugars; thus, it can be considered a modified secretory vesicle. These stored enzymes are used to lyse the outer coverings of the egg. In many species, such as sea urchins, a region

of globular actin molecules lies between the nucleus and the acrosomal vesicle. These proteins are used to extend a fingerlike **acrosomal process** from the sperm during the early stages of fertilization. In sea urchins and several other species, recognition between sperm and egg involves molecules on the acrosomal process. Together, the acrosome and nucleus constitute the head of the sperm.

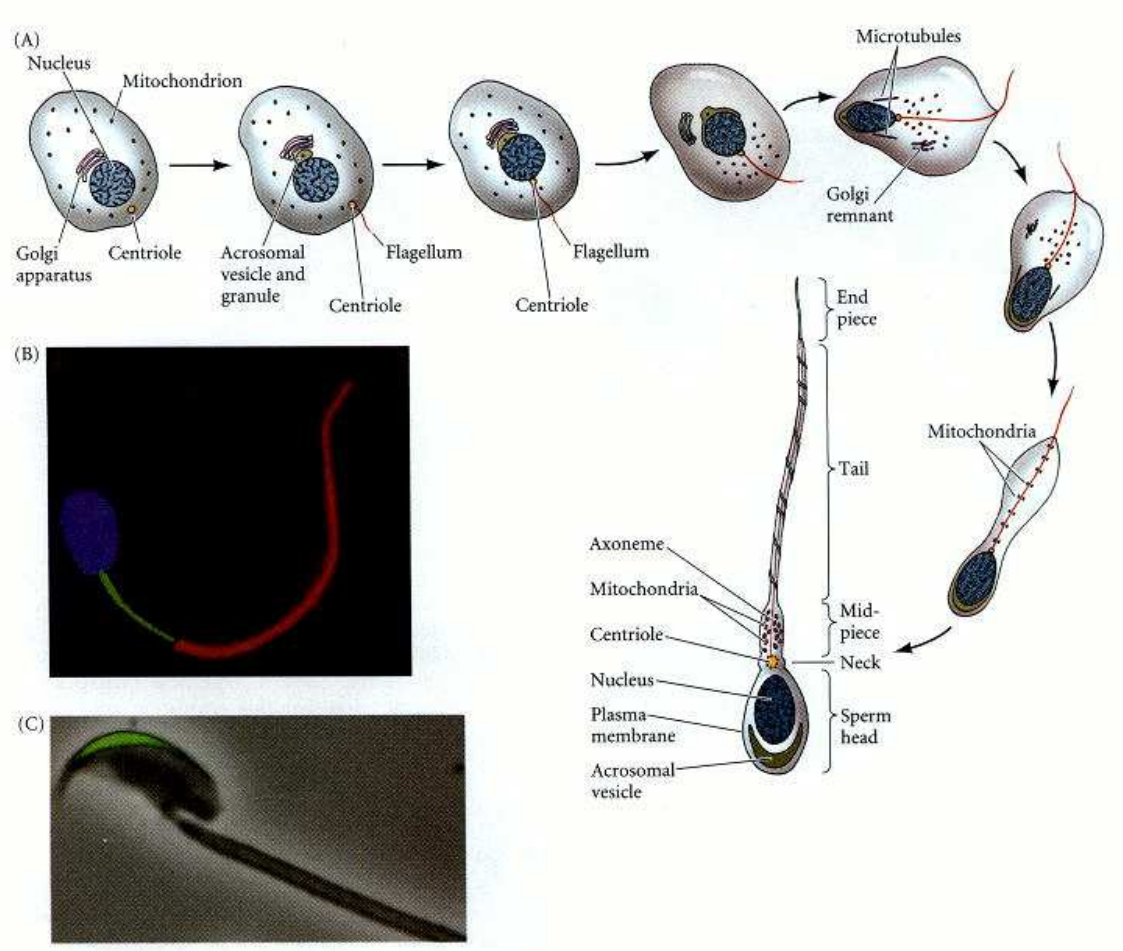


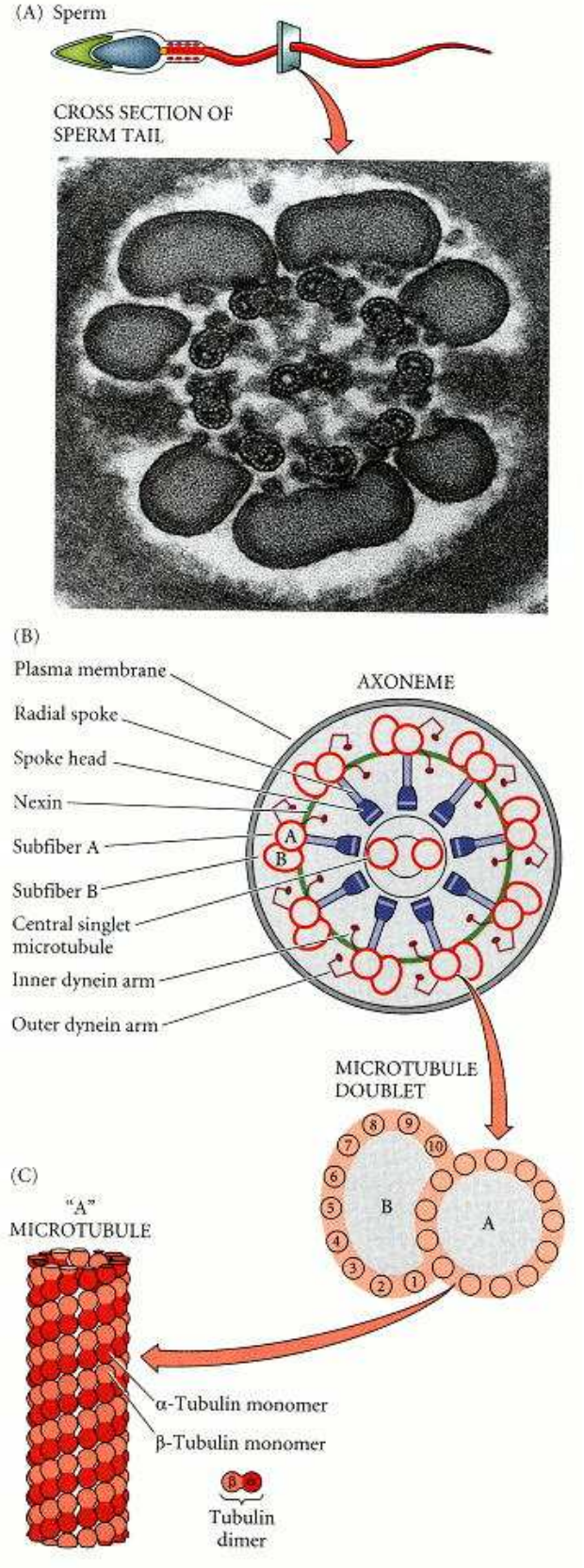
Figure7: Formation of Sperm head.

The means by which sperm are propelled vary according to how the species has adapted to environmental conditions. In some species (such as the parasitic roundworm *Ascaris*), the sperm travel by the amoeboid motion of lamellipodial extensions of the cell membrane. In most species, however, each sperm is able to travel long distances by whipping its **flagellum.** Flagella are complex structures. The major motor portion of the flagellum is called the **axoneme**. It is formed by microtubules emanating from the centriole at the base of the sperm nucleus (Figure 7)

The core of the axoneme consists of two central microtubules surrounded by a row of nine doublet microtubules. Actually, only one microtubule of each doublet is complete, having
13 protofilaments; the other is C-shaped and has only 11 protofilaments (Figure 7B). A three- dimensional model of a complete microtubule is shown in Figure 7C. Here we can see the 13
interconnected protofilaments, which are made exclusively of the dimeric protein tubulin.

Although tubulin is the basis for the structure of the flagellum, other proteins are also critical for flagellar function. The force for sperm propulsion is provided by **dynein**, a protein that is attached to the microtubules (Figure 7B). Dynein hydrolyzes molecules of ATP and can convert the released chemical energy into the mechanical energy that propels the sperm.
This energy allows the active sliding of the outer doublet microtubules, causing the flagellum to bend. The importance of dynein can be seen in individuals with the genetic syndrome called the Kartagener triad. These individuals lack dynein on all their

ciliated and flagellated cells, rendering these structures immotile.



Males with this disease are sterile (immotile sperm), are susceptible to bronchial infections (immotile respiratory cilia), and have a 50% chance of having the heart on the right side of the body

Another important flagellar protein appears to be histone H1.

This proteinis usually found inside the nucleus, where it folds the chromatin into tight clusters. However, Multigner and

colleagues (1992) found that this same protein stabilizes the flagellar microtubules so that they do not disassemble.

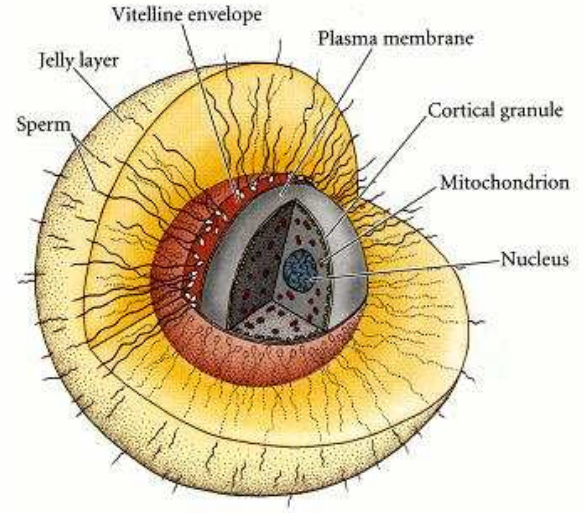
The "9 + 2" microtubule arrangement with the dynein arms has been conserved in axonemes throughout the eukaryotic kingdoms, suggesting that this arrangement is extremely well suited for transmitting energy for movement. The ATP needed to whip the flagellum and propel the sperm comes from rings of mitochondria located in the neck region of the sperm (see Figure 7).

In many species (notably mammals), a layer of dense fibers has interposed itself between the mitochondrial sheath and the axoneme. This fiber layer stiffens the sperm tail. Because the thickness of this layer decreases toward the tip, the fibers probably prevent the sperm head from being whipped around too suddenly. Thus, the sperm has undergone extensive modification for the transport of its nucleus to the egg.

The differentiation of mammalian sperm is not completed in the testes. After being expelled into the lumen of the seminiferous tubules, the sperm are stored in

the epididymis, where they acquire the ability to move. Motility is achieved through changes in the ATP-generating system (possibly through modification of dynein) as well as changes in the plasma membrane that make it more fluid (Yanagimachi 1994).

The sperm released during ejaculation are able to move, yet they do not yet have the capacity to bind to and fertilize an egg. These final stages of sperm maturation (called capacitation) do not occur until the sperm has been inside the female reproductive tract for a certain period of time.



3.1.2.The egg

All the material necessary for the beginning of growth and development must be stored in the mature egg (the **ovum**). Whereas the sperm has eliminated most of its cytoplasm, the developing egg (called the **oocyte** before it reaches the stage of meiosis at which it is fertilized) not only conserves its material, but is actively involved in accumulating more. The meiotic divisions that form the oocyte conserve its cytoplasm (rather than giving half of it away), and the oocyte either synthesizes or absorbs proteins, such as yolk, that act as food reservoirs for the developing embryo.

Thus, birds' eggs are enormous single cells, swollen with their accumulated yolk. Even eggs with relatively sparse yolk are comparatively large. The volume of a sea urchin egg is about 200 picoliters (2×10^{4} mm^3, more than 10,000 times the volume of the sperm) (Figure 7.4). So, while sperm and egg have equal haploid nuclear components, the egg also has a remarkable cytoplasmic storehouse that it has accumulated during its maturation. This cytoplasmic trove includes the following:*

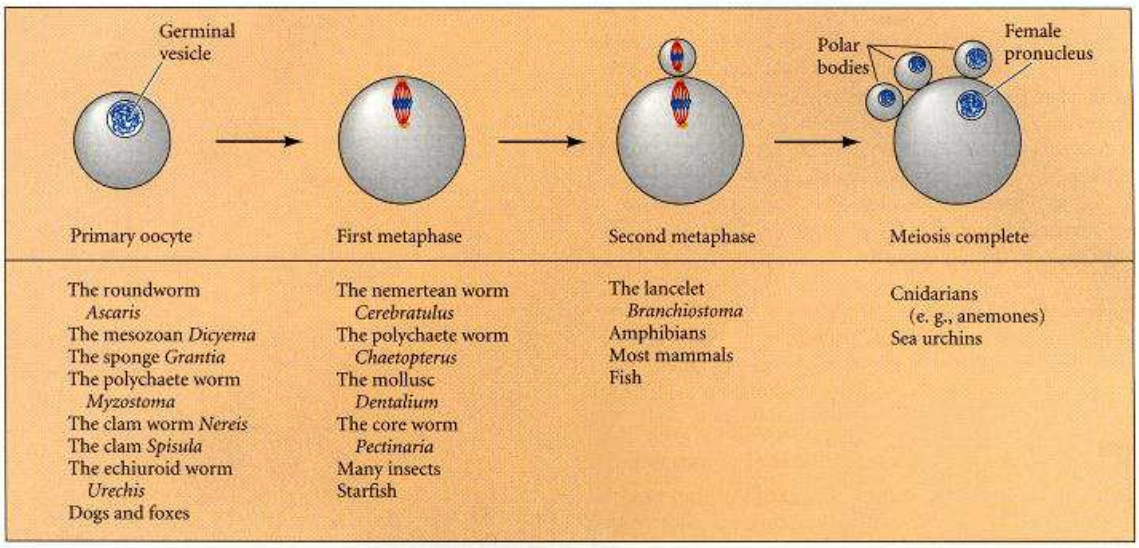
- **Proteins.** It will be a long while before the embryo is able to feed itself or obtain food from its mother. The early embryonic cells need a supply of energy and amino acids. In many species, this is accomplished by accumulating yolk proteins in the egg. Many of the yolk proteins are made in other organs (liver, fat body) and travel through the maternal blood to the egg.

- **Ribosomes and tRNA.** The early embryo needs to make many of its own proteins, and in some species, there is a burst of protein synthesis soon after fertilization. Protein synthesis is accomplished by ribosomes and tRNA, which exist in the egg. The developing egg has special mechanisms to synthesize ribosomes, and certain amphibian oocytes produce as many as 10^{12} ribosomes during their meiotic prophase.

- **Messenger RNA.** In most organisms, the instructions for proteins made during early development are already packaged in the oocyte. It is estimated that the eggs of sea urchins contain 25,000 to 50,000 different types of mRNA. This mRNA, however, remains dormant until after fertilization.
- **Morphogenetic factors.** Molecules that direct the differentiation of cells into certain cell types are present in the egg. They appear to be localized in different regions of the egg and become segregated into different cells during cleavage
- **Protective chemicals.** The embryo cannot run away from predators or move to a safer

environment, so it must come equipped to deal with threats. Many eggs contain ultraviolet filters and DNA repair enzymes that protect them from sunlight; some eggs contain molecules that potential predators find distasteful; and the yolk of bird eggs even contains antibodies.

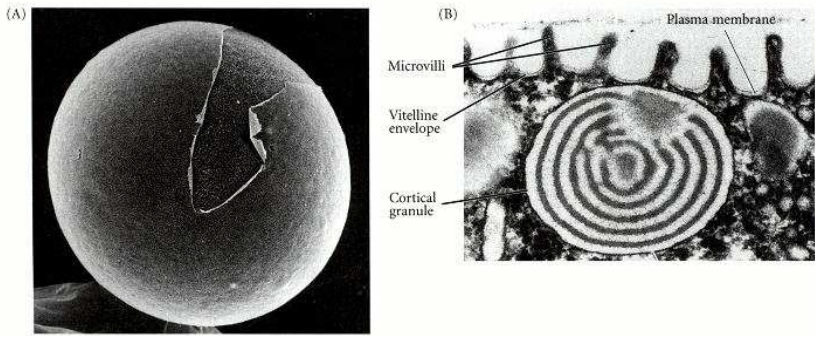
Within this enormous volume of cytoplasm resides a large nucleus. In some species (e.g., sea urchins), the nucleus is already haploid at the time of fertilization. In other species (including many worms and most mammals), the egg nucleus is still diploid, and the sperm enters before the meiotic divisions are completed. The stage of the egg nucleus at the time of sperm entry in different species is illustrated in figure below.

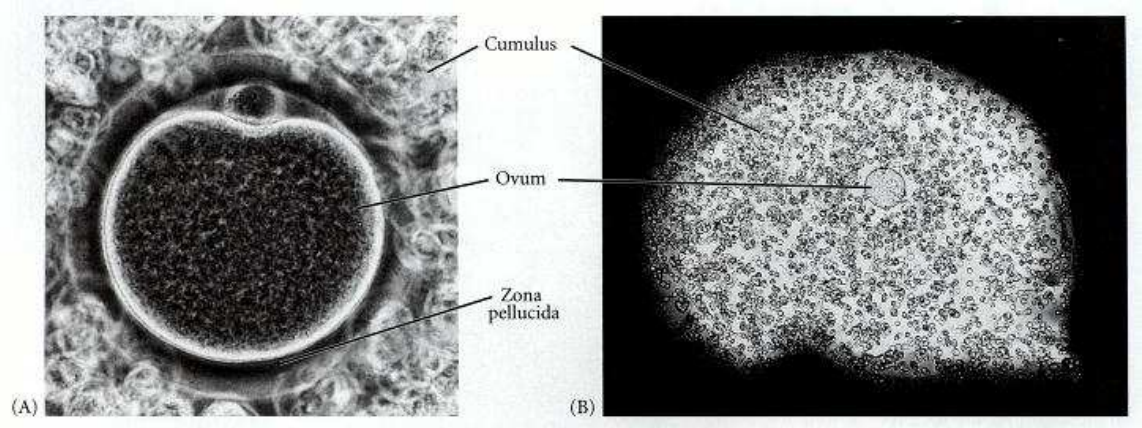


Enclosing the cytoplasm is the egg **plasma membrane**. This membrane must regulate the flow of certain ions during fertilization and must be capable of fusing with the sperm plasma membrane. Outside the plasma membrane is the **vitelline envelope,** which forms a fibrous mat around the egg. This envelope contains at least eight different glycoproteins and is often involved in sperm-egg recognition. It is supplemented by extensions of membrane glycoproteins from the plasma membrane and by proteinaceous vitelline posts that adhere the vitelline envelope to the membrane.

The vitelline envelope is essential for the species-specific binding of sperm. In mammals, the vitelline envelope is a separate and thick extracellular matrix called the **zona pellucida**.

The mammalian egg is also surrounded by a layer of cells called the **cumulus (**Figure 7), which is made up of the ovarian follicular cells that were nurturing the egg at the time of its release from the ovary. Mammalian sperm have to get past these cells to fertilize the egg. The innermost layer of cumulus cells, immediately adjacent to the zona pellucida, is called the **corona radiata.**





Lying immediately beneath the plasma membrane of the egg is a thin shell (about 5 μm) of gel-like cytoplasm called the **cortex**. The cytoplasm in this region is stiffer than the internal cytoplasm and contains high concentrations of globular actin molecules. During fertilization, these actin molecules polymerize to form long cables of actin known as **microfilaments**. Microfilaments are necessary for cell division, and they also are used to extend the egg surface into small projections called **microvilli**, which may aid sperm entry into the cell. Also within the cortex are the **cortical granules** (Fig 7b)These membrane-bound structures, which are homologous to the acrosomal vesicle of the sperm, are Golgi-derived organelles containing proteolytic enzymes. However, whereas each sperm contains one acrosomal vesicle, each sea urchin egg contains approximately 15,000 cortical granules. Moreover, in addition to digestive enzymes, the cortical granules contain mucopolysaccharides, adhesive glycoproteins, and hyaline protein. The enzymes and mucopolysaccharides are active in preventing other sperm from entering the egg after the first sperm has entered, and the hyalin and adhesive glycoproteins surround the early embryo and provide support for the cleavage-stage blastomeres.

Many types of eggs also have an **egg jelly** outside the vitelline envelope. This glycoprotein meshwork can have numerous functions, but most commonly is used either to attract or to activate sperm. The egg, then, is a cell specialized for receiving sperm and initiating development.

3.2 Recognition of Egg and Sperm

The interaction of sperm and egg generally proceeds according to five basic steps

1. The chemoattraction of the sperm to the egg by soluble molecules secreted by the egg

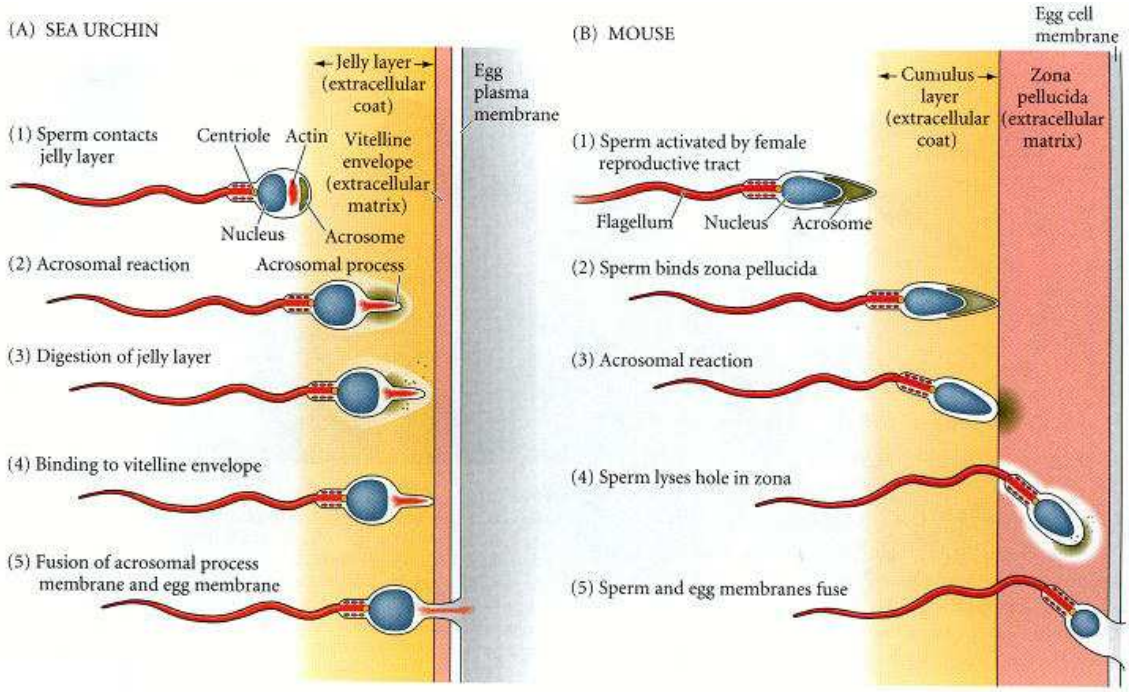
2. The exocytosis of the acrosomal vesicle to release its enzymes

3. The binding of the sperm to the extracellular envelope (vitelline layer or zona pellucida) of the egg

4. The passing of the sperm through this extracellular envelope

5. Fusion of egg and sperm cell plasma membranes

SEA URCHIN AND MOUSE



Sometimes steps 2 and 3 are reversed (as in mammalian fertilization) and the sperm binds to the egg before releasing the contents of the acrosome. After these five steps are accomplished, the haploid sperm and egg nuclei can meet, and the reactions that initiate development can begin.

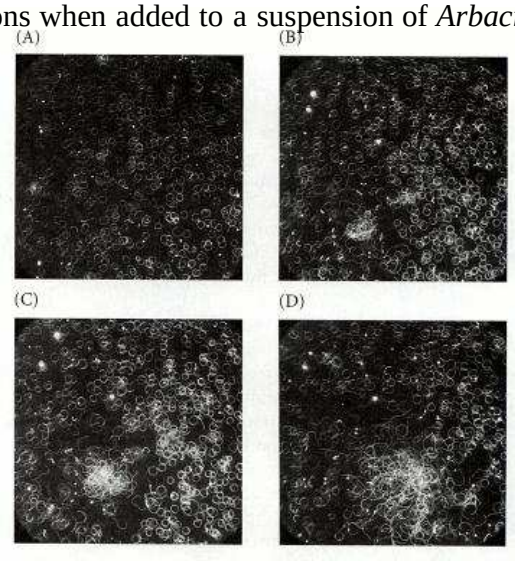
In many species, the meeting of sperm and egg is not a simple matter. Many marine organisms release their gametes into the environment. That environment may be as small as a tide pool or as large as an ocean. Moreover, it is shared with other species that may shed their sex cells at the same time. These organisms are faced with two problems: How can sperm and eggs meet in such a dilute concentration, and how can sperm be prevented from trying to fertilize eggs of another species? Two major mechanisms have evolved to solve these problems: species- specific attraction of sperm and species-specific sperm activation.

3.2.1. Sperm attraction: Action at a distance

Species-specific sperm attraction has been documented in numerous species, including cnidarians, molluscs, echinoderms, and urochordates. In many species, sperm are attracted toward eggs of their species by **chemotaxis**, that is, by following a gradient of a chemical secreted by the egg. In 1978, Miller demonstrated that the eggs of the cnidarian *Orthopyxis caliculata* not only secrete a chemotactic factor but also regulate the timing of its release. Developing oocytes at various stages in their maturation were fixed on microscope slides, and sperm were released at a certain distance from the eggs. Miller found that when sperm were added to oocytes that had not yet completed their second meiotic division, there was no attraction of sperm to eggs. However, after the second meiotic division was finished and the eggs were ready to be fertilized, the sperm migrated toward them. Thus, these oocytes control not only the type of sperm they attract, but also

the time at which they attract them.

The mechanisms of chemotaxis differamong species. One chemotactic molecule, a 14-amino acid peptide called **resact,** has been isolated from the egg jelly of the sea urchin *Arbacia punctulata.* Resact diffuses readily in seawater and has a profound effect at very low concentrations when added to a suspension of *Arbacia* sperm (Figure 7.). When a drop of seawater containing *Arbacia* sperm is placed on a microscope slide, the sperm generally swim in circles about 50 μm in diameter. Within seconds after a minute amount of resact is injected into the drop, sperm migrate into the region of the injection and congregate there.

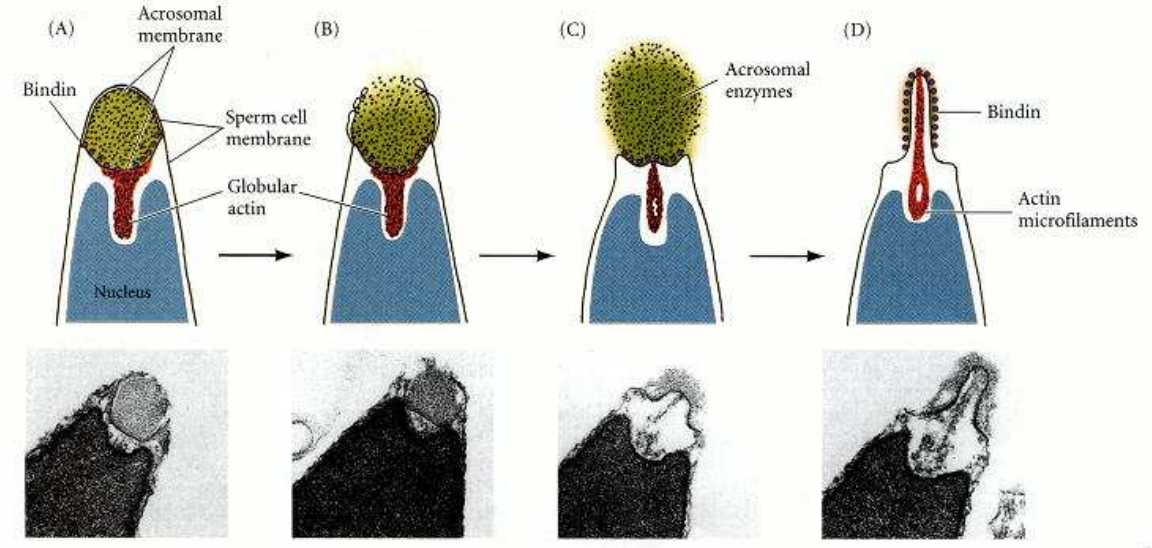


As resact continues to diffuse from the area of injection, more sperm are recruited into the growing cluster. Resact is specific for *A. punctulata* and does not attract sperm of other species. *A. punctulata* sperm have receptors in their plasma membranes that bind resact can swim up a concentration gradient of this compound until they reach the egg.

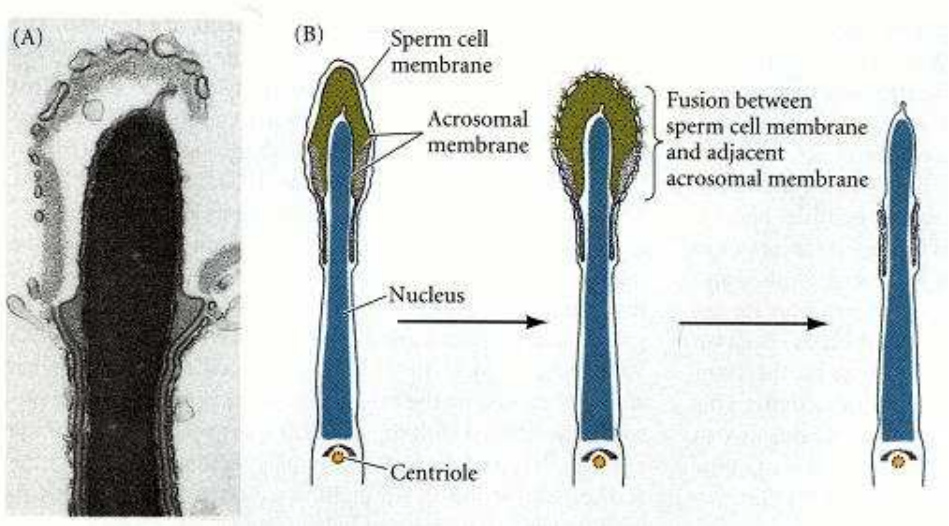
Resact also acts as a **sperm-activating peptide**. Sperm-activating peptides cause dramatic and immediate increases in mitochondrial respiration and sperm motility. The sperm receptor for resact is a transmembrane protein, and when it binds resact on the extracellular side, a conformational change on the cytoplasmic side activates the receptor's enzymatic activity. This activates the mitochondrial ATP-generating apparatus as well as the dynein ATPase that stimulates flagellar movement in the sperm .

3.3 Acrosomal Reaction in Sea-urchin

A second interaction between sperm and egg is the **acrosomal reaction.** In most marine invertebrates, the acrosomal reaction has two components: the fusion of the acrosomal vesicle with the sperm plasma membrane (an exocytosis that results in the release of the contents of the acrosomal vesicle) and the extension of the acrosomal process. The acrosomal reaction in sea urchins is initiated by contact of the sperm with the egg jelly. Contact with egg jelly causes the exocytosis of the sperm's acrosomal vesicle and the release of proteolytic enzymes that can digest a path through the jelly coat to the egg surface. The sequence of these events is outlined in Figure 7.10.



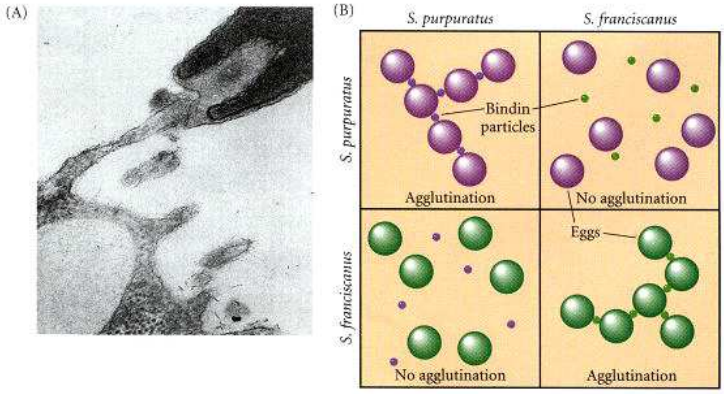
In sea urchins, the acrosomal reaction is thought to be initiated by a fucose-containing polysaccharide in the egg jelly that binds to the sperm and allows calcium to enter into the sperm head. The exocytosis of the acrosomal vesicle is caused by the calcium-mediated fusion of the acrosomal membrane with the adjacent sperm plasma membrane (Figures 7.10 and 7.11). The egg jelly factors that initiate the acrosomal reaction in sea urchins are often highly specific to each species*



The second part of the acrosomal reaction involves the extension of the acrosomal process (see Figure 7.10). This protrusion arises through the polymerization of globular actin molecules into actin filaments.

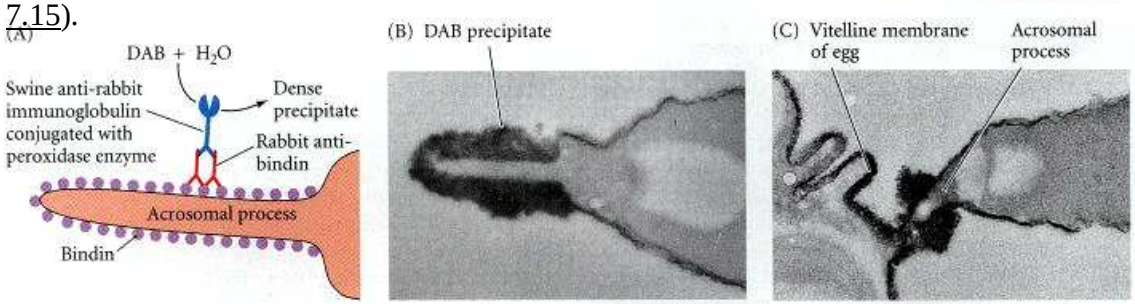
Species-specific recognition in sea urchins

Once the sea urchin sperm has penetrated the egg jelly, the acrosomal process of the sperm contacts the surface of the egg (Figure 7.14A). A major species-specific recognition step occurs at this point. The acrosomal protein mediating this recognition is called **bindin**. In 1977, Vacquier and co-workers isolated this nonsoluble 30,500-Da protein from the acrosome of *Strongylocentrotus purpuratus* and found it to be capable of binding to dejellied eggs of the same species (Figure 7.14B;). Further, its interaction with eggs is relatively species-specific binding isolated from the acrosomes of *S. purpuratus*

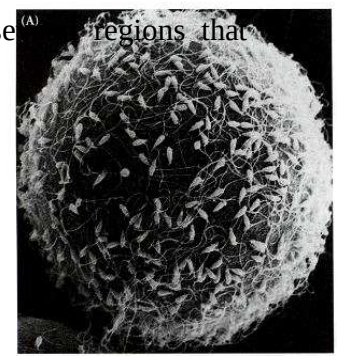


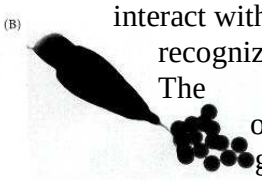
binds to its own dejellied eggs, but not to those of *Arbacia punctulata.*

Using immunological techniques, emonstrated that bindin is located specifically on the acrosomal process exactly where it should be for sperm-egg recognition (Figure 7.15).



Biochemical studies have shown that the bindins of closely related sea urchin species are indeed different. This finding implies the existence of species-specific bindin receptors on the egg, vitelline envelope, or plasma membrane. Such receptors were also suggested by the experiments of who saturated sea urchin eggs with sperm. As seen in Figure 7.16A, sperm binding does not occur over the entire egg surface. Even at saturating numbers of sperm (approximately 1500), there appears to be room on the ovum for more sperm heads, implying a limiting number of sperm-binding sites. The bindin receptor on the egg has recently been isolated. This 350-kDa protein may have several regions that interact with bindin. At least one of these sites recognizes only the bindin of the same species. The other site or sites appear to recognize a general bindin structure and can recognize the bindin of many species. The bindin receptors are thought to be aggregated into complexes on the egg cell surface, and hundreds of these complexes may be needed to tether the sperm to the egg (Figure 7.16B).





Thus, species-specific recognition of sea urchin gametes occurs at the levels of sperm attraction, sperm activation, and sperm adhesion to the egg surface.

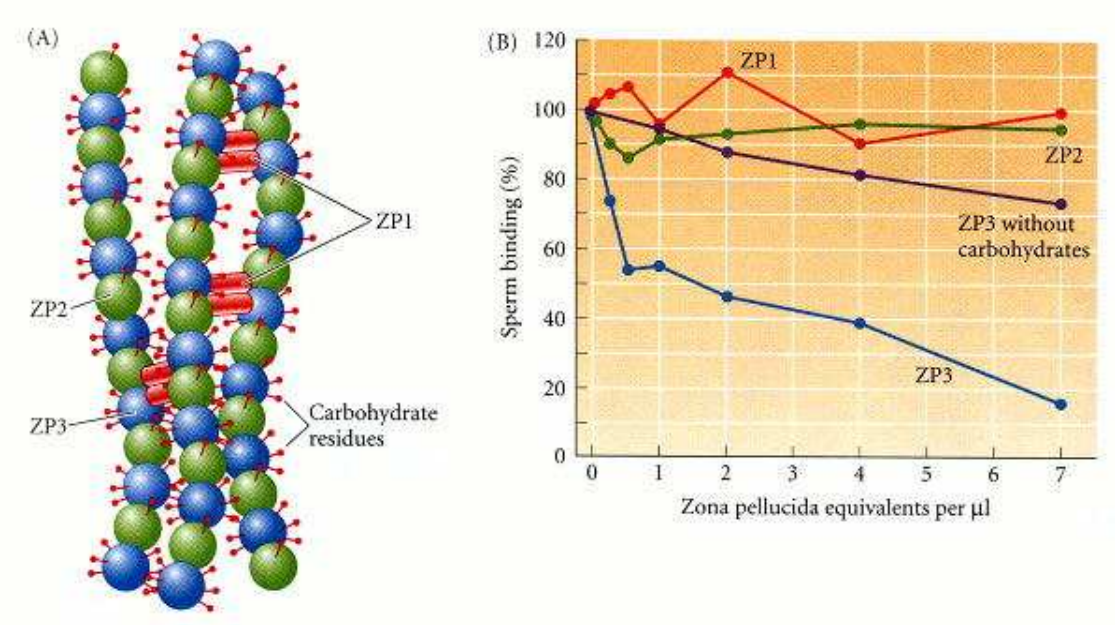
MAMMALS

3.4. Gamete binding and recognition in mammals

ZP3: the sperm-binding protein of the mouse zona pellucida.

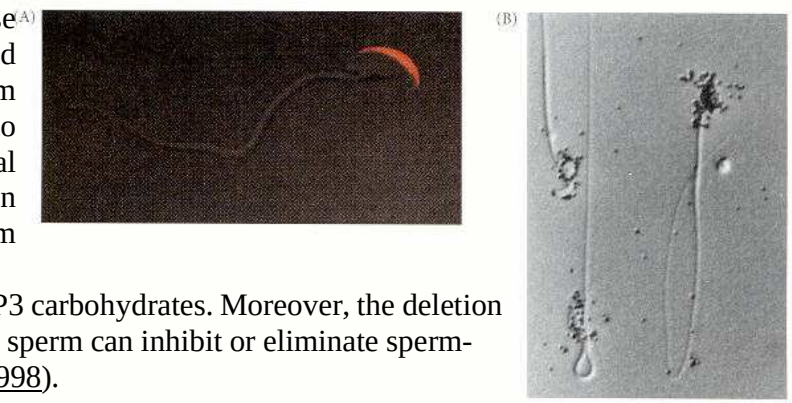
The zona pellucida in mammals plays a role analogous to that of the vitelline envelope in invertebrates. This glycoprotein matrix, which is synthesized and secreted by the growing oocyte, plays two major roles during fertilization: it binds the sperm, and it initiates the acrosomal reaction after the sperm is bound. The binding of sperm to the zona is relatively, but not absolutely, species-specific. (Species- specific gamete recognition is not a major problem when fertilization occurs internally.)

The binding of mouse sperm to the mouse zona pellucida can be inhibited by first incubating the sperm with zona glycoproteins. Bleil and Wassarman (1980, 1986 1988) isolated an 83-kDa glycoprotein, **ZP3**, from the mouse zona that was the active competitor for binding in this inhibition assay. The other two zona glycoproteins they found, ZP1 and ZP2, failed to compete for sperm binding (Figure 7.17). Moreover, they found that radiolabeled ZP3 bound to the heads of mouse sperm with intact acrosomes. Thus, ZP3 is the specific glycoprotein in the mouse zona pellucida to which the sperm bind. ZP3 also initiates the acrosomal reaction after sperm have bound to it. The mouse sperm can thereby concentrate its proteolytic enzymes directly at the point of attachment at the zona pellucida.



The molecular mechanism by which the zona pellucida and the mammalian sperm recognize each other is presently being studied. The current hypothesis of mammalian gamete binding postulates a set of proteins on the sperm capable of

recognizing specific carbohydrate regions of ZP3 (Figure 7.18A; Florman et al. 1984; Florman and Wassarman 1985; Wassarman 1987; Saling 1989).



Removal of these threonine- or serine-linked carbohydrate groups from ZP3 abolishes its ability to bind sperm. Several proteins have been identified on the sperm cell surface that specifically bind to the ZP3 carbohydrates. Moreover, the deletion of these proteins from the sperm can inhibit or eliminate sperm-zona binding (see Kopf 1998).

Induction of the mammalian acrosomal reaction by ZP3.

Unlike the sea urchin acrosomal reaction, the acrosomal reaction in mammals occurs only after the sperm has bound to the zona pellucida (Figure 7.8). The mouse sperm acrosomal reaction is induced by the crosslinking of ZP3 with the receptors for it on the sperm membrane. This crosslinking opens calcium channels to increase the concentration of calcium in the sperm. The mechanism by which ZP3 induces the opening of the calcium channels and the subsequent exocytosis of the acrosome remains controversial, but it may involve the receptor's activating a cation channel (for sodium, potassium, or calcium), which would change the resting potential of the sperm plasma membrane. The calcium channels in the membrane would be sensitive to this change in membrane potential, allowing calcium to enter the sperm.

The difference between the acrosomal reaction in sea urchins and mammals may be due to the thickness of the extracellular envelopes surrounding the egg. In the sea urchin, the vitelline envelope is very thin and porous. Once a sperm has bound there, it is very close to the egg plasma membrane, and, indeed, the bindin receptor may extend through the vitelline envelope. In mammals, however, the zona pellucida is a very thick matrix, so the sperm is far removed from the egg. By undergoing the acrosomal reaction directly on the zona, the sperm is able to concentrate its proteolytic enzymes to lyse a hole in this envelope. Indeed, sperm that undergo the acrosomal reaction before they reach the zona pellucida are unable to penetrate it.

Secondary binding of sperm to the zona pellucida.

During the acrosomal reaction, the anterior portion of the sperm plasma membrane is shed from the sperm (see Figure 7.11). This region is where the ZP3-binding proteins are located, and yet the sperm must still remain bound to the zona in order to lyse a path through it. In mice, it appears that secondary binding to the zona is accomplished by proteins in the inner acrosomal membrane that bind specifically to ZP2. Whereas acrosome-intact sperm will not bind to ZP2, acrosome-reacted sperm will. Moreover, antibodies against the ZP2 glycoprotein will not prevent the binding of acrosome-intact sperm to the zona, but will inhibit the attachment of acrosome-reacted sperm. The structure of the zona consists of repeating units of ZP3 and ZP2, occasionally crosslinked by ZP1 (Figure 7.18). It appears that the acrosome-reacted sperm transfer their binding from ZP3 to the adjacent ZP2 molecules. After a mouse sperm has entered the egg, the egg cortical granules release their contents. One of the proteins released by these granules is a protease that specifically alters ZP2. This inhibits other acrosome-reacted sperm from moving closer toward the egg.

In guinea pigs, secondary binding to the zona is thought to be mediated by the protein PH-20. Moreover, when this inner acrosomal membrane protein was injected into adult male or female guinea pigs, 100% of them became sterile for several months. The blood sera of these sterile guinea pigs had extremely high concentrations of antibodies to PH-20. The antiserum from guinea pigs sterilized in this manner not only bound specifically to PH-20, but also blocked sperm-zona adhesion in vitro. The contraceptive effect lasted several months, after which fertility was restored. These experiments show that the principle of immunological contraception is well founded.

*Such exocytotic reactions are seen in the release of insulin from pancreatic cells and in the release of neurotransmitters from synaptic terminals. In all cases, there is a calcium-mediated fusion between the secretory vesicle and the cell membrane. Indeed, the similarity of acrosomal vesicle exocytosis and synaptic vesicle exocytosis may actually be quite deep. Studies of acrosomal reactions in sea urchins and mammals suggest that when the receptors for the sperm-activating ligands bind these molecules, they cause a depolarization of the membrane that would open voltage-dependent calcium ion channels in a manner reminiscent of synaptic transmission. The proteins that dock the cortical granules of the egg to the plasma membrane also appear to be homologous to those used in the axon tip.

Bindin is probably the fastest evolving protein known. Closely related species may have near-identity of every other protein, but their bindins may have diverged significantly. For more information on bindin evolution,

3.4. Action at a Distance: Mammalian Gametes

It is very difficult to study the interactions that might be occurring between mammalian gametes prior to sperm-egg contact. One obvious reason for this is that mammalian fertilization occurs inside the oviducts of the female. While it is relatively easy to mimic the conditions surrounding sea urchin fertilization (using either natural or artificial seawater), we do not yet know the components of the various natural environments that mammalian sperm encounter as they travel to the egg. A second reason for this difficulty is that the sperm population ejaculated into the female is probably very heterogeneous, containing spermatozoa at different stages of maturation. Of the 280×10^6 human sperm normally ejaculated into the vagina, only about 200 reach the ampullary region of the oviduct, where fertilization takes place. Since fewer than 1 in 10,000 sperm get close to the egg, it is difficult to assay those molecules that might enable the sperm to swim toward the egg and become activated. There is a great deal of controversy concerning the mechanisms underlying the translocation of mammalian sperm to the oviduct, the possibility that the egg may be attracting the sperm through chemotaxis, and the capacitation and hyperactivation reactions that appear necessary for some species' sperm to bind with the egg.

