Sabbatical Report

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Purpose

The purpose of my sabbatical was instructional improvement by creating a laboratory atlas of developmental biology for classroom use. The atlas will be available for students to download to aid in their laboratory study of developmental biology.

Background and Project Description

Developmental Biology (Biology 427) introduces students to the amazing process by which a single fertilized egg, the zygote, develops into a complex, multicellular organism. The field of developmental biology field encompasses microscopic anatomy, experimental embryology and molecular mechanisms. I use a comparative approach to teach this course so that students are introduced to developmental processes in a variety of organisms including humans. I structure the course by first focusing on descriptive embryology followed by analyses of the molecular mechanisms that underlie developmental patterns. A full appreciation of the field requires an understanding of development at both microscopic and molecular levels. The course has both a lecture and a laboratory component and in the laboratory I focus primarily on microscopic observations of developing embryos or tissues involved in reproductive processes. The laboratory section of this course provides an excellent opportunity to engage students in science due to the dynamic nature and visual beauty of developing embryos.

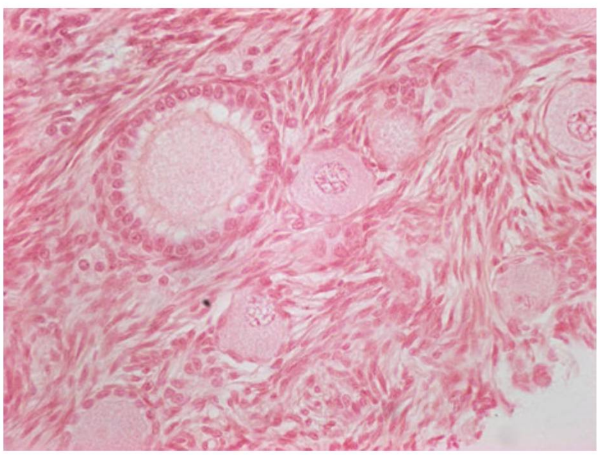
I have developed/written most of the lab exercises for this course and provide these as handouts. The handouts contain detailed descriptions of structures that students need to learn and identify, however, it is often difficult for students to match that textual description with what they observe under the microscope. In addition, although the Biology program has amassed a good collection of commercially prepared microscope slides; these slides are only available to students during the (relatively brief) period when they are in the teaching lab. For these reasons, I requested a sabbatical leave to work on a laboratory atlas for the developmental biology teaching laboratory.

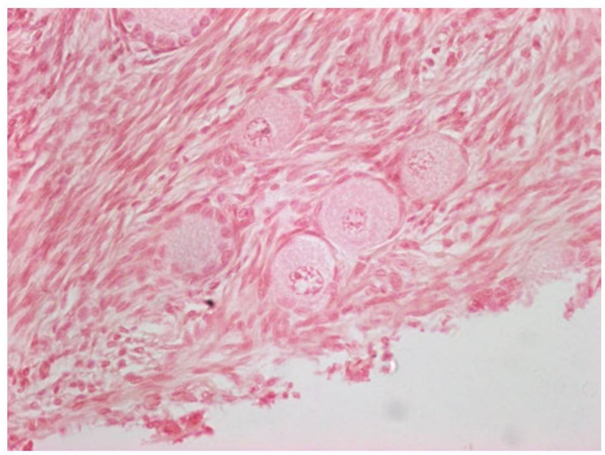
Outcomes

For my sabbatical, I worked on producing digital microscopic images of existing developmental biology slides from our collection. I then annotated these images by labeling micro-anatomical features of importance and wrote explanatory text to accompany the images. Students in the developmental biology course will now have this resource to use in the laboratory as they are examining slides and for studying outside of the laboratory. Two sample chapters are attached.

Biology 427 Laboratory

Laboratory manual and Atlas





Dr. Nancy Mozingo

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Oogenesis

LAB ACTIVITY #1: Examine slides of mammalian ovaries and identify the structures indicated below in **bold** .

Oogenesis in mammalian ovaries

Human females are born with ~700,000 oocytes which are arrested in prophase I of meiosis. After puberty and prior to menopause, a woman will ovulate ~ 400 eggs. The remaining eggs will undergo atresia or degeneration. Examine your section of ovary under low power. You should see a thin connective-tissue sheath called the **tunica albuginea** . How does this structure compare to the tunica albuginea in the testis? You should be able to distinguish two regions; an outer **cortex** and an inner **medula** . The cortex contains circular structures called follicles and the medulla contains connective tissue, nerves, lymph and blood vessels. A follicle consists of a single oocyte surrounded by follicle or nurse cells.