3.4.1Translocation and Capacitation

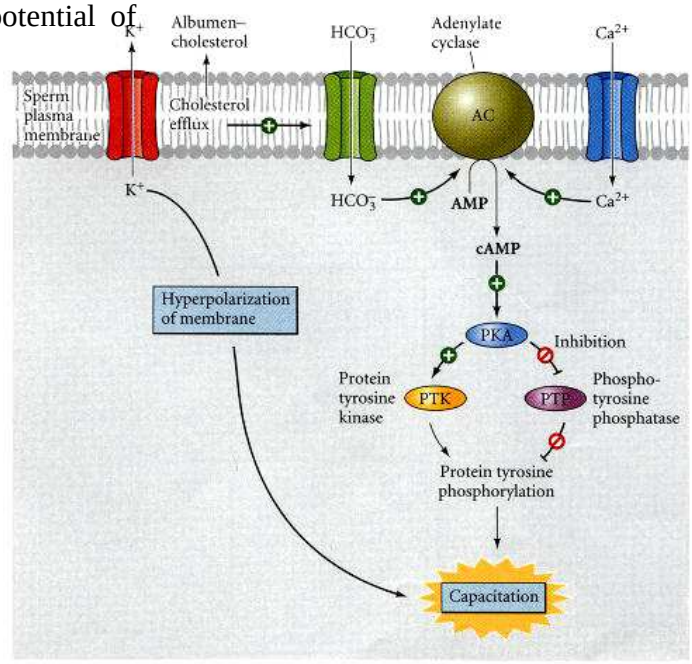
The reproductive tract of female mammals plays a very active role in the mammalian fertilization process. While sperm motility is required for mouse sperm to encounter the egg once it is in the oviduct, sperm motility is probably a minor factor in getting the sperm into the oviduct in the first place. Sperm are found in the oviducts of mice, hamsters, guinea pigs, cows, and humans within 30 minutes of sperm deposition in the vagina, a time "too short to have been attained by even the most Olympian sperm relying on their own flagellar power". Rather, the sperm appear to be transported to the

oviduct by the muscular activity of the uterus.

By whatever means, mammalian sperm pass through the uterus and oviduct, interacting with the cells and secretions of the female reproductive tract as they do so. These interactions are critical for their ability to interact with the egg. Newly ejaculated mammalian sperm are unable to undergo the acrosomal reaction without residing for some time in the female reproductive tract. The set of physiological changes that allow the sperm to be competent to fertilize the egg is called **capacitation**. The requirement for capacitation varies from species to species. Capacitation can be mimicked in vitro by incubating sperm in tissue culture media (containing calcium ions, bicarbonate, and serum albumin) or in fluid from the oviducts. Sperm that are not capacitated are "held up" in the cumulus and so do not reach the egg

As mentioned above (and contrary to the opening scenes of the *Look Who's Talking* movies), "the race is not always to the swiftest." Although some human sperm reach the ampullary region of the oviduct within a half hour after intercourse, those sperm may have little chance of fertilizing the egg. Wilcox and colleagues (1995) found that nearly all human pregnancies result from sexual intercourse during a 6-day period ending on the day of ovulation. This means that the fertilizing sperm could have taken as long as 6 days to make the journey. In hypothesis wherein capacitation is a transient event, and sperm are given a relatively brief window of competence in which they can successfully fertilize the egg. As the sperm reach the ampulla, they acquire competence, but if they stay around too long, they lose it. Sperm may also have different survival rates depending on their location within the reproductive tract, and this may allow some sperm to arrive late but with better chance of success than those that have arrived days earlier.

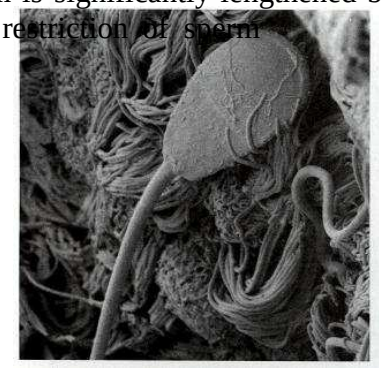
The molecular changes that account for capacitation are still unknown, but there are four sets of molecular changes that may be important. First, the fluidity of the sperm plasma membrane is altered by the removal of cholesterol by albumin proteins found in the female reproductive tract. If serum albumin is experimentally preloaded with cholesterol, it will not permit capacitation to occur in vitro. Second, particular proteins or carbohydrates on the sperm surface are lost during capacitation. It is possible that these compounds block the recognition sites for the proteins that bind to the zona pellucida. Third, the membrane potential of the sperm becomes more negative as potassium ions leave the sperm. This change in membrane potential may allow calcium channels to be opened and permit calcium to enter the sperm. Calcium and bicarbonate ions may be critical in activating cAMP production and in facilitating the membrane fusion events of the acrosomal reaction



Fourth, protein phosphorylation occurs. However, it is still uncertain whether these events are independent of one another

and to what extent each of them causes sperm capacitation (Figure 7.12).

There may be an important connection between sperm translocation and capacitation. Timothy have documented that before entering the ampulla of the oviduct (where mammalian fertilization occurs), the uncapacitated sperm bind actively to the membranes of the oviduct cells in the narrow passage (isthmus) preceding it (Figure 7.13). This binding is temporary and appears to be broken when the sperm become capacitated. Moreover, the life span of the sperm is significantly lengthened by this binding, and its capacitation is slowed down. This restriction of sperm entry into the ampulla, the slowing down of capacitation, and the expansion of sperm life span may have very important consequences. First, this binding may function as a block to polyspermy by preventing many sperm from reaching the egg at the same time. If the isthmus is excised in cows, a much higher rate of polyspermy results. Second, slowing the rate of sperm capacitation and extending the active life of sperm may maximize the probability of there being some sperm in the ampulla to meet the egg if ejaculation does not occur at the same time as ovulation.



3.4.2 Hyperactivation and chemotaxis

Different regions of the female reproductive tract may secrete different, regionally specific molecules. These factors may influence sperm motility as well as capacitation. For instance, when sperm of certain mammals (especially hamsters, guinea pigs, and some strains of mice) pass from the uterus into the oviducts, they become **hyperactivated**, swimming at higher velocities and generating greater force than before. Suarez and co-workers (1991) have shown that while this behavior is not conducive to traveling in low-viscosity fluids, it appears to be extremely well suited for linear sperm movement in the viscous fluid that sperm might encounter in the oviduct.

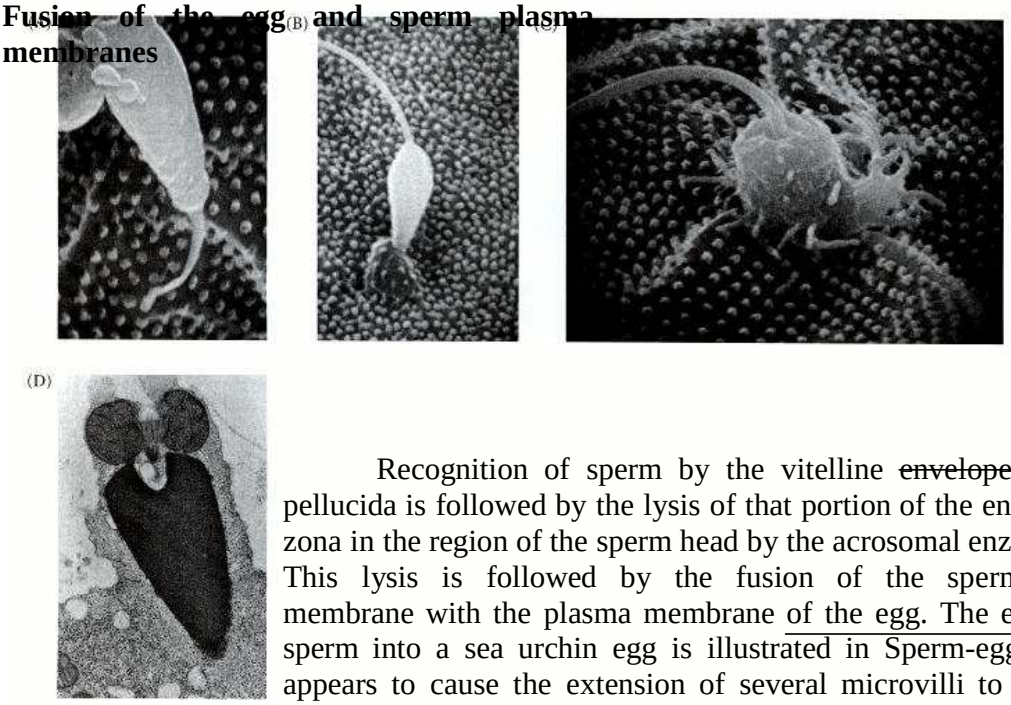
In addition to increasing the activity of sperm, soluble factors in the oviduct may also provide the directional component of sperm movement. There has been speculation that the ovum (or, more likely, the ovarian follicle in which it developed) may secrete chemotactic substances that attract the sperm toward the egg during the last stages of sperm migration tested this hypothesis using follicular fluid from human follicles whose eggs were being used for in vitro fertilization. Performing an experiment similar to the one described earlier with sea urchins, they microinjected a drop of follicular fluid into a larger drop of sperm suspension. When they did this, some of the sperm changed their direction to migrate toward the source of follicular fluid. Microinjection of other solutions did not have this effect. These studies did not rule out the possibility that the effect was due to a general stimulation of sperm movement or metabolism. However, these investigations uncovered a fascinating correlation: the fluid from only about half the follicles tested showed a chemotactic effect, and in nearly every case, the egg was fertilizable if, and only if, the fluid showed chemotactic ability ($P < 0.0001$). It is possible, then, that like certain invertebrate eggs, the human egg secretes a chemotactic factor only when it is capable of being fertilized.

The female reproductive tract, then, is not a passive conduit through which the sperm race, but a highly specialized set of tissues that regulate the timing of sperm

capacitation and access to the egg.

Gamete Fusion and the Prevention of Polyspermy

Fusion of the egg and sperm plasma membranes



Recognition of sperm by the vitelline ~~envelope or zona~~ pellucida is followed by the lysis of that portion of the envelope or zona in the region of the sperm head by the acrosomal enzymes This lysis is followed by the fusion of the sperm plasma membrane with the plasma membrane of the egg. The entry of a sperm into a sea urchin egg is illustrated in Sperm-egg binding appears to cause the extension of several microvilli to form the **fertilization cone** .Homology between the egg and the sperm is again demonstrated, because the transitory fertilization cone, like the acrosomal process, appears to be extended by the polymerization of actin. The sperm and egg plasma membranes then join together, and material from the sperm membrane can later be found on the egg membrane. The sperm nucleus and tail pass through the resulting cytoplasmic bridge, which is widened by the actin polymerization. A similar process occurs during the fusion of mammalian gametes

In the sea urchin, all regions of the egg plasma membrane are capable of fusing with sperm. In several other species, certain regions of the membrane are specialized for sperm recognition and fusion Fusion is an active process, often mediated by specific "fusogenic" proteins. It seems that bindin plays a second role as a fusogenic protein. Scientist has shown that sea urchin bindin will cause phospholipid vesicles to fuse together and that, like viral fusogenic proteins, bindin contains a long stretch of hydrophobic amino acids near its amino terminus. This region is able to fuse phospholipid vesicles.

In mammals, the **fertilin** proteins in the sperm plasma membrane are essential for sperm membrane-egg membrane fusion. Mouse fertilin is localized to the posterior plasma membrane of the sperm head. It adheres the sperm to the egg by binding to the 6β1 integrin protein on the egg plasma membrane. Moreover, like sea urchin bindin (to which it is not structurally related), fertilin has a hydrophobic region that could potentially mediate the union of the two membranes. Thus, fertilin appears to bind the sperm plasma membrane to the egg plasma membrane and then to fuse the two of them together. Mice homozygous for mutant fertilin have sperm with several defects, one of them being the inability to fuse with the egg plasma membrane. When the membranes are fused, the sperm nucleus, mitochondria, centriole, and flagellum can enter the egg.

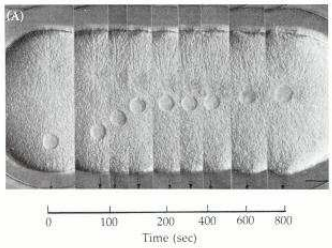
3.5 Fusion of the genetic material

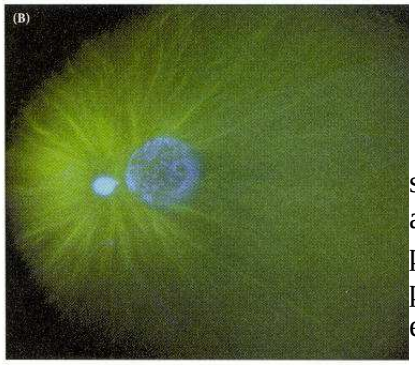
3.5.1 Fusion of genetic material in sea urchins

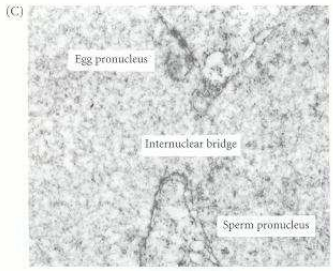
In sea urchins, the sperm nucleus enters the egg perpendicular to the egg surface. After fusion of the sperm and egg plasma membranes, the sperm nucleus and its centriole separate from the mitochondria and the flagellum. The mitochondria and the flagellum disintegrate inside the egg, so very few, if any, sperm-derived mitochondria are found in developing or adult organisms. In mice, it is estimated that only 1 out of every 10,000 mitochondria is sperm-derived. Thus, although each gamete contributes a haploid genome to the zygote, the mitochondrial genome is transmitted primarily by the maternal parent. Conversely, in almost all animals studied (the mouse being the major exception), the centrosome needed to produce the mitotic spindle of the subsequent divisions is derived from the sperm centriole.

The egg nucleus, once it is haploid, is called the **female pronucleus**. Once inside the egg, the sperm nucleus decondenses to form the **male pronucleus**. The sperm nucleus undergoes a dramatic transformation. The nuclear envelope vesiculates into small packets, thereby exposing the compact sperm chromatin to the egg cytoplasm. The proteins holding the sperm chromatin in its condensed, inactive state are exchanged for other proteins derived from the egg cytoplasm. This exchange permits the decondensation of the sperm chromatin. In sea urchins, decondensation appears to be initiated by the phosphorylation of two sperm-specific histones that bind tightly to the DNA. This process begins when the sperm comes into contact with a glycoprotein in the egg jelly that elevates the level of cAMP-dependent protein kinase activity. These protein kinases phosphorylate several of the basic residues of the sperm-specific histones and thereby interfere with their binding to DNA. This loosening is thought to facilitate the replacement of the sperm-specific histones with other histones that have been stored in the oocyte cytoplasm. Once decondensed, the DNA can begin transcription and replication.

After the sea urchin sperm enters the egg cytoplasm, the male pronucleus rotates 180° so that the sperm centriole is between the sperm pronucleus and the egg pronucleus. The sperm centriole then acts as a microtubule organizing center, extending its own microtubules and integrating them with egg microtubules to form an aster.*







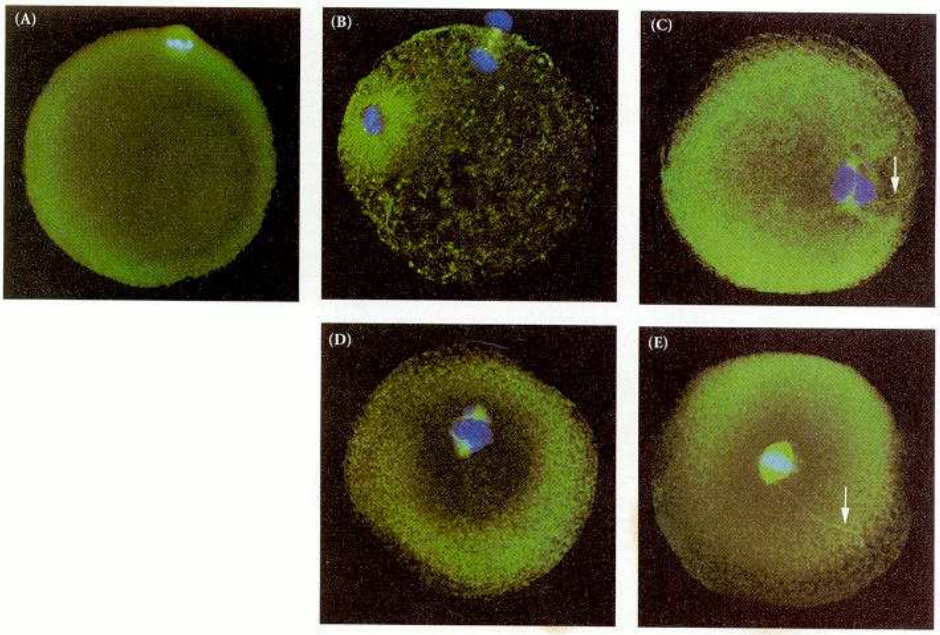
These microtubules extend throughout the egg and contact the female pronucleus, and the two pronuclei migrate toward each other

Their fusion forms the diploid **zygote nucleus** The initiation of DNA synthesis can occur either in the pronuclear stage (during migration) or after the formation of the zygote nucleus.

3.5.2. Fusion of genetic material in mammals

In mammals, the process of pronuclear migration takes about 12 hours, compared with less than 1 hour in the sea urchin. The mammalian sperm enters almost tangentially to the surface of the egg rather than approaching it perpendicularly, and it fuses with numerous microvilli.The mammalian sperm nucleus also breaks down as its chromatin decondenses and is then reconstructed by coalescing vesicles. The DNA of the sperm nucleus is bound by basic proteins called protamines, and these nuclear proteins are tightly compacted through disulfide bonds. In the egg cytoplasm, glutathione reduces these disulfide bonds and allows the uncoiling of the sperm chromatin. The mammalian male pronucleus enlarges while the oocyte nucleus completes its second meiotic division The centrosome (new centriole) accompanying the male pronucleus produces its asters (largely from proteins stored in the oocyte) and contacts the female pronucleus. Then each pronucleus migrates toward the other, replicating its DNA as it travels. Upon meeting, the two nuclear envelopes break down (FigureB). However, instead of producing a common zygote nucleus (as happens in sea urchin fertilization), the chromatin condenses into chromosomes that orient themselves on a common mitotic spindle (Figure C).

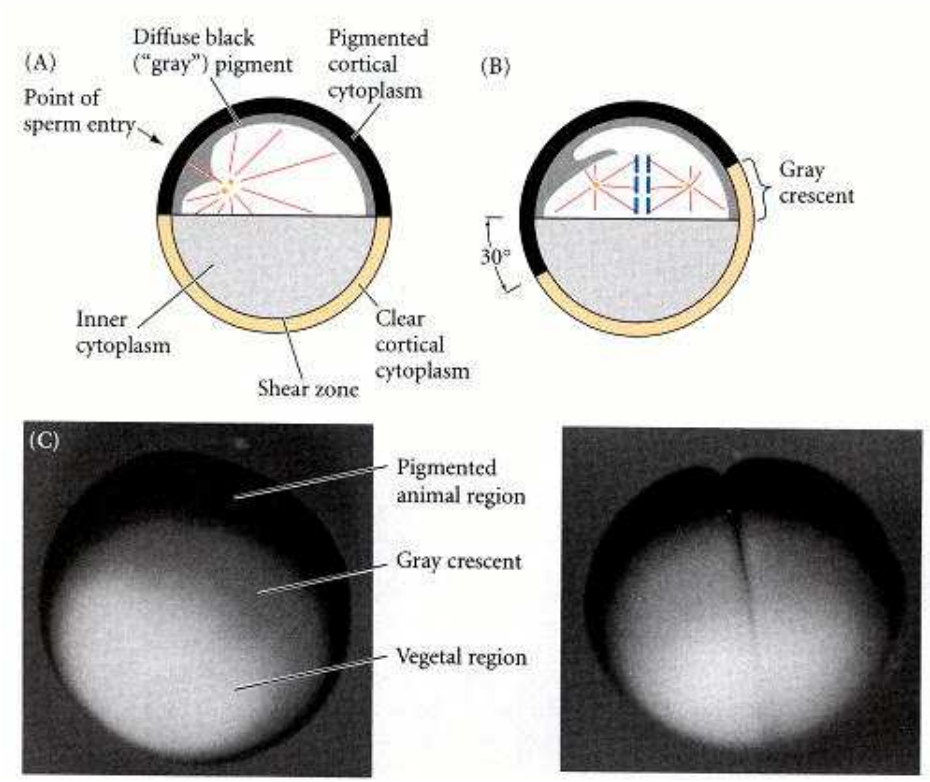
Thus, a true diploid nucleus in mammals is first seen not in the zygote, but at the 2-cell stage.



*When Oscar Hertwig observed this radial array of sperm asters forming in his newly fertilized sea urchin eggs, he called it "the sun in the egg," and he thought it the happy indication of a successful fertilization. More recently, found that certain types of human male infertility are due to defects in the centriole's ability to form these microtubular asters. This deficiency causes the failure of pronuclear migration and the cessation of further development.

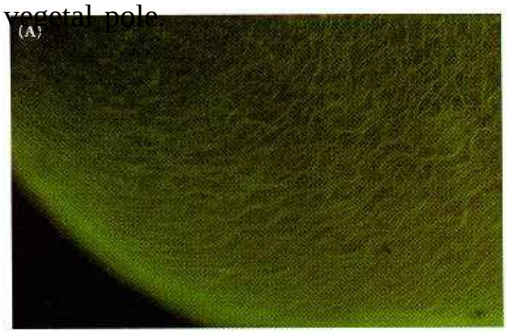
3.6 Rearrangement of the Egg Cytoplasm

Fertilization can initiate radical displacements of the egg's cytoplasmic materials. While these cytoplasmic movements are not obvious in mammalian or sea urchin eggs, there are several species in which these rearrangements of oocyte cytoplasm are crucial for cell differentiation later in development. In the eggs of tunicates, as we will see in, cytoplasmic rearrangements are particularly obvious because of the differing pigmentation of the different regions of the egg. Such cytoplasmic movements are also easy to see in amphibian eggs. In frogs, a single sperm can enter anywhere on the animal hemisphere of the egg; when it does, it changes the cytoplasmic pattern of the egg. Originally, the egg is radially symmetrical about the animal-vegetal axis. After sperm entry, however, the cortical (outer) cytoplasm shifts about 30° toward the point of sperm entry, relative to the inner cytoplasm . In some frogs (such as *Rana*), a region of the egg that was formerly covered by the dark cortical cytoplasm of the animal hemisphere is now exposed. This underlying cytoplasm, located near the equator on the side opposite the point of sperm entry, contains diffuse pigment granules and therefore appears gray. Thus, this region has been referred to as the **gray crescent** As we will see in subsequent chapters, the gray crescent marks the region where gastrulation is initiated in amphibian embryos.



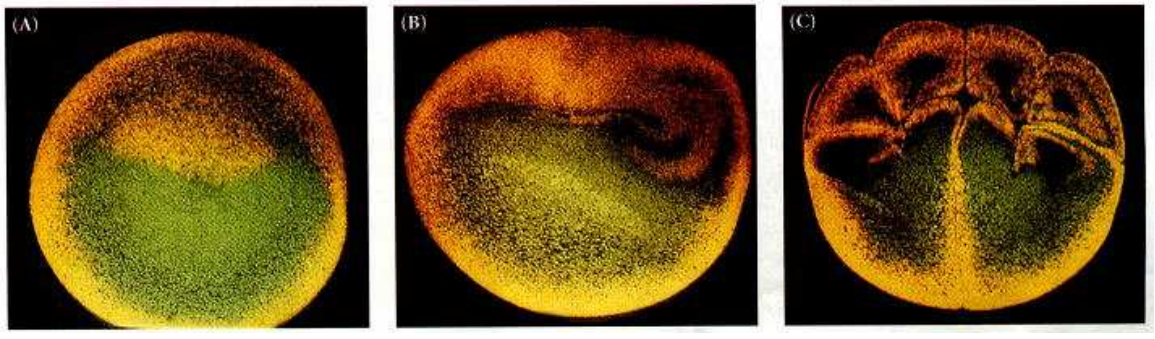
In frogs such as *Xenopus*, in which no gray crescent appears, dye labeling confirms that the cortical cytoplasm rotates 30° relative to the internal, subcortical cytoplasm (Vincent et al. 1986). The motor for these cytoplasmic movements in amphibian eggs appears to be an array of parallel microtubules that form between the cortical and inner cytoplasm of the vegetal hemisphere parallel to the direction of cytoplasmic rotation. These microtubular tracks are first seen immediately before the rotation commences, and they disappear when rotation ceases

Treating the egg with colchicine or ultraviolet radiation at the beginning of rotation stops the formation of these microtubules, thereby inhibiting the cytoplasmic rotation. Using antibodies that bind to the microtubules, Houliston and Elinson (1991a) found that these tracks are formed from both sperm- and egg-derived microtubules, and that the sperm centriole directs the polymerization of the microtubules so that they grow into the vegetal region of the egg. Upon reaching the vegetal cortex, the microtubules angle away from the point of sperm entry, toward the vegetal pole.



The off-center position of the sperm centriole as it initiates microtubule polymerization provides the directionality to the rotation. The motive force for the rotation may be provided by the ATPase **kinesin**. Like dynein and myosin, kinesin is able to attach to fibers and produce energy through ATP hydrolysis. This ATPase is located on the vegetal microtubules and the membranes of the cortical endoplasmic reticulum.

The movement of the cortical cytoplasm with respect to the inner cytoplasm causes profound changes within the inner cytoplasm have labeled yolk platelets with Nile blue and watched their movement by fluorescent microscopy (the bound dye fluoresces red). During the middle part of the first cell cycle, a mass of central egg cytoplasm flows from the presumptive ventral (belly) to the future dorsal (back) side of the embryo. By the end of first division, the cytoplasm of the prospective dorsal side of the embryo is distinctly different from that of the prospective ventral side. What had been a radially symmetrical embryo is now a bilaterally symmetrical embryo. These cytoplasmic movements initiate a cascade of events that determine the dorsal-ventral axis of the frog. Indeed, the parallel microtubules that allow these rearrangements to stretch along what will become the dorsal-ventral axis of the frog



3.6.1.Preparation for cleavage

The increase in intracellular free calcium ions that activates DNA and protein synthesis also sets in motion the apparatus for cell division. The mechanisms by which cleavage is initiated probably differ among species, depending on the stage of meiosis at which fertilization occurs. However, in all species studied, the rhythm of cell divisions is regulated by the synthesis and degradation of a protein called **cyclin**. As we will see in cyclin keeps cells in metaphase, and the breakdown of cyclin enables the cells to return to interphase. In addition to their other activities, calcium ions appear to initiate the degradation of cyclin. Once the cyclin is degraded, the cycles of cell division can begin anew.

Cleavage has a special relationship to the egg regions established by the cytoplasmic movements described above. In tunicate embryos, the first cleavage bisects the egg, with its established cytoplasmic pattern, into mirror-image duplicates. From that stage on, every division on one side of the cleavage furrow has a mirror-image division on the opposite side. Similarly, the gray crescent is bisected by the first cleavage furrow in amphibian eggs (Figure 7.33D). Thus, the position of the first cleavage is not random, but tends to be specified by the point of sperm entry and the subsequent rotation of the egg cytoplasm. The coordination of cleavage plane and cytoplasmic rearrangements is probably mediated through the microtubules of the sperm aster.

Toward the end of the first cell cycle, then, the cytoplasm is rearranged, the pronuclei have met, DNA is replicating, and new proteins are being translated. The stage is set for the development of a multicellular organism.

4.0 CONCLUSION

In this unit you learnt how gametes is formed with the structure it forms before and after fertilization, with specific reference to seaurchin and mammals. Also, you learnt about acrosomal reaction of sperm and egg in sea urchin and mammals and finally fusion of genetic materials in both sea urchin and mammal.

5.0 SUMMARY

Fertilization accomplishes two separate activities: sex (the combining of genes derived from two parents) and reproduction (the creation of a new organism).

The events of conception usually include: (1) contact and recognition between sperm and egg; (2) regulation of sperm entry into the egg; (3) fusion of genetic material from the two gametes; and (4) activation of egg metabolism to start development.

The sperm head consists of a haploid nucleus and an acrosome. The acrosome is derived from the Golgi apparatus and contains enzymes needed to digest extracellular coats surrounding the egg. The neck of the sperm contains the mitochondria and the centriole that generates the microtubules of the flagellum. Energy for flagellar motion comes from mitochondrial ATP and a dynein ATPase in the flagellum.

The egg contains a haploid nucleus, and an enlarged cytoplasm storing ribosomes, mRNAs, and nutritive proteins. Other mRNAs and proteins, used as morphogenetic

factors, are also stored in the egg. Cortical granules lie beneath the egg's plasma membrane. Many eggs also contain protective agents needed for survival in their particular environment.

Surrounding the egg plasma membrane is an extracellular layer often used in sperm recognition. In most animals, this extracellular layer is the vitelline envelope. In mammals, it is the much thicker zona pellucida. In many species, eggs secrete diffusible molecules that attract and activate the sperm.

In sea urchins, the acrosome reaction is initiated by compounds in the egg jelly. The acrosomal vesicle undergoes exocytosis to release its enzymes. Globular actin polymerizes to extend the acrosomal process. Bindin on the acrosomal process is recognized by a protein complex on the
sea urchin egg surface.

In mammals, sperm must be capacitated in the female reproductive tract before they are capable of fertilizing the egg. Mammalian sperm bind to the zona pellucida before undergoing the acrosome reaction. In the mouse, this binding is mediated by ZP3 (zona protein 3) and one or many sperm proteins that recognize it. The mammalian acrosome reaction is initiated on the zona pellucida, and the acrosomal enzymes are concentrated there.

Fusion between sperm and egg is mediated by protein molecules whose hydrophobic groups can merge the sperm and egg plasma membranes. In sea urchins, bindin may mediate gamete fusion. In mammals, fertilin proteins in the sperm bind to integrins in the egg and allow the membranes to fuse.

The male pronucleus and the female pronucleus migrate toward each other, replicating DNA as they move. In sea urchins, the two pronuclei merge and a diploid zygote nucleus is formed. In mammals, the pronuclei disintegrate as they approach each other, and their chromosomes gather around a common metaphase plate.

Some genes are transmitted differently depending on whether they are from the egg or the sperm. Methylation differences determine if these genes are to be expressed in the early embryo.Microtubular changes cause cytoplasmic movements. These rearrangements of cytoplasm can be critical in specifying which portions of the egg are going to develop into which organs.

6.0 TUTOR-MARKED ASSIGNMENT

1 Discuss the structure of gametes in sea urchin and mammals

2 Explain acrosomal reaction in sea urchin and mammals

3Differentiate between sea urchin and mammals gamete structure

7.0 REFERENCES/FURTHER

Professor Scott Gilbert, Developmental Biology, 6th Edition.

MODULE 3: FERTILIZATION AND CLEAVAGE FORMATION IN ANIMALS

UNIT 1: FERTILIZATION IN ANIMALS

CONTENT

1.0 INTRODUCTON

Fertilization is the fusion of gametes to produce a new organism. It is also known as conception, fecundation and syngamy. In animals, fertilization is the fusion of a sperm cell with an egg cell. The penetration of the egg cell by the chromosome-containing part of the sperm cell causes a reaction, which prevents additional sperm cells from entering the egg. The egg and sperm each contribute half of the new organism's genetic material. A fertilized egg cell is known as a zygote. The zygote undergoes continuous cell division, which eventually produces a new multicellular organism. Fertilization in humans occurs in oviducts (fallopian tubes) of the female reproductive tract and takes place within hours following sexual intercourse. Only one of the approximately 300 million sperm released into a female's vagina during intercourse can fertilize the single female egg cell. The successful sperm cell must enter the uterus and swim up the fallopian tube to meet the egg cell, where it passes through the thick coating surrounding the egg. This coating, consisting of sugars and proteins, is known as the zona pellucida. The tip of the head of the sperm cell

contains enzymes which break through the zona pellucida and aid the penetration of the sperm into the egg. Once the head of the sperm is inside the egg, the tail of the sperm falls off, and the perimeter of the egg thickens to prevent another sperm from entering.

The sperm and the egg each contain only half the normal number of chromosomes, a condition known as haploid. When the genetic material of the two cells fuses, the fertilization is complete.

In humans, a number of variables affect whether or not fertilization occurs following intercourse. One factor is a woman's ovulatory cycle. Human eggs can only be fertilized a few days after ovulation, which usually occurs only once every 28 days. In other species, fertilization occurs either internally (as above) or externally, depending on the species involved.

2.0 OBJECTIVES

By the end of the unit, the student should be able to:

1. Explain what you understand by fertilization
2. Explain with examples internal and external fertilization with example in animals
3. Explain fertilization with a specific example in (a) Mammals (b) Human

3.0 MAIN CONTENT

3.0 Types of Fertilization: There two methods of fertilization; external and internal fertilization.

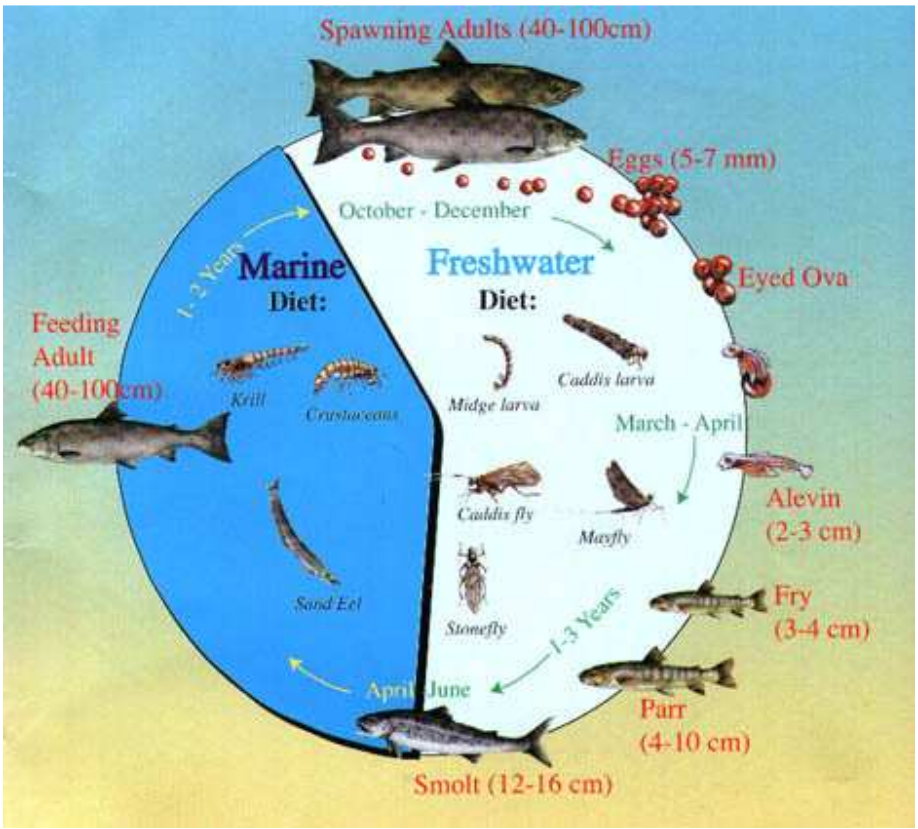
3.1 External Fertilization

External fertilization occurs mostly in wet environments and requires both the male and the female to release their gametes into their surroundings (usually water). An advantage of external fertilization is that it results in the production of a large number of offspring. One disadvantage is that environmental hazards such as predators greatly reduce the chance of surviving into adulthood.

The entire process of development of new individuals is called procreation, the act of species reproduction. Consideration as to whether an animal (more specifically a vertebrate) uses internal or external fertilisation is often dependent on the method of birth. Fertilization outside of the animal's body occurs in aquatic animals such as sea urchins, fish, and frogs. In sea urchins, several billion sperm are released into the water and swim towards eggs

released in the same area, in most of fish and in many amphibians, the eggs and sperms are released in the water around the animals and fertilization takes place there. When the fish are about to reproduce, the males and females swim close together.

Oviparous animals laying eggs with thick calcium shells, such as chickens, or thick leathery shells generally reproduce via internal fertilisation so that the sperm fertilise the egg without having to pass through the thick, protective, tertiary layer of the egg. Ovoviviparous and euviviparous animals also use internal fertilisation. It is important to note that although some organisms reproduce via amplexus, they may still use internal fertilisation, as with some salamanders. Advantages to internal fertilisation include: minimal waste of gametes; greater chance of individual egg fertilisation, relatively "longer" time period of egg protection, and selective fertilisation; many females have the ability to store sperm for extended periods of time and can fertilize their eggs at their own desire, minimal contact and transmission of bodily fluids; decreasing the risk of disease transmission, and greater genetic variation (especially during broadcast spawning external fertilisation methods).



External Fertilization

3.1.1.Aquatic Animals with External fertilization

1 **The sea urchin**

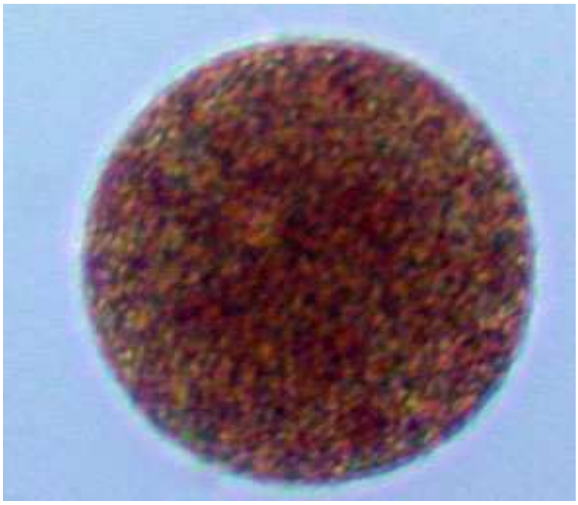
To illustrate the gametes and fertilization process in animals, we will begin with the sea urchin, an aquatic invertebrate.

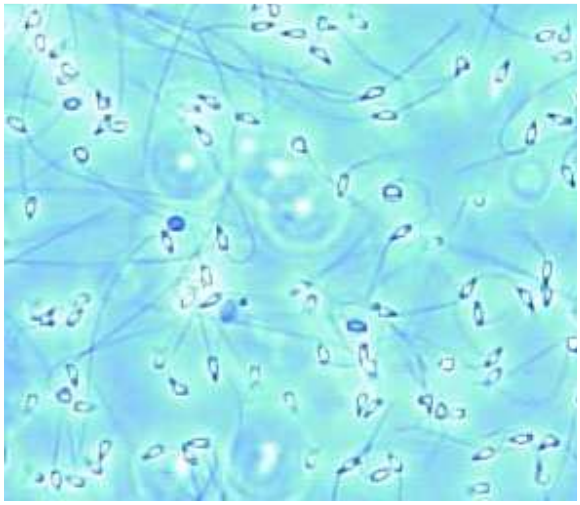
Sea urchins are echinoderms that live in marine environments throughout the world. We are using the sea urchin *Arbacia punctulata* from the Florida coast. Like most aquatic animals, the sea urchin sheds its gametes into the water and fertilization is external.

Gametes are produced by meiosis as described in Animal Development I. The production of sperm by the male (spermatogenesis) entails the usual meiotic divisions to produce haploid daughter cells. However, these cells are not sperm until they undergo morphological changes. Review the structure of sperm before proceeding. The production of eggs by the female (oogenesis) is also by meiosis, but there are several deviations from the "classic" meiotic process. A major feature of oogenesis is the arrest of meiosis at the first meiotic prophase during which large quantities of yolk and other substances are stored within the cell cytoplasm. When these large cells divide at telophase I and telophase II, they divide unequally so that all stored materials are retained within one cell. Thus oogenesis of a mother cell produces one large egg and 3 tiny (non-functional) cells called polar bodies. Also, eggs are surrounded by protective coverings; a vitelline envelope and jelly coat in the case of sea urchins. Review oogenesis and egg structure before proceeding to the fertilization experiment.

We can perform fertilization in the laboratory by inducing fertile sea urchins to release their gametes. Play the following video to see how this is done, then observe the eggs and sperm as they appear using the light microscope.

Sperm are obtained using the same procedure, except that the milky sperm suspension is collected from the urchin's body surface and diluted in a small amount of sea water. The resulting sperm suspension can be viewed in the video below.





Unfertilized sea urchin egg **Suspension of sea urchin sperm**

Note the difference in size of egg vs. sperm (micrographs are at similar magnification)

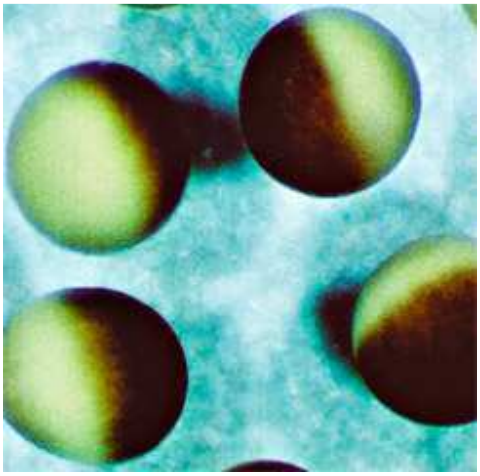
To view fertilization, a drop of sperm is added to the slide containing eggs, and the eggs are observed under the microscope as shown in the following video. Note that shortly after a sperm has contacted the egg surface, the surrounding vitelline envelope rises creating what is called the "fertilization membrane". Prior to fertilization, the vitelline membrane adheres to the egg surface and is not visible as a separate structure. The fertilization membrane soon hardens to provide a protective covering for the developing embryo. The rising fertilization membrane also pushes other sperm away from the egg and forms a permanent block to polyspermy (i.e. no additional sperm can enter the egg).

You have learned from the topic Animal Development I that the sperm of one species canno usually t fertilize the eggs of a another species. We will now put this to the test. The following video shows a slide containing eggs from two species of sea urchin. The eggs of *Lytechinus pictus* are large and those of *Strongylocentrotus purpuratus* are small and brown. Sperm from *Lytechinus pictus* are added to the mixture at the beginning of the video.

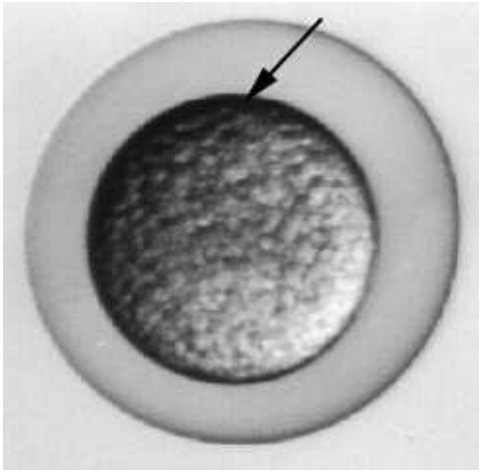
To complete the fertilization process, the nucleus of sperm and egg must fuse. The compacted DNA within the sperm nucleus expands and can be seen within the egg. The fusing nuclei are called pronuclei and the resulting diploid cell is called a zygote. Observe pronuclear fusion in the following video.

2 **Frog and Zebrafish eggs**

Many aquatic species produce eggs containing more yolk than the sea urchin. Thus these eggs are much larger, relative to the size of the adult animal. Two examples of large, yolk-filled eggs are shown below. Note that the yolk is asymmetrically distributed within these eggs, whereas in the sea urchin egg the relative small amount of yolk is present as small granules throughout the egg cytoplasm.



Several frog eggs are shown here. They are quite large, about 1 mm in diameter.Tthe brown part of the egg is mainly cytoplasm and the yellow part is mainly yolk. Frog eggs have relatively more yolk than sea urchin eggs, and the frog embryo develops longer before hatching. Thus the newly hatched larva of the frog embryo is more mature than that of the sea urchin. The frog egg is surrounded by a vitelline membrane which lies so close to the egg surface that it is not visible as a separate structure.



This zebrafish egg is physically smaller than the frog egg and must be viewed under a microscope. However, the adult fish is smaller than a frog, so the egg is actually larger than the frog egg relative to animal size. Like all fish eggs, it contains a huge amount of yolk. In fact the entire egg, except for a small patch near the arrow, is yolk. With so much yolk, the embryo develops into a mature-looking larva before hatching. The covering around the egg is called a "chorion" but is analogous to the vitelline membrane of other species.

3.2 Internal Fertilization

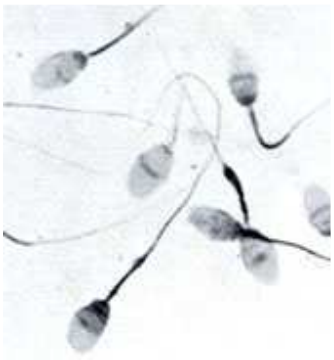
Animals that use internal fertilization specialize in the protection of the developing egg. For example, reptiles and birds secrete eggs that are covered by a protective shell that is resistant to water loss and damage. Mammals, with the exception of monotremes, take this idea of protection a step further by allowing the embryo to develop within the mother. This extra protection increases the chances of survival because mom supplies everything that the embryo needs. In fact, most mammalian mothers continue to care for their young for several years after birth.

3.2.1Mammals and internal fertilization

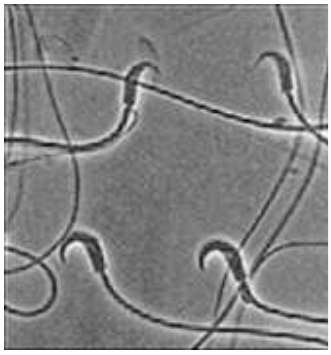
To complete our activity on animal gametogenesis and fertilization, we will now examine these processes in mammals. Gametogenesis in mammals is unique in that the resulting eggs do not contain yolk (why?). Thus, they are smaller than the eggs of most other animals. Since mammals live on land, gametes cannot be shed into water, so fertilization is internal. As compared to external fertilization, a relatively small number of sperm arrive at the location of the egg, so the block to polyspermy is less robust and slower to take effect.

Gametes

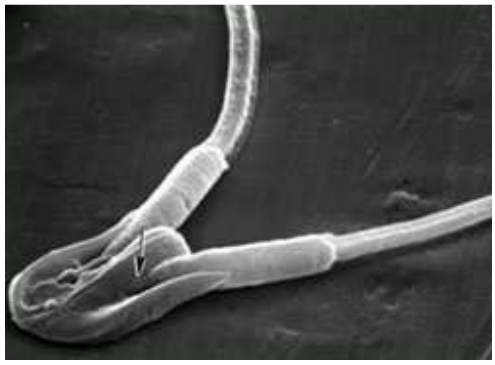
While all sperm have the same basic structure, there are often distinguishing features in different species. Note the different shape of the sperm head and/or acrosome in these sperm:



Human

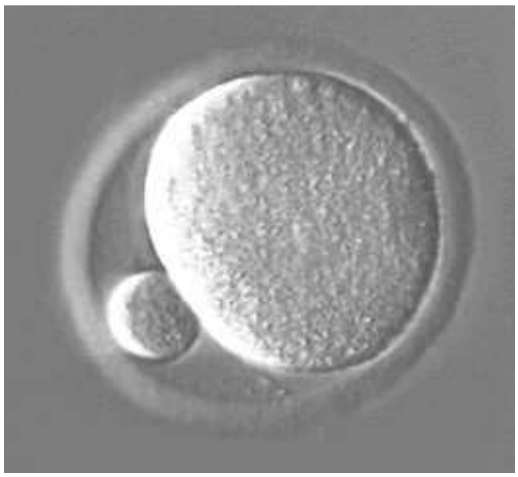


Hamster



Opossum

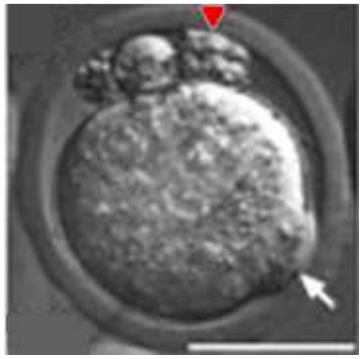
Did you notice that sperm of the opossum have two tails? This condition is extremely rare and the reason for it is unknown. One might wonder if these sperm can swim. Watch the following video of opossum sperm and decide for yourself.



This light micrograph shows a human egg. It is typical of mammalian eggs and contains no yolk. The first meiotic division has been completed and the first polar body is visible. As in many mammalian species, the second meiotic division will not occur until the egg is fertilized. The covering that surrounds the egg and polar body is called the zona pellucida. It bears sperm binding sites and is analogous to the vitelline membrane of non-mammalian eggs.

Fertilization in mammals occurs internally, as is typical of terrestrial animals. The sperm utilizes the acrosomal reaction to penetrate through the zona pellucida and is then pulled into the egg cytoplasm. After the second meiotic division is complete, the sperm and egg pronuclei fuse as in all animal species.

Sperm within uterus
Sperm will traverse the uterus and enter a fallopian tube



Fertilization
Red arrow: a polar body, white arrow: entry point of the sperm

Pronuclear fusion
Circle surrounds the sperm and egg pronuclei

3.0 Conclusion

In this unit you were introduce to the term fertilization, types with specific examples to sea urchin , frog and zebra. Other example were also explain in mammal as internal fertilization. External fertilization is also found in aquatic organism e.g fish etc.

4.0 Summary

Fertilisation (also known as conception, fecundation and syngamy), is the fusion of gametes to produce a new organism. There are two types of fertilization, internal and external. In animals, the process involves a sperm fusing with an ovum, which eventually leads to the development of an embryo. Depending on the animal species, the process can occur within the body of the female in internal fertilisation, or outside in the case of external fertilisation. The fertilized egg cell is known as the zygote. Examples of internal and external fertilization in mammal and sea-urchin and frog are explained respectively.

5.0 Tutor-marked Assignment

1 Explain what you understand by fertilization
2 Explain with examples internal and external fertilization with example in animals
3 Explain fertilization with a specific example in (a) Mammals (b) Human

7.0 References

Regina Bailey, 1977. Sexual Reproduction: Fertilization About.com Guide, 1997

http://biology.northwestcollege.edu/biology/b1010lab/mitwfish.htm

Forgács, G. & Newman, Stuart A. (2005). "Cleavage and blastula formation". *Biological physics of the developing embryo*. Cambridge University Press. I

UNIT 2: CLEAVAGE AND BLASTOCYST FORMATION

CONTENT

1.0 INTRODUCTION

Remarkable as it is, fertilization is but the initiating step in development. The zygote, with its new genetic potential and its new arrangement of cytoplasm, now begins the production of a multicellular organism. Between these events of fertilization and the events of organ formation are two critical stages: cleavage and gastrulation. During cleavage, rapid cell divisions divide the cytoplasm of the fertilized egg into numerous cells. These cells then undergo dramatic displacements during gastrulation, a process whereby they move to different parts of the embryo and acquire new neighbors (see Chapter 2). During cleavage and gastrulation, the major axes of the embryo are determined, and the cells begin to acquire their respective fates.

While cleavage always precedes gastrulation, axis formation can begin as early as oocyte formation. It can be completed during cleavage (as is the case with *Drosophila*) or extend all the way through gastrulation (as it does in *Xenopus*). There are three axes that need to be specified: the anterior-posterior (head-anus) axis, the dorsal-ventral (back-belly) axis, and the left-right axis. Different species specify these axes at different times, using different mechanisms

2.0 OBJECTIVES

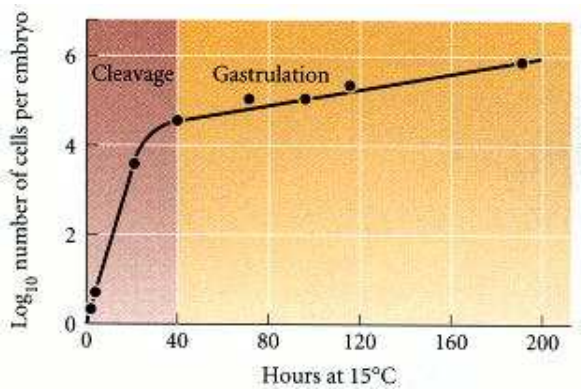
At the end of this unit the student should be able to:

1.0 Explain cleavage and the significance mitosis promoting factor in cleavage formation.
2.0 State and explain the different pattern of embryonic cleavage

3.0 MAIN CONTENT

3.1 Cleavage

After fertilization, the development of a multicellular organism proceeds by a process called **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 (mammals being the chief exception), 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. The zygotic genome, transmitted by mitosis to all the new cells, does not function in early-cleavage embryos. Few, if any, mRNAs are made until relatively late in cleavage, and the embryo can divide properly even when chemicals are used experimentally to inhibit transcription. During cleavage, however, cytoplasmic volume does not increase. Rather, the enormous volume of zygote cytoplasm is divided into increasingly smaller cells. First the egg is divided in half, then quarters, then eighths, and so forth. This division of egg cytoplasm without increasing its volume is accomplished by abolishing the growth period between cell divisions (that is, the G_1 and G_2 phases of the cell cycle). Meanwhile, the cleavage of nuclei occurs at a rapid rate never seen again (not even in tumor cells). A frog egg, for example, can divide into 37,000 cells in just 43 hours. Mitosis in cleavage-stage *Drosophila* embryos occurs every 10 minutes for over 2 hours and in just 12 hours forms some 50,000 cells.



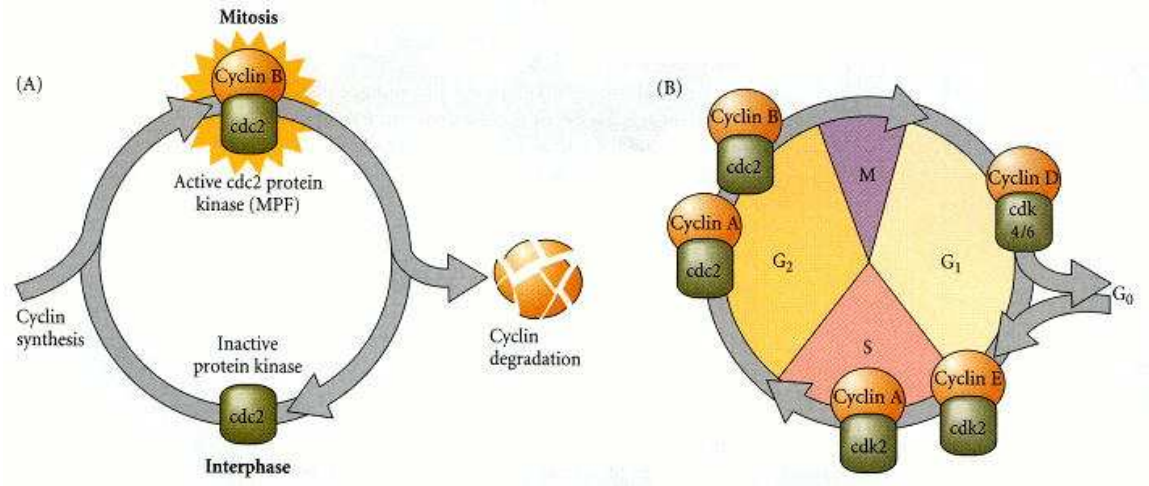
This dramatic increase in cell number can be appreciated by comparing cleavage with other stages of development. Figure 8.1 shows the logarithm of cell number in a frog embryo plotted against the time of development. It illustrates a sharp discontinuity between cleavage and gastrulation.

One consequence of this rapid cell division is that the ratio of cytoplasmic to nuclear volume gets increasingly smaller as cleavage progresses. In many types of embryos (such as those of *Xenopus* and *Drosophila,* but not those of *C. elegans* or mammals), 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 **mid-blastula transition**. It is thought that some factor in the egg is being titrated by the newly made chromatin, because the time of

this transition can be changed by experimentally altering the ratio of chromatin to cytoplasm in the cell. Thus, cleavage begins soon after fertilization and ends shortly after the stage when the embryo achieves a new balance between nucleus and cytoplasm.

3.1.1From fertilization to cleavage

The transition from fertilization to cleavage is caused by the activation of **mitosis promoting factor** (**MPF**). MPF was first discovered as the major factor responsible for the resumption of meiotic cell divisions in the ovulated frog egg. It continues to play a role after fertilization, regulating the biphasic cell cycle of early blastomeres. Blastomeres generally progress through a cell cycle consisting of just two steps: M (mitosis) and S (DNA synthesis). MPF undergoes cyclical changes in its level of activity in mitotic cells. The MPF activity of early blastomeres is highest during M and undetectable during demonstrated that DNA replication (S) and mitosis (M) are driven solely by the gain and loss of MPF activity. Cleaving cells can be experimentally trapped in S phase by incubating them in an inhibitor of protein synthesis. When MPF is microinjected into these cells, they enter M. Their nuclear envelope breaks down and their chromatin condenses into chromosomes. After an hour, MPF is degraded and the chromosomes return to S phase.



What causes this cyclic activity of MPF? Mitosis-promoting factor contains two subunits. The large subunit is called **cyclin B**. It is this component that shows a periodic behavior, accumulating during S and then being degraded after the cells have reached. Cyclin B is often encoded by mRNAs stored in the oocyte cytoplasm, and if the translation of this message is specifically inhibited, the cell will not enter mitosis. The presence of cyclin B depends upon its synthesis and its degradation. Cyclin B regulates the small subunit of MPF, the **cyclin-dependent kinase**.

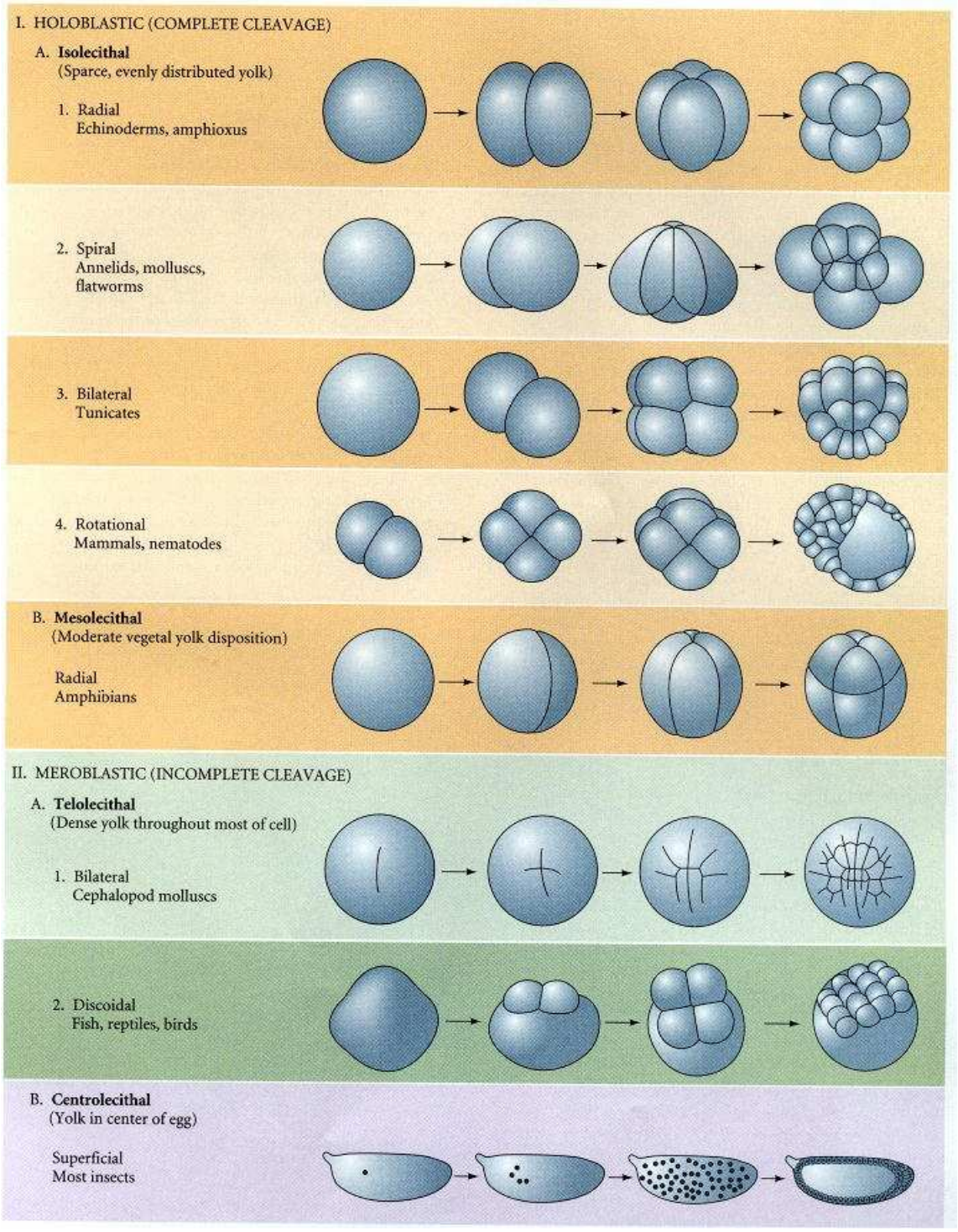
This kinase activates mitosis by phosphorylating several target proteins, including histones, the nuclear envelope lamin proteins, and the regulatory subunit of cytoplasmic myosin. This brings about chromatin condensation, nuclear envelope depolymerization, and the organization of the mitotic spindle.

Without cyclin, the cyclin-dependent kinase will not function. The presence of cyclin is controlled by several proteins that ensure its periodic synthesis and degradation. In most species studied, the regulators of cyclin (and thus, of MPF) are stored in the egg cytoplasm. Therefore, the cell cycle is independent of the nuclear genome for

numerous cell divisions. These early divisions tend to be rapid and synchronous. However, as the cytoplasmic components are used up, the nucleus begins to synthesize them. The embryo now enters the mid-blastula transition, in which several new phenomena are added to the biphasic cell divisions of the embryo. First, the growth stages (G_1 and G_2) are added to the cell cycle, permitting the cells to grow. Before this time, the egg cytoplasm was being divided into smaller and smaller cells, but the total volume of the organism remained unchanged. *Xenopus* embryos add those phases to the cell cycle shortly after the twelfth cleavage. *Drosophila* adds G_2 during cycle 14 and G_1 during cycle 17. Second, the synchronicity of cell division is lost, as different cells synthesize different regulators of MPF. Third, new mRNAs are transcribed. Many of these messages encode proteins that will become necessary for gastrulation. If transcription is blocked, cell division will occur at normal rates and at normal times in many species, but the embryo will not be able to initiate gastrulation.

3.1.2. Pattern of embryonic cleavage

The cell, manifestly, entertains a very different opinion." Indeed, different organisms undergo cleavage in distinctly different ways. The pattern of embryonic cleavage particular to a species is determined by two major parameters: the amount and distribution of yolk protein within the cytoplasm, and factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation.



The amount and distribution of yolk determines where cleavage can occur and the relative size of the blastomeres. When one pole of the egg is relatively yolk-free, the cellular divisions occur there at a faster rate than at the opposite pole. The yolk-rich pole is referred to as the **vegetal pole**; the yolk concentration in the **animal pole** is relatively low. The zygote nucleus is freuently displaced toward the animal pole. In general, yolk inhibits cleavage.Figure8provides a classification of cleavage types and shows the influence of yolk on cleavage symmetry and pattern.

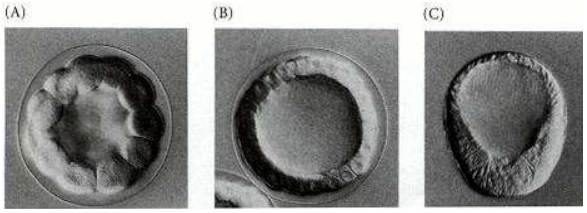
At one extreme are the eggs of sea urchins, mammals, and snails. These eggs have sparse, equally spaced yolk and are thus **isolecithal** (Greek, "equal yolk"). In these species, cleavage is **holoblastic** (Greek *holos,* "complete"). meaning that the cleavage furrow extends through the entire egg. These embryos must have some other way of obtaining food. Most will generate a voracious larval form, while mammals get their nutrition from the placenta.

At the other extreme are the eggs of insects, fishes, reptiles, and birds. Most of their cell volumes are made up of yolk. The yolk must be sufficient to nourish these animals. Zygotes containing large accumulations of yolk undergo **meroblastic** cleavage, wherein only a portion of the cytoplasm is cleaved. The cleavage furrow does not penetrate into the yolky portion of the cytoplasm. The eggs of insects have their yolk in the center (i.e., they are **centrolecithal**), and the divisions of the cytoplasm occur only in the rim of cytoplasm around the periphery of the cell (i.e., **superficial** cleavage). The eggs of birds and fishes have only one small area of the egg that is free of yolk (**telolecithal** eggs), and therefore, the cell divisions occur only in this small disc of cytoplasm, giving rise to the **discoidal** pattern of cleavage. These are general rules, however, and closely related species can evolve different patterns of cleavage in a different environment.

However, the yolk is just one factor influencing a species' pattern of cleavage. There are also inherited patterns of cell division that are superimposed upon the constraints of the yolk. This can readily be seen in isolecithal eggs, in which very little yolk is present. In the absence of a large concentration of yolk, four major cleavage types can be observed: radial holoblastic, spiral holoblastic, bilateral holoblastic, and rotational holoblastic cleavage. We will see examples of these cleavage patterns below when we take a more detailed look at the early development of four different invertebrate groups.

3.2 Blastula formation

The **blastula** stage of sea urchin development begins at the 128-cell stage. Here the cells form a hollow sphere surrounding a central cavity, or **blastocoel** (Figure 8.). By this time, all the cells are the same size, the micromeres having slowed down their cell division. Every cell is in contact with the proteinaceous fluid of the blastocoel on the inside and with the hyaline layer on the outside. At this time, tight junctions unite the once loosely connected blastomeres into a seamless epithelial sheet that completely encircles the blastocoels. As the cells continue to divide, the blastula remains one cell layer thick, thinning out as it expands. This is accomplished by the adhesion of the blastomeres to the hyaline layer and by an influx of water that expands the blastocoels.



These rapid and invariant cell cleavages last through the ninth or tenth cell division, depending upon the species. After that time, there is a mid-blastula transition, when the synchrony of cell division ends, new genes become expressed, and many of the nondividing cells develop cilia on their outer surfaces. The ciliated blastula begins to rotate within the fertilization envelope. Soon afterward, differences are seen in the cells. The cells at the vegetal pole of the blastula begin to thicken, forming a **vegetal plate**. The cells of the animal half synthesize and secrete a hatching enzyme that digests the fertilization envelop. The embryo is now a free-swimming hatched blastula.

4.0 CONCLUSION

In this unit you leaent a briefly introduction to cleavage as the next stage from fertilization and how mitosis promoting factor accelerate the formation of cleavage. Also, different pattern of cleavage were shown structurally and explain.

5.0 SUMMARY

This unit introduce you to cleavage as the next stage from fertilization and how mitosis promoting factor accelerate the formation of cleavage. Also, different pattern of cleavage were shown structurally and explain. E.g holoblastic (complete cleavage) and meroblastic(incomplete cleavage).

6.0 TUTOR-MARKED ASSIGNMENT

1.0 Briefly explain cleavage and the significance mitosis promoting factor in cleavage formation .

2.0 State and explain the different pattern of embryonic cleavage

7.0 REFERENCES

Professor Scott Gilbert, Developmental Biology, 6th Edition.

UNIT 3: CLEAVAGE PATTERNS IN MAJOR GROUPS OF ORGANISMS

CONTENT

1.0 INTRODUCTION

Fertilization is the initiating step in development. The zygote, with its new genetic potential and its new arrangement of cytoplasm, now begins the production of a multicellular organism. Between these events of fertilization and the events of organ formation are two critical stages: cleavage and gastrulation. During cleavage, rapid cell divisions divide the cytoplasm of the fertilized egg into numerous cells. These cells then undergo dramatic displacements during gastrulation, a process whereby they move to different parts of the embryo and acquire new neighbors. During cleavage and gastrulation, the major axes of the embryo are determined, and the cells begin to acquire their respective fates. While cleavage always precedes gastrulation, axis formation can begin as early as oocyte formation. It can be completed during cleavage (as is the case with *Drosophila*) or extend all the way through gastrulation (as it does in *Xenopus*). There are three axes that need to be specified: the anterior-posterior (head-anus) axis, the dorsal-ventral (back-belly) axis, and the left-right axis. Different species specify these axes at different times, using different mechanisms

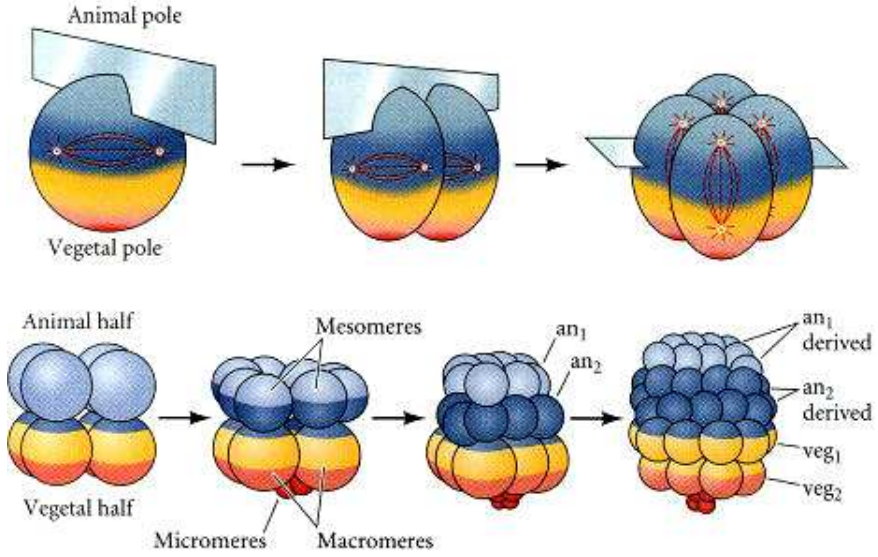
2.0 OBJECTIVE

3.0 MAIN CONTENT

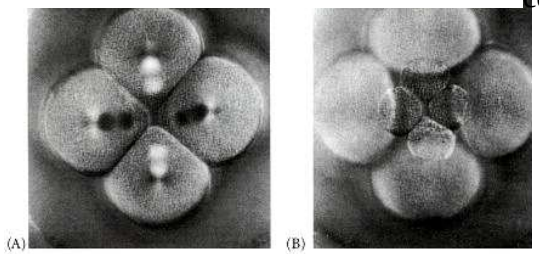
3.1 Cleavage formation in Sea urchin

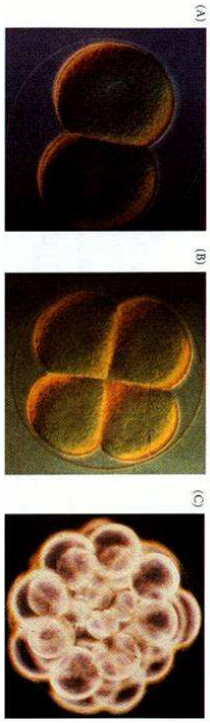
Sea urchins exhibit **radial holoblastic cleavage**. The first and second cleavages are both meridional and are perpendicular to each other. That is to say, the cleavage furrows pass through the animal and vegetal poles. The third cleavage is equatorial, perpendicular to the first two cleavage planes, and separates the animal and vegetal hemispheres from one another. The fourth cleavage, however, is very different from the first three.

The four cells of the animal tier divide meridionally into eight blastomeres, each with the same volume. These cells are called **mesomeres**. The vegetal tier, however, undergoes an *unequal* equatorial cleavage to produce four large cells, the **macromeres**, and four smaller **micromeres** at the vegetal pole. As the 16-cell embryo cleaves, the eight mesomeres divide to produce two "animal" tiers, an1 and an2, one staggered above the other. The macromeres divide meridionally, forming a tier of eight cells below an2. The micromeres also divide, albeit somewhat later, producing a small cluster beneath the larger tier. All the cleavage furrows of the sixth division are equatorial, and the seventh division is meridional, producing a 128-



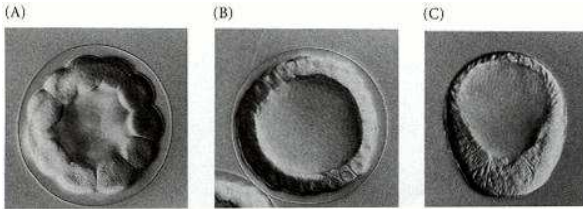
cell blastula.





3.2 Blastula formationin sea urchin

The **blastula** stage of sea urchin development begins at the 128-cell stage. Here the cells form a hollow sphere surrounding a central cavity, or **blastocoel** (Figure 8.11A). By this time, all the cells are the same size, the micromeres having slowed down their cell division. Every cell is in contact with the proteinaceous fluid of the blastocoel on the inside and with the hyaline layer on the outside. At this time, tight junctions unite the once loosely connected blastomeres into a seamless epithelial sheet that completely encircles the blastocoel As the cells continue to divide, the blastula remains one cell layer thick, thinning out. as it expands. This is accomplished by the adhesion of the blastomeres to the hyaline layer and by an influx of water that expands the blastocoel.



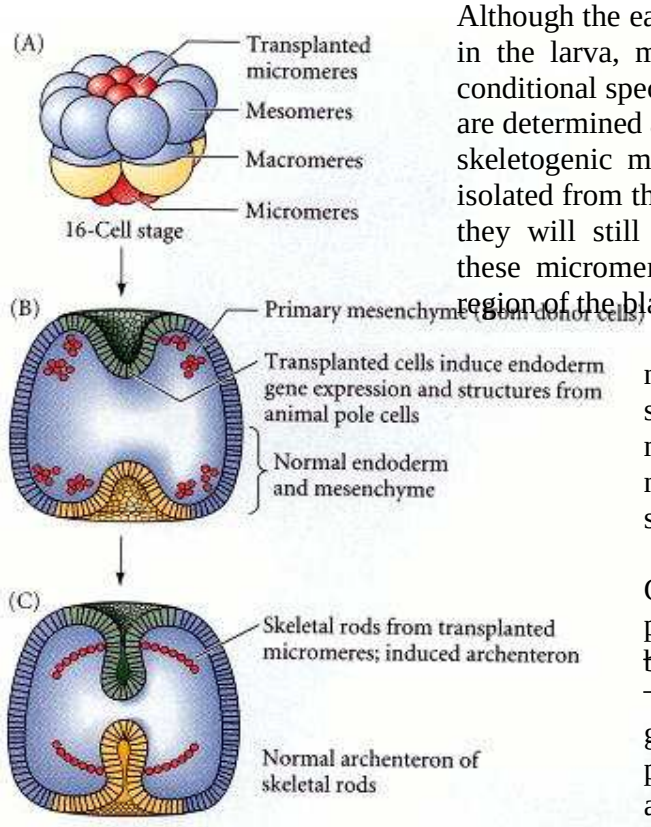
These rapid and invariant cell cleavages last through the ninth or tenth cell division, depending upon the species. After that time, there is a mid-blastula transition, when the synchrony of cell division ends, new genes become expressed, and many of the nondividing cells develop cilia on their outer surfaces; The ciliated blastula begins to rotate within the fertilization envelope. Soon afterward, differences are seen in the cells. The cells at the vegetal pole of the blastula begin to thicken, forming a **vegetal plate**. The cells of the animal half synthesize and secrete a hatching enzyme that digests the fertilization envelope. The embryo is now a free-swimming hatched blastula.

3.2.1 Fate maps and the determination of sea urchin blastomeres

Cell Fate Determination

The fate map of the sea urchin embryo was originally created by observing each of the cell layers and what its descendants became. More recent investigations have refined these maps by following the fates of individual cells injected with fluorescent dyes such as dil. These studies have shown that by the 60-cell stage, most of the embryonic cell fates are specified, but that the cells are not irreversibly committed. In other words, particular blastomeres consistently produce the same cell types in each embryo, but these cells remain pluripotent and can give rise to other cell types if experimentally placed in a different part of the embryo.

A fate map of the 60-cell sea urchin embryo is shown below. The animal half of the embryo consistently gives rise to the ectoderm the larval skin and its neurons. The veg1 layer produces cells that can enter into either the ectodermal or endodermal organs. The veg2 layer gives rise to cells that can populate three different structures the endoderm, the coelom (body wall), and secondary mesenchyme (pigment cells, immunocytes, and muscle cells). The first tier of micromeres produces the primary mesenchyme cells that form the larval skeleton, while the second tier of micromeres contributes cells to the coelom.

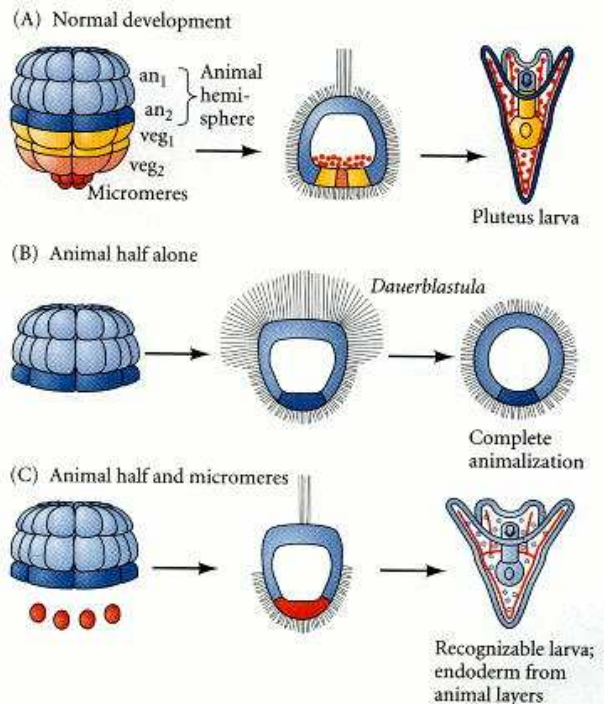


Although the early blastomeres have consistent fates in the larva, most of these fates are achieved by conditional specification. The only cells whose fates are determined autonomously are the skeletogenic micromeres. If these micromeres are isolated from the embryo and placed in test tubes, they will still form skeletal spicules. Moreover, if these micromeres are transplanted into the animal region of the blastula, not only will their descendants form skeletal spicules, but the transplanted micromeres will alter the fates of nearby cells by inducing a secondary site for gastrulation.

Cells that would normally have produced ectodermal skin cells will ~~be respecified~~ as endoderm and will produce a secondary gut. The micromeres appear to produce a signal that tells the cells adjacent to them to become endoderm and induces them to invaginate into the embryo.

Their ability to reorganize the embryonic cells is so pronounced that if the isolated micromeres are recombined with an isolated animal cap (the top two animal tiers), the animal cap cells will generate endoderm, and a more or less normal larva will develop.

In a normal embryo, the veg_2 cells become specified by the micromeres, and they, in turn, help specify the veg_1 layer. Without the veg_2 layer, the veg_1 cells are able to produce endoderm, but the endoderm is not specified as foregut, midgut, or hindgut. Thus, there appears to be a cascade wherein the vegetal pole micromeres induce the cells above them to become the veg_2 cells, and the veg_2 cells induce the cells above them to assume the veg_1 fates.Thus, the micromeres undergo autonomous specification to become skeletogenic mesenchyme, and these micromeres produce the initial signals that specify the other tiers of cells.



The identities of the signaling molecules involved in this process are just now becoming known. The molecule responsible for specifying the micromeres (and their ability to induce the neighboring cells) appears to be β-catenin. As we saw in β-catenin is a transcription factor that is often activated by the Wnt pathway, and several pieces of evidence suggest it for this role. First, during normal sea urchin development, β-catenin accumulates in the nuclei of those cells fated to become endoderm and mesoderm accumulation is autonomous and can occur even if the micromere precursors are separated from the rest of the embryo. Second, this accumulation appears to be responsible for specifying the vegetal half of the embryo.

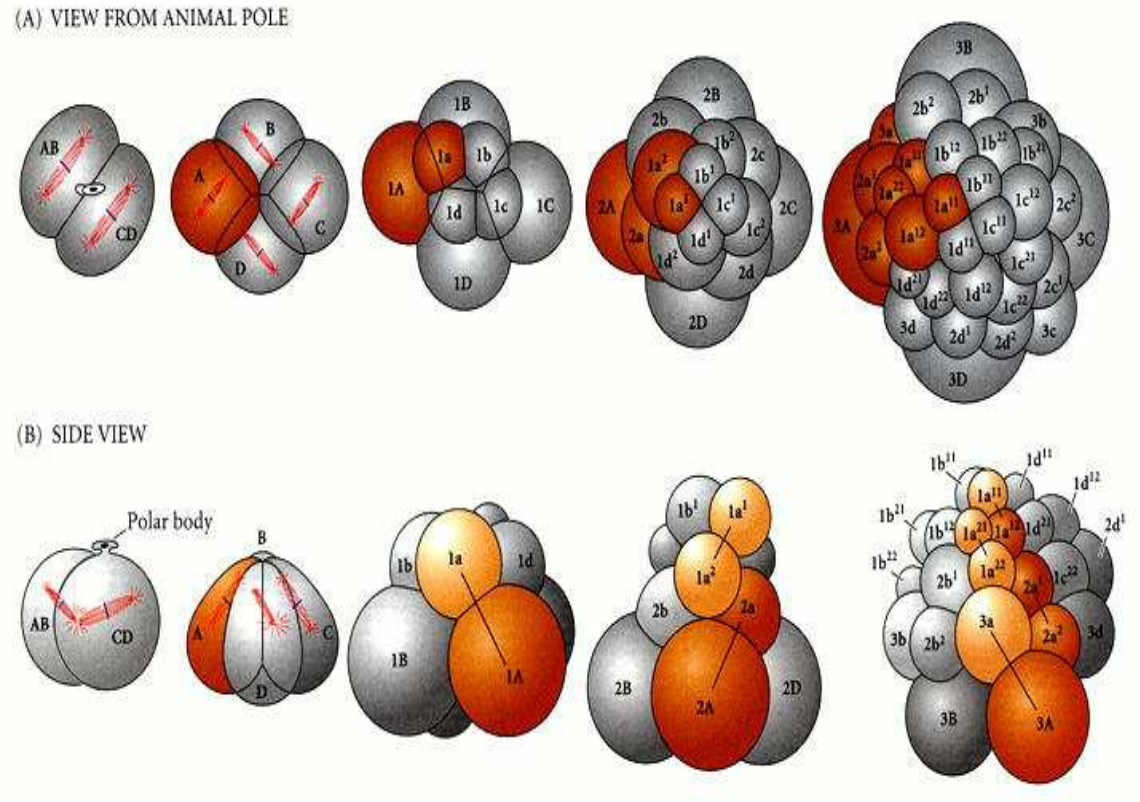
3.1.2 Axis specification

In the sea urchin blastula, the cell fates line up along the animal-vegetal axis established in the egg cytoplasm prior to fertilization. The animal-vegetal axis also appears to structure the future anterior-posterior axis, with the vegetal region sequestering those maternal components necessary for posterior development.

In most sea urchins, the dorsal-ventral and left-right axes are specified after fertilization, but the manner of their specification is not well understood. Since the first cleavage plane can be either parallel, perpendicular, or oblique with respect to the eventual dorsal-ventral axis, it is probable that the dorsal-ventral axis is not specified until the 8-cell stage, when there are cell boundaries that correspond to these positions. Interestingly, in those sea urchins that bypass the larval stage to develop directly into juveniles, the dorsal- ventral axis is specified maternally in the egg cytoplasm.

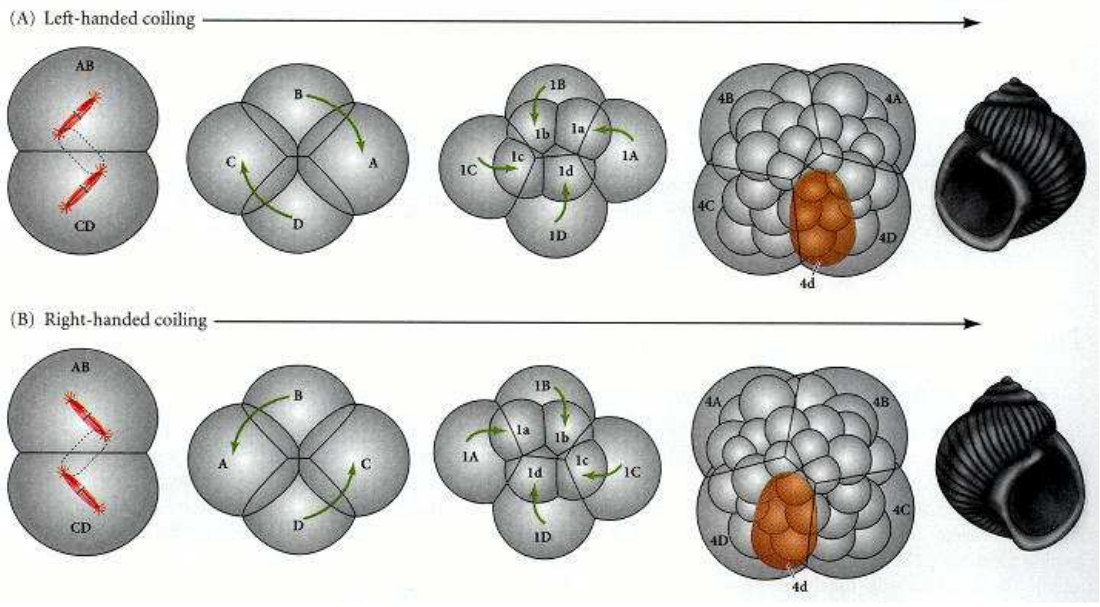
4.0 Cleavage in Snail eggs

Spiral holoblastic cleavage is characteristic of several animal groups, including annelid worms, some flatworms, and most molluscs. It differs from radial cleavage in numerous ways. First, the cleavage planes are not parallel or perpendicular to the animal-vegetal axis of the egg; rather, cleavage is at oblique angles, forming a "spiral" arrangement of daughter blastomeres. Second, the cells touch one another at more places than do those of radially cleaving embryos. In fact, they assume the most thermodynamically stable packing orientation, much like that of adjacent soap bubbles. Third, spirally cleaving embryos usually undergo fewer divisions before they begin gastrulation, making it possible to follow the fate of each cell of the blastula. When the fates of the individual blastomeres from annelid, flatworm, and mollusc embryos were compared, many of the same cells were seen in the same places, and their general fates were identical. Blastulae produced by radial cleavage have no blastocoel and are called stereoblastulae.



The figure above the cleavage of mollusc embryos. The first two cleavages are nearly meridional, producing four large macromeres (labeled A, B, C, and D). In many species, the blastomeres are different sizes (D being the largest), a characteristic that allows them to be individually identified. In each successive cleavage, each macromere buds off a small micromere at its animal pole. Each successive quartet of micromeres is displaced to the right or to the left of its sister macromere, creating the characteristic spiral pattern. Looking down on the embryo from the animal pole, the upper ends of the mitotic spindles appear to alternate clockwise and counterclockwise. This causes alternate micromeres to form obliquely to the left and to the right of their macromere. At the third cleavage, the A macromere gives rise to two daughter cells, macromere 1A and micromere 1a. The B, C, and D cells behave similarly, producing the first quartet of micromeres. In most species, the micromeres are to the right of their macromeres (looking down on the animal pole). At the fourth cleavage, macromere 1A divides to form macromere 2A and micromere 2a; and micromere 1a divides to form two more micromeres, $1a^1$ and $1a^2$. Further cleavage yields blastomeres 3A and 3a from macromere 2A, and micromere $1a^2$ divides to produce cells $1a^{21}$ and $1a^{22}$.

The orientation of the cleavage plane to the left or to the right is controlled by cytoplasmic factors within the oocyte. This was discovered by analyzing mutations of snail coiling. Some snails have their coils opening to the right of their shells (**dextral** coiling), whereas other snails have their coils opening to the left (**sinistral** coiling). Usually, the direction of coiling is the same for all members of a given species. Occasionally, though, mutants are found. For instance, in species in which the coils open on the right, some individuals will be found with coils that open on the left. He analyzed the embryos of such aberrant snails and found that their early cleavage differed from the norm. The orientation of the cells after the second cleavage was different in the sinistrally coiling snails owing to a different orientation of the mitotic apparatus. All subsequent divisions in left-coiling embryos are mirror images of those in dextrally coiling embryos. In Figure 8, one can see that the position of the
4d blastomere (which is extremely important, as its progeny will form the mesodermal organs) is different in the two types of spiraling embryos. Eventually, two snails are formed, with their bodies on different sides of the coil opening.



The direction of snail shell coiling is controlled by a single pair of genes.

In the snail *Limnaea peregra*, most individuals are dextrally coiled. Rare mutants exhibiting sinistral coiling were found and mated with wild-type snails. These matings showed that there is a right-coiling allele *D*, which is dominant to the left-coiling allele *d*. However, the direction of cleavage is determined not by the genotype of the developing snail, but by the genotype of the snail's mother. A *dd* female snail can produce only sinistrally coiling offspring, even if the offspring's genotype is *Dd*. A *Dd* individual will coil either left or right, depending on the genotype of its mother. Such matings produce a chart like this:

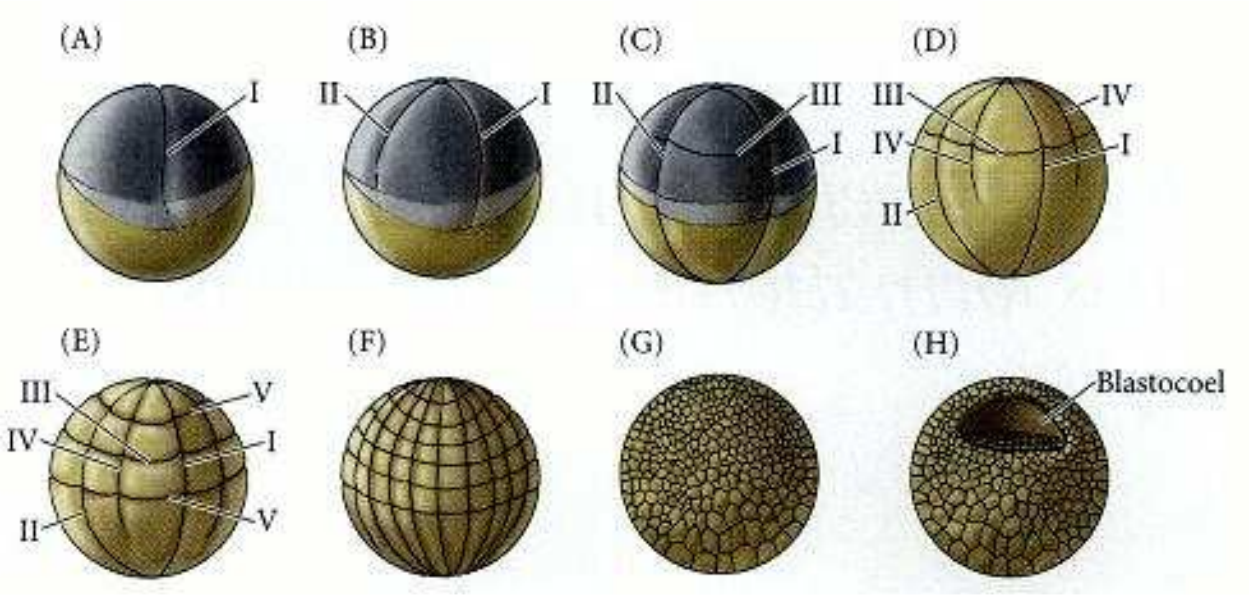
		Genotype	Phenotype
DD ♀ × *dd* ♂	→	*Dd*	All right-coiling
DD ♂ × *dd* ♀	→	*Dd*	All left-coiling
Dd × *Dd*	→	*1DD:2Dd:1dd*	All right-coiling

The genetic factors involved in snail coiling are brought to the embryo by the oocyte cytoplasm. It is the genotype of the ovary in which the oocyte develops that determines which orientation cleavage will take. When injected a small amount of cytoplasm from dextrally coiling snails into the eggs of *dd* mothers, the resulting embryos coiled to the right. Cytoplasm from sinistrally coiling snails did not affect the right-coiling embryos. These findings confirmed that the wild-type mothers were placing a factor into their eggs that was absent or defective in the *dd* mothers.

Cleavage in Early Amphibian Development

3.4 Cleavage in Amphibians

Cleavage in most frog and salamander embryos is radially symmetrical and holoblastic, just like echinoderm cleavage. The amphibian egg, however, contains much more yolk. This yolk, which is concentrated in the vegetal hemisphere, is an impediment to cleavage. Thus, the first division begins at the animal pole and slowly extends down into the vegetal region (Figure). In the axolotl salamander, the cleavage furrow extends through the animal hemisphere at a rate close to 1 mm per minute. The cleavage furrow bisects the gray crescent and then slows down to a mere 0.02 / 0.03 mm per minute as it approaches the vegetal pole.


(A)
I
(B)
II
I
(C)
II
III
I
(D)
III
IV
IV
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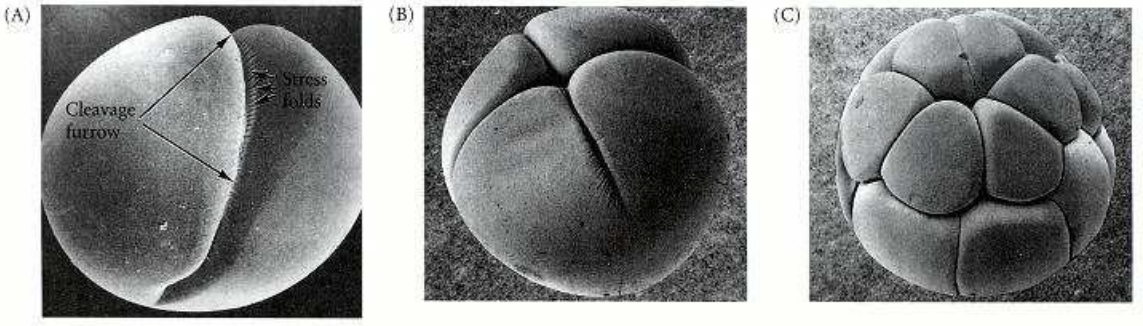
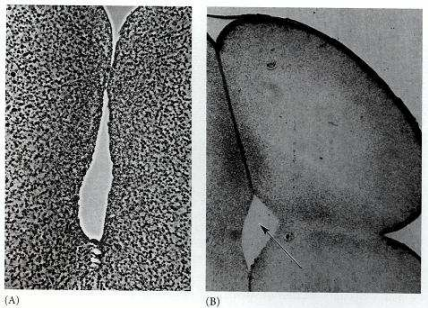
II
(E)
III
V
IV
I
II
V
(F)
(G)
(H)
Blastocoel

Figure 10 is a scanning electron micrograph showing the first cleavage in a frog egg. One can see the difference in the furrow between the animal and the vegetal hemispheres. Figure shows that while the first cleavage furrow is still cleaving the yolky cytoplasm of the vegetal hemisphere, the second cleavage has already started near the animal pole. This cleavage is at right angles to the first one and is also meridional.