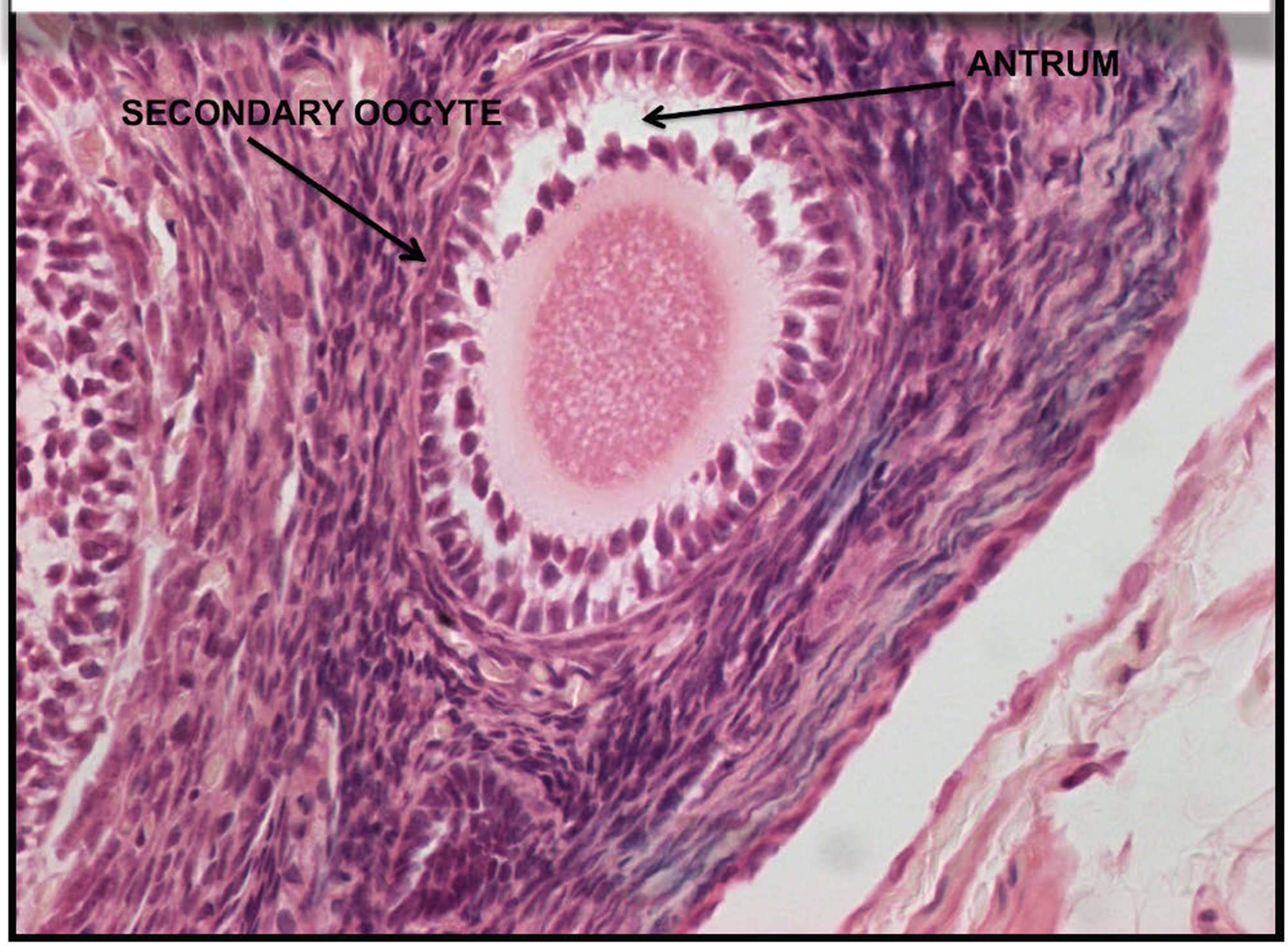
Follicles

First under low power and then under high power, examine the follicles. You should be able to distinguish follicles in various stages of development.