The third cleavage, as expected, is equatorial. However, because of the vegetally placed yolk, this cleavage furrow in amphibian eggs is not actually at the equator, but is displaced toward the animal pole. It divides the frog embryo into four small animal blastomeres (micromeres) and four large blastomeres (macromeres) in the vegetal region. This unequal holoblastic cleavage establishes two major embryonic regions: a rapidly dividing region of micromeres near the animal pole and a more slowly dividing vegetal macromere area.(Figure). As cleavage progresses, the animal region becomes packed with numerous small cells, while the vegetal region contains only a relatively small number of large, yolk-laden macromeres.

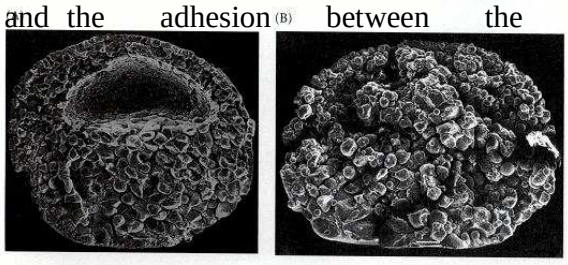
An amphibian embryo containing 16 to 64 cells is commonly called a **morula** (plural: **morulae;** from the Latin, "mulberry," whose shape it vaguely resembles).



At the 128-cell stage, the blastocoel becomes apparent, and the embryo is considered a blastula. Actually, the formation of the blastocoel has been traced back to the very first cleavage furrow. demonstrated that in the frog *Xenopus laevis,* the first cleavage furrow widens in the animal hemisphere to create a small intercellular cavity that is sealed off from the outside by tight intercellular junctions (Figure 10). This cavity expands during subsequent cleavages to become the blastocoel.

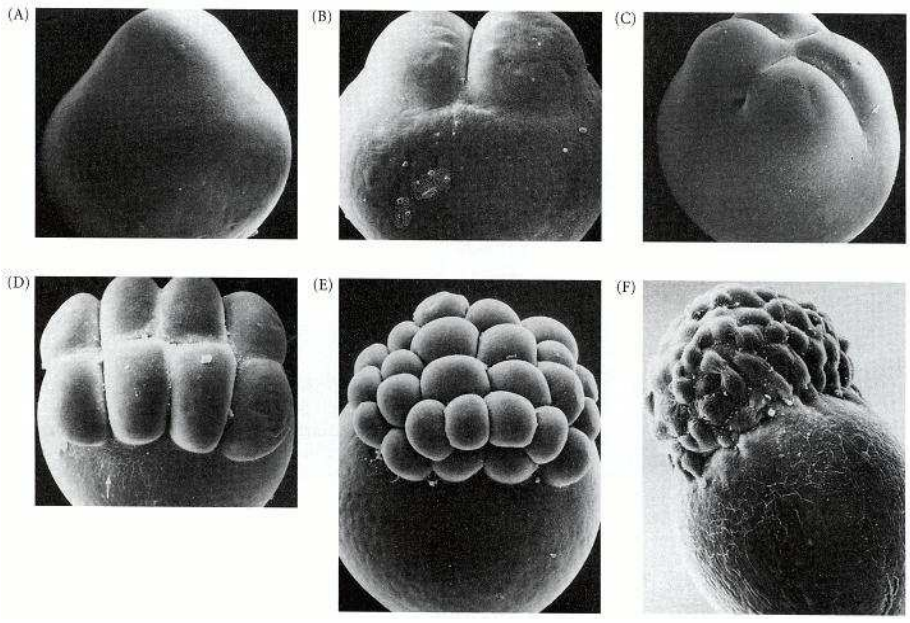
The blastocoel probably serves two major functions in frog embryos:
(1) it permits cell migration during gastrulation, and (2) it prevents the cells beneath it from interacting prematurely with the cells above it. When took embryonic newt cells from the roof of the blastocoel, in the animal hemisphere, and placed them next to the yolky vegetal cells from the base of the blastocoel, these animal cells differentiated into mesodermal tissue instead of ectoderm. Because mesodermal tissue is normally formed from those animal cells that are adjacent to the vegetal endoderm precursors, it seems plausible that the vegetal cells influence adjacent cells to differentiate into mesodermal tissues. Thus, the blastocoel appears to prevent the contact of the vegetal cells destined to become endoderm with those cells fated to give rise to the skin and nerves.

While these cells are dividing, numerous cell adhesion molecules keep the blastomeres together. One of the most important of these molecules is EP-cadherin. The mRNA for this protein is supplied in the oocyte cytoplasm. If this message is destroyed (by injecting antisense oligonucleotides complementary to this mRNA into the oocyte), the EP-cadherin is not made, and the adhesion between the blastomeres is dramatically reduced, resulting in the obliteration of the blastocoel.



3.5 Cleavage in Fish Eggs

In fish eggs, cleavage occurs only in the **blastodisc**, a thin region of yolk-free cytoplasm at the animal cap of the egg. Most of the egg cell is full of yolk. The cell divisions do not completely divide the egg, so this type of cleavage is called **meroblastic** (Greek, *meros,* "part"). Since only the cytoplasm of the blastodisc becomes the embryo, this type of meroblastic cleavage is called **discoidal**. Scanning electron micrographs show beautifully the incomplete nature of discoidal meroblastic cleavage in fish eggs (Figure 11). The calcium waves initiated at fertilization stimulate the contraction of the actin cytoskeleton to squeeze non-yolky cytoplasm into the animal pole of the egg. This converts the spherical egg into a more pear-shaped structure, with an apical blastodisc . Early cleavage divisions follow a highly reproducible pattern of meridional and equatorial cleavages.



These divisions are rapid, taking about 15 minutes each. The first 12 divisions occur synchronously, forming a mound of cells that sits at the animal pole of a large **yolk cell**. These cells constitute the **blastoderm**. Initially, all the cells maintain some open connection with one another and with the underlying yolk cell so that moderately sized (17-kDa) molecules can pass freely from one blastomere to the next.

Beginning at about the tenth cell division, the onset of the midblastula transition can be detected: zygotic gene transcription begins, cell divisions slow, and cell movement becomes evident. At this time, three distinct cell populations can be distinguished. The first of these is the **yolk syncytial layer** (**YSL**). The YSL is formed at the ninth or tenth cell cycle, when the cells at the vegetal edge of the blastoderm fuse with the underlying yolk cell. This fusion produces a ring of nuclei within the part of the yolk cell cytoplasm that sits just beneath the blastoderm. Later, as the blastoderm expands vegetally to surround the yolk cell, some of the yolk syncytial nuclei will move under the blastoderm to form the **internal YSL**, and some of the nuclei will move vegetally, staying ahead of the blastoderm margin, to form **external YSL** (Figure 11). The YSL will be important for directing some of the cell movements of gastrulation. The second cell population distinguished at the midblastula transition is the **enveloping layer.** It is made up of the most superficial cells of the blastoderm, which form an epithelial sheet a single cell layer thick. The EVL eventually becomes the **periderm**, an extraembryonic protective covering that is sloughed off during later development.

Between the EVL and the YSL are the **deep cells**. These are the cells that give rise to the embryo proper. The fates of the early blastoderm cells are not determined, and cell lineage studies (in which a nondiffusible fluorescent dye is injected into one of the cells so that the descendants of that cell can be followed) show that there is much cell mixing during cleavage. Moreover, any one of these cells can give rise to an unpredictable variety of tissue descendants. The fate of the blastoderm cells appears to be fixed shortly before gastrulation begins.

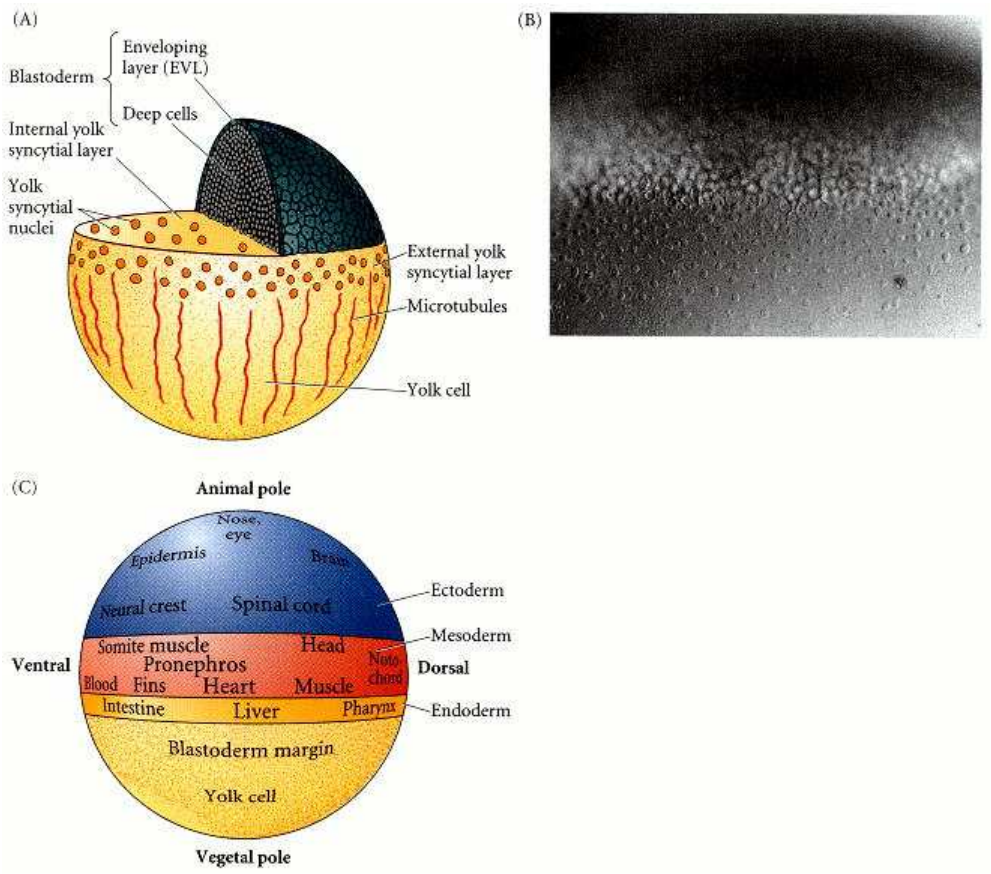


Fig 11

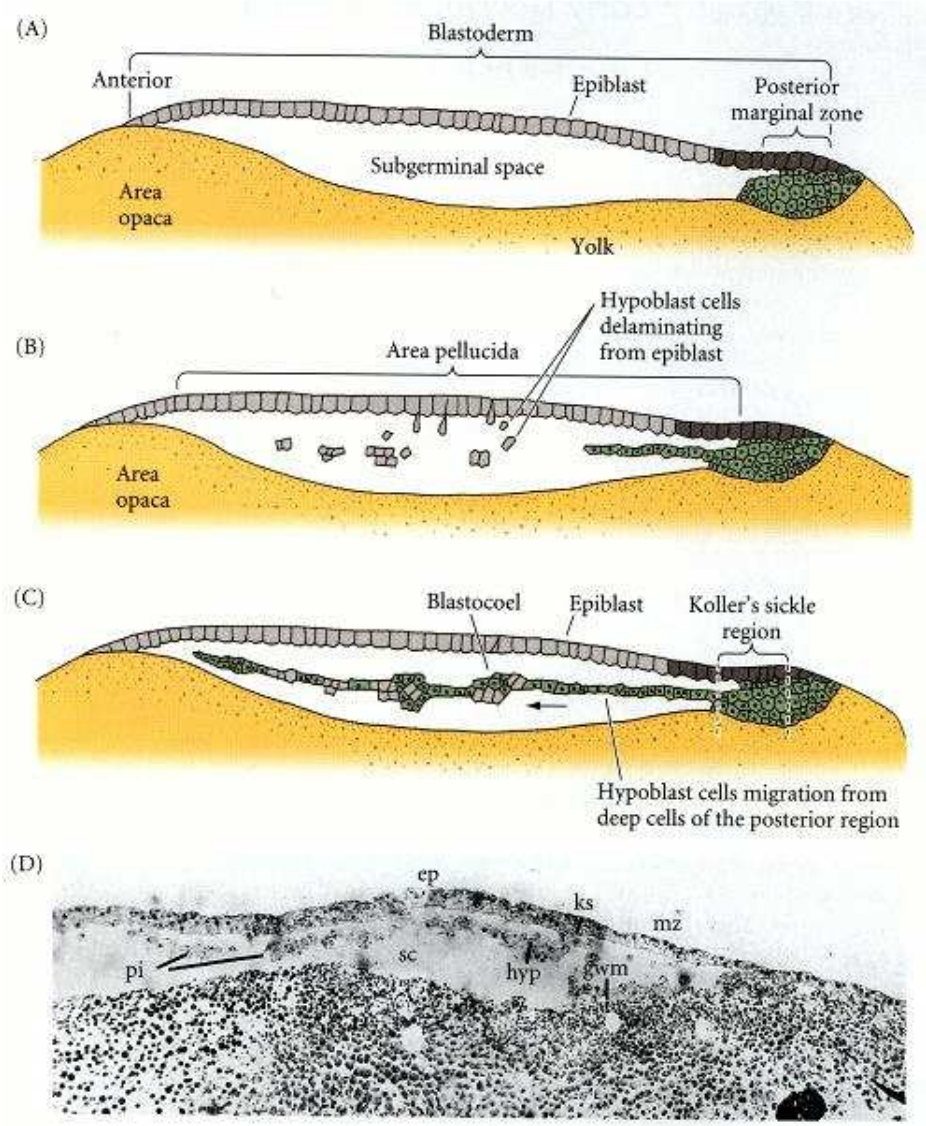
At this time, cells in specific regions of the embryo give rise to certain tissues in a highly predictable manner, allowing a fate map to be made (Figure11)

3.6 Cleavage in Bird

Ever since Aristotle first followed its 3-week development, the domestic chicken has been a favorite organism for embryological studies. It is accessible all year and is easily raised. Moreover, at any particular temperature, its developmental stage can be accurately predicted. Thus, large numbers of embryos can be obtained at the same stage. The chick embryo can be surgically manipulated and, since it forms most of its organs in ways very similarly to those of mammals, it has often served as a surrogate for human embryos.

Fertilization of the chick egg occurs in the oviduct, before the albumen and the shell are secreted upon it. The egg is telolecithal (like that of the fish), with a small disc of cytoplasm sitting atop a large yolk. Like fish eggs, the yolky eggs of birds undergo discoidal meroblastic cleavage. Cleavage occurs only in the blastodisc, a small disc of cytoplasm 23 mm in diameter at the animal pole of the egg cell. The first cleavage furrow appears centrally in the blastodisc, and other cleavages follow to create a single-layered blastoderm. As in the fish embryo, these cleavages do not extend into the yolky cytoplasm, so the early-cleavage cells are continuous with each other and with the yolk at their bases. Thereafter, equatorial and vertical cleavages divide the blastoderm into a tissue five to six cell layers thick. These cells become linked together by tight junctions . Between the blastoderm and the yolk is a space called the **subgerminal cavity**. This space is created when the blastoderm cells absorb fluid from the albumin ("egg white") and secrete it between themselves and the yolk.

At this stage, the deep cells in the center of the blastoderm are shed and die, leaving behind a one- cell-thick **area pellucida**. This part of the blastoderm forms most of the actual embryo. The peripheral ring of blastoderm cells that have not shed their deep cells constitutes the **area opaca**.

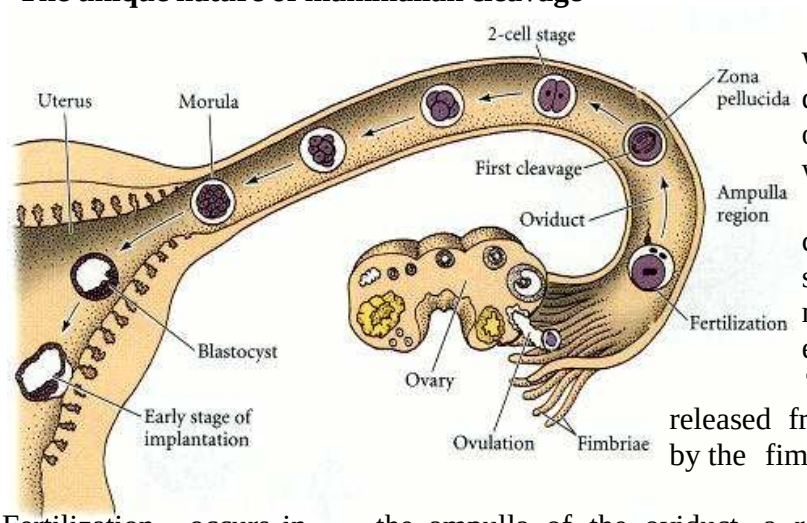


Between the area pellucida and the area opaca is a thin layer of cells called the **marginal zone** (or **marginal belt**)

3.7 Cleavage in Mammal

It is not surprising that mammalian cleavage has been the most difficult to study. Mammalian eggs are among the smallest in the animal kingdom, making them hard to manipulate experimentally. The human zygote, for instance, is only 100 μm in diameter barely visible to the eye and less than one-thousandth the volume of a *Xenopus* egg. Also, mammalian zygotes are not produced in numbers comparable to sea urchin or frog zygotes, so it is difficult to obtain enough material for biochemical studies. Usually, fewer than ten eggs are ovulated by a female at a given time. As a final hurdle, the development of mammalian embryos is accomplished within another organism, rather than in the external environment. Only recently has it been possible to duplicate some of these internal conditions and observe development in vitro.

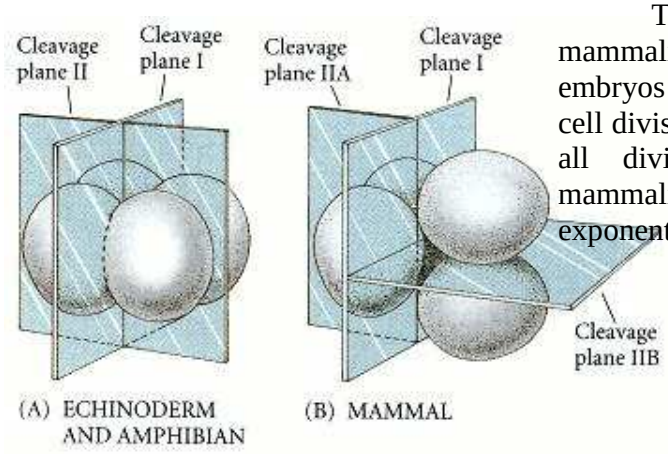
The unique nature of mammalian cleavage



With all these difficulties, knowledge of mammalian cleavage was worth waiting for, as mammalian cleavage turned out to be strikingly different from most other patterns of embryonic cell division.

The mammalian oocyte is released from the ovary and swept by the fimbriae into the oviduct . Fertilization occurs in the ampulla of the oviduct, a region close to the ovary. Meiosis is completed at this time, and first cleavage begins about a day later. Cleavages in mammalian eggs are among the slowest in the animal kingdom about 12/24 hours apart. Meanwhile, the cilia in the oviduct push the embryo toward the uterus; the first cleavages occur along this journey.

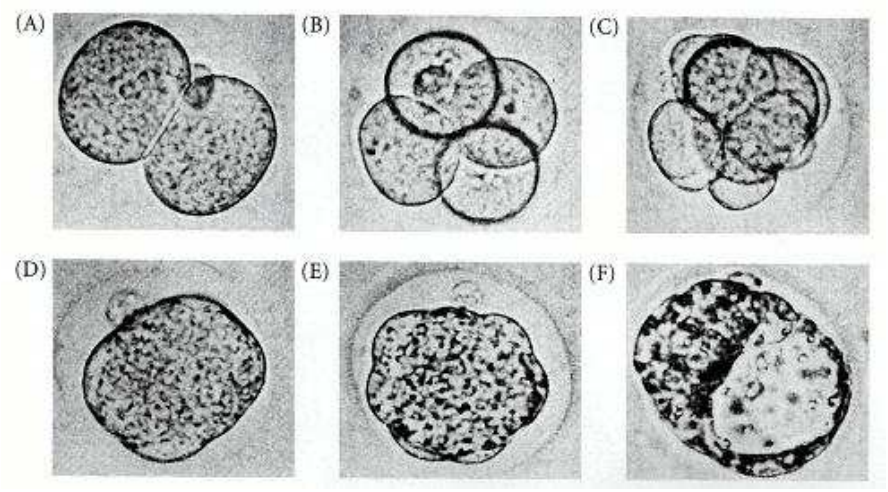
In addition to the slowness of cell division, there are several other features of mammalian cleavage that distinguish it from other cleavage types. The second of these differences is the unique orientation of mammalian blastomeres with relation to one another. The first cleavage is a normal meridional division; however, in the second cleavage, one of the two blastomeres divides meridionally and the other divides equatorially. This type of cleavage is called **rotational cleavage** .



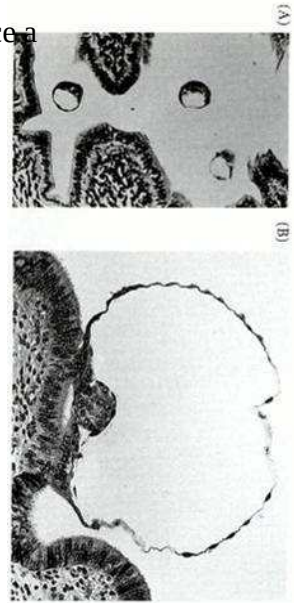
The third major difference between mammalian cleavage and that of most other embryos is the marked asynchrony of early cell division. Mammalian blastomeres do not all divide at the same time. Thus, mammalian embryos do not increase exponentially from 2- to 4- to 8-cell stages, but frequently contain odd numbers of cells. Fourth, unlike almost all other animal genomes, the mammalian genome is activated during early cleavage, and produces the proteins necessary for cleavage to occur. In the mouse andgoat, the switch from maternal to zygotic control occurs at the 2-cell stage.

Most research on mammalian development has focused on the mouse embryo, since mice are relatively easy to breed throughout the year, have large litters, and can be housed easily. Thus, most of the studies discussed here will concern **murine** (mouse) development.

Compaction

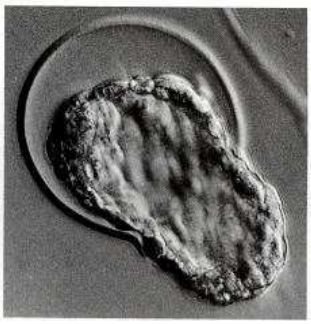


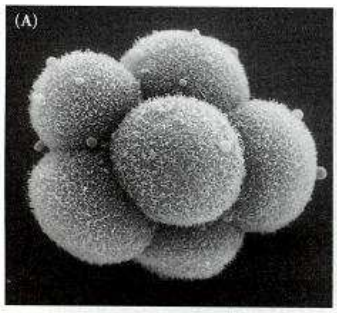
The fifth, and perhaps the most crucial, difference between mammalian cleavage and all other types involves the phenomenon of **compaction**. As seen in mouse blastomeres through the 8-cell stage form a loose arrangement with plenty of space between them. Following the third cleavage, however, the blastomeres undergo a spectacular change in their behavior. They suddenly huddle together, maximizing their contact with one another and forming a compact ball of cells. This tightly packed arrangement is stabilized by tight junctions that form between the outside cells of the ball, sealing off the inside of the sphere. The cells within the sphere form gap junctions, thereby enabling small molecules and ions to pass between them.

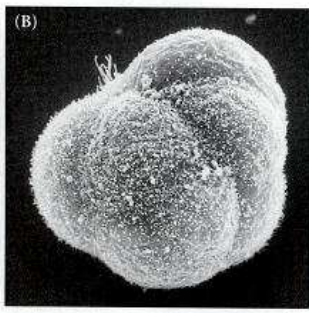


The cells of the compacted 8-cell embryo divide to produce a 16-cell **morula**. The morula consists of a small group of internal cells surrounded by a larger group of external cells. Most of the descendants of the external cells become the **trophoblast** (**trophectoderm**) cells. This group of cells produces no embryonic structures. Rather, it forms the tissue of the **chorion**, the embryonic portion of the **placenta**. The chorion enables the fetus to get oxygen and nourishment from the mother. It also secretes hormones that cause the mother's uterus to retain the fetus, and produces regulators of the immune response so that the mother will not reject the embryo as she would an organ graft. The mouse embryo proper is derived from the descendants of the inner cells of the 16-cell stage, supplemented by cells dividing from the trophoblast during the transition to the 32-cell stage. These cells generate the **inner cell mass** (**ICM**), which will give rise to the embryo and its associated yolk sac, allantois, and amnion. By the 64-cell stage, the inner cell mass (approximately 13 cells) and the trophoblast cells have become separate cell layers, neither contributing cells to the other group. Thus, the distinction between trophoblast and inner cell mass blastomeres represents the first differentiation event in mammalian development. This differentiation is required for the early mammalian embryo to adhere to the uterus. The development of the embryo proper can wait until after that attachment occurs. The inner cell mass actively supports the trophoblast, secreting proteins that cause the trophoblast cells to divide.

Initially, the morula does not have an internal cavity. However, during a process called **cavitation**, the trophoblast cells secrete fluid into the morula to create a blastocoel. The inner cell mass is positioned on one side of the ring of trophoblast cells.
The resulting structure, called the **blastocyst**, is another hallmark of mammalian cleavage.







Escape from the Zona Pellucida

While the embryo is moving through the oviduct en route to the uterus, the blastocyst expands within the **zona pellucida** (the extracellular matrix of the egg that was essential for sperm binding during fertilization. The plasma membranes of the trophoblast cells contain a sodium pump (a Na^+/K^+-ATPase) facing the blastocoel, and these proteins pump sodium ions into the central cavity. This accumulation of sodium ions draws in water osmotically, thus enlarging the blastocoel. During this time, the zona pellucida prevents the blastocyst from adhering to the oviduct walls. When such adherence does take place in humans, it is called an ectopic or **tubal pregnancy**. This is a dangerous condition because the implantation of the embryo into the oviduct can cause a life-threatening hemorrhage. When the embryo reaches the uterus, however, it must "hatch" from the zona so that it can adhere to the uterine wall.

The mouse blastocyst hatches from the zona by lysing a small hole in it and squeezing through that hole as the blastocyst expands. A trypsin-like protease, **strypsin**, is located on the trophoblast cell membranes and lyses a hole in the fibrillar matrix of the zona. Once out, the blastocyst can make direct contact with the uterus. The uterine epithelium (**endometrium**) "catches" the blastocyst on an extracellular matrix containing collagen, laminin, fibronectin, hyaluronic acid, and heparan sulfate receptors. The trophoblast cells contain integrins that will bind to the uterine collagen, fibronectin, and laminin, and they synthesize heparan sulfate proteoglycan precisely prior to implantation. Once in contact with the endometrium, the trophoblast secretes another set of proteases, including collagenase, stromelysin, and plasminogen activator. These protein-digesting enzymes digest the extracellular matrix of the uterine tissue, enabling the blastocyst to bury itself within the uterine wall.

4.0 CONCLUSION

In this unit you learnt about cleavage formation in animals with specific example to sea urchin, snail, amphibian, bird and mammal.

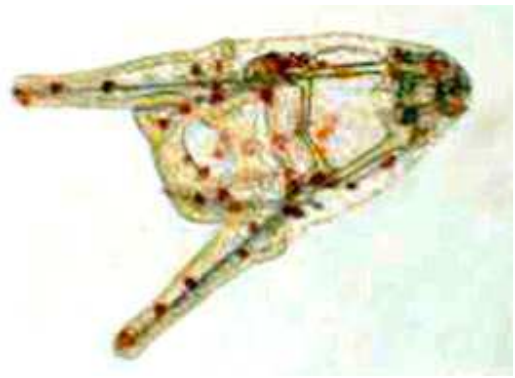
5.0 SUMMARY

Sea urchin embryos

In all animal embryos, the initial divisions of the zygote are called cleavages. As cleavage proceeds, the mitotic divisions are rapid with no time for cell growth between divisions. Thus the cells become smaller after each cleavage. As the blastula begins to form, the cleavage rate slows down. Future cell divisions are slower, allowing the cells to grow. The formation of a mature blastula marks the end of the cleavage period. Observe cleavage and blastula formation in the video below.

When the blastula is fully formed, gastrulation begins. In almost all animal embryos, gastrulation forms a new internal cavity that becomes the digestive tract, and additional cells move inside the embryo to form mesoderm. The mesoderm eventually gives rise to internal organs such as the heart, kidneys, and reproductive tract. Gastrulation is accomplished in various ways by different animal groups. In echinoderms (such as the sea urchin), it is a 2-step process. Study the following video and micrographs to learn how mesoderm and the digestive tract are formed in the sea urchin.

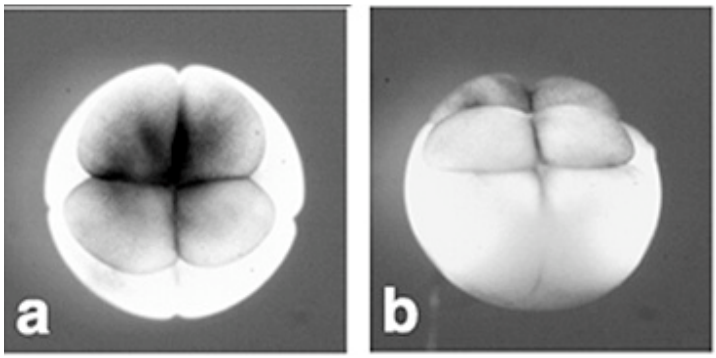
When gastrulation is complete, a mouth forms at the end of the digestive tract opposite to the anus, and spicules (the larval skeleton) are secreted by mesoderm cells. The embryo then changes into the larval body form which is known as a pluteus larva. The larva can swim and feed. After a few weeks of growth and further morphological changes, it undergoes metamorphosis to the adult sea urchin body form. Observe transformation of the gastrula into a pluteus in the animation and further growth of the larva in the micrographs below.



Young pluteus larva **2-Week old pluteus larva** (1 mm in length)

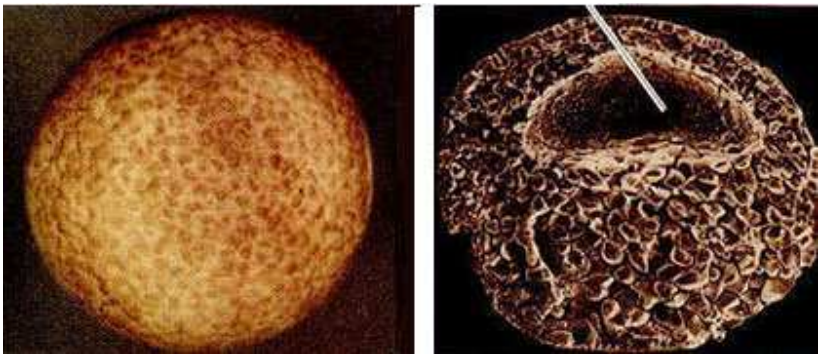
Amphibian embryos

All animal embryos go through cleavage in which the cells are reduced in size. In eggs that have a large amount of unequally distributed yolk, the cleavage pattern is asymmetrical and not all cells are the same size. In amphibian embryos, such as the frog, those cells containing mainly yolk divide more slowly and thus are larger than cells contain mainly cytoplasm during most of the cleavage period.



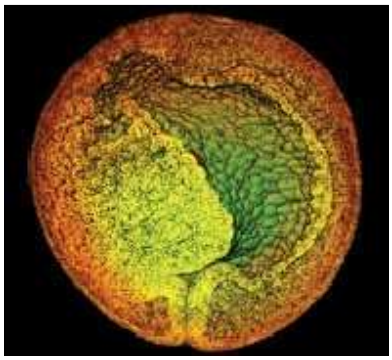
8-Cell frog embryo: " a" is viewed from the top and "b" from the side. Cells containing yolk are lighter in color.

Study the following video of frog development from the first cleavage division to the gastrula stage. Note the similarities and differences between frog and sea urchin gastrulation. Examine the micrographs of the blastula and gastrula stages to compare an external vs. internal view of the embryo. The blastula stage is difficult to detect unless the blastocoel cavity can be seen within the embryo.



Blastula (external view) **Cut blastula showing blastocoel cavity**

Gastrula (external view)



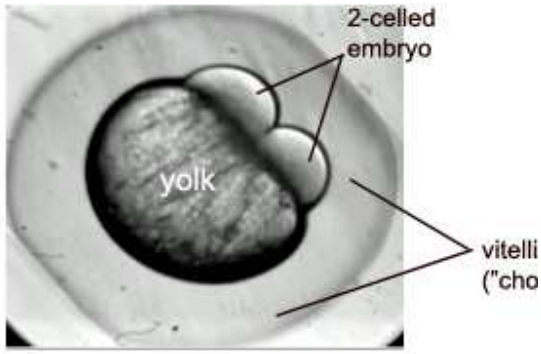
Cut gastrula showing new internal cavity

Now we will examine further development of the frog embryo to the neurula stage and then to a hatched tadpole larva. First view the short black-and-white video that shows gastrulation with the blastopore facing forward, followed by formation of the neural tube. Remember that the neural tube is extremely important in vertebrate animals because it forms the brain and spinal cord. Then study the longer color video that begins with neurulation and ends with a tadpole larva. Note that development of the embryo occurs within the tough vitelline membrane which can be clearly seen at later developmental stages. The emergence of the larva from the vitelline sac constitutes "hatching"

Zebrafish embryos

The embryos of fish develop from eggs with so much yolk that cytoplasm is segregated into a small patch at the egg periphery. This is the only part of the egg that divides during the cleavage period. As a result, the developing embryo lies on top of the yolk mass throughout development.

Adult zebrafish in an aquarium



2-Cell stage of zebrafish embryo

Now view this time lapse video of zebrafish development. The entire embryonic period from the 2-cell stage to larva takes only 48 hours in this fast-developing species.

Cleavage to Late Fetal Stage in Mammals

The development of mammalian embryos has some unique characteristics. Because there is no yolk in the egg, the entire egg divides and cleavage cells are the same size. The blastula

of mammals is called a blastocyst. It is unique in that it contains an inner cell mass from which the embryonic body develops and an outer ring of cells, the trophoblast, which will assist in implantation and form part of the placenta. Remember that implantation of the embryo within the uterine wall occurs at the blastocyst stage.

Early cleavage **Late cleavage** **Early blastocyst** **Late blastocyst**

The inner cell mass forms a disk which develops into the embryonic body. Gastrulation is a bit different than in the other embryos we have studied, but neurulation is essentially the same in all vertebrate embryos so is like that seen in the frog. View the following animation. It is a realistic view of the human embryo from fertilization to the late fetal stage.

6.0 TUTOR-MARKED ASSIGNMENT

1 Discuss the cleavage formation in aquatic animal (sea urchin and fish)

2 Discuss the cleavage formation in in amphibian ,bird and mammal

7.0 **7.0References**

Professor Scott Gilbert, Developmental Biology, 6th Edition.

Module 4: Gastrulation, Invagination and Organogenesis Formation

Unit 1: Gastrulation and Invagination in major groups of organisms

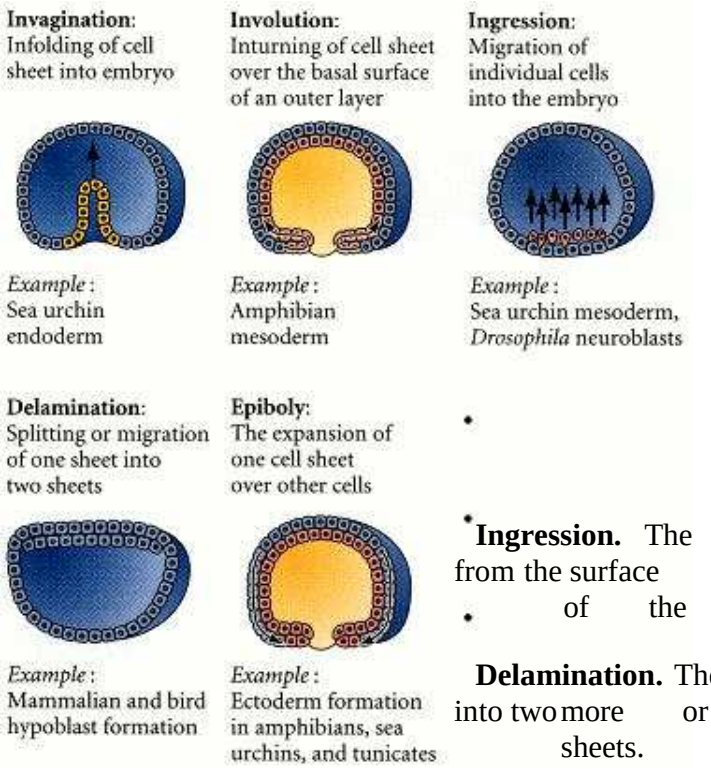
CONTENT

1.0 INTRODUCTION

Gastrulation is the process of highly coordinated cell and tissue movements whereby the cells of the blastula are dramatically rearranged. The blastula consists of numerous cells, the positions of which were established during cleavage. During gastrulation, these cells are given new positions and new neighbors, and the multilayered body plan of the organism is established. The cells that will form the endodermal and mesodermal organs are brought inside the embryo, while the cells that will form the skin and nervous system are spread over its outside surface. Thus, the three germ layers outer ectoderm, inner endoderm, and interstitial mesoderm are first produced during gastrulation. In addition, the stage is set for the interactions of these newly positioned tissues.

The movements of gastrulation involve the entire embryo, and cell migrations in one part of the gastrulating embryo must be intimately coordinated with other movements occurring simultaneously. Although the patterns of gastrulation vary enormously throughout the animal kingdom, there are only a few basic types of cell movements.

Gastrulation usually involves some combination of the following types of movements:



- **Invagination.** The infolding of a region of cells, much like the indenting of a soft rubber ball when it is poked.

- **Involution.** The inturning or inward movement of an expanding outer layer so that it spreads over the internal surface of the remaining external cells.

- **Ingression.** The migration of individual cells from the surface layer into the interior of the embryo.

- **Delamination.** The splitting of one cellular sheet into two more or less parallel sheets.

- **Epiboly.** The movement of epithelial sheets (usually of ectodermal cells) that spread as a unit, rather than individually, to enclose the deeper layers of the embryo.

As we look at gastrulation in different types of embryos, we should keep in mind the following questions.

- **What is the unit of migration?** Is migration dependent on the movements of individual cells, or are the cells part of a migrating sheet or region?

- **Is the spreading or folding of a cell sheet due to intrinsic factors within the sheet or to extrinsic forces stretching or distorting it?** It is essential to know the answer to this question if we are to understand how the various cell movements of gastrulation are integrated. For instance, do involuting cells pull epibolizing cells down toward them, or are the two movements independent?

- **Is there active spreading of the whole tissue, or does the leading edge expand and drag the rest of a cell sheet passively along?**

- **Are changes in cell shape and motility during gastrulation the consequence of changes in cell surface properties,** such as adhesiveness to the substrate or to other cells?

Contrary to expectations, some regional migrational properties may be totally controlled by cytoplasmic factors that are independent of cellularization. Many of the events of early development occurred even in the absence of cells. The cytoplasm of the zygote separated into defined regions, and cilia differentiated in the appropriate parts of the egg. Moreover, the outermost clear cytoplasm migrated down over the vegetal regions in a manner specifically reminiscent of the epiboly of animal hemisphere cells during normal development.

This occurred at precisely the time that epiboly would have taken place during normal gastrulation. Thus, epiboly may be (at least in some respects) independent of the cells that form the migrating region.

2.0 OBJECTIVES

At the end of this unit the student should be able to:

1 Explain gastrulation and invagination in sea urchin, snai and fish

2 Describe the process of gaastrulation in amphibian, aves and mammal.

4.0 MAIN CONTENT

3.1 Gastrulation

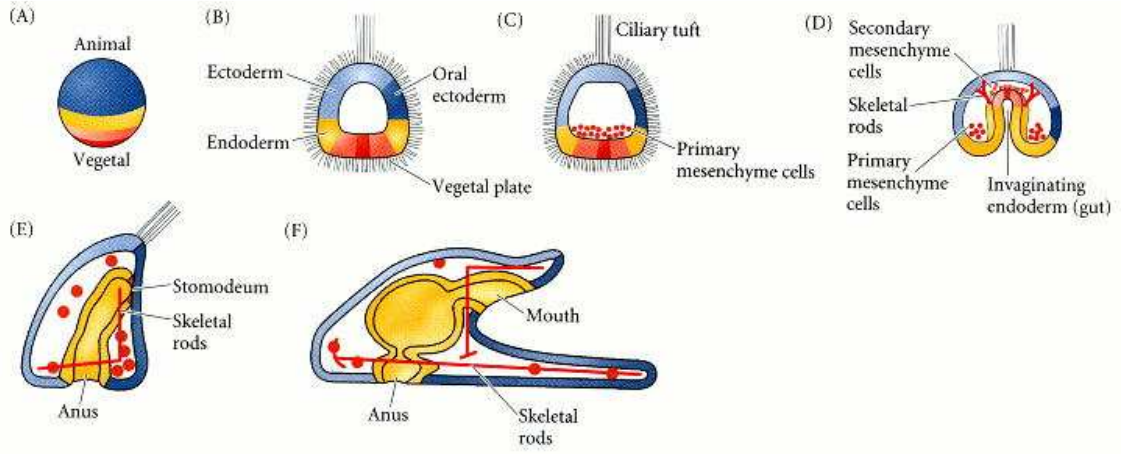
Gastrulation is the process of highly coordinated cell and tissue movements whereby the cells of the blastula are dramatically rearranged. The blastula consists of numerous cells, the positions of which were established during cleavage. During gastrulation, these cells are given new positions and new neighbors, and the multilayered body plan of the organism is established. The cells that will form the endodermal and mesodermal organs are brought inside the embryo, while the cells that will form the skin and nervous system are spread over its outside surface. Thus, the three germ layers outer ectoderm, inner endoderm, and interstitial mesoderm are first produced during gastrulation. In addition, the stage is set for the interactions of these newly positioned tissues.

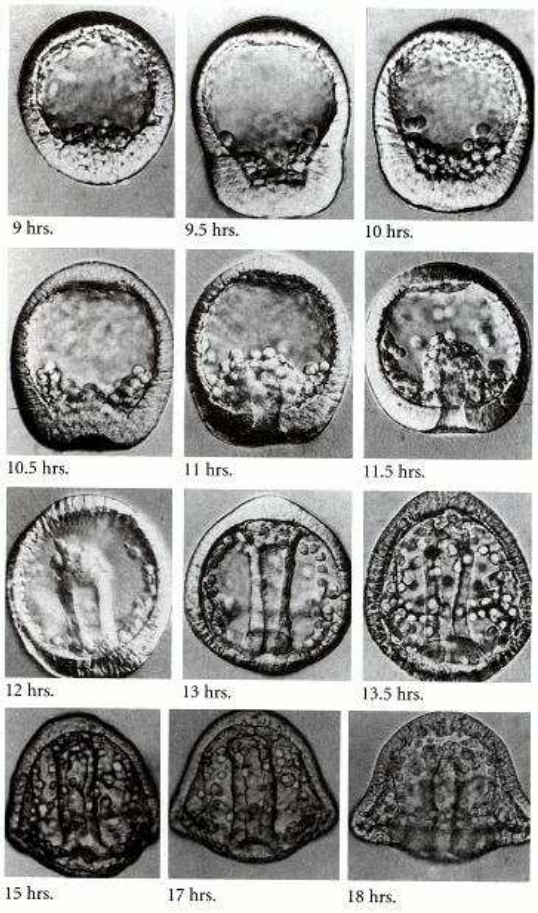
The movements of gastrulation involve the entire embryo, and cell migrations in one part of the gastrulating embryo must be intimately coordinated with other movements occurring simultaneously. Although the patterns of gastrulation vary enormously throughout the animal kingdom, there are only a few basic types of cell movements. Gastrulation usually involves some combination of the following types of movements (Figure 8.6):

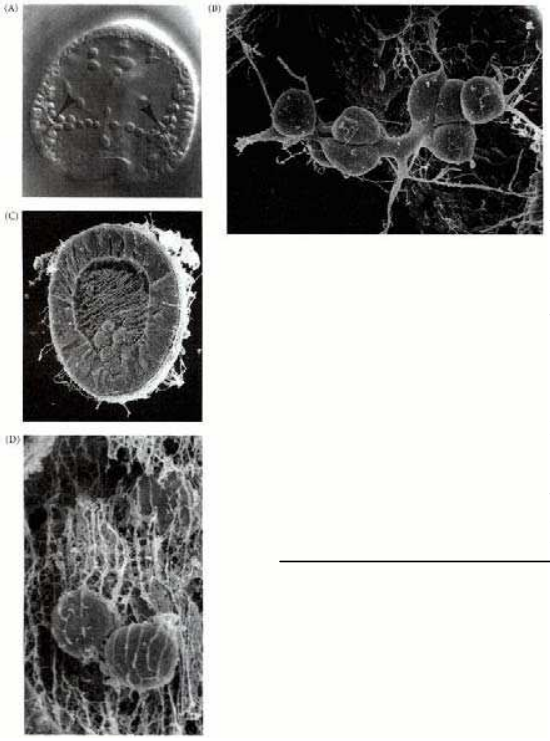
3.1.1 Gastrulation in Sea Urchin

The late sea urchin blastula consists of single layer of about 1000 cells that form a

hollow ball, somewhat flattened at the vegetal end. The blastomeres, derived from different regions of the zygote, have different sizes and properties. Figures 8.16 and 8.17 show the fates of the various regions of the blastula as it develops through gastrulation to the **pluteus larva** stage characteristic of sea urchins. The fate of each cell layer can be seen through its movements during gastrulation.



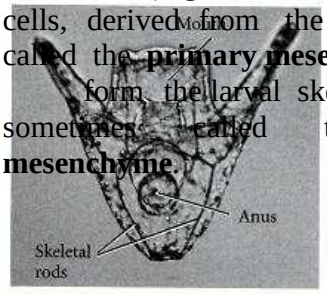




sIngression of primary mesenchyme

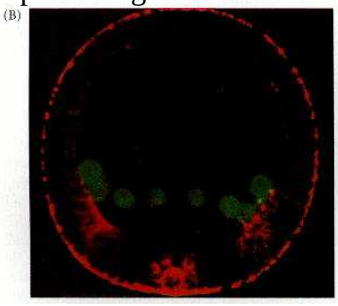
Function of primary mesenchyme cells

Shortly after the blastula hatches from its fertilization envelope, the vegetal side of the spherical blastula begins to thicken and flatten. At the center of this flat vegetal plate, a cluster of small cells begins to change. These cells begin extending and contracting long, thin (30 × 5 μm) processes called **filopodia** from their inner surfaces. The cells then dissociate from the epithelial monolayer and ingress into the blastocoel (Figure 8. 9 10 hours). These cells, derived from the micromeres, are called the **primary mesenchyme**. Theywill form the larval skeleton, so they are sometimes called the **skeletogenic mesenchyme**.



At first the cells appear to move randomly along the inner blastocoel surface, actively making and breaking filopodial connections to the wall of the blastocoel. Eventually, however, they become localized within theprospective ventrolateral region of the blastocoel. Here they fuse into syncytial cables, which will form the axis of the calcium carbonate spicules of the larval skeleton.

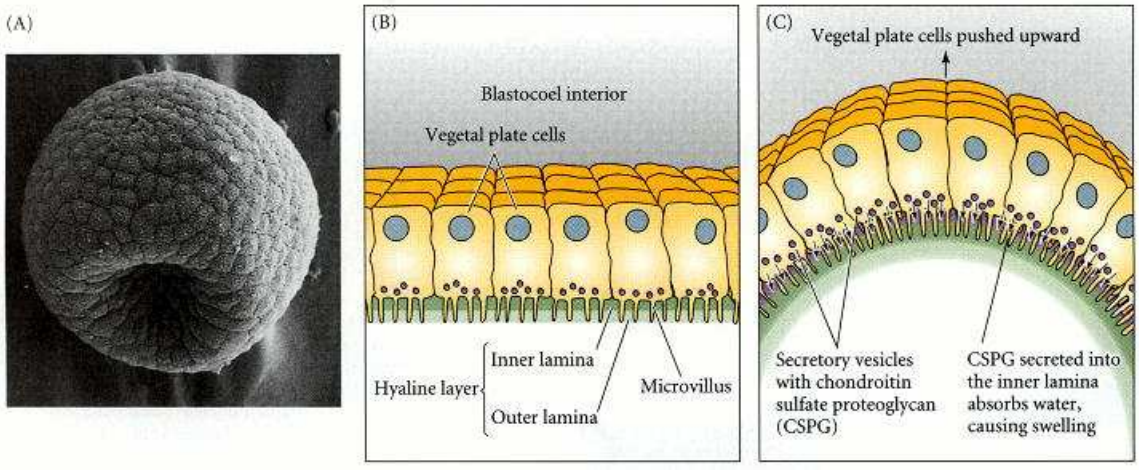
But these guidance cues cannot be sufficient, since the migrating cells "know" when to stop their movement and form spicules near the equator of the blastocoel. The primary mesenchyme cells arrange themselves in a ring at a specific position along the animal-vegetal axis. At two sites near the future ventral side of the larva, many of these primary mesenchyme cells cluster together, fuse with each other, and initiate spicule formation. If a labeled micromere from another embryo is injected into the blastocoel of a gastrulating sea urchin embryo, it migrates to the correct location and contributes to the formation of the embryonic spicules. It is thought that this positional information is provided by the prospective ectodermal cells and their basal laminae. Only the primary mesenchyme cells (and not other cell types or latex beads) are capable of responding to these patterning cues. The existence of extremely fine (0.3-µm diameter) filopodia on the skeleton-forming mesenchyme. These filopodia are not thought to function in locomotion; rather, they appear to explore and sense the blastocoel wall and may be responsible for picking up dorsal-ventral and animal-vegetal patterning cues from the ectoderm .



3.2 Invagination

3. 2.1First stage of archenteron invagination

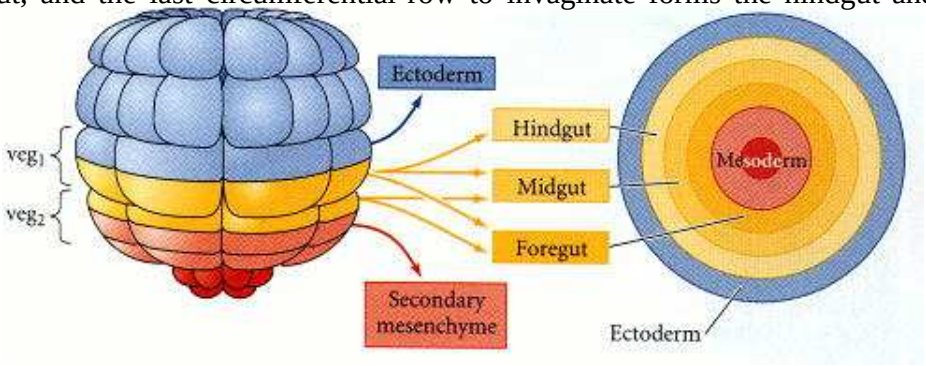
As the ring of primary mesenchyme cells leaves the vegetal region of the blastocoel, important changes are occurring in the cells that remain at the vegetal plate. These cells remain bound to one another and to the hyaline layer of the egg, and they move to fill the gaps caused by the ingression of the primary mesenchyme. Moreover, the vegetal plate bends inward and invaginates about one-fourth to one- half the way into the blastocoel (Figure 8. – 8.17, 10.5 11.5 hours). Then invagination suddenly ceases. The invaginated region is called the **archenteron** (primitive gut), and the opening of the archenteron at the vegetal region is called the **blastopore**.



Scientist have provided evidence that the mechansim of this invagination is similar to that of the buckling produced by heating a bimetallic strip. The hyaline layer is actually made up of two layers, an outer lamina made primarily of hyalin protein and an inner lamina composed of fibropellin proteins.

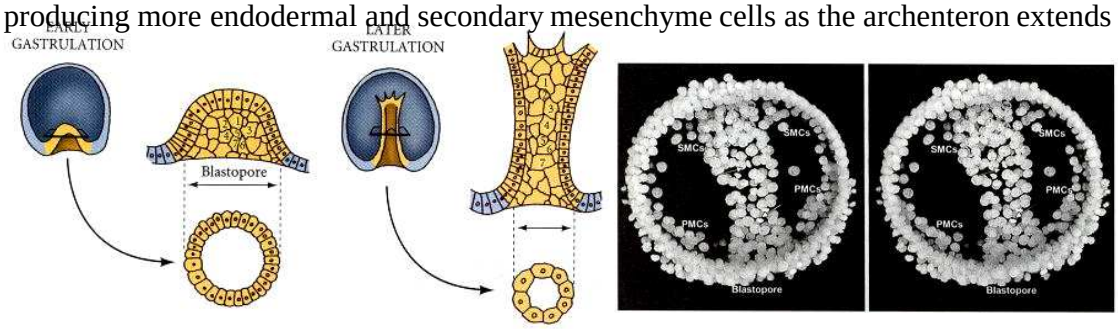
Fibropellins are stored in secretory granules within the oocyte, and are secreted from those granules after cortical granule exocytosis releases the hyalin protein. By the blastula stage, the fibropellins have formed a meshlike network over the embryo surface. At the time of invagination, the vegetal plate cells (and only those cells) secrete a chondroitin sulfate proteoglycan into the inner lamina of the hyaline layer directly beneath them. This hygroscopic (water-absorbing) molecule swells the inner lamina, but not the outer lamina. This causes the vegetal region of the hyaline layer to buckle (Figure 8.21C). Slightly later, a second force arising from the movements of epithelial cells adjacent to the vegetal plate may facilitate this invagination by drawing the buckled layer inward

At the stage when the skeletogenic mesenchyme cells begin ingressing into the blastocoel, the fates of the vegetal plate cells have already been specified. The endodermal cells adjacent to the micromere-derived mesenchyme become foregut, migrating the farthest distance into the blastocoel. The next layer of endodermal cells becomes midgut, and the last circumferential row to invaginate forms the hindgut and anus.



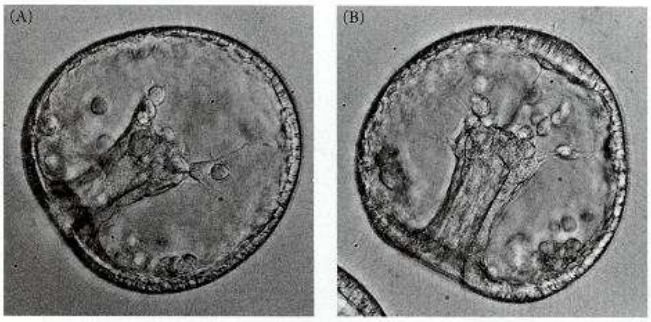
3.2.2 Second and third stages of archenteron invagination

The invagination of the vegetal cells occurs in three discrete stages. After a brief pause, the second phase of archenteron formation begins. During this time, the archenteron extends dramatically, sometimes tripling its length. In this process of extension, the wide, short gut rudiment is transformed into a long, thin tube (see Figure 8.17, 12 hours; Figure 8.23). To accomplish this extension, the cells of the archenteron rearrange themselves by migrating over one another and by flattening themselves. This phenomenon, wherein cells intercalate to narrow the tissue and at the same time move it forward, is called **convergent extension**. Moreover, cell division continues, producing more endodermal and secondary mesenchyme cells as the archenteron extends



In at least some species of sea urchins, a third stage of archenteron elongation occurs. This last phase is initiated by the tension provided by secondary mesenchyme cells, which form at the tip of the archenteron and remain there. Filopodia are extended from these cells through the blastocoel fluid to contact the inner surface of the

blastocoel wall. The filopodia attach to the wall at the junctions between the blastoderm cells and then shorten, pulling up the archenteron. The secondary mesenchyme cells with a laser, with the result that the archenteron could elongate to only about two-thirds of the normal length. If a few secondary mesenchyme cells were left, elongation continued,
although at a slower rate. The secondary mesenchyme cells, then, play an essential role in pulling the archenteron up to the blastocoel wall during the last phase of invagination.



But can the secondary mesenchyme filopodia attach to any part of the blastocoel wall, or is there a specific target in the animal hemisphere that must be present for attachment to occur? Is there a region of the blastocoel wall that is already committed to becoming the ventral side of the larva? Studies show that there is a specific "target" site for the filopodia that differs from other regions of the animal hemisphere. The filopodia extend, touch the blastocoel wall at random sites, and then retract. However, when the filopodia contact a particular region of the wall, they remain attached there, flatten out against this region, and pull the archenteron toward it.

When Hardin and McClay poked in the other side of the blastocoel wall so that the contacts were made most readily with that region, the filopodia continued to extend and retract after touching it. Only when the filopodia found their "target" did they cease these movements. If the gastrula was constricted so that filopodia never reached the target area, the secondary mesenchyme cells continued to explore until they eventually moved off the archenteron and found the target tissue as freely migrating cells. There appears, then, to be a target region on what is to become the ventral side of the larva that is recognized by the secondary mesenchyme cells, and which positions the archenteron in the region where the mouth will form.

As the top of the archenteron meets the blastocoel wall in the target region, the secondary mesenchyme cells disperse into the blastocoel, where they proliferate to form the mesodermal organs. Where the archenteron contacts the wall, a mouth is eventually formed. The mouth fuses with the archenteron to create a continuous digestive tube. Thus, as is characteristic of deuterostomes, the blastopore marks the position of the anus.

3.3 Gastrulation in Snails

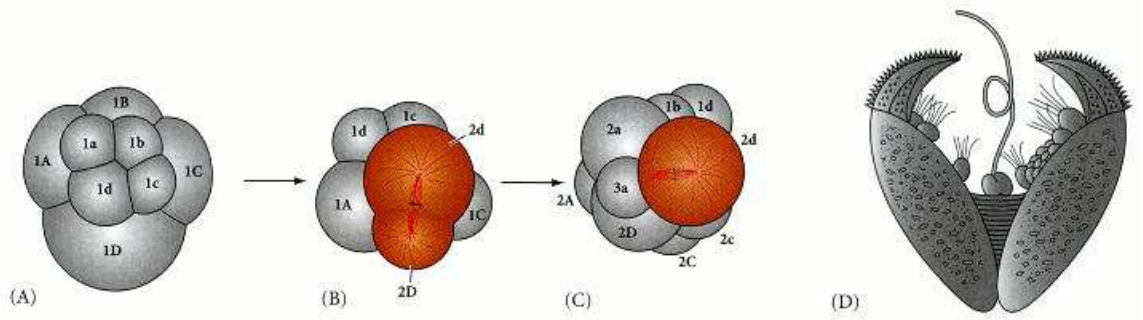
The snail stereoblastula is relatively small, and its cell fates have already been determined by the D series of macromeres. Gastrulation is accomplished primarily by epiboly, wherein the micromeres at the animal cap multiply and "overgrow" the vegetal macromeres. Eventually, the micromeres will cover the entire embryo, leaving a small slit at the vegetal pole.

The shell gland is an ectodermal organ formed through induction by mesodermal cells. Without the mesoderm, no cells are present to induce the competent ectoderm. Here we see an example of limited induction within a mosaic embryo. For more information on the formation of snail embryos.

Adaptation by Modifying Embryonic Cleavage

Evolution is caused by the hereditary alteration of embryonic development. Sometimes we are able to identify a modification of embryogenesis that has enabled the organism to survive in an otherwise inhospitable environment. One such modification, discovered by Frank Lillie in
1898, is brought about by altering the typical pattern of spiral cleavage in the unionid family of clams.

Unlike most clams, *Unio* and its relatives live in swift-flowing streams. Streams create a problem for the dispersal of larvae: because the adults are sedentary, free-swimming larvae would always be carried downstream by the current. These clams, however, have adapted to this environment by effecting two changes in their development. The first alters embryonic cleavage. In the typical cleavage of molluscs, either all the macromeres are equal in size or the 2D blastomere is the largest cell at that embryonic stage. However, the division of *Unio* is such that the 2d blastomere gets the largest amount of cytoplasm. This cell divides to produce most of the larval structures, including a



gland capable of producing a large shell.

The resulting larvae (called **glochidia**) resemble tiny bear traps; they have sensitive hairs that cause the valves of the shell to snap shut when they are touched by the gills or fins of a wandering fish. They attach themselves to the fish and "hitchhike" with it until they are ready to drop off and metamorphose into adult clams. In this manner, they can spread upstream.

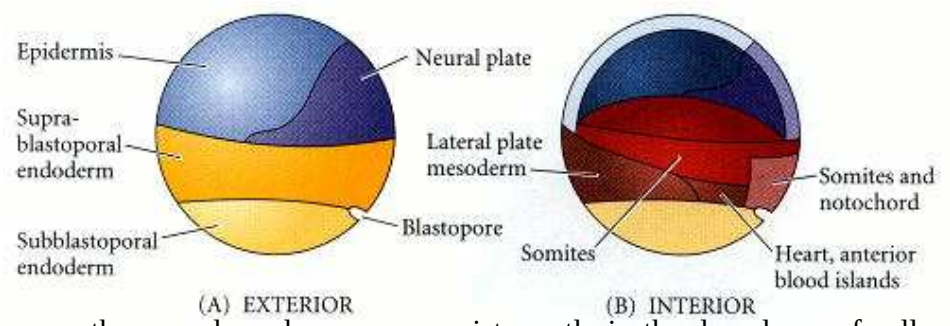
In some species, glochidia are released from the female's brood pouch and merely wait for a fish to come wandering by. Some other species, such as *Lampsilis ventricosa,* have increased the chances of their larvae finding a fish by yet another modification of their development any clams develop a thin mantle that flaps around the shell and surrounds the brood pouch. In some unionids, the shape of the brood pouch (marsupium) and the undulations of the mantle mimic the shape and swimming behavior of a minnow. To make the deception all the better, they develop a black "eyespot" on one end and a flaring "tail" on the other. The "fish" seen in Figure 8.30 is not a fish at all, but the brood pouch and mantle of the clam beneath it. When a predatory fish is lured within range, the clam discharges the glochidia from the brood pouch. Thus, the modification of existing developmental patterns has permitted unionid clams to survive in challenging environments

3.3 Gastrulation in Amphibian

The study of amphibian gastrulation is both one of the oldest and one of the newest areas of experimental embryology.Even though amphibian gastrulation has been extensively studied for the past century, most of our theories concerning the mechanisms of these developmental movements have been revised over the past decade. The study of amphibian gastrulation has been complicated by the fact that there is no single way amphibians gastrulate. Different species employ different means toward the same goal. In recent years, the most intensive investigations have focused on the frog *Xenopus laevis,* so we will concentrate on its mode of gastrulation.

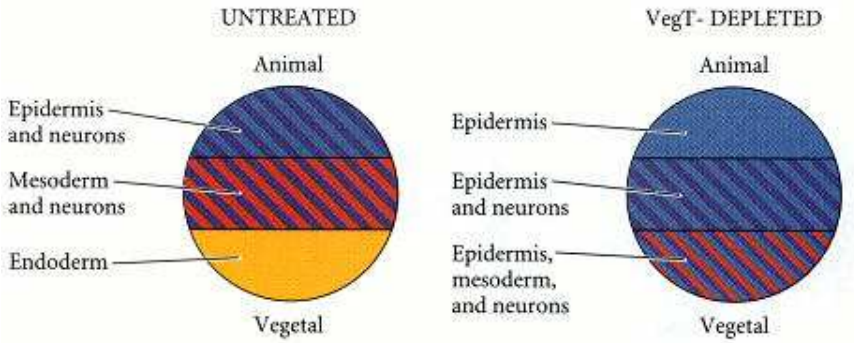
3.4.1.The fate map of Xenopus

Amphibian blastulae are faced with the same tasks as the invertebrate blastulae to bring inside the embryo those areas destined to form the endodermal organs, to surround the embryo with cells capable of forming the ectoderm, and to place the mesodermal cells in the proper positions between them. The movements whereby this is accomplished can be visualized by the technique of vital dye staining.
Fate mapping has shown that cells of the *Xenopus* blastula have different fates depending on whether they are located in the deep or the superficial layers of the embryo.



In *Xenopus*, the mesodermal precursors exist mostly in the deep layer of cells, while the ectoderm and endoderm arise from the superficial layer on the surface of the embryo. Most of the precursors for the notochord and other mesodermal tissues are located beneath the surface in the equatorial (marginal) region of the embryo. In urodeles (salamanders such as *Triturus* and *Ambystoma*) and in some frogs other than *Xenopus*, many more of the notochord and mesoderm precursors are among the surface cells.

As we have seen, the unfertilized egg has a polarity along the animal-vegetal axis. Thus, the germ layers can be mapped onto the oocyte even before fertilization. The surface of the animal hemisphere will become the cells of the ectoderm (skin and nerves), the vegetal hemisphere surface will form the cells of the gut and associated organs (endoderm), and the mesodermal cells will form from the internal cytoplasm around the equator. This general fate map is thought to be imposed upon the egg by the transcription factor **VegT** and the paracrine factor **Vg1**. The mRNAs for these proteins are located in the cortex of the vegetal hemisphere of *Xenopus* oocytes, and they are apportioned to the vegetal cells during cleavage. By using antisense oligonucleotides,were able to deplete maternal VegT protein in early embryos. The resulting embryos lacked the normal fate map. The animal third of the embryo produced only ventral epidermis, while the marginal cells (which normally produced mesoderm) generated epidermal and neural tissue. The vegetal third (which usually produces endoderm) produced a mixture of ectoderm and mesoderm demonstrated that embryos that lacked functional Vg1 lacked endoderm and dorsal mesoderm.

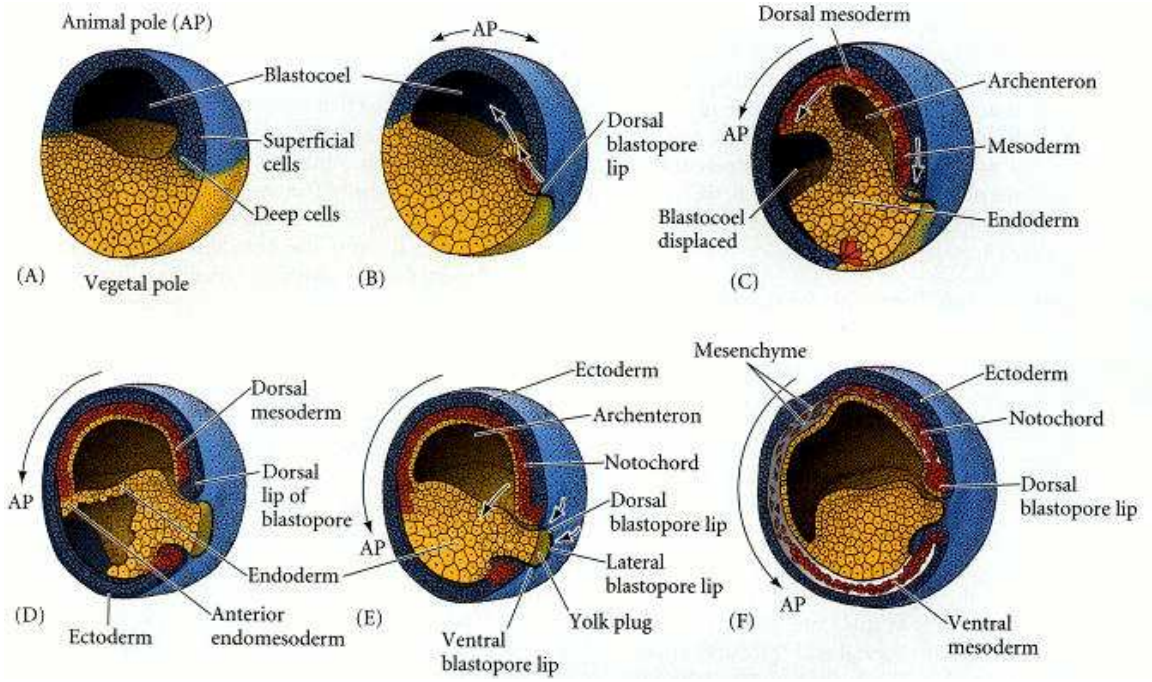


These findings tell us nothing, however, about which part of the egg will form the belly and which the back. The anterior-posterior, dorsal-ventral, and left-right axes are specified by the events of fertilization and are realized during gastrulation.

Cell movements during amphibian gastrulation

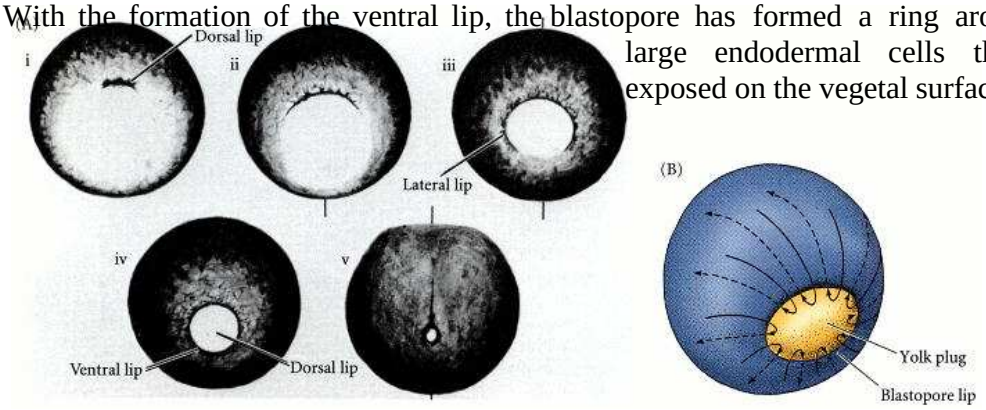
Before we look at the process of gastrulation in detail, let us first trace the movement patterns of the germ layers. Gastrulation in frog embryos is initiated on the future dorsal side of the embryo, just below the equator in the region of the gray crescent

Here, the cells invaginate to form a slitlike blastopore. These cells change their shape dramatically. The main body of each cell is displaced toward the inside of the embryo while the cell maintains contact with the outside surface by way of a slender neck). These **bottle cells** line the archenteron as it forms. Thus, as in the gastrulating sea urchin, an invagination of cells initiates archenteron formation. However, unlike gastrulation in sea urchins, gastrulation in the frog begins not at the most vegetal region, but in the **marginal zone:** the zone surrounding the equator of the blastula, where the animal and vegetal hemispheres meet. Here the endodermal cells are not as large or as yolky as the most vegetal blastomeres.



The next phase of gastrulation involves the involution of the marginal zone cells hile the animal cells undergo epiboly and converge at the blastopore. When the migrating marginal cells reach the **dorsal lip** of the blastopore, they turn inward and travel along the inner surface of the outer animal hemisphere cells. Thus, the cells constituting the lip of the blastopore are constantly changing. The first cells to compose the dorsal blastopore lip are the bottle cells that invaginated to form the leading edge of the archenteron. These cells later become the pharyngeal cells of the foregut. As these first cells pass into the interior of the embryo, the dorsal blastopore lip becomes composed of cells that involute into the embryo to become the **prechordal plate** (the precursor of the head mesoderm). The next cells involuting into the embryo through the dorsal blastopore lip are called the **chordamesoderm** cells. These cells will form the **notochord**, a transient mesodermal "backbone" that plays an important role in distinguishing and patterning the nervous system.

As the new cells enter the embryo, the blastocoel is displaced to the side opposite the dorsal lip of the blastopore. Meanwhile, the blastopore lip expands laterally and ventrally as the processes of bottle cell formation and involution continue about the blastopore. The widening blastopore "crescent" develops lateral lips and finally a ventral lip over which additional mesodermal and endodermal precursor cells pass. With the formation of the ventral lip, the blastopore has formed a ring around the large endodermal cells that remain exposed on the vegetal surface.



This remaining patch of endoderm is called the **yolk plug**; it, too, is eventually internalized (Figure 10.9). At that point, all the endodermal precursors have been brought into the interior of the embryo, the ectoderm has encircled the surface, and the mesoderm has been brought between them.

3.4.2.The midblastula transition: preparing for gastrulation

Now that we have seen an overview of amphibian gastrulation, we can look more deeply into its mechanisms. The first precondition for gastrulation is the activation of the genome. In *Xenopus*, the nuclear genes are not transcribed until late in the twelfth cell cycle. At that time, different genes begin to be transcribed in different cells, and the blastomeres acquire the capacity to become motile. This dramatic change is called the midblastula transition. It is thought that different transcription factors (such as the VegT protein, mentioned above) become active in different cells at this time, giving the cells new properties. For instance, the vegetal cells (probably under the direction of the maternal VegT protein) become the endoderm and begin secreting the factors that cause the cells above them to become the mesoderm.

Positioning the blastopore

The vegetal cells are critical in determining the location of the blastopore, as is the point of sperm entry. The microtubules of the sperm direct cytoplasmic movements that empower the vegetal cells opposite the point of sperm entry to induce the blastopore in the mesoderm above them. This region of cells opposite the point of sperm entry will form the blastopore and become the dorsal portion of the body.

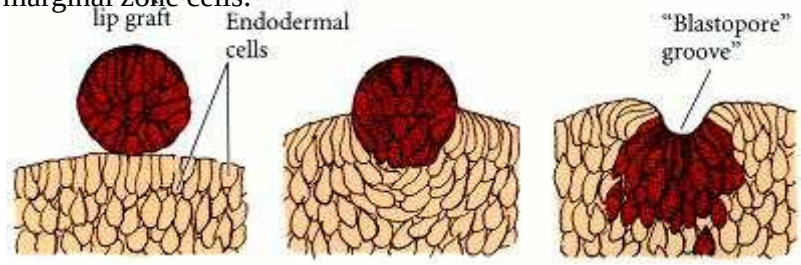
we saw that the internal cytoplasm of the fertilized egg remains oriented with respect to gravity because of its dense yolk accumulation, while the cortical cytoplasm actively rotates 30 degrees animally ("upward"), toward the point of sperm entry (see Figure
7.33). In this way, a new state of symmetry is acquired. Whereas the unfertilized egg was radially symmetrical about the animal-vegetal axis, the fertilized egg now has a dorsal-ventral axis. It has become bilaterally symmetrical (having right and left sides). The inner cytoplasm moves as well. Fluorescence microscopy of early embryos has shown that the cytoplasm of the presumptive dorsal cells differs from that of the presumptive ventral cells. These cytoplasmic movements activate the cytoplasm opposite the point of sperm entry, enabling it to initiate gastrulation. The side where the sperm enters marks the future ventral surface of the embryo; the opposite side, where gastrulation is initiated, marks the future dorsum of the embryo. If cortical rotation is blocked, there is no dorsal development, and the embryo dies as a mass of ventral (primarily gut) cells.

Although the sperm is not needed to induce these movements in the egg cytoplasm, it is important in determining the *direction* of the rotation. If an egg is artificially activated, the cortical rotation still takes place at the correct time. However, the direction of this movement is unpredictable. The directional bias provided by the point of sperm entry can be overridden by mechanically redirecting the spatial relationship between the cortical and internal cytoplasms. When a *Xenopus* egg is turned 90 degrees, so that the point of sperm entry faces upward, the cytoplasm rotates such that

the embryo initiates gastrulation on the *same* side as sperm entry. One can even produce two gastrulation initiation sites by combining the natural sperm-oriented rotation with an artificially induced rotation of the egg.

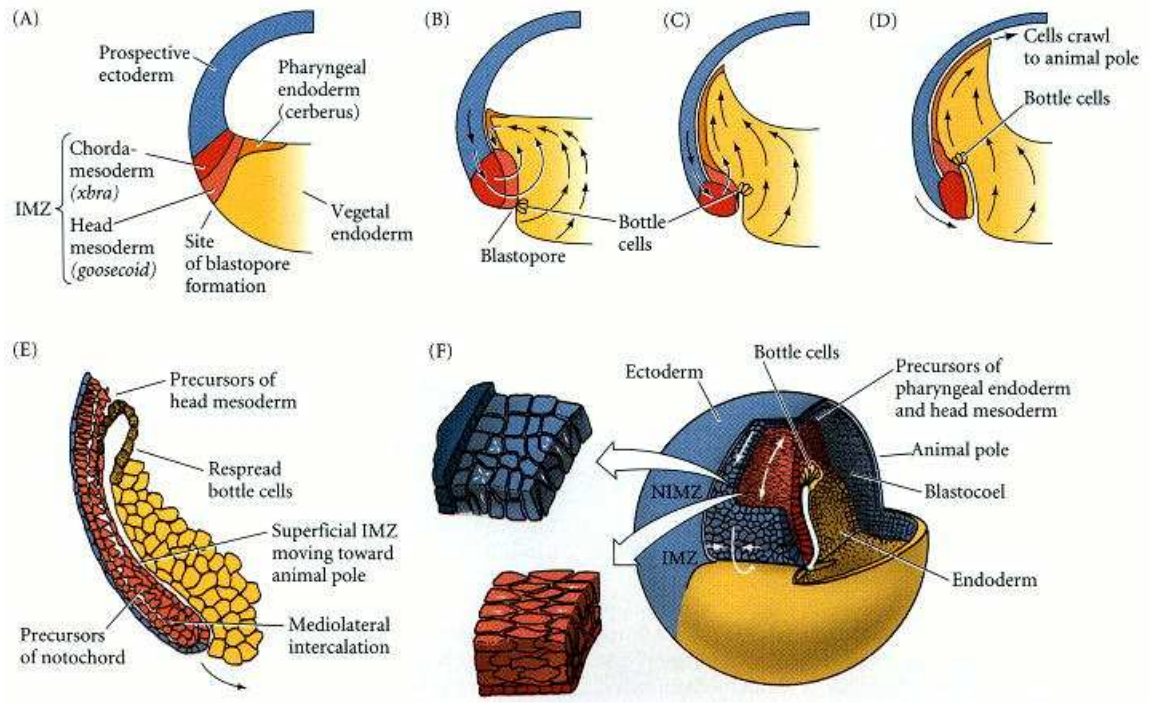
3.4 Invagination and involution in Amphibian

Amphibian gastrulation is first visible when a group of marginal endoderm cells on the dorsal surface of the blastula sinks into the embryo. The outer (**apical**) surfaces of these cells contract dramatically, while their inner (**basal**) ends expand. The apical-basal length of these cells greatly increases to yield the characteristic "bottle" shape. In salamanders, these bottle cells appear to have an active role in the early movements of gastrulation, found that bottle cells from early salamander gastrulae could attach to glass coverslips and lead the movement of those cells attached to them. Even more convincing were Holtfreter's recombination experiments. When dorsal marginal zone cells (which would normally give rise to the dorsal lip of the blastopore) were excised and placed on inner prospective endoderm tissue, they formed bottle cells and sank below the surface of the inner endoderm. Moreover, as they sank, they created a depression reminiscent of the early blastopore. Thus, Holtfreter claimed that the ability to invaginate into the deep endoderm is an innate property of the dorsal marginal zone cells.



The situation in the frog embryo is somewhat different. The peculiar shape change of the bottle cells is needed to initiate gastrulation; it is the constriction of these cells that first forms the slit-like blastopore. However, after starting these movements, the *Xenopus* bottle cells are no longer needed for gastrulation. When bottle cells are removed after their formation, involution and blastopore formation and closure continue.

The major factor in the movement of cells into the embryo appears to be the involution of the subsurface marginal cells, rather than the superficial ones. The movements of the vegetal endoderm place the prospective pharyngeal endoderm adjacent to the roof of the blastocoel. This places the prospective pharyngeal endoderm immediately ahead of the migrating mesoderm. The cells then migrate along the basal surface of the blastocoel roof. The superficial layer of marginal cells is pulled inward to form the endodermal lining of the archenteron, merely because it is attached to the actively migrating deep cells. While experimental removal of the bottle cells does not affect the involution of the deep or superficial marginal zone cells into the embryo, the removal of the deep involuting marginal zone (IMZ) cells and their replacement with animal region cells (which do not normally undergo involution) stops archenteron formation.

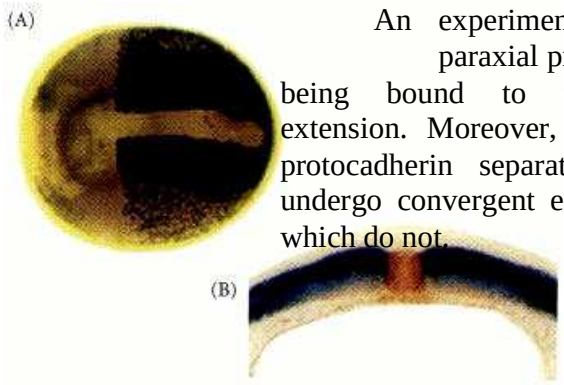


3.5.1 The convergent extension of the dorsal mesoderm

Involution begins dorsally, led by the pharyngeal endomesoderm* and the prechordal plate. These tissues will migrate most anteriorly beneath the surface ectoderm. The next tissues to enter the dorsal blastopore lip contain notochord and somite precursors. Meanwhile, as the lip of the blastopore expands to have dorsolateral, lateral, and ventral sides, the prosepective heart mesoderm, kidney mesoderm, and ventral mesoderm enter into the embryo.

Figures F depict the behavior of the IMZ cells at successive stages of *Xenopus* gastrulation. The IMZ is originally several layers thick. Shortly before their involution through the blastopore lip, the several layers of deep IMZ cells intercalate radially to form one thin, broad layer. This intercalation further extends the IMZ vegetally. At the same time, the superficial cells spread out by dividing and flattening. When the deep cells reach the blastopore lip, they involute into the embryo and initiate a second type of intercalation. This intercalation causes a **convergent extension** along the mediolateral axis that integrates several mesodermal streams to form a long, narrow band. This is reminiscent of traffic on a highway when several lanes must merge to form a single lane. The anterior part of this band migrates toward the animal cap. Thus, the mesodermal stream continues to migrate toward the animal pole, and the overlying layer of superficial cells (including the bottle cells) is passively pulled toward the animal pole, thereby forming the endodermal roof of the archenteron. The radial and mediolateral intercalations of the deep layer of cells appear to be responsible for the continued movement of mesoderm into the embryo.

The adhesive changes driving convergent extension appear to be directed by two cell adhesion molecules, **paraxial protocadherin** and **axial protocadherin**. The former is initially found throughout the dorsal mesoderm, but then is turned off in the precursors of the notochord. At that time, axial protocadherin becomes expressed in the notochordal tissue



An experimental dominant negative form of paraxial protocadherin (which is secreted instead of being bound to the cell membrane) prevents convergent extension. Moreover, the expression domain of paraxial protocadherin separates the trunk mesodermal cells, which undergo convergent extension, from the head mesodermal cells, which do not.

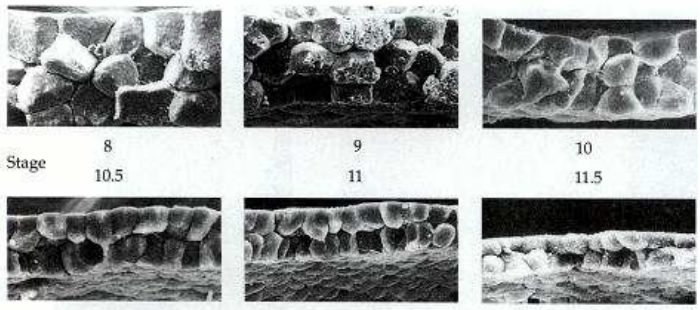
Migration of the involuting mesoderm

3.5.2 Migration of the involuting mesoderm

As mesodermal movement progresses, convergent extension continues to narrow and lengthen the involuting marginal zone. The IMZ contains the prospective endodermal roof of the archenteron in its superficial layer (IMZ_S) and the prospective mesodermal cells, including those of the notochord, in its deep region (IMZ_D). During the middle third of gastrulation, the expanding sheet of mesoderm converges toward the midline of the embryo. This process is driven by the continued mediolateral intercalation of cells along the anterior-posterior axis, thereby further narrowing the band. Toward the end of gastrulation, the centrally located notochord separates from the somitic mesoderm on either side of it, and the notochord cells elongate separately. This may in part be a consequence of the different protocadherins in the axial and paraxial mesoderms. This convergent extension of the mesoderm appears to be autonomous, because the movements of these cells occur even if this region of the embryo is experimentally isolated from the rest of the embry.

During gastrulation, the **animal cap** and **noninvoluting marginal zone (NIMZ)** cells expand by epiboly to cover the entire embryo. The dorsal portion of the NIMZ extends more rapidly toward the blastopore than the ventral portion, thus causing the blastopore lips to move toward the ventral side. While those mesodermal cells entering through the dorsal lip of the blastopore give rise to the dorsal axial mesoderm (notochord and somites), the remainder of the body mesoderm (which forms the heart, kidneys, blood, bones, and parts of several other organs) enters through the ventral and lateral blastopore lips to create the **mesodermal mantle.** The endoderm is derived from the IMZ_S cells that form the lining of the archenteron roof and from the subblastoporal vegetal cells that become the archenteron floor.

3.5.3 Epiboly of the ectoderm



While involution is occurring at the blastopore lips,the ectodermal precursors are expanding over theentire embryo. have used scanning electron microscopy to observe the changes in both the superficial cells and the deep cells of the animal and marginal regions. The major mechanism of epiboly in *Xenopus* gastrulation appears to be an increase in cell number (through division) coupled with a concurrent integration of several deep layers into one.

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During early gastrulation, three rounds of cell division increase the number of the deep cell layers in the animal hemisphere. At the same time, complete integration of the numerous deep cells into one layer occurs. The most superficial layer expands by cell division and flattening. The spreading of cells in the dorsal and ventral marginal zones appears to proceed by the same mechanism, although changes in cell shape appear to play a greater role than in the animal region. The result of these expansions is the epiboly of the superficial and deep cells of the animal cap and NIMZ over the surface of the embryo. Most of the marginal zone cells, as previously mentioned, involute to join the mesodermal cell stream within the embryo. As the ectoderm epibolizes over the entire embryo, it eventually internalizes all the endoderm within it. At this point, the ectoderm covers the embryo, the endoderm is located within the embryo, and the mesoderm is positioned between them.

*The pharyngeal endoderm and head mesoderm cannot be separated experimentally at this stage, so they are therefore sometimes referred to collectively as the pharyngeal endomesoderm. The notochord is the basic unit of the dorsal mesoderm, but it is thought that the dorsal portion of the somites may also have similar properties.

†Dominant negative proteins are mutated forms of the wild-type protein that interfere with the normal functioning of the wild-type protein. Thus, a dominant negative protein will have an effect similar to a loss-of-function mutation in the gene encoding the particular protein.

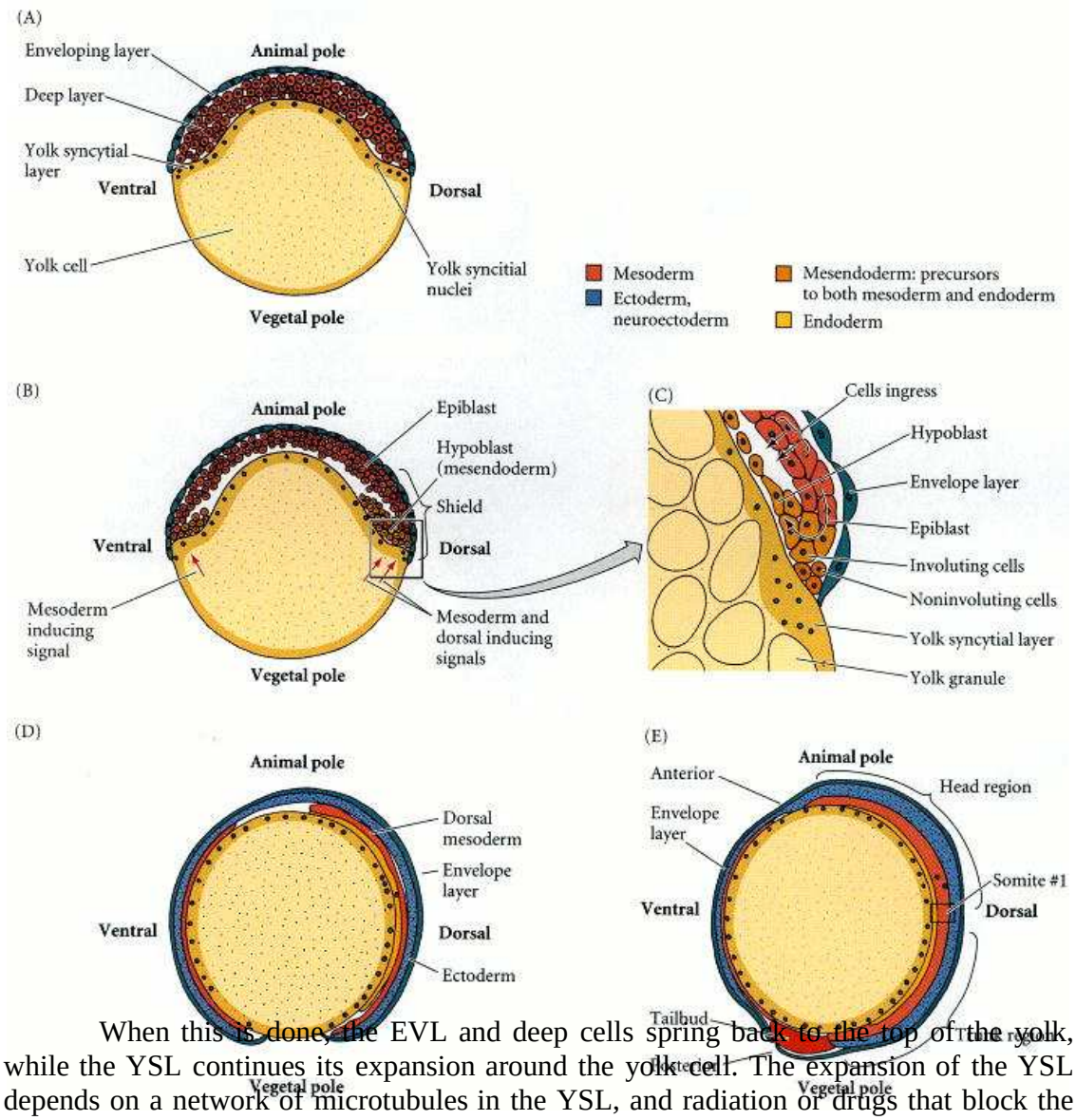
3.6 Gastrulation in Fish Embryo

The first cell movement of fish gastrulation is the epiboly of the blastoderm cells over the yolk. In the initial phase, the deep blastoderm cells move outwardly to intercalate with the more superficial cells. Later, these cells move over the surface of the yolk to envelop it completely.

This movement is not due to the active crawling of the blastomeres, however.

Rather, the movement is provided by the autonomously expanding YSL "within" the animal pole yolk cytoplasm. The EVL is tightly joined to the YSL and is dragged along with it. The deep cells of the blastoderm then fill in the space between the YSL and the EVL as epiboly proceeds. This can be demonstrated by severing the

attachments between the YSL and the EVL.



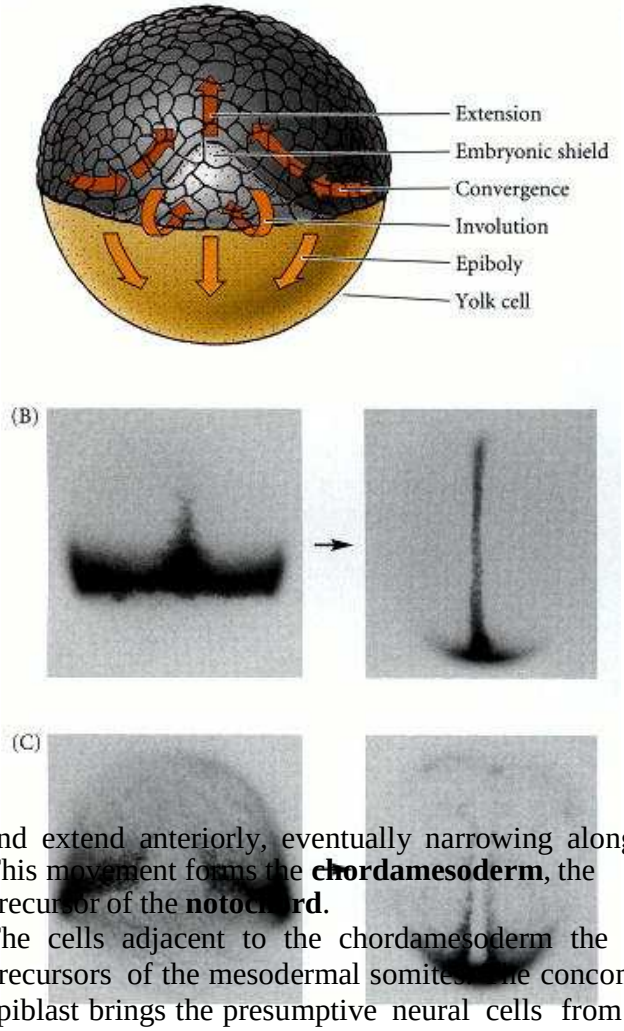
When this is done, the EVL and deep cells spring back to the top of the yolk, while the YSL continues its expansion around the yolk cell. The expansion of the YSL depends on a network of microtubules in the YSL, and radiation or drugs that block the polymerization of tubulin inhibit epiboly. During migration, one side of the blastoderm becomes noticeably thicker than the other. Cell-labeling experiments indicate that the thicker side marks the site of the future dorsal surface of the embryo.