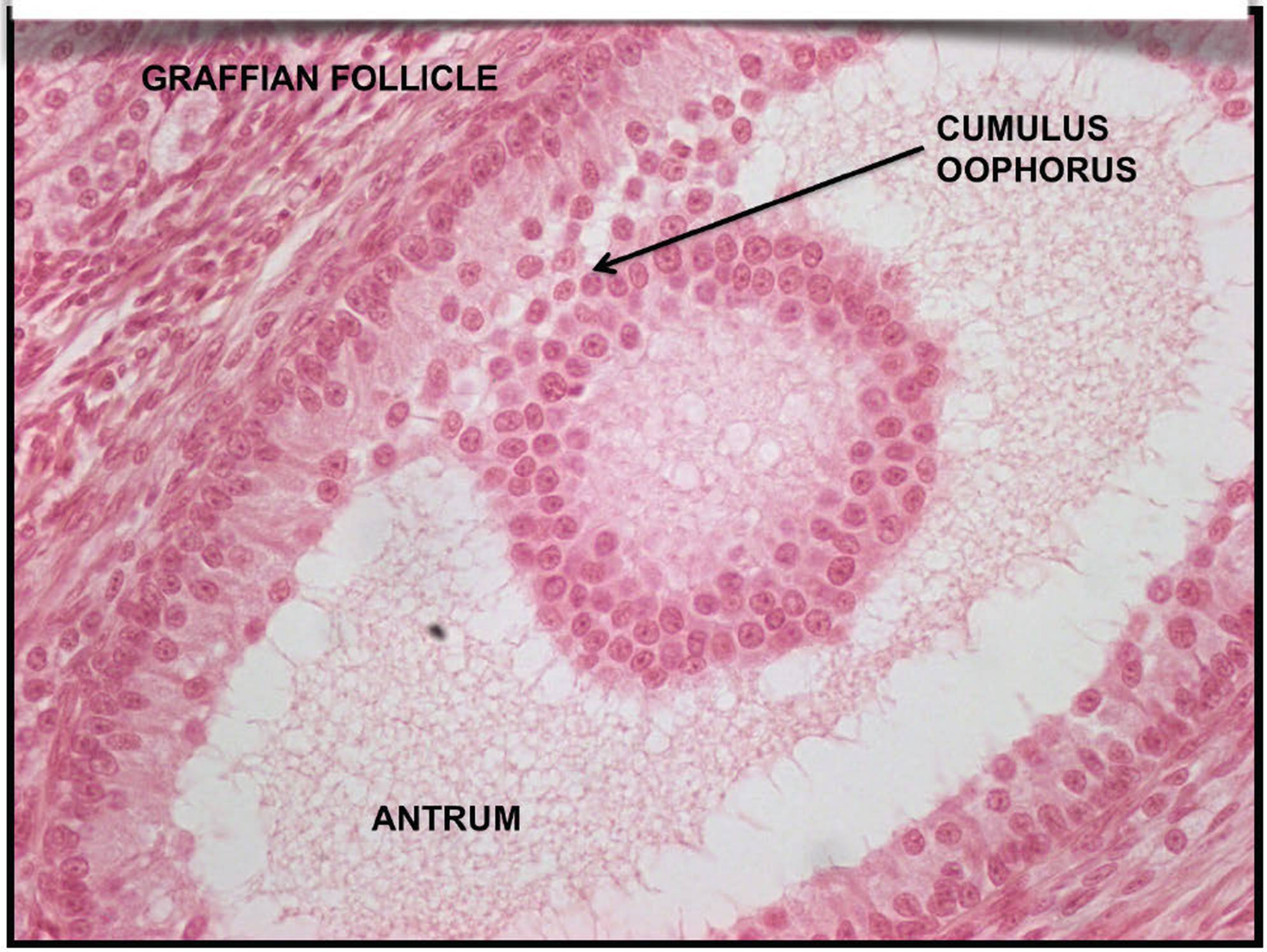
Primordial follicles: Primordial follicles : a follicle containing a primary oocyte surrounded by a single layer of squamous follicle cells. Primordial follicles are numerous.