3.6.1 formation of germ layers

After the blastoderm cells have covered about half the zebrafish yolk cell (and earlier in fish eggs with larger yolks), a thickening occurs throughout the margin of the epibolizing blastoderm. This thickening is called the **germ ring**, and it is composed of a superficial layer, the **epiblast**, and an inner layer, the **hypoblast**. We do not understand how the hypoblast is made. Some research groups claim that the hypoblast is

formed by the *involution* of superficial cells under the margin followed by their migration toward the animal pole. The involution begins at the future dorsal portion of the embryo, but occurs all around the margin. Other laboratories claim that these superficial cells *ingress* to form the hypoblast. It is possible that both mechanisms are at work, with different modes of hypoblast formation predominating in different species. Once formed, however, the cells of both the epiblast and hypoblast intercalate on the future dorsal side of the embryo to form a localized thickening, the **embryonic shield**.

As we will see, this shield is functionally equivalent to the dorsal blastopore lip of amphibians, since it can organize a secondary embryonic axis when transplanted to a host embryo. Thus, as the cells undergo epiboly around the yolk, they are also involuting at the margins and converging anteriorly and dorsally toward the embryonic shield. The hypoblast cells of the embryonic shield converge



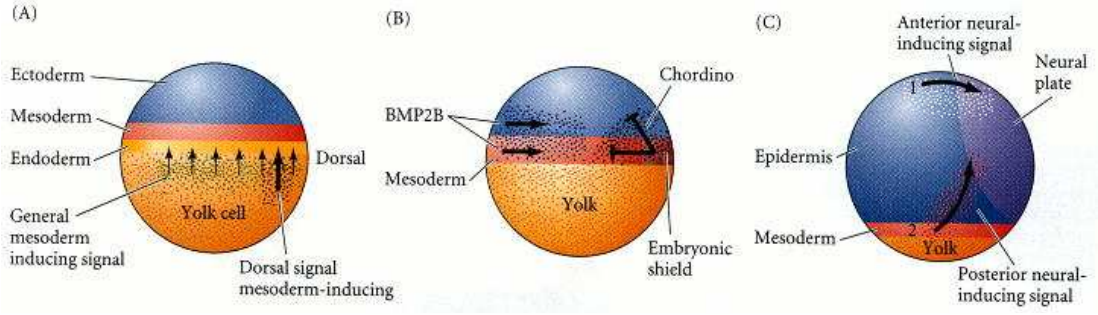
and extend anteriorly, eventually narrowing along the dorsal midline of the hypoblast. This movement forms the **chordamesoderm**, the precursor of the **notochord**.

The cells adjacent to the chordamesoderm the **paraxial mesoderm** cells, are the precursors of the mesodermal somites. The concomitant convergence and extension in the epiblast brings the presumptive neural cells from all over the epiblast into the dorsal midline, where they form the **neural keel**. The rest of the epiblast becomes the skin of the fish.

The zebrafish fate map, then, is not much different from that of the frog or other vertebrates (as we will soon see).

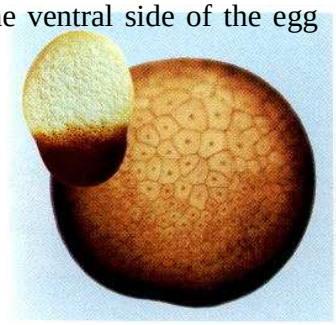
If one conceptually opens a *Xenopus* blastula at the vegetal pole and stretches the opening into a marginal ring, the resulting fate map closely resembles that of the zebrafish embryo at the stage when half of the yolk has been covered by the blastoderm.

Like the amphibian dorsal blastopore lip, the embryonic shield forms the prechordal plate and the notochord of the developing embryo. The precursors of these two regions are responsible for inducing the ectoderm to become neural ectoderm. Moreover, the presumptive notochord and prechordal plate appear to do this in a manner very much like that of their homologous structures in amphibians.* In both fishes and amphibians, BMP proteins made in the ventral and lateral regions of the embryo would normally cause the ectoderm to become epidermis. The notochord of both fishes and amphibians secretes factors that block this induction and thereby allow the ectoderm to become neural. In fishes, the BMP that ventralizes the embryo is **BMP2B**. The protein secreted by the chordamesoderm that binds with and inactivates BMP2B is a chordin-like paracrine factor called **Chordino.**



If the *chordino* gene is mutated, the neural tube fails to form. It is hypothesized that different concentrations of BMP2B pattern the ventral and lateral regions of the zebrafish ectoderm and mesoderm, and that the ratio between Chordino and BMP2B may specify the position along the dorsal-ventral axis. In fishes, however, the notochord may not be the only structure capable of producing the proteins that block BMP2B. If the notochord fails to form (as in the *floating head* or *no tail* mutations), the neural tube will still be produced. It is possible that the notochordal precursor cells (which are produced in these mutations) are still able to induce the neural tube, or that the dorsal portion of the somite precursors can compensate for the lack of a notochord.

The embryonic shield appears to acquire its organizing ability in much the same way as its amphibian counterparts. In amphibians, the endoderm cells beneath the dorsal blastopore lip (i.e., the Nieuwkoop center) accumulate β-catenin. This protein is critical in amphibians for the ability of the endoderm to induce the cells above them to become the dorsal lip (organizer) cells. In zebrafish, the nuclei in that part of the yolk syncytial layer that lies beneath the cells that will become the embryonic shield similarly accumulate β-catenin. This protein distinguishes the dorsal YSL from the lateral and ventral YSL regions. Inducing β-catenin accumulation on the ventral side of the egg causes dorsalization and a second embryonic axis. In addition, just prior to gastrulation, the cells of the dorsal blastopore margin synthesize and secrete Nodal-related proteins. These induce the precursors of the notochord and prechordal plate to activate *goosecoid* and other genes. Thus, the embryonic shield is considered equivalent to the amphibian organizer, and the dorsal part of the yolk cell can be thought of as the Nieuwkoop center of the fish embryo.



Anterior-posterior axis formation: two signaling centers

As is evident from figure above when a second dorsal-ventral axis is experimentally induced in zebrafish eggs, both the regular and the induced axes have the same anterior-posterior polarity. Both heads are at the former animal cap, and both tails are located vegetally. Indeed, the anterior-posterior axis is specified during oogenesis, and the animal cap marks the anterior of the embryo. This axis becomes stabilized during gastrulation through two distinct signaling centers. First, a small group of anterior neural cells at the border between the neural and surface ectoderm (a region that become the pituitary gland, nasal placode, and anterior forebrain) secrete compounds that cause anterior development. If these anterior neural cells are experimentally placed more posteriorly in the embryo, they will cause the neural cells near them to assume the characteristics of forebrain neurons. The second signaling center, in the posterior of the embryo, consists of lateral mesendoderm precursors at the margin of the gastrulating blastoderm. These cells produce caudalizing compounds , most likely Nodal-related proteins and activin.. If transplanted adjacent to anterior neural ectoderm, this tissue will transform the presumptive forebrain tissue into hindbrain-like structures.

3.7 Gastrulation of the Avian Embryo

The hypoblast

By the time a hen has laid an egg, the blastoderm contains some 20,000 cells. At this time, most of the cells of the area pellucida remain at the surface, forming the epiblast, while other area pellucida cells have delaminated and migrated individually into the subgerminal cavity to form the **polyinvagination islands** (**primary hypoblast**), an archipelago of disconnected clusters containing 5 20 cells each. Shortly thereafter, a sheet of cells from the *posterior* margin of the blastoderm (distinguished from the other regions of the margin by **Koller's sickle**, a local thickening) migrates anteriorly to join the polyinvagination islands,

thereby forming the **secondary hypoblast**. The two-layered blastoderm (epiblast and hypoblast) is joined together at the margin of the area opaca, and the space between the layers forms a blastocoel. Thus, although the shape and formation of the avian blastodisc differ from those of the amphibian, fish, or echinoderm blastula, the overall spatial relationships are retained.

The avian embryo comes entirely from the epiblast. The hypoblast does not contribute any cells to the developing embryo. Rather, the hypoblast cells form portions of the external membranes, especially the yolk sac and the stalk that links the yolk mass to the endodermal digestive tube. All three germ layers of the embryo proper (plus a considerable amount of extraembryonic membrane) are formed from the epiblastic cells. Fate maps of the chick epiblast.

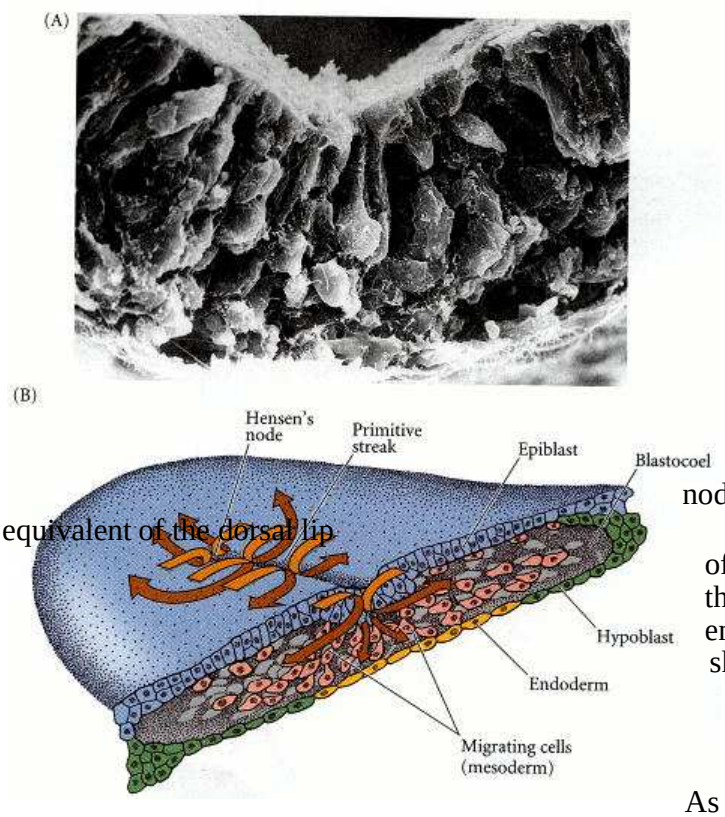
The primitive streak

The major structural characteristic of avian, reptilian, and mammalian gastrulation is the **primitive streak**. This streak is first visible as a thickening of the epiblast at the posterior region of the embryo, just anterior to Koller's sickle . This thickening is caused by the ingression of endodermal precursors from the epiblast into the blastocoel and by the migration of cells from the lateral region of the posterior epiblast toward the center. As these cells enter the primitive streak, the streak elongates toward the future head region. At the same time, the secondary hypoblast cells continue to migrate anteriorly from the posterior margin of the blastoderm. The elongation of the primitive streak appears to be coextensive with the anterior migration of these secondary hypoblast cells. The streak eventually extends 60 75% of the length of the area pellucida.

The primitive streak defines the axes of the embryo. It extends from *posterior* to *anterior*; migrating cells enter through its *dorsal* side and move to its *ventral* side; and it separates the *left* portion of the embryo from the *right*. Those elements close to the streak will be the **medial** (central) structures, while those farther from it will be the **distal** (lateral) structures.

As cells converge to form the primitive streak, a depression forms within the streak. This depression is called the **primitive groove**, and it serves as an opening through which migrating cells pass into the blastocoel. Thus, the primitive groove is analogous to the amphibian blastopore. At the anterior end of the primitive streak is a regional thickening of cells called the **primitive knot** or **Hensen's node**. The center of this node contains a funnel-shaped depression (sometimes called the **primitive pit**) through

which cells can pass into the blastocoel. Hensen's



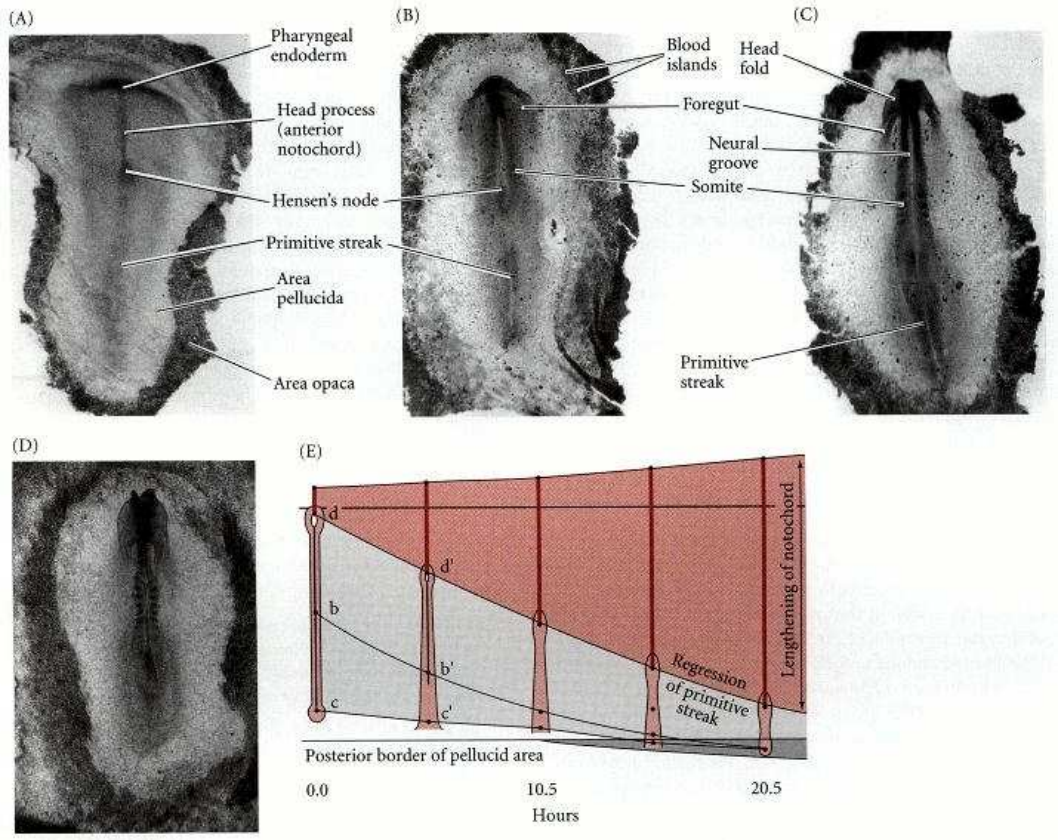
node is the functional equivalent of the dorsal lip of the amphibian blastopore (i.e., the organizer) and the fish embryonic shield.

As soon as the primitive streak has formed, epiblast cells begin to migrate through it and into the blastocoel
The primitive streak has a continually changing cell population. Cells migrating through Hensen's node pass down into the blastocoel and migrate anteriorly, forming foregut, head mesoderm, and notochord; cells passing through the lateral portions of the primitive streak give rise to the majority of endodermal and mesodermal tissues. Unlike the *Xenopus* mesoderm, which migrates as sheets of cells into the blastocoel, cells entering the inside of the avian embryo ingress as individuals afterundergoing an epithelial-to-mesenchymal transformation. At Hensen's node and throughout the primitive streak, the breakdown of the basal lamina and the release of these cells into the embryo is thought to be accomplished by **scatter factor**, a 190-kDa protein secreted by the cells as they enter the streak. Scatter factor can convert epithelial sheets into mesenchymal cells in several ways, and it is probably involved both in downregulating E-cadherin expression and in preventing E- cadherin from functioning

Migration through the primitive streak: formation of endoderm and mesoderm.

The first cells to migrate through Hensen's node are those destined to become the pharyngial endoderm of the foregut. Once inside the blastocoel, these endodermal cells migrate anteriorly and eventually displace the hypoblast cells, causing the hypoblast cells to be confined to a region in the anterior portion of the area pellucida.

This region, the **germinal crescent**, does not form any embryonic structures, but it does contain the precursors of the germ cells, which later migrate through the blood vessels to the gonads ..



The next cells entering the blastocoel through Hensen's node also move anteriorly, but they do not move as far ventrally as the presumptive foregut endodermal cells. Rather, they remain between the endoderm and the epiblast to form the **head mesenchyme** and the **prechordal plate mesoderm**. These early-ingressing cells all move anteriorly, pushing up the anterior midline region of the epiblast to form the **head process** (Figure 11.. Thus, the head of the avian embryo forms anterior (**rostral**) to Hensen's node. The next cells migrating through Hensen's node become chordamesoderm (notochord) cells. These cells extend up to the presumptive midbrain, where they meet the prechordal plate. The hindbrain and trunk form from the chordamesoderm at the level of Hensen's node and caudal to it.

Meanwhile, cells continue migrating inwardly through the lateral portion of the primitive streak. As they enter the blastocoel, these cells separate into two layers. The deep layer joins the hypoblast along its midline and displaces the hypoblast cells to the sides. These deep-moving cells give rise to all the endodermal organs of the embryo as well as to most of the extraembryonic membranes (the hypoblast forms the rest). The second migrating layer spreads between this endoderm and the epiblast, forming a loose layer of cells. These middle layer cells generate the mesodermal portions of the embryo and extraembryonic membranes. By 22 hours of incubation, most of the presumptive endodermal cells are in the interior of the embryo, although presumptive mesodermal cells continue to migrate inward for a longer time.

Regression of the primitive streak.

Now a new phase of gastrulation begins. While mesodermal ingression continues, the primitive streak starts to regress, moving Hensen's node from near the center of the area pellucida to a more posterior position. It leaves in its wake the dorsal axis of the embryo and the notochord. As the node moves posteriorly, the notochord is laid down, starting at the level of the future midbrain. While the anterior portion of the notochord is formed by the ingression of cells through Hensen's node, the posterior notochord (after somite 17 in the chick) forms from the condensation of mesodermal tissue that has ingressed through the primitive streak (i.e., not through Hensen's node). This portion of the notochord extends posteriorly to form the tail of the embryo Finally, Hensen's node regresses to its most posterior position, forming the anal region. At this time, all the presumptive endodermal and mesodermal cells have entered the embryo, and the epiblast is composed entirely of presumptive ectodermal cells.

As a consequence of the sequence by which the head mesoderm and notochord are established, avian (and mammalian) embryos exhibit a distinct anterior-to-posterior gradient of developmental maturity. While cells of the posterior portions of the embryo are undergoing gastrulation, cells at the anterior end are already starting to form organs . For the next several days, the anterior end of the embryo is more advanced in its development (having had a "head start," if you will) than the posterior end.

Epiboly of the ectoderm

While the presumptive mesodermal and endodermal cells are moving inward, the ectodermal precursors proliferate. Moreover, the ectodermal cells migrate to surround the yolk by epiboly. The enclosure of the yolk by the ectoderm (again reminiscent of the epiboly of amphibian ectoderm) is a Herculean task that takes the greater part of 4 days to complete. It involves the continuous production of new cellular material and the migration of the presumptive ectodermal cells along the underside of the vitelline envelope. Interestingly, only the cells of the outer edge of the area opaca attach firmly to the vitelline envelope. These cells are inherently different from the other blastoderm cells, as they can extend enormous (500 μm) cytoplasmic processes onto the vitelline envelope. These elongated filopodia are believed to be the locomotor apparatus of these marginal cells, by which they pull the other ectodermal cells around the yolk. The filopodia appear to bind to fibronectin, a laminar protein that is a component of the chick vitelline envelope. If the contact between the marginal cells and the fibronectin is experimentally broken (by adding a soluble polypeptide similar to fibronectin), the filopodia retract, and epidermal migration ceases.

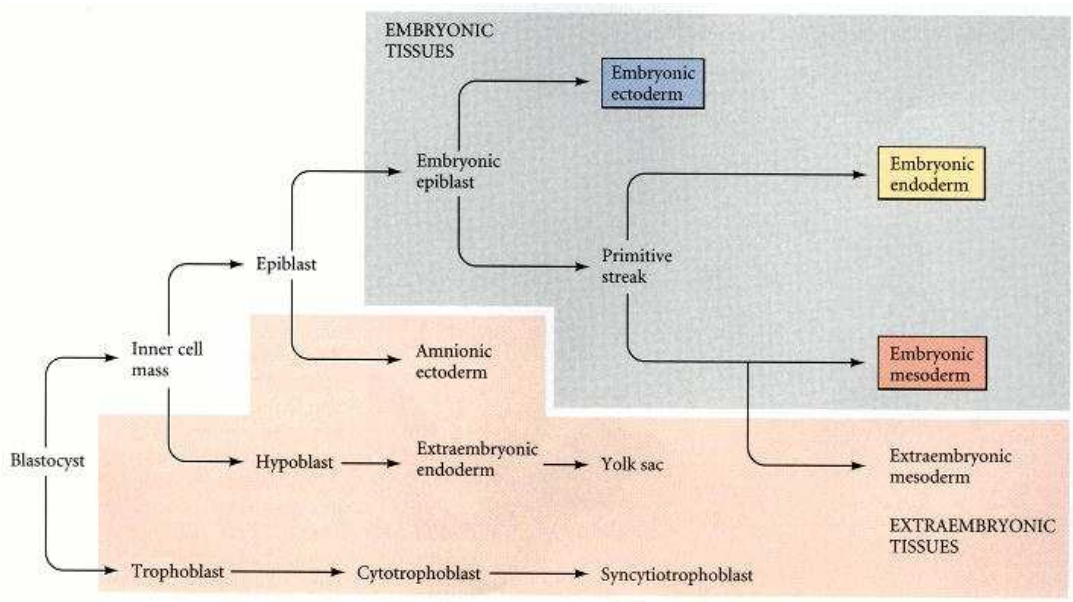
Thus, as avian gastrulation draws to a close, the ectoderm has surrounded the yolk, the endoderm has replaced the hypoblast, and the mesoderm has positioned itself between these two regions. We have identified many of the processes involved in avian gastrulation, but we remain ignorant as to the mechanisms by which many of these processes are carried out.

3.8 Gastrulation in Mammals

Birds and mammals are both descendants of reptilian species. Therefore, it is not surprising that mammalian development parallels that of reptiles and birds. What is surprising is that the gastrulation movements of reptilian and avian embryos, which evolved as an adaptation to yolky eggs, are retained even in the absence of large amounts of yolk in the mammalian embryo. The mammalian inner cell mass can be envisioned as sitting atop an imaginary ball of yolk, following instructions that seem more appropriate to its reptilian ancestors.

Modifications for development within another organism

The mammalian embryo obtains nutrients directly from its mother and does not rely on stored yolk. This adaptation has entailed a dramatic restructuring of the maternal anatomy (such as expansion of the oviduct to form the uterus) as well as the development of a fetal organ capable of absorbing maternal nutrients. This fetal organ the chorion is derived primarily from embryonic trophoblast cells, supplemented with mesodermal cells derived from the inner cell mass. The chorion forms the fetal portion of the placenta. It will induce the uterine cells to form the maternal portion of the placenta, the **decidua**. The decidua becomes rich in the blood vessels that will provide oxygen and nutrients to the embryo.



The origins of early mammalian tissues are summarized in Figure 11.. The first segregation of cells within the inner cell mass results in the formation of the hypoblast (sometimes called the **primitive endoderm**) layer (Figure 11.). The hypoblast cells delaminate from the inner cell mass to line the blastocoel cavity, where they give rise to the **extraembryonic endoderm**, which forms the yolk sac. As in avian embryos, these cells do not produce any part of the newborn organism. The remaining inner cell mass tissue above the hypoblast is now referred to as the epiblast. The epiblast cell layer is split by small clefts that eventually coalesce to separate the **embryonic epiblast** from the other epiblast cells, which form the **amnionic cavity** . Once the lining of the amnion is completed, it fills with a secretion called **amnionic** (**amniotic**) **fluid**, which serves as a shock absorber for the developing embryo while preventing its desiccation. The

embryonic epiblast is believed to contain all the cells that will generate the actual embryo, and it is similar in many ways to the avian epiblast.

By labeling individual cells of the epiblast with horseradish peroxidase, a detailed fate map of the mouse epiblast was constructed. Gastrulation begins at the posterior end of the embryo, and this is where the **node** forms . Like the chick epiblast cells, the mammalian mesoderm and endoderm migrate through a primitive streak, and like their avian counterparts, the migrating cells of the mammalian epiblast lose E-cadherin, detach from their neighbors, and migrate through the streak as individual cells.

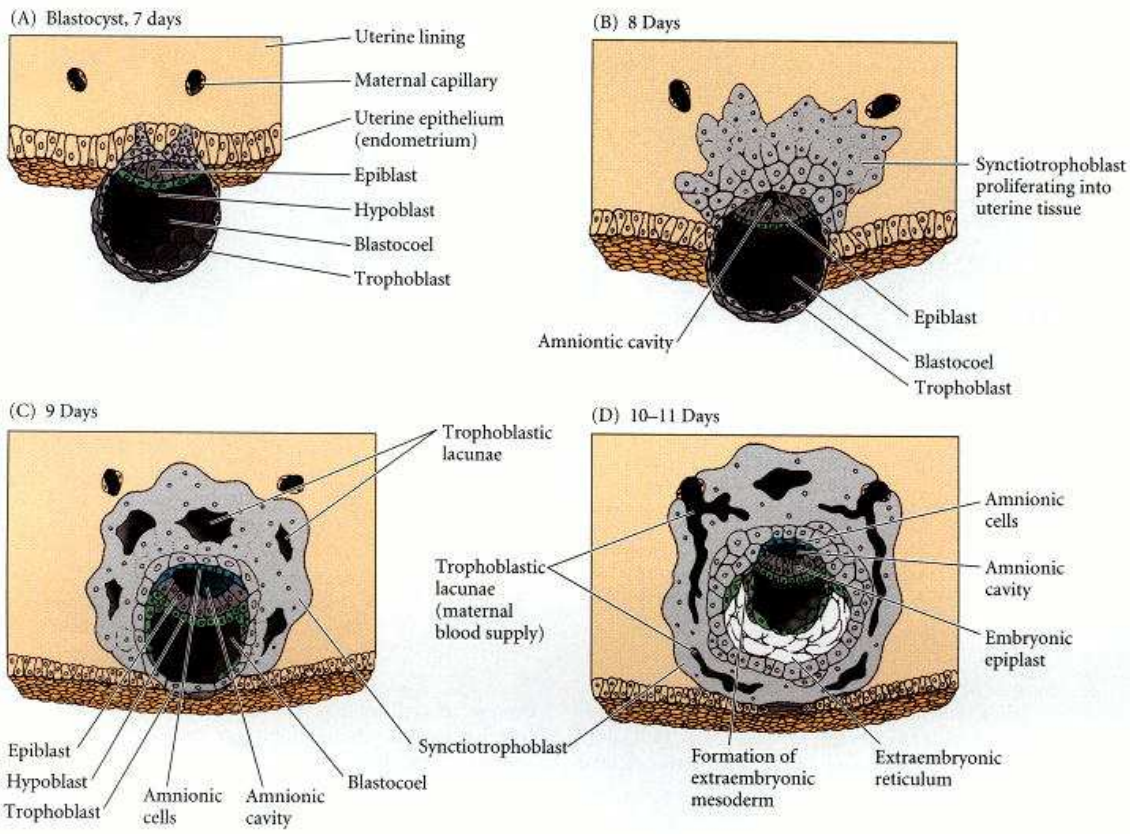


Fig.11.

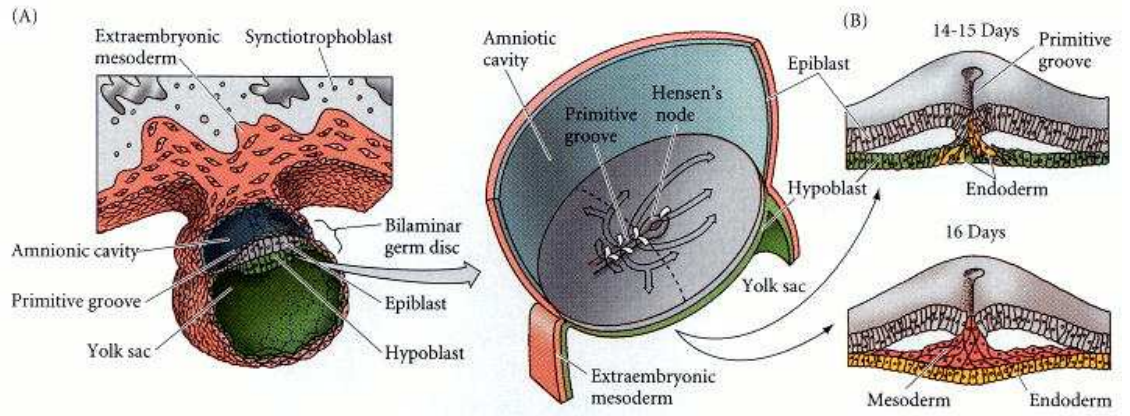
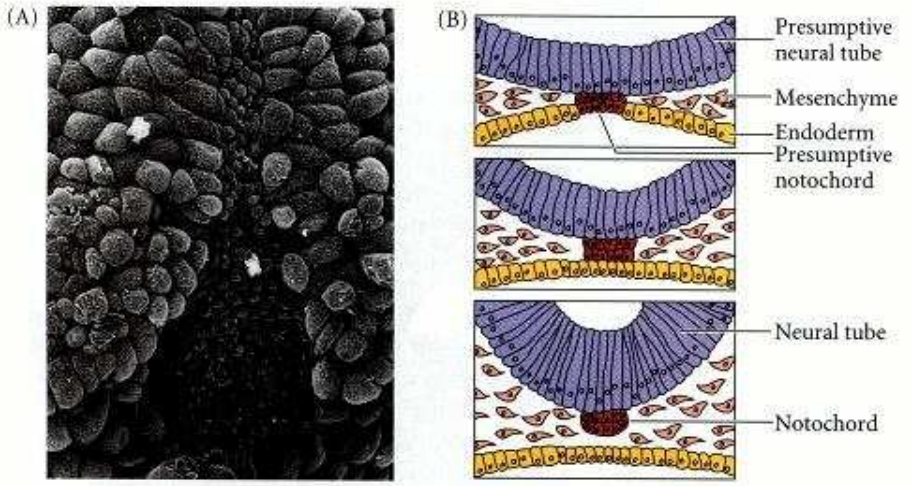


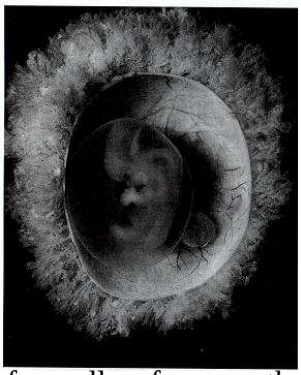
Fig. 11.28

Those cells migrating through the node give rise to the notochord. However, in contrast to notochord formation in the chick, the cells that form the mouse notochord are thought to become integrated into the endoderm of the primitive gut. These cells can be seen as a band of small, ciliated cells extending rostrally from the node (Figure 11.29). They form the notochord by converging medially and folding off in a dorsal direction from the roof of the gut.



The ectodermal precursors end up anterior to the fully extended primitive streak, as in the chick epiblast; but whereas the mesoderm of the chick forms from cells posterior to the farthest extent of the streak, the mouse mesoderm forms from cells anterior to the primitive streak. In some instances, a single cell gives rise to descendants in more than one germ layer, or to both embryonic and extraembryonic derivatives. Thus, at the epiblast stage, these lineages have not become separate from one another. As in avian embryos, the cells migrating in between the hypoblast and epiblast layers are coated with hyaluronic acid, which they synthesize as they leave the primitive streak. This acts to keep them separate while they migrate. It is thought that the replacement of human hypoblast cells by endoderm precursors occurs on days 14 15 of gestation, while the migration of cells forming the mesoderm does not start until day 16.

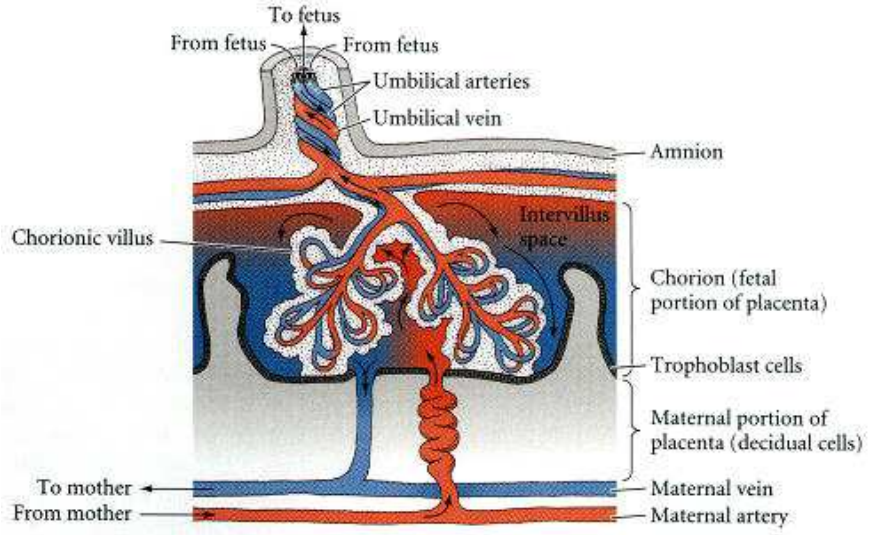
Formation of extraembryonic membranes



While the embryonic epiblast is undergoing cell movements reminiscent of those seen in reptilian or avian gastrulation, the extraembryonic cells are making the distinctly mammalian tissues that enable the fetus to survive within the maternal uterus. Although the initial trophoblast cells of mice and humans divide like most other cells of the body, they give rise to a population of cells wherein nuclear division occurs in the absence of cytokinesis.
The original type of trophoblast cells constitute a layer called the **cytotrophoblast**, whereas the multinucleated type of cell forms the **syncytiotrophoblast**. The cytotrophoblast initially adheres to the endometrium through a series of adhesion molecules. Moreover, these cells also contain proteolytic enzymes that enable them to enter the uterine wall and remodel the uterine blood vessels so that the maternal blood bathes fetal blood vessels. The syncytiotrophoblast tissue is thought to further the progression of the embryo into the uterine wall by digesting uterine tissue. The uterus, in turn, sends blood vessels into this area, where they eventually contact the syncytiotrophoblast. Shortly thereafter, mesodermal tissue extends outward from the gastrulating embryo . Studies of human and rhesus monkey embryos have suggested that the yolk sac (and hence the hypoblast) is the source of this extraembryonic mesoderm.

The extraembryonic mesoderm joins the trophoblastic extensions and gives rise to the blood vessels that carry nutrients from the mother to the embryo. The narrow connecting stalk of extraembryonic mesoderm that links the embryo to the trophoblast eventually forms the vessels of the **umbilical cord**. The fully developed extraembryonic organ, consisting of trophoblast tissue and blood vessel-containing mesoderm, is called the chorion, and it fuses with the uterine wall to create the placenta. Thus, the placenta has both a maternal portion (the uterine endometrium, which is modified during pregnancy) and a fetal component (the chorion). The chorion may be very closely apposed to maternal tissues while still being readily separable from them (as in the contact placenta of the pig), or it may be so intimately integrated with maternal tissues that the two cannot be separated without damage to both the mother and the developing fetus (as in the deciduous placenta of most mammals, including humans).

Figure 11.30 shows the relationships between the embryonic and extraembryonic tissues of a 6-week human embryo. The embryo is seen encased in the amnion and is further shielded by
the chorion.



The blood vessels extending to and from the chorion are readily observable, as are the villi that project from the outer surface of the chorion. These villi contain the blood vessels and allow the chorion to have a large area exposed to the maternal blood. Although fetal and maternal circulatory systems normally never merge, diffusion of soluble substances can occur through the villi (Figure 11.31). In this manner, the mother provides the fetus with nutrients and oxygen, and the fetus sends its waste products (mainly carbon dioxide and urea) into the maternal circulation. The maternal and fetal blood cells, however, usually do not mix.

4.0 CONCLUSION

In this unit you learnt about formation of gastulation and invagination in some animals. For example; sea urchin, snail and amphibian, fish, aves and mammal.

5.0 SUMMMARY

Gastrulation and invagination is the next stage of development in animal after cleavage and blastula formation. Gastrulation is highly coordinated cell and tissue movements whereby the cells of the blastula are dramatically rearranged. The blastula consists of numerous cells, the positions of which were established during cleavage. During gastrulation, these cells are given new positions and new neighbors, and the multilayered body plan of the organism is established.

The cells that will form the endodermal and mesodermal organs are brought inside the embryo, while the cells that will form the skin and nervous system are spread over its outside surface. Thus, the three germ layers outer ectoderm, inner endoderm, and interstitial mesoderm are first produced during gastrulation (cell movement).The infolding of cells into the embryo is called invagination.

6.0 **TUTOR-MARKED ASSIGNMENT**

1.0 Explain gastrulation and invagination in sea urchin, snai and fish
2.0 Describe the process of gaastrulation in amphibian, aves and mammal.

7.0 REFERENCES

Professor Scott Gilbert, Developmental Biology, 6th Edition.

Unit 2: Organogenesis

1.0 INTRODUCTION

In animal development, organogenesis (organo-genesis, compound of the Greek words *όργανον* "that with which one works, and *γένεσις* "origin, creation, generation") is the process by which the ectoderm, endoderm, and mesoderm develop into the internal organs of the organism. Internal organs initiate development in humans within the 3rd to 8th weeks in utero. The germ layers in organogenesis differ by three processes: folds, splits, and condensation. Developing early during this stage in chordate animals are the neural tube and notochord. Vertebrate animals all differentiate from the gastrula the same way. Vertebrates develop a neural crest that differentiates into many structures, including some bones, muscles, and components of the peripheral nervous system. The coelom of the body forms from a split of the mesoderm along the somite axis.

2.0 OBJECTIVE

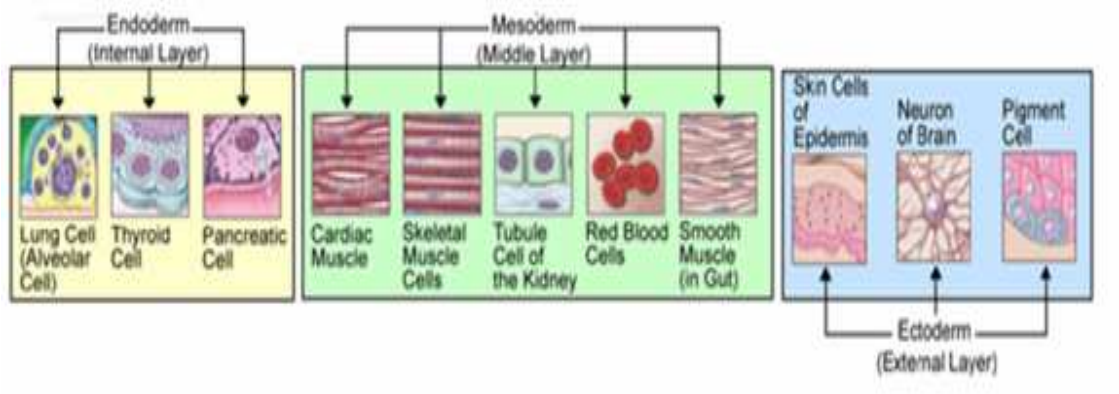
3.0 MAIN CONTENT

3.1 Organogenesis in animal

At some point after the different germ layers are defined, organogenesis begins. The first stage in vertebrates is called neurulation, where the neural plate folds forming the neural tube. Other common organs or structures which arise at this time include the heart and somites, but from now on embryogenesis follows no common pattern among the different taxa of the animal kingdom. In most animals organogenesis along with morphogenesis will result in a larva. The hatching of the larva, which must then undergo metamorphosis, marks the end of embryonic development.

3.1.1Product of Three Germ Layer

The proceeding graph below represents the products produced by the three germ layers.



The endoderm produces tissue within the lungs, thyroid, and pancreas. The mesoderm aids in the production of cardiac muscle, skeletal muscle, smooth muscle, tissues within the kidneys, and

red blood cells. The ectoderm produces tissues within the epidermis and aids in the formation of neurons within the brain, and melanocytes.

Germ Layer	Category	Product
Endoderm	General	Gastrointestinal tract
Endoderm	General	Respiratory tract
Endoderm	General	Endocrine glands and organs (liver and pancreas)
Mesoderm	General	Bones
Mesoderm	General	Most of circulatory system
Mesoderm	General	Connective tissues of the gut and integuments
Mesoderm	General	Excretory tract
Mesoderm	General	Mesenchyme
Mesoderm	General	Mesothelium
Mesoderm	General	Muscles
Mesoderm	General	Peritoneum
Mesoderm	General	Reproductive system
Mesoderm	General	Urinary System
Mesoderm	Vertebrate	Chordamesoderm
Mesoderm	Vertebrate	Paraxial mesoderm
Mesoderm	Vertebrate	Intermediate mesoderm
Mesoderm	Vertebrate	Lateral plate mesoderm
Ectoderm	General	Outer part of integument

Ectoderm	General	Skin (along with glands, hair,nails)
Ectoderm	Vertebrate	Epithelium of the mouth and nasal cavity
Ectoderm	Vertebrate	Lens and cornea of the eye
Ectoderm	Vertebrate	Melanocytes
Ectoderm	Vertebrate	Peripheral nervous system
Ectoderm	Vertebrate	Facial cartilage
Ectoderm	Vertebrate	Dentin(in teeth)
Ectoderm	Vertebrate	Brain (rhombencephalon, mesencephalon and prosencephalon)
Ectoderm	Vertebrate	Spinal cord and motor neuron
Ectoderm	Vertebrate	Retina
Ectoderm	Vertebrate	Posterior pituitary

3.1.2 Formation of the germ layers

The embryonic disk becomes oval and then pear-shaped, the wider end being directed forward. Near the narrow, posterior end, an opaque streak, called the primitive streak, makes its appearance and extends along the middle of the disk for about one-half of its length; at the anterior end of the streak there is a knob-like thickening termed Hensen's knot. A shallow groove, the primitive groove, appears on the surface of the streak, and the anterior end of this groove communicates by means of an aperture, the blastopore, with the yolk sac. The primitive streak is produced by a thickening of the axial part of the ectoderm, the cells of which multiply, grow downward, and blend with those of the subjacent entoderm. From the sides of the primitive streak a third layer of cells, the mesoderm, extends lateralward between the ectoderm and entoderm; the caudal end of the primitive streak forms the cloacal membrane. The blastoderm now consists of three layers, named from without inward: ectoderm, mesoderm, and entoderm; each has distinctive characteristics and gives rise to certain tissues of the body. For many

mammals, it is sometime during formation of the germ layers that implantation of the embryo in the uterus of the mother occurs.

3.1.3 Formation of the early nervous system - neural groove, tube and notochord

In front of the primitive streak, two longitudinal ridges, caused by a folding up of the ectoderm, make their appearance, one on either side of the middle line formed by the streak. These are named the neural folds; they commence some little distance behind the anterior end of the embryonic disk, where they are continuous with each other, and from there gradually extend backward, one on either side of the anterior end of the primitive streak. Between these folds is a shallow median groove, the neural groove. The groove gradually deepens as the neural folds become elevated, and ultimately the folds meet and coalesce in the middle line and convert the groove into a closed tube, the neural tube or canal, the ectodermal wall of which forms the rudiment of the nervous system. After the coalescence of the neural folds over the anterior end of the primitive streak, the blastopore no longer opens on the surface but into the closed canal of the neural tube, and thus a transitory communication, the neurenteric canal, is established between the neural tube and the primitive digestive tube. The coalescence of the neural folds occurs first in the region of the hind brain, and from there extends forward and backward; toward the end of the third week, the front opening (anterior neuropore) of the tube finally closes at the anterior end of the future brain, and forms a recess which is in contact, for a time, with the overlying ectoderm; the hinder part of the neural groove presents for a time a rhomboidal shape, and to this expanded portion the term sinus rhomboidalis has been applied. Before the neural groove is closed, a ridge of ectodermal cells appears along the prominent margin of each neural fold; this is termed the neural crest or ganglion ridge, and from it the spinal and cranial nerve ganglia and the ganglia of the sympathetic nervous system are developed. By the upward growth of the mesoderm, the neural tube is ultimately separated from the overlying ectoderm.

The cephalic end of the neural groove exhibits several dilatations which, when the tube is closed, assume the form of three vesicles; these constitute the three primary cerebral vesicles, and correspond respectively to the future **fore-brain** (prosencephalon), **mid-brain** (mesencephalon), and **hind-brain** (rhombencephalon). The walls of the vesicles are developed into the nervous tissue and neuroglia of the brain, and their cavities are modified to form its ventricles. The

remainder of the tube forms the medulla spinalis or spinal cord; from its ectodermal wall the nervous and neuroglial elements of the medulla spinalis are developed, while the cavity persists as the central canal.

3.1.4 Formation of the early septum

The extension of the mesoderm takes place throughout the whole of the embryonic and extra-embryonic areas of the ovum, except in certain regions. One of these is seen immediately in front of the neural tube. Here the mesoderm extends forward in the form of two crescentic masses, which meet in the middle line so as to enclose behind them an area which is devoid of mesoderm. Over this area, the ectoderm and entoderm come into direct contact with each other and constitute a thin membrane, the buccopharyngeal membrane, which forms a septum between the primitive mouth and pharynx.

3.1.5. Early formation of the heart and other primitive structures

In front of the buccopharyngeal area, where the lateral crescents of mesoderm fuse in the middle line, the pericardium is afterward developed, and this region is therefore designated the pericardial area. A second region where the mesoderm is absent, at least for a time, is that immediately in front of the pericardial area. This is termed the proamniotic area, and is the region where the proamnion is developed; in humans, however, a proamnion is apparently never formed. A third region is at the hind end of the embryo where the ectoderm and entoderm come into apposition and form the cloacal membrane.

3.1.6 Somitogenesis

Somitogenesis is the process by which somites are produced. These segmented tissue blocks differentiate into skeletal muscle, vertebrae, and dermis of all vertebrates.

Somitogenesis begins with the formation of somitomeres (whorls of concentric mesoderm) marking the future somites in the presomitic mesoderm (unsegmented paraxial). The presomitic mesoderm gives rise to successive pairs of somites, identical in appearance and which differentiate into the same cell types but the structures formed by the cells vary depending upon

the anteroposterior (e.g. the thoracic vertebrates have ribs, the lumbar vertebrates do not). Somites have unique positional values along this axis and it is thought that these are specified by the Hox (homeotic) genes.

Toward the end of the second week after fertilization, transverse segmentation of the paraxial mesoderm begins, and it is converted into a series of well-defined, more or less cubical masses, also known as the **primitive segments**, which occupy the entire length of the trunk on either side of the middle line from the occipital region of the head. Each segment contains a central cavity (known as a myocoel) which, however, is soon filled with angular and spindle-shaped cells. The primitive segments lie immediately under the ectoderm on the lateral aspect of the neural tube and notochord, and are connected to the lateral mesoderm by the intermediate cell mass. Those of the trunk may be arranged in the following groups, viz.: cervical 8, thoracic 12, lumbar 5, sacral 5, and coccygeal from 5 to 8. Those of the occipital region of the head are usually described as being four in number. In mammals, primitive segments of the head can be recognized only in the occipital region, but a study of the lower vertebrates leads to the belief that they are present also in the anterior part of the head and that, altogether, nine segments are represented in the cephalic region.

4.0 CONCLUSION

In this unit you learnt about organogenesis as process of organ formation from three germ layer(product of gastrulation). The ectoderm, endoderm and mesoderm develop into internal organs of the organism.

5.0 SUMMARY

The process by which the ectoderm, endoderm, and mesoderm develop into the internal organs of the organism is called organogenesis.The internal organs initiate development in humans within the 3rd to 8th weeks in utero. The germ layers in organogenesis differ by three processes: folds, splits, and condensation. Developing early during this stage in chordate animals are the neural tube and notochord.

6.0 TUTOR-MARKED ASSIGNMENT

1 Explain the term organogenesis

2 Explain the germ layers with the aid of a suitable diagram in animal development

3 Explain

7.0REFERENCES

Professor Scott Gilbert, Developmental Biology, 6th Edition.

MODULE 5: GENERAL EMBRYOLOGY

Unit 1: General Embryology

CONTENT

1.0 INTRODUCTION

The literal meaning of embryology is the study of the developmental changes (structurally and physiologically) that a zygote undergoes till the formation of an adult form. The study of all these sequential orderly processes comprises developmental biology or embryology. Developmental biology includes the study of (i) ontogenic development, which involves transformation of fertilized egg into a new adult. (ii) phylogenetic development, which involves gradual transformation of forms of life. Embryonic development involves gametogenesis, fertilization, cleavage, blastulation, gastrulation and organogenesis.

2.0 OBJECTIVES

At the end of this unit the student should be able to:

1 Explain the formation of embryo through the developmental processes
2 Decribe and explain the pattern of cleavage from A-H

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3.0 MAIN CONTENT

3.1 GAMETOGENESIS

It involves the fusion of gametes to form a diploid zygote, already discussed in previous chapter. Yolk is a morphological term used to describe the reserve food in the oocyte, formed of proteins, neutral fats, phospholipids, carbohydrates and glycogen. The process of synthesis and accumulation of yolk is called as **vitellogenesis**. Based on the distribution and amount of yolk the eggs are classified into following categories-

I. Based on Amount of Yolk.

1. ***Microlecithal.*** Small sized egg with only a small amount of yolk or reserve food. E.g. Amphioxus, sea urchin, starfish. According to some scientists, **alecithal** term is also used, meaning no yolk, but this term is not appropriate as no egg is without yolk.
2. ***Mesolecithal.*** Eggs containing moderate amount of yolk. E.g. amphibians.
3. ***Macrolecithal.*** Eggs containing enormous amount of yolk. E.g. reptiles, birds.

II. Based on Distribution of Yolk.

1. ***Isolecithal.*** In microlecithal eggs the amount of yolk is so little that it is evenly distributed throughout the egg cytoplasm. E.g. protochordates and echinoderms.
2. ***Telolecithal.*** In mesolecithal and macrolecithal eggs the yolk is concentrated in the lower part of the egg (vegetal pole), while the upper part is yolk free called animal pole. E.g. reptiles and birds.
3. ***Centrolecithal.*** In macrolecithal eggs the yolk is concentrated in the centre of the egg with the cytoplasm surrounding it. E.g. insects.

3.2 Types of Egg Membranes.

- **Primary Egg Membranes.** Formed outside the plasma membrane of the oocyte secreted by the follicle cells. These are of following types:

1. ***Vitelline membrane-*** Closely applied to the plasma membrane, found in insects, molluscs, amphibians and birds formed of mucopolysaccharides and fibrous proteins.
2. ***Zona radiata-*** It is striated in appearance or perforated by minute pores, found in sharks, bony fishes, some amphibians and reptiles.
3. ***Zona pellucida-*** It is unstriated in appearance, formed by the secretions of the ovum and the follicle cells, found in mammals.
4. ***Jelly envelope-*** It is jelly like and thicker in appearance also known as jelly coat, found in echinoderms and marine invertebrates.

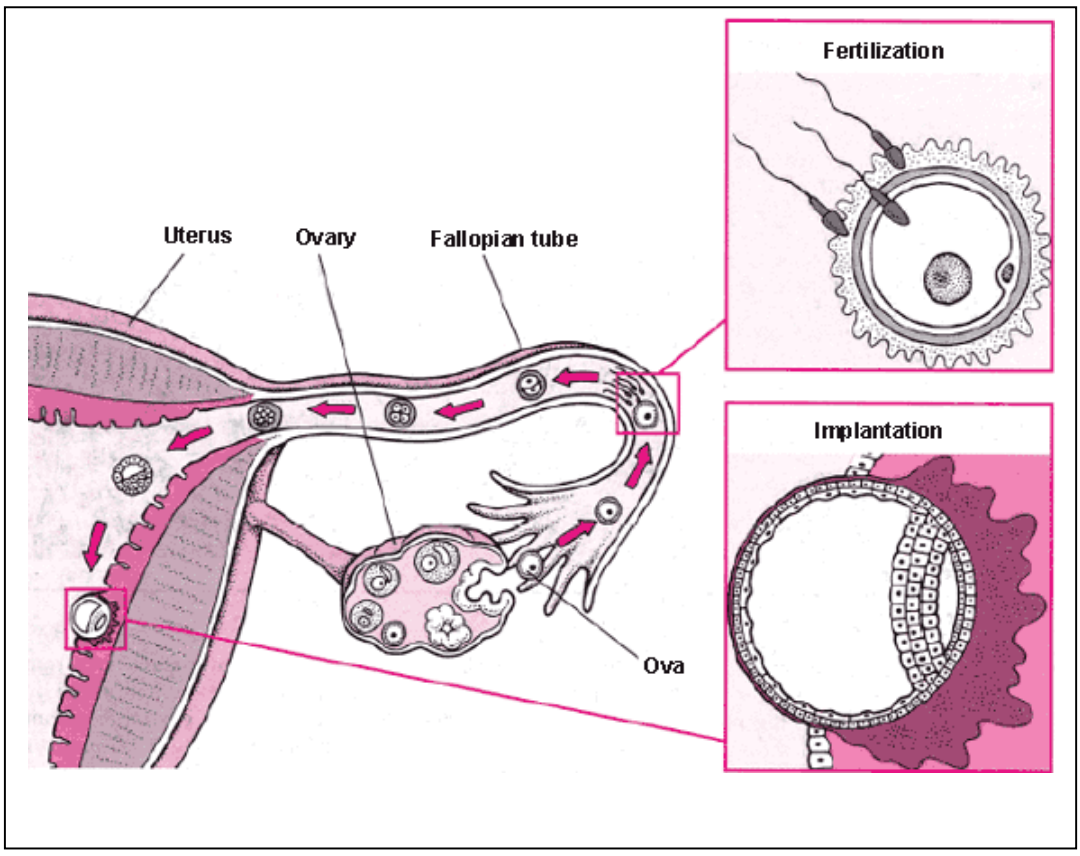
- **Secondary Egg Membranes.** Secreted outside the primary egg membranes by the follicle cells surrounding the oocyte. E.g. chorion present around an egg of insect. In mammalian eggs the secondary egg membrane is absent; instead a layer of follicle cells surrounds the zona pellucida known as **corona radiata**. It is not a true membrane because its cells are peeled off as the egg passes through the oviduct.

- **Tertiary Egg Membranes.** Secreted either by the oviduct or some other accessory parts of the maternal genital tract when the egg passes down through the oviduct to the exterior. E.g. outermost calcareous shell of hen's egg, jelly coat around the amphibian oocyte.

3.2 FERTILIZATION

All mammals rely on internal fertilization through **copulation**. To deliver the sperm to the female, the male inserts his sexual organ, the penis, into the opening of the vagina; the passage into the female's other sexual organs. Once the male ejaculates, a large number of sperm cells swim from the upper vagina through the cervix and across the length of the uterus toward the ovum- a considerable distance compared to the size of the sperm cell. The spermatozoon and the oocyte meet and interact in the **ampulla** of the fallopian tube. It is probable that chemotaxis is involved in directing the sperm to the egg, but the mechanism has yet to be worked out. **Human fertilization** is the union of a human egg and sperm, usually occurring in the ampulla of the fallopian tube. There is a specific sequence of events that occur in fertilization:

- The sperm passes through the corona radiata, the outermost cell layer of the egg.
- The sperm breaks through the zona pellucida. This occurs with the aid of several enzymes possessed by the sperm that break down the proteins of the zona pellucida, the most important one being acrosin. When the sperm penetrates the zona pellucida, the cortical reaction occurs. This makes the egg impermeable to any other sperms and prevents fertilization by more than one sperm.
- The cell membranes of the egg and sperm fuse together.
- The female egg, also called a secondary oocyte at this stage, completes its second meiotic division. This results in a mature ovum.
- The sperm's tail and mitochondria degenerate with the formation of the male pronucleus. This is why all mitochondria in humans are of maternal origin.
- The male and female pronuclei fuse to form a new nucleus that is a combination of the genetic material from both the sperm and egg.



3.2.1.Mechanism of Fertilization.

(a) Approximation of Sperm and ovum. This is done by fertilizin-antifertilizin compatibility reaction. A chemical substance, the fertilizin secreted from the cortical region of the egg cytoplasm interacts with the antifertilizin of the sperm of the same species. This makes the sperms stick to the surface of the egg. Both fertilizin and antifertilizin are species specific; fertilizin acts as a receptor for antifertilizin and makes the sperm capable of fertilizing the egg of the same species. This process is called as **capacitation**.

(b) Acrosomal Reaction. The acrosome is a highly modified lysosome derived from the golgi apparatus during spermiogenesis. The sperm, after attachment to the egg surface has to penetrate the egg membranes so as to reach the egg plasma membrane. Acrosome contains following enzymes which assists in fertilization:

- **Zona lysins.** These are proteolytic enzymes produced from the acrosome and are capable of dissolving the zona pellucida and clear the path for sperm to reach the plasma membrane of the egg.
- **Hyaluronidase.** The mammalian egg is covered by a membrane called corona radiata, formed of single layer of follicular cells, connected together by an adhesive substance, the hyaluronic acid. Hyaluronidase dissolves the hyaluronic acid, separates the follicular cells of corona radiata so that the sperm can propel inward. The acrosome is not able to release hyaluronidase and zona lysins till it has undergone capacitation.
- **Neuraminidase.** It is a hydrolytic enzyme which checks the entry of more than one sperm from entering the ovum and thus prevents polyspermy.

(c) Activation of Ovum. As the sperm enters the ovum (actually a secondary oocyte), it gets activated and undergoes second meiotic division forming a haploid sperm and a secondary polar body. This is followed by the breakdown of nuclear membrane of sperm and ova at their pint of contact and their contents gets surrounded by a common nuclear membrane forming the diploid zygote nucleus, which contains the maternal and paternal chromosomes. The zygote begins to divide and form a blastocyst and when it reaches the uterus, it implants in the endometrium. At this point the female is said to be **pregnant**. If the embryo emplants in any tissue other than the uterine wall, an **ectopic pregnancy** results, which can be fatal to the mother.

3.3 CLEAVAGE

Immediately after fertilization, the fertilized egg undergoes a series of repeated mitotic cell divisions to form a multicellular blastula. Cleavage can be defined as "*the process of progressive subdivision of the zygote by mitotic cell divisions into an increasing number of cells of progressively decreasing size*". Cleavage differs from mitosis in the following aspects:

- ✓ There is no growth phase between the successive divisions, so the resulting blastomeres are half the original size. Since, the growth phase is absent, interphase is very short.
- ✓ The metabolic activity is very high including high consumption of oxygen and rapid DNA synthesis.

Cleavage continues till the reserves are exhausted and the average size of the daughter cells (blastomeres) reaches the characteristic size of the differentiated somatic cells of parent organisms. The pace of cleavage is determined by the cytoplasm rather than by the nucleus. The nuclear-cytoplasmic ratio is very high at the beginning of the cleavage; it gradually increases during cleavage and at the end it is brought to the same level as is found in the ordinary somatic cells. The rate of cleavage is rapid during early development and is synchronous, but it becomes very slow and asynchronous by the completion of blastula.

3.3.1. Types of Cleavage.

Yolk is a non-living component of the egg which do not participate in cleavage or formation of embryo. Its function is only to provide nourishment to the developing embryo. Yolk has a pronounced influence on the process of cleavage; retarding and interfering with it. The cleavage occurs more rapidly inactive yolk-free cytoplasm than in yolk-laden cytoplasm, because the yolk granules remain inert and passive during cleavage. Therefore, the blastomeres which are richer in yolk remain larger in size than those having less yolk. Depending on the amount and distribution of yolk, cleavage may be holoblastic and meroblastic.

- **Holoblastic.** In this type of cleavage, the cleavage pass through the entire egg, dividing it completely into equal or almost equal blastomeres. It occurs in alecithal, microlecithal and mesolecithal eggs. It is of two types: ***Holoblastic equal-*** It occurs in mesolecithal or telolecithal eggs, where the yolk is distributed along vegetal animal axis and the blastomeres are of unequal size e.g. Amphioxus, marsupials and placental mammals. ***Holoblastic unequal-*** It occurs in microlecithal or isolecithal eggs and the blastomeres are of equal size called micromeres (small) and macromeres (large) e.g. amphibians and lower fishes.

- **Meroblastic.** Such type of cleavage occurs in megalecithal or heavily telolecithal eggs, which have enormous amount of yolk. The active portion of the egg is confined to a small cytoplasmic region at the animal pole, called the **germinal disc** or **blastodisc**. The cleavage furrow do not completely pass through the egg and remains confined to the germinal disc as the yolk provides restriction to cleavage e.g. insects, reptiles.

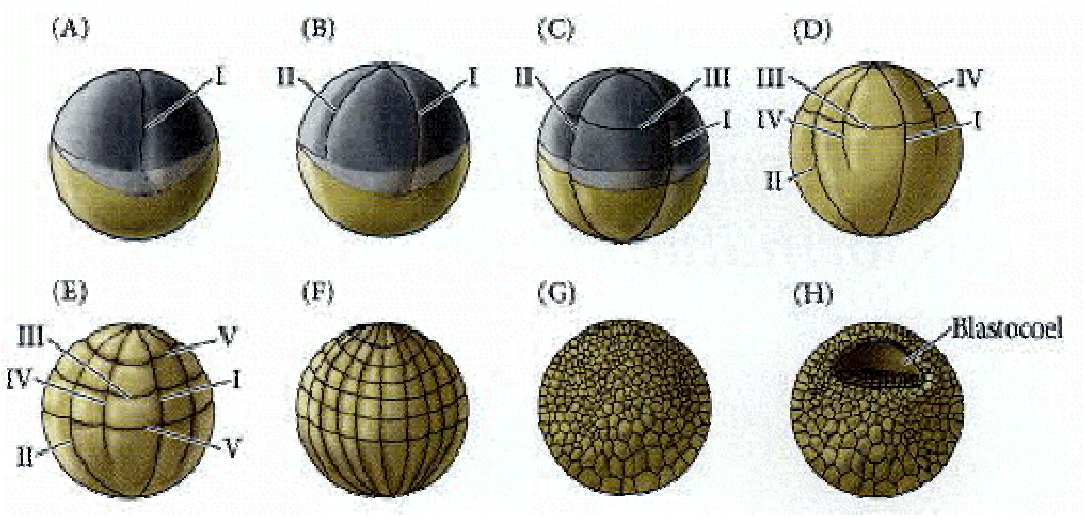


Fig. 13.2. (A) First vertical cleavage; (B) Second vertical cleavage; (C) Third latitudinal cleavage; (D) Fourth vertical cleavage; (E) Fifth equatorial cleavage; (F) Irregular

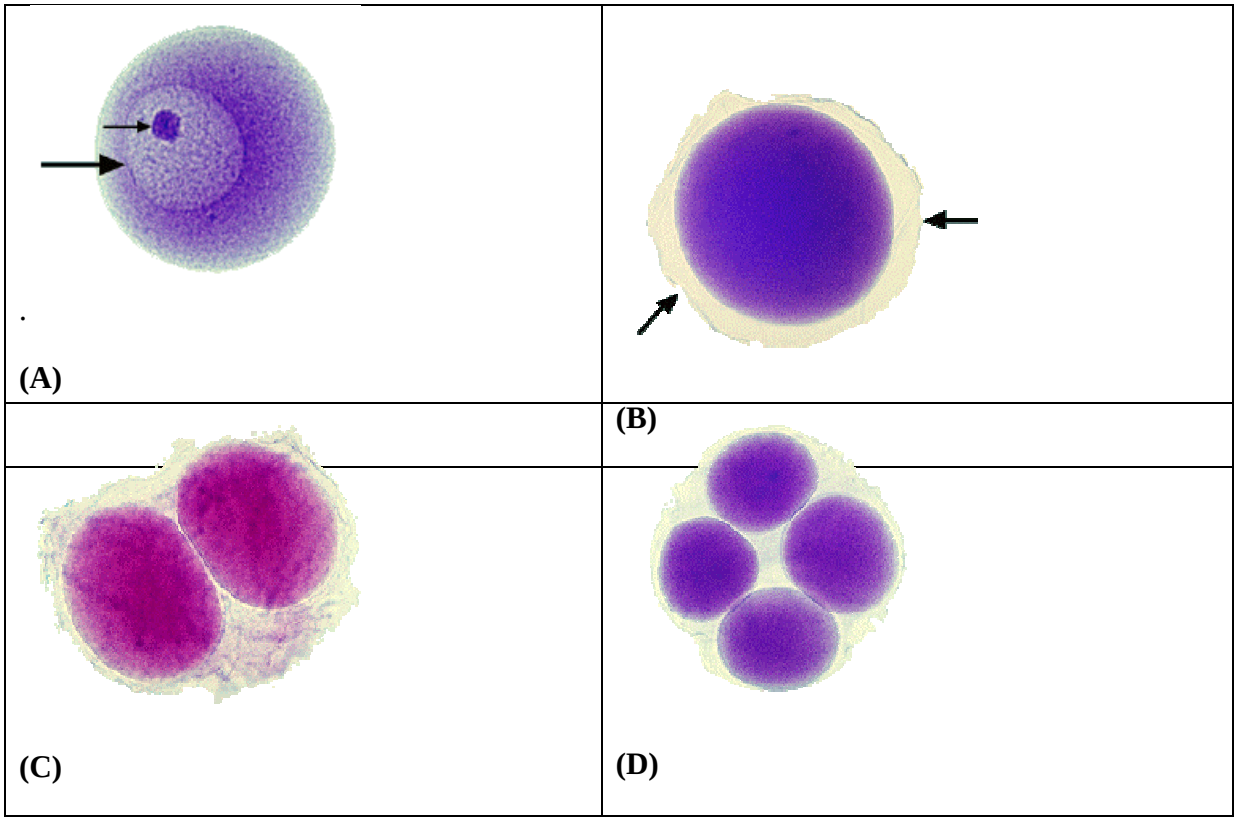
3.3.2 Planes of Cleavage.

- ***Meridional.*** The cleavage furrow passes through the polar axis of the egg and bisects both the animal and vegetal pole through the middle e.g. amphibians.
- ***Vertical.*** The cleavage furrow passes through the animal and vegetal pole but not through the median axis. It passes either through the left or right of the axis e.g. chick.
- ***Equatorial.*** The cleavage furrow is horizontal and is laid down in the equatorial plane at right angles to the main axis between the animal and vegetal poles e.g. mammals.
- ***Latitudinal.*** The cleavage furrow is laid down transversely or horizontally not on the equator but on either side of it e.g. Amphioxus.

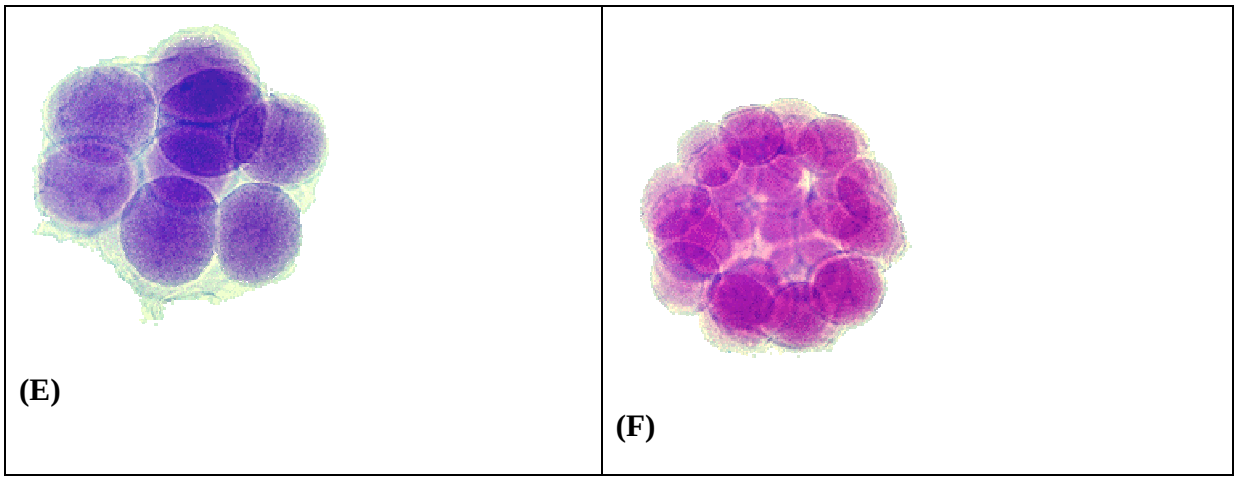
3.3.3.Patterns of Cleavage.

- ***Radial.*** Successive cleavage planes cut straight through the egg, at right angles to each other so that the resultant blastomeres are arranged radially e.g. *Synapta paracentrotus*.
- ***Biradial.*** The first three division planes do not cut straight the axis of the egg and are not laid down at right angle to each other e.g. Ctenophora.
- ***Spiral.*** It is a modified form of radial cleavage where there is rotational movement of cell parts around the egg axis leading to displacement of mitotic spindle, so that the four blastomeres of upper tier do not lie over the corresponding blastomeres of the lower tier but between them e.g. annelids, molluscs, nematodes, turbullerians.

- ***Bilateral.*** It is also a modified form of radial cleavage where the two of the four blastomeres are smaller than the other two and thus remain bilaterally arranged at four cell-stage e.g. tunicates and nematodes.



(A) **(B)** **(C)** **(D)**



(E) **(F)**

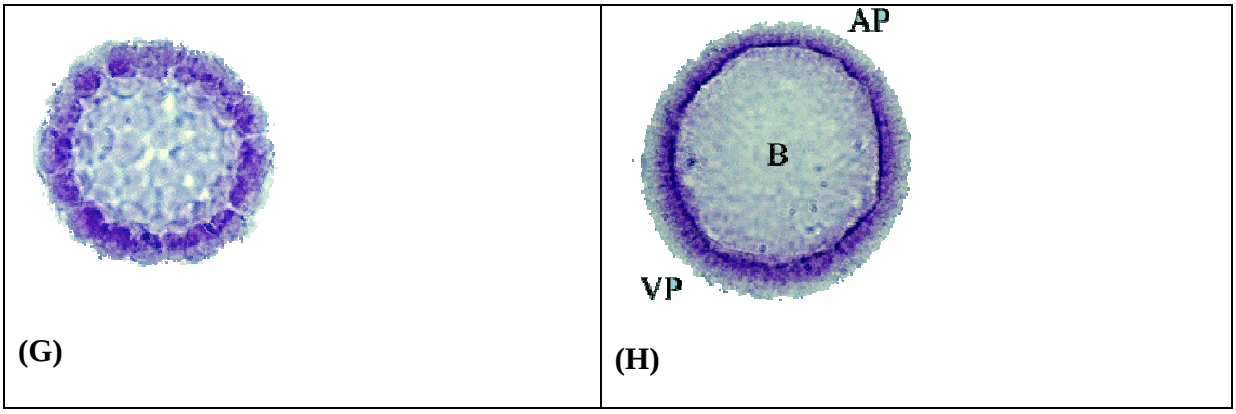


Fig. 13. (A) An unfertilized egg, differentiated from zygote by the presence of a large, conspicuous nucleus (large arrow) with obvious nucleolus (smaller arrow) and lack of a fertilization membrane.

(B) A zygote, recognized by the presence of the fertilization membrane (arrows) surrounding it and the peripheral, fluid-filled previtelline space.

(C) Cleavage showing 2-cell stage. The zygote has completed its first cleavage (equal and holoblastic). The division (cleavage) has passed through the animal-vegetal axis, producing two similar blastomeres.

(D) The 4-cell stage. The second cleavage also passes through the animal-vegetal axis, but perpendicular to the first cleavage. Four equal-sized blastomeres are formed at the end.

(E) The 8-cell stage. The third cleavage has occurred in the equatorial plane (perpendicular to the first two cleavages and the animal-vegetal axis). Note that the upper four blastomeres are slightly smaller than the lower four blastomeres. NOTE: The 16 cell stage is not shown - its division is once again vertical, along the animal-vegetal axis.

(F) The 32- cell stage. After the 16-cell stage, the cleavages become more difficult to follow, due to the increasing number of cells and to the division of blastomeres becoming asynchronous. Cleavage continues, forming a mass of cells which organizes itself into the blastula. The lighter area in the centre of the embryo is the beginning of the blastocoel.

(G) The early blastula. With continuing cleavage, the cells in centre begin to lose contact with one another, and a central fluid-filled cavity (the blastocoel) forms. This blastocoel is surrounded by a single layer of cells, forming the hollow sphere know as the blastula.

(H) The late blastula, characterized by a single layer of cells surrounding the blastocoel (B). The blastomeres are seen to be smaller and are individually not as obvious. The

blastomeres at the vegetal pole (VP) are taller than those at the animal pole (AP), making the vegetal pole appears slightly thicker.

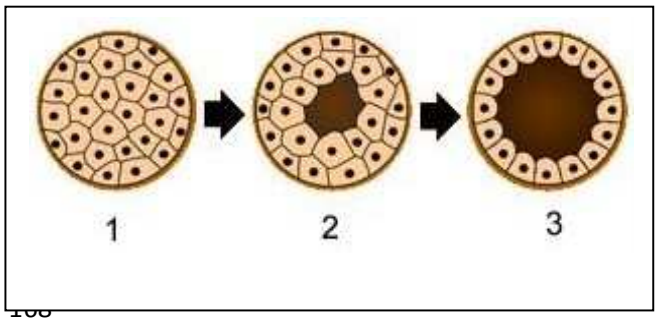
Cleavage in mammalian ovum takes place about 14 hours after fertilization, during its passage through the fallopian tube to the uterus. Cleavage results in smaller cells, the blastomeres. During early cleavage the blastomeres maintain spherical shape but during later stage those surfaces of blastomeres that remain in contact with one another, become flattened because of compression. However, the free outer and inner surfaces remain spherical. As a result, the external surface of the developing embryo assumes a characteristic mulberry-like appearance. The embryo at this stage is called as **morula** which takes approximately 3 days in humans. It reaches the uterus about 4-6 days after fertilization. A *true morula is absent in all vertebrates* because the blastocoel appears very soon in the early cleavage stages whereas a morula is regarded to be a solid ball of cells as found in most invertebrates.

According to the development abilities of the egg cytoplasm and fate of the blastomeres, cleavage is classified as determinate and indeterminate.

- ***Determinate.*** In this type of cleavage the blastomeres have a predetermined future i.e. the fate of blastomeres is fixed and determined to give rise to specific parts of the embryo e.g. annelida, mollusca.
- ***Indeterminate.*** In this type of cleavage, the fate of blastomeres is not predetermined having no characteristic position and alterable fate. For example, each of the two blastomeres of a zygote, if separated after the first cleavage, can produce one complete embryo e.g. echinoderms and chordates.

3.4 BLASTULATION

The formation of a segmentation cavity or **blastocoel** within a mass of cleaving blastomeres and rearrangement of blastomeres around this cavity in such a way as to form the type of definitive blastula is a characteristic of each species. The **blastocoel** originates as an intercellular space, which sometimes arises as early as the four or eight-cell stage. *Thus blastulation is initiated during early cleavage stages, and formation of the*



definitive blastula is thought to terminate cleavage and to initiate gastrulation.

The blastula is an early stage of embryonic development in animals. It is also called **blastosphere**. It is produced by cleavage of a fertilized ovum and consists of a spherical layer of around 128 cells surrounding a central fluid-filled cavity called the **blastocoel**. The blastula follows the morula and precedes the gastrula in the developmental sequence. The blastula is usually a hollow sphere. Its wall may vary from one to several cells in thickness. In eggs, which contain considerable amounts of yolk, the blastocoel may be eccentric in position, i.e. shifted

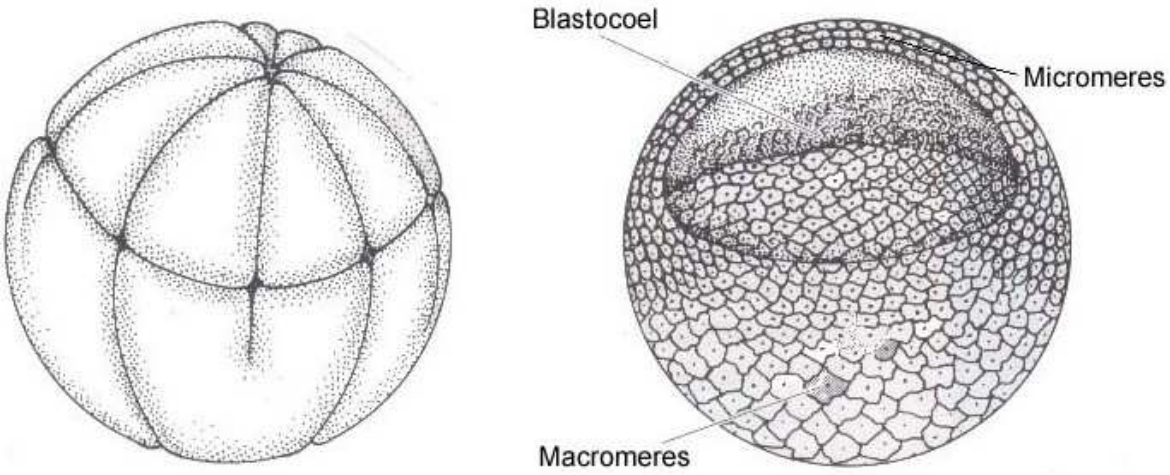
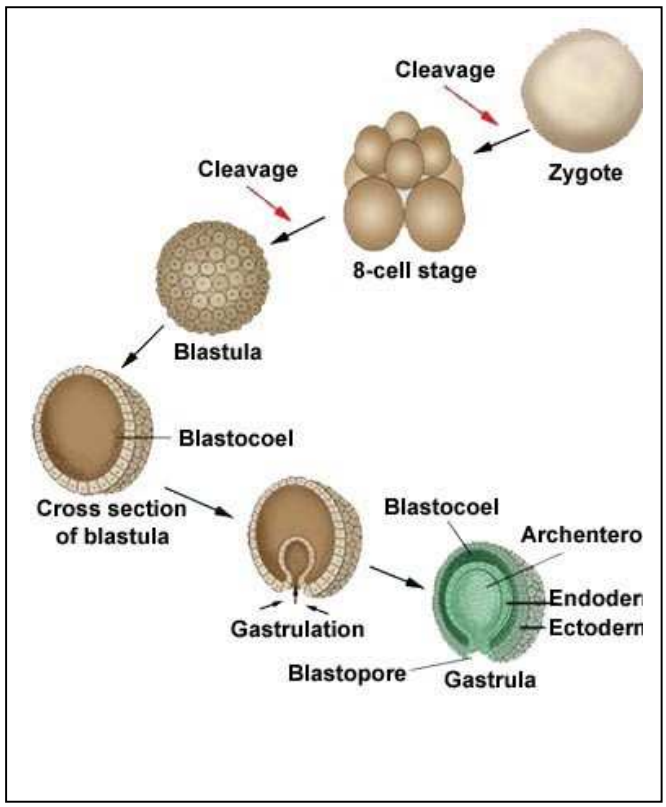


Fig. 13.5. (A) Blastula- exterior view. (B) Blastula-exterior view.

toward the animal pole. The animal portion of its wall is always completely divided into relatively small cells, whereas the vegetative portion tends to be composed of relatively large cells and may be incompletely cellulated in certain species. The blastocoel contains a gelatinous or jellylike fluid, which originates in part as a secretion by the blastomeres and in part by passage of water through the blastomeres or intercellular material, or both, into the blastocoel



In mammals, blastulation leads to the formation of the **blastocyst**, which must not be confused with the blastula; even though they are similar in structure, their cells have different fates. The blastocyst consists of three parts: the

inner cell mass, the **trophoblast** and the **blastocoel**. The inner cell mass is the inner cluster of blastomeres, which is the source of embryonic stem cells and gives rise to all later structures of the adult organism. The trophoblast combines with the maternal endometrium to form the placenta in eutherian mammals.

3.4 GASTRULATION

Prior to gastrulation, the embryo implants in the uterus. The blastocyst hatches from the zona pellucida at about the time it enters the uterus. The trophoblast cells attach to the **endometrial cells** of the uterus by adhering to extracellular matrix molecules such as fibronectin, collagen, hyaluronic acid, and heparan sulphate receptors. After contact, the cells directly in contact with the endometrium fuse to form **syncytiotrophoblast** while the remaining trophoblast cells are called **cytotrophoblast** cells. The syncytiotrophoblast cells produce enzymes that allow for the invasion of the embryo into the uterine wall and to induce the necessary changes in the uterine tissue. The inner cell mass, meanwhile, divides into two layers: the **epiblast** and **hypoblast**. The hypoblast spreads out and covers the blastocoel to form the **yolk sac**. The yolk sac is an extraembryonic tissue that produces blood cells similar to the structure that surrounds the yolk in birds. The epiblast further divides into two more layers. The **amnion** layer forms the fluid filled cavity to surround and protect the embryo during pregnancy. The **embryonic epiblast** undergoes gastrulation.

Gastrulation is the morphogenetic movements of the cells of the blastoderm, present on the surface of blastula to their specific positions where these occur in the adult. It is the differentiation of three primary germinal layers i.e. the **ectoderm**, **mesoderm** and **endoderm** formed by a single layer of cells, the **blastoderm**. The cells of inner cell mass nearest to the blastocoel split off to form the endoderm. This is known as **delamination**. The endoderm cells spread rapidly and line the blastocyst as a relatively large sac. Its cavity is known as **yolk-sac** or **gastrocoel**. Mesoderm is formed fro the cells moving sideways from the streak between ectoderm and endoderm. During gastrulation a cavity called the **archenterone** occurs inside the gastrula forming the future alimentary canal. All the morphogenetic movements in gastrulation are divided into following categories:

(a) Epiboly. It is the spreading of ectodermal cells (micromeres) over the inwardly moving endodermal and mesodermal cells (macromeres). The micromeres divide rapidly to form large number of cells which migrate downwards and spread over the macromeres.

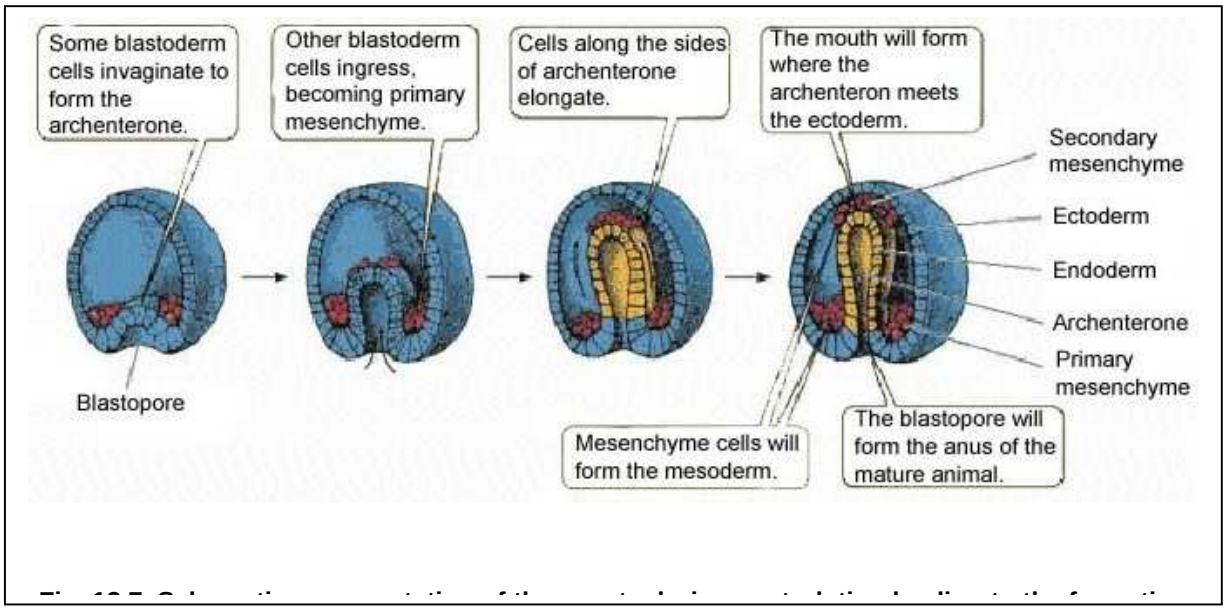
(b) Emboly. It involves the inward movement of mesodermal and endodermal cells, which are different in different animals, as follows-

(c) Invagination. It is the inward folding of endodermal cells to form the archenterone.

(d) Involution. It is the inward rotation of mesodermal and endodermal cells from the surface of the blastula to go into the blastocoel.

(e) Delamination. Cells split off from the pre-existing layer of mass into the blastocoel. These separated cells form the hypoblast and the blastopore is not formed.

Gastrulation is a very significant stage in the development bringing the presumptive organs rudiments from the external surface of the blastula to their prospective normal positions,



which will develop into the organs of the adults. Once the presumptive organ rudiments have reached their future position in the gastrula, these induce the development of different organ systems.

3.5 ORGANOGENESIS

Gastrulation results in the establishment of three primary germ layers namely, ectoderm, mesoderm and endoderm. All the organs of the embryo are formed from these layers. The development of morphologically recognizable tissues and organs of the new individual is called **organogenesis**.

	ECTODERM	MESODERM	ENDODERM
S	***Systems:*** Central Nervous	***Systems:*** Circulatory, Excretory, Respiratory,	***Linings of:*** respiratory system, urinary bladder,

U **M** **M** **A** **R** **Y**	System, Sense organs. ***Glands:*** Adrenal medulla, Pituitary, Pineal. ***Others:*** Epidermis, Receptors.	Reproductive. ***Glands:*** Adrenal cortex, Gonads. ***Others:*** Vertebral Column, muscles, Skeleton, Dermis, Mesenteries, Peritoneum, Connective tissue.	vagina, digestive epithelium, middle ear. ***Glands:*** Thyroid, Parathyroid, Pituitary, Thymus, digestive.
D **E** **T** **A** **I** **L** **S**	Epidermis, epidermal derivatives (hair, nails, etc.) internal ear, auditory vesicles, lens of eye, nervous system, adrenal medulla, intermediate and posterior lobe of pituitary, pineal gland, lining of foregut and hindgut.	Dermis of skin, vertebral column, skeletal muscles, kidneys, adrenal cortex, gonads, excretory and reproductive ducts, heart, blood and lymph vessels, spleen, gut wall, parietal and visceral peritoneum, mesenteries, body cavity.	Lining of mid gut, gastric glands (liver, pancreas), intestinal glands, anterior lobe of pituitary, thyroid gland, parathyroid gland, thymus gland, lining of vagina, urethra, reproductive glands, lining of lungs and middle ear.

Table. 13.1. Structures formed by the three germ layers.

4.0 CONCLUSION

In this unit you learnt about general embryology, development from gamete to embryo through the following process; gametogenesis, fertilization, cleavage formation,blastula, gastrulation, and organogenesis. The last stage is embryo

5.0 SUMMARY

Embryology is the formation embryo through different processes of development; for example, gametogenesis, fertilization, cleavage formation,blastula, gastrulation, and organogenesis. The last stage is embryo formation.

TUTOR-MARKED ASSIGNMENT

1 Explain the formation of embryo through the developmental processes

2 Decribe and explain the pattern of cleavage from A-H

REFERENCES

Professor Scott Gilbert, Developmental Biology, 6th Edition.

Unit 2: Embryonic membrane and Placenta

CONTENT

1.0 INTRODUCTION

Embryogenesis is the process by which the embryo is formed and develops, until it develops into a fetus. It starts with the fertilization of the ovum (or egg) by sperm. The fertilized ovum is referred to as a zygote. The zygote undergoes rapid mitotic divisions with no significant growth

(a process known as cleavage) and cellular differentiation, leading to development of an embryo. Although embryogenesis occurs in both animal and plant development, this article addresses the common features among different animals, with some emphasis on the embryonic development of vertebrates and mammals.

2.0 OBJECTIVES

At the end of this unit the student should be able to:

1Explain and describe the structure of embryonic membrane.

2 Explain the structure and function of placenta

3 Which method and treatment is suggest for infertility in male and female

4 How can you control birth?

3.0 MAIN CONTENT

3.1 EXTRAEMBRYONIC MEMBRANES

The extraembryonic membranes or foetal membranes are formed of embryonic tissue, the **trophoblast** that lies outside the embryo. The embryos of amniotes, i.e. reptiles, birds and mammals produce four extraembryonic membranes, the **amnion**, **yolk sac**, **chorion** and

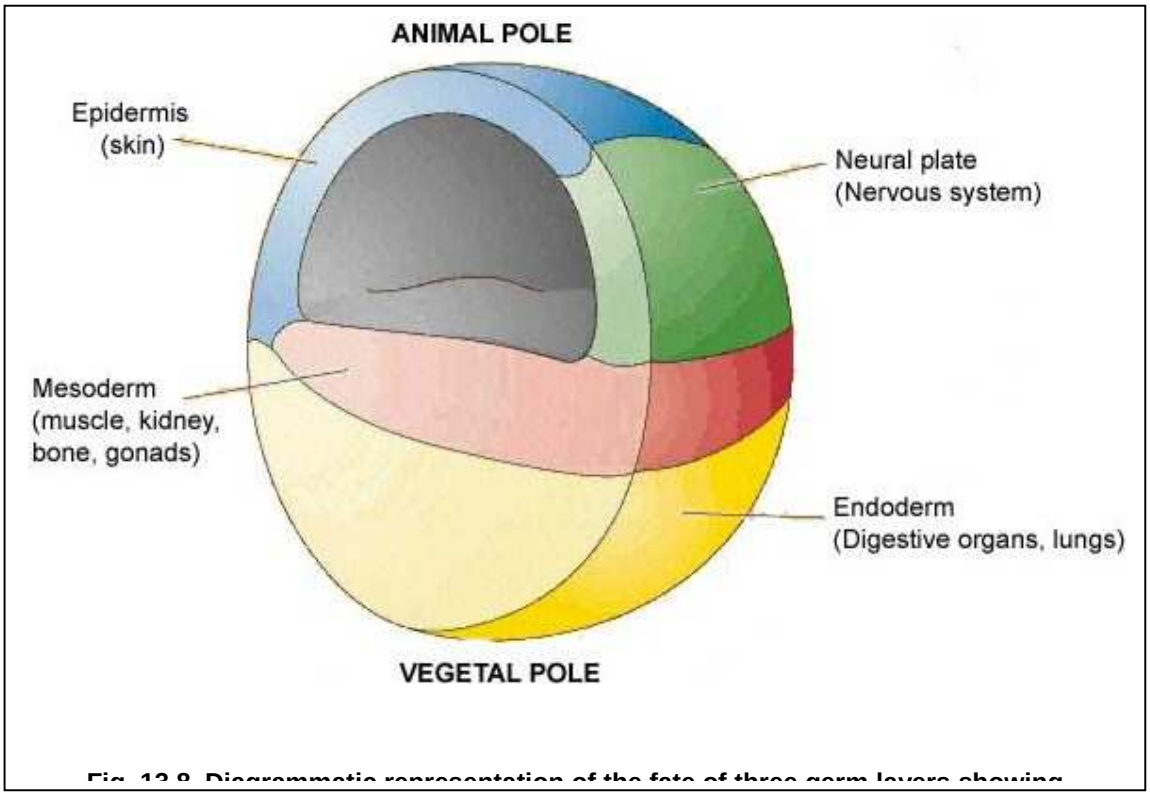
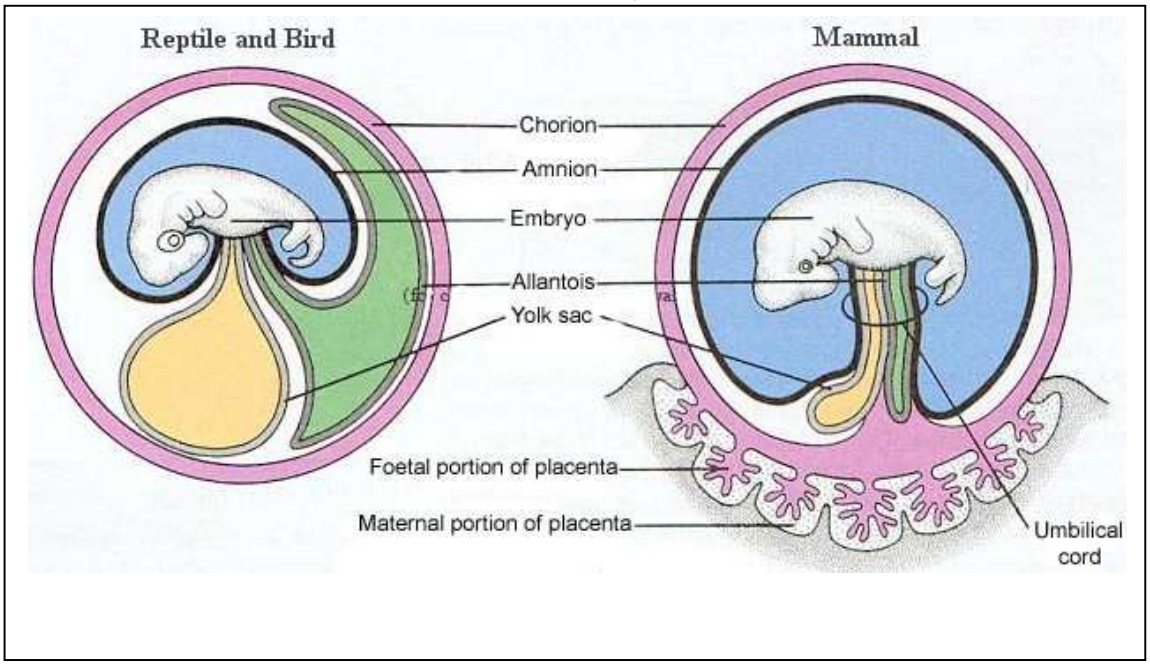


Fig. 13.8. Diagrammatic representation of the fate of three germ layers showing

allantois. In birds and most reptiles, the embryo with its extraembryonic membranes develops within a shelled egg.

1. **Amnion**. It is formed of mesoderm on outside and ectoderm inside and is devoid of blood vessels. The space between amnion and foetus is called the amniotic cavity filled with amniotic fluid. The embryo is fully immersed and bathed in the amniotic fluid which serves as an efficient shock absorber. Being viscous and gelatinous, it protects the delicate embryo from external as well as internal mechanical pressures.
2. **Chorion**. It is formed of ectoderm externally and mesoderm inside. It forms the placenta along with the allantois. It participates in the exchange of gases between the embryo and the outside air and also provides nourishment to the developing embryo.
3. **Allantois**. It is formed of mesoderm externally and endoderm inside. It is the precursor of the mature umbilical cord. It is primarily found in the blastocyst stage of early embryological development, and its purpose is to collect liquid waste from the embryo. This sac-like structure is primarily involved in respiration and excretion, and is webbed with blood vessels.
4. **Yolk sac.** The yolk sac is situated on the ventral aspect of the embryo; it is lined by endoderm, outside of which is a layer of mesoderm. In mammals yolk sac begins to form during early gastrulation. As compared to the yolk sac of birds, the mammalian yolk sac is not filled with yolk as a nutritive material because in mammals the function of nutrition is performed by the placenta. It is filled with fluid, the **vitelline fluid,** which possibly may be utilized for the nourishment of the embryo during the earlier stages of its existence. Blood is conveyed to the wall of the sac by the primitive aorta, and after circulating through a wide-meshed capillary plexus, is returned by the vitelline veins to the tubular heart of the embryo. This constitutes the **vitelline circulation,** and by means of it nutritive material is absorbed from the yolk sac and conveyed to the embryo. In mammals the yolk sac functions as the site of blood cell formation until about the sixth week, when the liver takes over this role. There

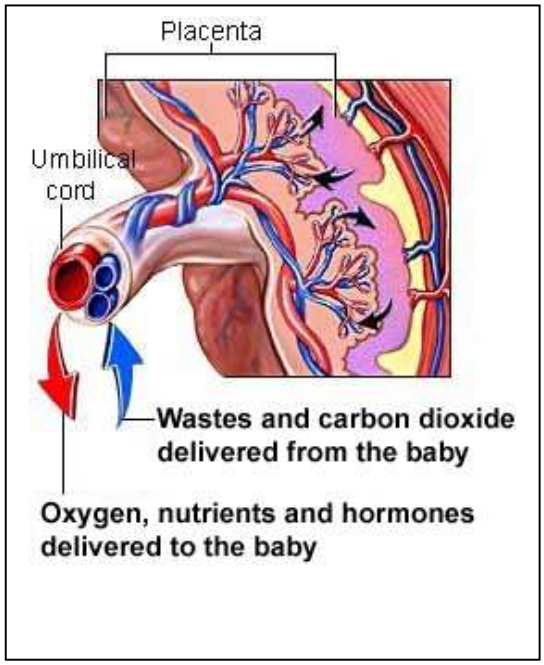


after the yolk sac starts to shrink.

With these four membranes, the developing embryo is able to carry on essential metabolism while sealed within the egg. Surrounded by amniotic fluid, the embryo is kept as moist as a fish embryo in a pond. Although most mammals do not make a shelled egg, they do also enclose their embryo in an amnion. For this reason, the reptiles, birds, and mammals are collectively referred to as the **amniota**. In placental mammals, the extraembryonic membranes form a **placenta** and **umbilical cord**, which connect the embryo to the mother's uterus in a more elaborate and efficient way. The blood supply of the developing fetus is continuous with that of the placenta. The placenta extracts food and oxygen from the uterus. Carbon dioxide and other wastes (e.g., urea) are transferred to the mother for disposal by her excretory organs.

3.2 PLACENTA

In viviparous animals, the egg has no reserve food material and the developing embryo depends completely on the mother for its nourishment. So the embryo gets attached to the uterine wall and obtains nourishment from her until birth. This organic connection between the foetus and uterine wall is called as **placenta** which develops at the point of implantation. It is formed of both foetal and maternal tissues; the allantois gives rise to umbilical cord which contains blood vessels connecting foetus and placenta. Since, the placenta is formed only after implantation, i.e. after 8-10 days, so before implantation, the embryo (blastocyst) is nourished by secretion of uterine glands called the **uterine milk**.



The most primitive type of true placenta is formed of following layers of foetal and maternal tissues:

Foetal layers: 1. Foetal blood capillaries; 2. Foetal connective tissue; 3. Foetal chorionic epithelium.

Maternal layers: 1. Uterine epithelium; 2. Uterine connective tissue; 3. Maternal blood capillaries.

3.2.1 Types of Placenta.

I. Based on Histology.

1. ***Epitheliochorial.*** It is the most primitive type of placenta with all the six barriers between foetal and maternal blood. E.g. marsupials, ungulates (pig, horse, etc.).
2. ***Syndesmochorial.*** The chorionic villi erode the uterine wall and so the uterine epithelium is ruptured, with only five barriers left. E.g. sheep, cow (i.e. ruminant ungulates).
3. ***Endotheliochorial.*** The chorionic villi erode not only the uterine epithelium but also the uterine connective tissue, with only four barriers left. E.g. lion, tiger, dog, cat and other carnivores.
4. ***Haemochorial.*** Here all the three layers of maternal part is eroded, so the placenta is left with three barriers only. E.g. humans, apes.
5. ***Haemoendothelial.*** All the barriers except the foetal capillaries are eroded. E.g. rat, rabbit.

II. Based on Degree of Association.

1. ***Non-deciduate.*** The implantation of the embryo in the uterus is superficial. At the time of birth, no part of uterine portion of placenta is broken off and no bleeding occurs. E.g. pigs, cattle, horse and other ruminants.
2. ***Deciduate.*** In this case the degree of contact between foetal and maternal tissue is more intimate. At the time of birth the foetal part of placenta separates from the uterine part of placenta as a result of which a portion of uterine tissue called **decidua** is detached and passes out at birth. Therefore it causes tearing of tissues from the uterine wall and extensive haemorrhage. E.g. man, rabbit, dog, cat and most of the mammals.
3. ***Contradeciduate.*** The degree of contact between foetal and maternal tissue is same as in deciduate type but at the time of birth the maternal and even the foetal part of the placenta is retained to provide nourishment. E.g. *Talpa* (mole), *Parameles*.

III. Based on Distribution of Villi.

1. ***Diffused.*** Villi distributed evenly all over the surface of the chorion. E.g. ungulates (horse, pig).
2. ***Cotyledonary.*** Villi distributed in the form of isolated patches. E.g. ruminants (sheep, cow).
3. ***Zonary.*** Villi arranged in definite transverse bands or girdles. E.g. carnivores (cat, dog, lion).

4. ***Discoidal.*** Villi confined to one (monodiscoidal) or two (bidiscoidal) disc-like areas. E.g. rat,

rabbit, monkey, apes, man.

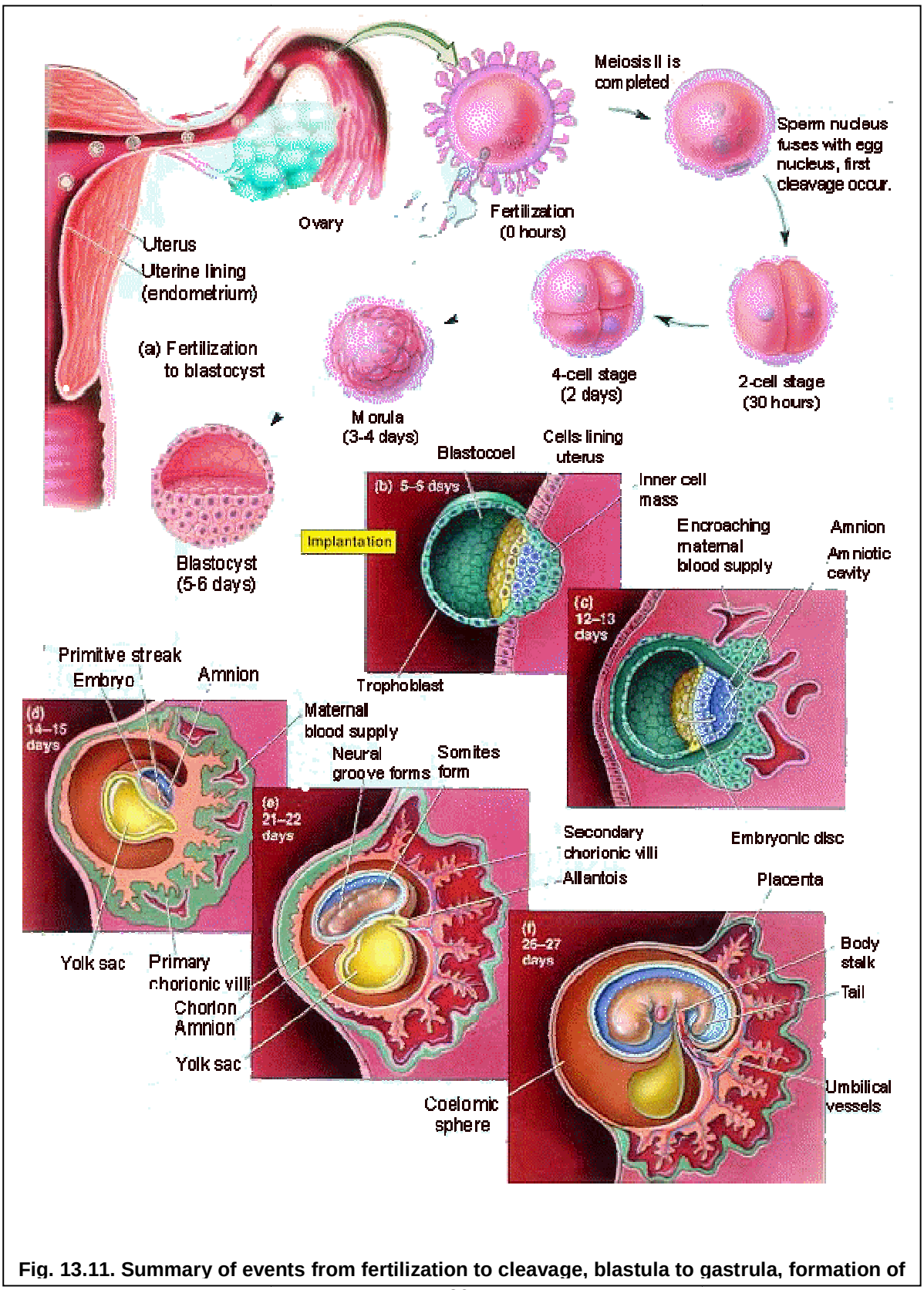


Fig. 13.11. Summary of events from fertilization to cleavage, blastula to gastrula, formation of

3.2.2.Functions of Placenta.

1. **Filtration and transfer.** The placenta receives nutrients, oxygen, antibodies and hormones from the mother's blood and passes out waste. It forms a barrier, the placental barrier, which filters out some substances which could harm the fetus. Many substances are not filtered out, however, including alcohol and some chemicals associated with smoking cigarettes. Several types of viruses, such as Human Cytomegalovirus, may also cross this barrier; this often leads to various degrees of birth defects in the infant.
2. **Metabolic and endocrine activity.** In addition to the transfer of gases and nutrients, the placenta also has metabolic and endocrine activity. It produces, amongst other hormones, progesterone, which is important in maintaining the pregnancy; somatomammotropin (also known as placental lactogen),

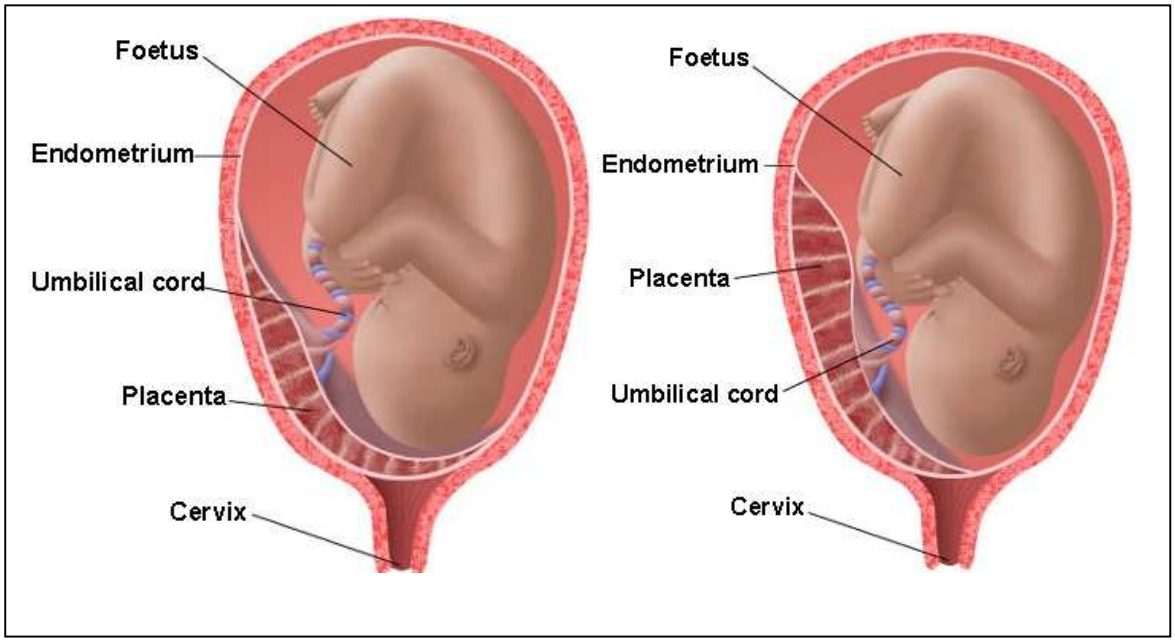
which acts to increase the amount of glucose and lipids in the maternal blood; oestrogen; relaxin, and human chorionic gonadotrophin HCG. This results in increased transfer of these nutrients to the fetus and is also the main cause of the increased blood sugar levels seen in pregnancy.

3. **After delivery.** When the fetus is delivered, the placenta is delivered afterwards (and for this reason is often called the **afterbirth**). After delivery of the placenta the umbilical cord is usually clamped and severed or may be left attached to fall off naturally which is referred to as a Lotus Birth. In most mammalian species, the mother bites through the cord and consumes the placenta, primarily for the benefit of prostaglandin on the uterus after birth. This is known as **placentophagy**. The site of the former umbilical cord attachment in the center of the front of the abdomen is known as the umbilicus, navel, or belly-button.

3.3.PLACENTA PREVIA

Bleeding may occur at various times in pregnancy. Although it is alarming, it may or may not be a serious complication. The time of bleeding in the pregnancy, the amount and whether or not there is pain may vary depending on the cause. Bleeding in the first trimester of pregnancy is quite common and may be due to implantation of the placenta in the uterus, miscarriage (pregnancy loss), ectopic pregnancy (pregnancy in the fallopian tube), gestational trophoblastic disease (a rare condition that may be cancerous in which a grape-like mass of fetal and placental tissues develops) or infection. Bleeding in late pregnancy (after about 20 weeks) may be due to **placenta previa** (placenta is near or covers the cervical opening) or **placental abruption** (placenta detaches prematurely from the uterus).

Placenta previa is a condition in which the placenta is attached close to or covering the cervix. Placenta previa occurs in about one in every 200 live births. There are three types of placenta previa:



1. **Total placenta previa** - the placenta completely covers the cervix.
2. **Partial placenta previa** - the placenta is partially over the cervix.
3. **Marginal placenta previa** - the placenta is near the edge of the cervix.

The cause of placenta previa is unknown, but it is associated with certain conditions including the following:

- Women who have scarring of the uterine wall from previous pregnancies.
- Women who have fibroids or other abnormalities of the uterus.
- Women who have had previous uterine surgeries or cesarean deliveries.
- Older mothers (over age 35).
- Cigarette smoking.
- Placenta previa in a previous pregnancy.

The greatest risk of placenta previa is bleeding (or hemorrhage). Bleeding often occurs as the lower part of the uterus thins during the third trimester of pregnancy in preparation for labour. This causes the area of the placenta over the cervix to bleed. The more of the placenta that covers the cervical, the greater the risk for bleeding. Other risks include the following:

- Abnormal implantation of the placenta.
- Slowed fetal growth.
- Preterm birth.
- Birth defects.
- Infection after delivery.

The most common symptom of placenta previa is **vaginal bleeding** that is bright red and not associated with abdominal tenderness or pain, especially in the third trimester of pregnancy. However, each woman may exhibit different symptoms of the condition or symptoms may resemble other conditions or medical problems.

Treatment.

Specific treatment for placenta previa will be determined by a physician based on (a) pregnancy, overall health, and medical history; (b) extent of the condition; (c) ttolerance for specific medications, procedures, or therapies. There is no treatment to change the position of the placenta. Once placenta previa is diagnosed, additional ultrasound examinations are often performed to track its location. It may be necessary to deliver the baby, depending on the amount of bleeding, the gestational age, and condition of the foetus. Cesarean delivery is necessary for most cases of placenta previa. Severe blood loss may require a blood transfusion.

3.4 PLACENTAL ABRUPTION

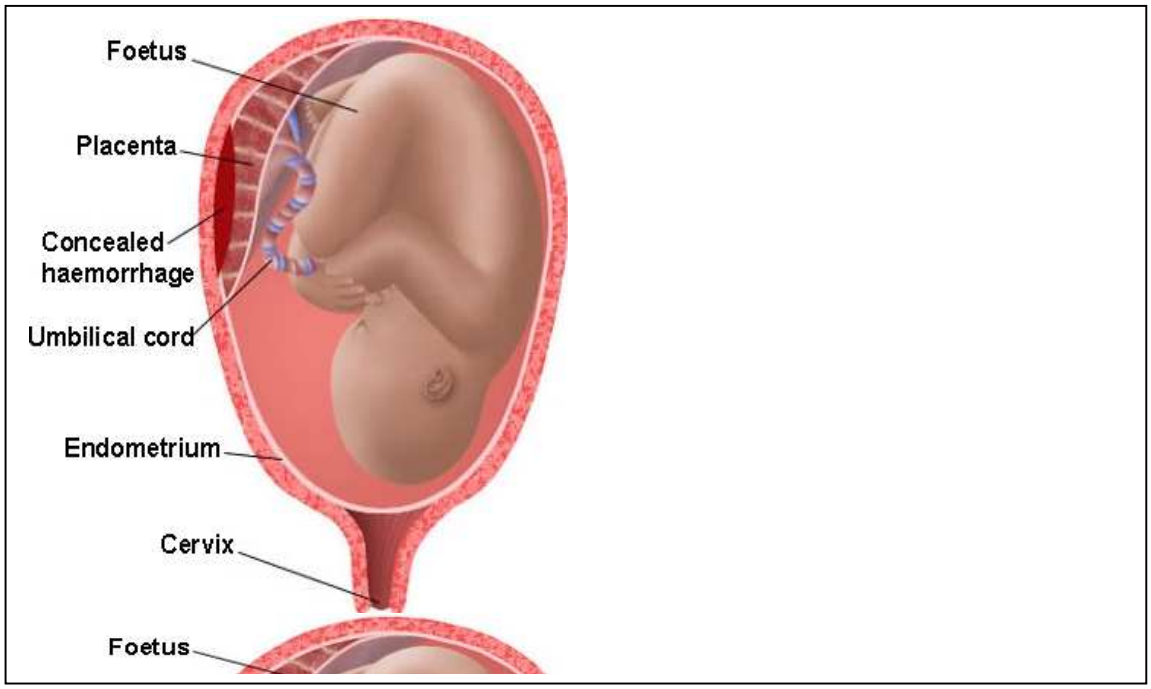
Placental abruption is the premature separation of a placenta from its implantation in the uterus. Within the placenta are many blood vessels that allow the transfer of nutrients to the foetus from the mother. If the placenta begins to detach during pregnancy, there is bleeding from these vessels. The larger the area that detaches, the greater the amount of bleeding. Placental abruption occurs about once in every 120 births. It is also called **abruptio placenta**.

Other than direct trauma to the uterus such as in a motor vehicle accident, the cause of placental abruption is unknown. It is, however, associated with certain conditions, including the following:

- Previous pregnancy with placental abruption.
- Hypertension (high blood pressure).
- Cigarette smoking.
- Multiple pregnancies.

Placental abruption is dangerous because of the risk of uncontrolled bleeding (haemorrhage). Although severe placental abruption is rare, other complications may include hemorrhage and shock, disseminated vascular coagulation (DIC) - a serious blood clotting complication, poor blood flow and damage to maternal and/or foetal kidneys or brain, stillbirth, postpartum (after delivery) haemorrhage.

The most common symptom of placental abruption is dark red **vaginal bleeding** with pain during the third trimester of pregnancy. It also can occur during labour. However, each woman may experience symptoms differently. Symptoms may include vaginal bleeding,



abdominal pain, uterine contractions that do not relax, blood in amniotic fluid, nausea, thirst, faint feeling and decreased foetal movements.

The diagnosis of placental abruption is usually made by the symptoms and the amount of bleeding and pain. Ultrasound may also be used to show the location of the bleeding and to check the foetus. There are three grades of placental abruption, including the following:

Grade 1 - small amount of vaginal bleeding and some uterine contractions, no signs of foetal distress or low blood pressure in the mother.

Grade 2 - mild to moderate amount of bleeding, uterine contractions, the foetal heart rate may shows signs of distress.

Grade 3 - moderate to severe bleeding or concealed (hidden) bleeding, uterine contractions that do not relax (called tetany), abdominal pain, low blood pressure, foetal death.

Sometimes placental abruption is not diagnosed until after delivery, when an area of clotted blood is found behind the placenta.

Treatment.

Specific treatment for placental abruption will be determined by a physician based on (a) pregnancy, overall health, and medical history; (b) extent of the condition; (c) ttolerance for specific medications, procedures, or therapies. There is no treatment to stop placental abruption or reattach the placenta. Once placental abruption is diagnosed, a woman's care depends on the amount of bleeding, the gestational age and condition of the foetus. Vaginal delivery is still possible when placental abruption occurs, however, emergent caesarean delivery may be necessary if severe hemorrhage or foetal heart abnormalities occur. Severe blood loss may require a blood transfusion.

4.0 BIRTH CONTROL METHODS

All women and men should have control over if and when they become parents. Making decisions about birth control, or contraception is not easy– there are many things to think about. Learning about birth control methods can use to prevent pregnancy and talking with a doctor are two good ways to get started. There is no "best" method of birth control. Each method has its own pros and cons. Some methods work better than others do at preventing pregnancy. Researchers are always working to develop or improve birth control methods.

It must be noted that no method of birth control prevents pregnancy all of the time. Birth control methods can fail, but a couple can greatly increase a method's success rate by using it correctly all of the time. The only way to be sure women never get pregnant is to not have sex (**abstinence**). There are many methods of birth control that a woman can use. Most birth control does NOT protect from HIV or other sexually transmitted diseases (STDs) like *gonorrhea*, *herpes*, and *chlamydia*. Other than not having sex, the best protection against STDs and HIV is the male latex condom. The female condom may give some STD protection. Here is a list of birth control methods with estimates of effectiveness, or how well they work in preventing pregnancy when used correctly, for each method:

1. **Continuous Abstinence** – This means not having sexual intercourse at any time. It is the only sure way to prevent pregnancy and protect against HIV and other STDs. This method is 100% effective at preventing pregnancy and STDs.

2. **Periodic Abstinence or Fertility Awareness Methods** – A woman who has a regular menstrual cycle has about seven or more fertile days or days when she is able to get pregnant, each month. Periodic abstinence means that she do not have sex on the days that she may be fertile. These fertile days are approximately 5 days before ovulation, the day of ovulation, and one or more days after ovulation. Fertility awareness means that she can be

abstinent or have sex but use a “barrier” method of birth control to keep sperm from getting to the egg. Barrier methods include condoms, diaphragms, or cervical caps, used together with spermicides, which kill sperm. These methods are 75 to 99% effective at preventing pregnancy.

3. **The Male Condom** – Condoms are called barrier methods of birth control because they put up a block, or barrier, which keeps the sperm from reaching the egg. Only latex or polyurethane (because some people are allergic to latex) condoms are proven to help protect against STDs, including HIV. "Natural” or “lambskin” condoms made from animal products also are available, but lambskin condoms are not recommended for STD prevention because they have tiny pores that may allow for the passage of viruses like HIV, hepatitis B and herpes. Male condoms are 84 to 98% effective at preventing pregnancy.

4. **Oral Contraceptives** – Also called “the pill,” contains the hormones estrogen and progesterone and is available in different hormone dosages. A pill is taken daily to block the release of eggs from the ovaries. Oral contraceptives lighten the flow of period and can reduce the risk of pelvic inflammatory disease (PID), ovarian cancer, benign ovarian cysts, endometrial cancer, and iron deficiency anemia. It does not protect against STDs or HIV. The pill may add to risk of heart disease, including high blood pressure, blood clots, and blockage of the arteries, especially if a woman smoke. Women over the age of 35 smoke, or have a history of blood clots or breast, liver, or endometrial cancer, they are advised not to take the pill. The pill is 95 to 99.9% effective at preventing pregnancy. Some antibiotics may reduce the effectiveness of the pill in some women.

5. **The Mini-Pill** – Unlike the pill, the mini-pill only has one hormone, progesterone, instead of both estrogen and progesterone. Taken daily, the mini-pill thickens cervical mucus to prevent sperm from reaching the egg. It also prevents a fertilized egg from implanting in the uterus (womb). The mini-pill also can decrease the flow of period and protect against PID and ovarian and endometrial cancer. Mothers who breastfeed can use it because it will not affect their milk supply. The mini-pill is a good option for women who can’t take estrogen, are over 35, or have a risk of blood clots. The mini-pill does not protect against STDs or HIV. Mini-pills are 92 to 99.9% effective at preventing pregnancy if used correctly.

6. **Copper T IUD (Intrauterine Device)** – An IUD is a small device that is shaped in the form of a “T.” An expert gynaecologist places it inside the uterus. The arms of the Copper T IUD contain some copper, which stops fertilization by preventing sperm from making their way up through the uterus into the fallopian tubes. If fertilization does occur, the IUD would prevent the fertilized egg from implanting in the lining of the uterus. The Copper T IUD can stay in uterus for up to 12 years. It does not protect against STDs or HIV. This IUD is 99% effective at preventing pregnancy.

7. **Progestasert IUD (Intrauterine Device)** –This IUD is a small plastic T- shaped device that is placed inside the uterus by a doctor. It contains the hormone progesterone, the same hormone produced by a woman's ovaries during the monthly menstrual cycle. The progesterone causes the cervical mucus to thicken so sperm cannot reach the egg, and it changes the lining of the uterus so that a fertilized egg cannot successfully implant. The Progestasert IUD can stay in uterus for one year. This IUD is 98% effective at preventing pregnancy.

8. **Intrauterine System or IUS (Mirena)** – The IUS is a small T-shaped device like the IUD and is placed inside the uterus by a doctor. Each day, it releases a small amount of a hormone similar to progesterone called levonorgestrel that causes the cervical mucus to thicken so sperm cannot reach the egg. The IUS stays in uterus for up to 5 years. It does not protect against STDs or HIV. The IUS is 99% effective. The Food and Drug Administration approved this method in December 2000.

9. **The Female Condom** – Worn by the woman, this barrier method keeps sperm from getting into her body. It is made of polyurethane, is packaged with a lubricant, and may protect against STDs, including HIV. It can be inserted up to 24 hours prior to sexual intercourse. Female condoms are 79 to 95% effective at preventing pregnancy. There is only one kind of female condom, called **Reality**.

10. **Depo-Provera** – With this method women get injections of the hormone progesterone in the buttocks or arm every 3 months. It does not protect against STDs or HIV. Women should not use Depo-Provera for more than 2 years in a row because it can cause a temporary loss of bone density that increases the longer this method is used. The bone does start to grow after this method is stopped, but it may increase the risk of fracture and osteoporosis if used for a long time. It is 97% effective at preventing pregnancy.

11. **Diaphragm, Cervical Cap or Shield** – These are barrier methods of birth control, where the sperm are blocked from entering the cervix and reaching the egg. The diaphragm is shaped like a shallow latex cup. The cervical cap is a thimble-shaped latex cup. The cervical shield is a silicone cup that has a one-way valve that creates suction and helps it fit against the cervix. Before sexual intercourse, they are layered with spermicide (to block or kill sperm) and placed up inside the vagina to cover the cervix (the opening of womb). The diaphragm is 84 to 94% effective at preventing pregnancy. The cervical cap is 84 to 91% effective at preventing pregnancy for women who have not had a child and 68 to 74% for women who have had a child. The cervical shield is 85% effective at preventing pregnancy. Barrier methods must be left in place for 6 to 8 hours after intercourse to prevent pregnancy and removed by 24 hours for the diaphragm and 48 for cap and shield.

12. **Contraceptive Sponge** - This is a barrier method of birth control that was re-approved by the Food and Drug Administration in 2005. It is a soft, disk shaped device, with a loop for

removal. It is made out of polyurethane foam and contains the spermicide nonoxynol-9. Before intercourse, the sponge is made wet and place it, loop side down, up inside the vagina to cover the cervix. The sponge is 84 to 91% effective at preventing pregnancy in women who have not had a child and 68 to 80% for women who have had a child. The sponge is effective for more than one act of intercourse for up 24 hours. It needs to be left in for at least six hours after intercourse to prevent pregnancy and must be removed within 30 hours after it is inserted. There is a risk of getting Toxic Shock syndrome or TSS if the sponge is left in for more than 30 hours. The sponge does not protect against STDs or HIV.

13. **The Patch (Ortho Evra)** –This is a skin patch worn on the lower abdomen, buttocks, or upper body. It releases the hormones progesterone and estrogen into the bloodstream. One may put on a new patch once a week for three weeks, and then do not wear a patch during the fourth week in order to have a menstrual period. The patch is 98 to 99% effective at preventing pregnancy, but appears to be less effective in women who weigh more than 198 pounds. It does not protect against STDs or HIV.

14. **The Hormonal Vaginal Contraceptive Ring (NuvaRing)** – The NuvaRing is a ring that releases the hormones progesterone and estrogen. It is squeezed between the thumb and index finger and inserted into the vagina. One may wear the ring for three weeks, take it out for the week to have period, and then put in a new ring. The ring is 98 to 99% effective at preventing pregnancy. This birth control method is not recommended while breastfeeding because the hormone estrogen may decrease breast milk production.

15. **Surgical Sterilization (Tubal Ligation or Vasectomy)** – These surgical methods are meant for people who want a permanent method of birth control. In other words, they never want to have a child or they do not want more children. Tubal ligation or “tying tubes” is done on the woman to stop eggs from going down to her uterus where they can be fertilized. The man has a vasectomy to keep sperm from going to his penis, so his ejaculate never has any sperm in it. They are 99.9% effective at preventing pregnancy.

16. **Nonsurgical Sterilization (Essure Permanent Birth Control System)** – This is the first non-surgical method of sterilizing women. A thin tube is used to thread a tiny spring-like device through the vagina and uterus into each fallopian tube. Flexible coils temporarily anchor it inside the fallopian tube. A Dacron-like mesh material embedded in the coils irritates the lining of the fallopian tubes to cause scar tissue to grow and eventually permanently plug the tubes. It can take about three months for the scar tissue to grow, so it is important to use another form of birth control during this time. Essure has been shown to be 99.8 % effective in preventing pregnancy.

17. **Emergency Contraception** – This is not a regular method of birth control and should never be used as one. Emergency contraception, or emergency birth control, is used to keep a woman from getting pregnant when she has had unprotected vaginal intercourse. Emergency

contraception consists of taking two doses of hormonal pills taken 12 hours apart and started within three days after having unprotected sex. These are sometimes wrongly called the “morning after pill.” The pills are 75 to 89% effective at preventing pregnancy. Another type of emergency contraception is having the Copper T IUD put into uterus within seven days of unprotected sex. This method is 99.9% effective at preventing pregnancy. Neither method of emergency contraception protects against STDs or HIV.

4.1 INFERTILITY

Most experts define infertility as not being able to get pregnant after at least one year of trying. Women who are able to get pregnant but then have repeat miscarriages are also said to be infertile. Infertility is not always a woman's problem. In only about one-third of cases is infertility due to woman (female factors). In another one third of cases, infertility is due to man (male factors). The remaining cases are caused by a mixture of male and female factors or by unknown factors. Pregnancy is the result of a complex chain of events. In order to get pregnant:

- A woman must release an egg from one of her ovaries (ovulation).
- The egg must go through a fallopian tube toward the uterus (womb).
- A man’s sperm must join with (fertilize) the egg along the way.
- The fertilized egg must attach to the inside of the uterus (implantation).

Infertility can result from problems that interfere with any of these steps.

Male Infertility.

Infertility in men is most often caused by:

1. Problems making sperm; producing too few sperm or none at all
2. Problems with the sperm's ability to reach the egg and fertilize it; abnormal sperm shape or structure prevent it from moving correctly
3. Sometimes a man is born with the problems that affect his sperm. Other times problems start later in life due to illness or injury. For example, cystic fibrosis often causes infertility in men.

Female Infertility.

Infertility in men is most often caused by:

1. Problems with ovulation account for most cases of infertility in women. Without ovulation, there are no eggs to be fertilized. Some signs that a woman is not ovulating normally include irregular or absent menstrual periods.
2. Less common causes of fertility problems in women include:

3. Blocked fallopian tubes due to pelvic inflammatory disease, endometriosis, or surgery for an ectopic pregnancy
4. Physical problems with the uterus
5. Uterine fibroids
6. Affect of female's age.

More and more women are waiting until their 30s and 40s to have children. Actually, about 20 percent of women now have their first child after age 35. So age is an increasingly common cause of fertility problems. About one third of couples in which the woman is over 35 have fertility problems. Aging decreases a woman's chances of having a baby in the following ways:

(i) The ability of a woman's ovaries to release eggs ready for fertilization declines with age.
(ii) The health of a woman's eggs declines with age.
(iii) As a woman ages she is more likely to have health problems that can interfere with fertility.
(iv) As a women ages, her risk of having a miscarriage increases.

Some health issues also increase the risk of fertility problems. So women with the following issues should speak to their doctors as soon as possible:

- Irregular periods or no menstrual periods.
- Very painful periods.
- Endometriosis.
- Pelvic inflammatory disease.
- More than one miscarriage.

Combined Infertility.

In some cases, both the man and woman may be infertile or sub-fertile, and the couple's infertility arises from the combination of these conditions. In other cases, the cause is suspected to be immunological or genetic; it may be that each partner is independently fertile but the couple cannot conceive together without assistance.

Unexplained Infertility.

In about 15% of cases the infertility investigation will show no abnormalities. In these cases abnormalities are likely to be present but not detected by current methods. Possible problems could be that the egg is not released at the optimum time for fertilization, that it may not enter the fallopian tube, sperm may not be able to reach the egg, fertilization may fail to occur, transport of the zygote may be disturbed, or implantation fails. It is increasingly recognized that egg quality is of critical importance and women of advanced maternal age have eggs of reduced capacity for normal and successful fertilization.

4.2 Treatment.

Infertility can be treated with medicine, surgery, artificial insemination or assisted reproductive technology. Many times these treatments are combined. About two-thirds of couples that are treated for infertility are able to have a baby. In most cases infertility is treated with drugs or surgery. Doctors recommend specific treatments for infertility based on (a) test results, (b) how long the couple has been trying to get pregnant, (c) the age of both the man and woman, (d) the overall health of the partners, and (e) preference of the partners.

4.1.2.Treatment of Male Infertility.

- **Sexual problems:** If the man is impotent or has problems with premature ejaculation, doctors can help him address these issues. Behavioral therapy and/or medicines can be used in these cases.
- **Too few sperm:** If the man produces too few sperm, sometimes surgery can correct this problem. In other cases, doctors can surgically remove sperm from the male reproductive tract. Antibiotics can also be used to clear up infections affecting sperm count.

4.1.3.Treatment of Female Infertility.

- **Intrauterine insemination** (IUI) is another type of treatment for infertility. IUI is known by most people as artificial insemination. In this procedure, the woman is injected with specially prepared sperm. Some common medicines used to treat infertility in women include:
- **Clomiphene citrate (*Clomid*):** This medicine causes ovulation by acting on the pituitary gland. It is often used in women who have Polycystic Ovarian Syndrome (PCOS) or other problems with ovulation. This medicine is taken by mouth.
- **Human menopausal gonadotropin or hMG (*Repronex, Pergonal*):** This medicine is often used for women who don't ovulate due to problems with their pituitary gland. hMG acts directly on the ovaries to stimulate ovulation. It is an injected medicine.
- **Follicle-stimulating hormone or FSH (*Gonal-F, Follistim*):** FSH works much like hMG. It causes the ovaries to begin the process of ovulation. These medicines are usually injected.
- **Gonadotropin-releasing hormone (Gn-RH) analog:** These medicines are often used for women who don't ovulate regularly each month. Women who ovulate before the egg is ready can also use these medicines. Gn-RH analogs act on the pituitary gland to change when the body ovulates. These medicines are usually injected or given with a nasal spray.
- **Metformin (*Glucophage*):** Doctors use this medicine for women who have insulin resistance and/or Polycystic Ovarian Syndrome (PCOS). This drug helps lower the high levels of male hormones in women with these conditions. This helps the body to ovulate. Sometimes clomiphene citrate or FSH is combined with metformin. This medicine is usually taken by mouth.
- **Bromocriptine (*Parlodel*):** This medicine is used for women with ovulation problems due to high levels of prolactin. Prolactin is a hormone that causes milk production.

4. 3 Prevention of Infertility.

A. Male Infertility. Some cases of male infertility may be avoided by doing the following:

- Avoid drugs and medications known to cause fertility problems, like steriods and some antifungal medications.
- Avoid excessive exercise.
- Avoid exposure to environmental hazards such as pesticides.
- Avoid frequent hot baths or use of hot tubs.
- Avoid tight underwear or pants.
- Eat a diet with adequate folic acid, and vitamine C and zinc loaded food.
- Get early treatment for sexually transmitted diseases.
- Have regular physical examinations to detect early signs of infections or abnormalities.
- Keep diseases, such as diabetes and hypothyroidism, under control.
- Practice safer sex to avoid sexually transmitted diseases.
- Take a lycopene supplement.
- Wear protection over the scrotum during athletic activities.

B. Female Infertility. Some cases of female infertility may be prevented by taking the following steps:

- Avoid excessive exercise.
- Avoid smoking.
- Control diseases such as diabetes and hypothyroidism
- Follow good weight management guidelines.
- Get early treatment for sexually transmitted diseases.
- Have regular physical examinations to detect early signs of infections or abnormalities.
- Limit caffeine and alcohol intake.
- Use birth control to prevent unwanted pregnancy and abortions.

Assisted Reproductive Technology.

Assisted reproductive technology (ART) is a term that describes several different methods used to help infertile couples. ART involves removing eggs from a woman's body, mixing them with sperm in the laboratory and putting the embryos back into a woman’s body. ART can be expensive and time-consuming. But it has allowed many couples to have children that otherwise would not have been conceived. The most common complication of ART is multiple foetuses. Common methods of ART include:

1. **In vitro fertilization (IVF)** means fertilization outside of the body. IVF is the most effective ART. It is often used when a woman's fallopian tubes are blocked or when a man produces too few sperm. Doctors treat the woman with a drug that causes the ovaries to produce multiple eggs. Once mature, the eggs are removed from the woman. They are put in a dish in

the lab along with the man's sperm for fertilization. After 3 to 5 days, healthy embryos are implanted in the woman's uterus.

2. **Zygote intrafallopian transfer (ZIFT)** or **Tubal Embryo Transfer** is similar to IVF. Fertilization occurs in the laboratory. Then the very young embryo is transferred to the fallopian tube instead of the uterus.
3. **Gamete intrafallopian transfer (GIFT)** involves transferring eggs and sperm into the woman's fallopian tube. So fertilization occurs in the woman's body. Few practices offer GIFT as an option.
4. **Intracytoplasmic sperm injection (ICSI)** is often used for couples in which there are serious problems with the sperm. Sometimes it is also used for older couples or for those with failed IVF attempts. In ICSI, a single sperm is injected into a mature egg. Then the embryo is transferred to the uterus or fallopian tube.

4.3 ARTIFICIAL INSEMINATION

In humans, artificial insemination is usually a part of infertility treatment; either the woman's partner's sperm (artificial insemination by husband, **AIH**) or donor sperm (artificial insemination by donor, **AID**) can be used. Earlier, a popular form of artificial insemination was AIC, in which the sperm of the husband and a donor were mixed. The advantage of this procedure was that it could not be conclusively stated that the husband was not the father of the child. The woman's menstrual cycle is closely observed, using ovulation kits, ultrasounds or blood tests. When an ovum is released, semen from a donor is inserted into her body. Just as with in vitro fertilization, the male donor is recommended not to ejaculate for a few days before the procedure. This is to ensure a higher sperm count. After the donation the sperm must immediately be "washed" in a laboratory. The process of "washing" the sperm increases the chances of fertilization and removes any chemicals in the semen that may cause discomfort for the woman. A chemical is added to the sperm that will separate the most active sperm in the sample. If the procedure is successful, she conceives and bears to term a baby as normal, making her both the genetic and gestational mother.

Of course, there are various gradations of treatment, and more technical procedures are sometimes needed. For example, semen can be injected directly into a woman's uterus to improve the chance of conception in a process called intrauterine insemination.

4.4. IN-VITRO FERTILIZATION (IVF).

In vitro fertilization is a medical technique in which a woman's egg is placed with her husband's sperm in a laboratory environment to promote fertilization. The result is a so-called **test-tube baby**. The first IVF baby in the world was born on July 25 of 1978 at Bourne Hall, in Cambridge, England, named **Louise Brown**. **Subhash Mukhopadhyay** became the first physician in **India**, and the second in the world after **Steptoe** and **Edwards**, to perform *in vitro* fertilization resulting in a test tube baby **Durga** (alias **Kanupriya Agarwal**) on October 3, 1978. Facing social ostracism, bureaucratic negligence, reprimand and insult instead of recognition from the Marxist West Bengal government and refusal of the Government of India to allow him to attend international conferences, he committed suicide in his Calcutta residence in 1981.

Initially IVF was developed to overcome infertility due to problems of the fallopian tube, but it turned out that it was successful in many other infertility situations as well. The introduction of **intracytoplasmic sperm injection (ICSI)** addresses the problem of male infertility to a large extent. Thus, for IVF to be successful it may be easier to say that it requires healthy ova, sperm that can fertilize, and a uterus that can maintain a pregnancy.

IVF Technique. The main medical techniques of in vitro fertilization are:

1. An egg (ovum or oocyte) is taken from the ovaries of the mother.
2. It is then placed with the sperm of the husband in a laboratory test tube.
3. The fertilized egg is transferred to the uterus of the mother, usually 2 or 3 days later.
4. In 9 months, the delivery of a healthy baby, a test-tube baby occurs.
   The fertilization rates of 70-80% are currently being achieved, with healthy pregnancies and deliveries. These advanced fertility treatments are minor outpatient procedures and do not involve hospitalization. The cost is between $3,000 and $10,000.

Variations.

The mother usually undergoes ovarian stimulation for a week with fertility medications so that several mature eggs develop. In order to determine the best time to stop the fertility medications and recover the eggs, ultrasound examinations and blood tests are performed.

- **GIFT (Gamete Intra-Fallopian Transfer)**, is to place the eggs and sperm into the wife's fallopian tubes directly, instead of in a laboratory test tube.
- **ICSI (Intra-Cytoplasmic Sperm Injection)**, it is a micromanipulation technique to help achieve fertilization for couples with severe male factor infertility, in which sperm counts or motility are low but there are enough to allow fertilization in the laboratory: The semen sample is prepared by centrifuging (spinning the sperm cells through a special medium), to separate live sperm from debris and death sperm. Then, the micromanipulation specialist picks up the single live sperm in a glass needle and injects it directly into the egg in the laboratory.

Who can benefit of the IVF?

1. Women with problems in the uterus: Endometriosis, polyps, malformations, amenorrhea.
2. Problems in the ovaries: Polycystic ovarian syndrome, advanced female age with egg quality problems.
3. Women with problems in the fallopian tubes, or tubal ligation, or block due to infections, scars, tumors, congenital.
4. Genetic diseases that result in miscarriage or abnormal births.
5. Men with vasectomy (ligation of the tube from the testicle to the prostate), or with problems of the tubes due to infections, tumors, scars, or congenital, not solved surgically.
6. A male with low sperm counts or motility.
7. Infertility secondary to sperm antibodies.
8. Unexplained infertility that has not responded to other treatments.

Complications.

The major complication of IVF is the risk of multiple births. This is directly related to the practice of transferring multiple embryos at embryo transfer. Multiple births are related to increased risk of pregnancy loss, obstetrical complications, prematurity, and neonatal morbidity with the potential for long term damage. **Spontaneous splitting** of embryos in the womb after transfer does occur, but is rare and would lead to identical twins. Recent evidence suggest that singleton offspring after IVF is at higher risk for **lower birth weight** for unknown reasons. Another risk of ovarian stimulation is the development of **ovarian hyperstimulation syndrome**. If the underlying infertility is related to abnormalities in spermatogenesis, it is plausible, but too early to examine that male offspring is at higher risk for **sperm abnormalities**.

Social Problems.

(i) If the egg is taken from a woman different of the wife, the baby born has no hereditary characteristics of the wife, but those of the woman who donated the egg, even if the embryo is placed in the uterus of the wife, and the wife delivers him. This procedure is against the ethics. It is like the husband having a child from another woman, but without sex.
(ii) If the semen is taken from a man different than the husband, the child will have no hereditary characteristics of the husband, but of the one who donated the sperm. This is also against the ethics. It is a wife having a baby from another man, but without sex.
(iii) If the egg is from the wife and the sperm from the husband, and the uterus used is the one of another woman, the hereditary characteristics of the child will be those of the husband and wife, and none of the woman who had the pregnancy and delivered the baby. This procedure goes along with the ethics, though the surrogate mother may feel the baby is hers.

5.0 CONCLUSION

In this unit you learnt about the embryonic membrane and placental processes and function in embryo development.Also, birth control and child birth, infertility in bothmale and female,artificial insemination and invitro fertilization.

SUMMARY

The extraembryonic membranes or foetal membranes are formed of embryonic tissue, the **trophoblast** that lies outside the embryo. The embryos of amniotes, i.e. reptiles, birds and mammals produce four extraembryonic membranes, the **amnion**, **yolk sac**, **chorion** and **allantois**. In birds and most reptiles, the embryo with its extraembryonic membranes develops within a shelled egg. With these four membranes, the developing embryo is able to carry on essential metabolism while sealed within the egg.

This organic connection between the foetus and uterine wall is called as **placenta** which develops at the point of implantation. It is formed of both foetal and maternal tissues; the allantois gives rise to umbilical cord which contains blood vessels connecting foetus and placenta.

All women and men should have control over if and when they become parents. Making decisions about birth control, or contraception is not easy– there are many things to think about. Learning about birth control methods can use to prevent pregnancy and talking with a doctor are two good ways to get started. Some other methods are discussed in the unit.

7.0 TUTOR-MARKED ASSIGNMENT

1Explain and describe the structure of embryonic membrane.

2 Explain the structure and function of placenta

3 Which method and treatment is suggest for infertility in male and female

4 How can you control birth?

REFERENCES

Professor Scott Gilbert, Developmental Biology, 6th Edition.