Primary follicles : follicle consisting of a primary oocyte surrounded by multiple layers of follicle cells. In humans, 20-50 primordial follicles mature into primary follicles each month under the influence of follicle stimulating hormone. The follicle cells become cuboidal/ columnar and increase in number. The multiple layers of follicle cells is referred to as the **stratum granulosum** .

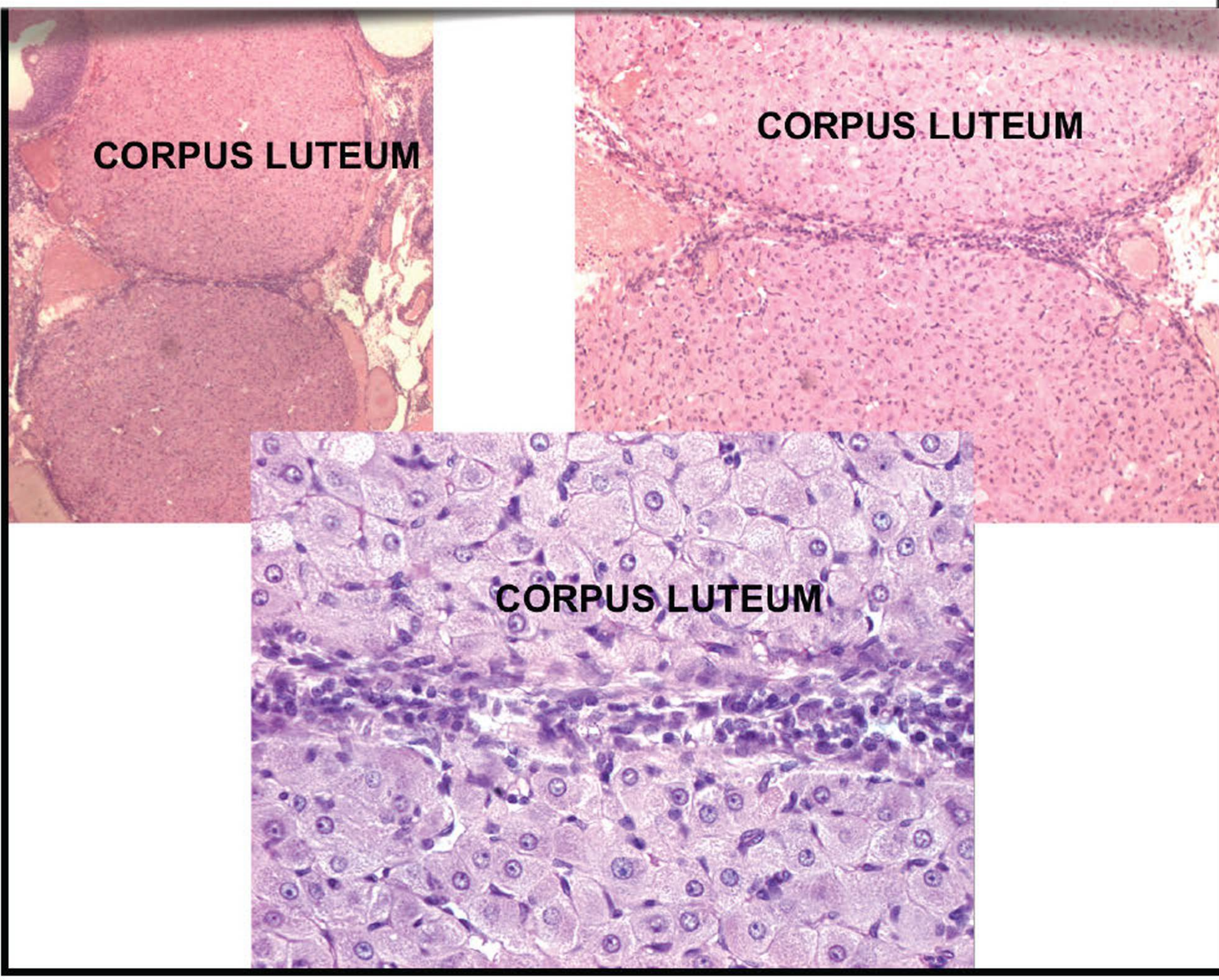
Secondary follicle : Follicle cells secrete a fluid which accumulates in spaces between follicle cells. These spaces are referred to as the **antrum** and once these spaces appear, the follicle is called a secondary follicle.



Graffian follicle : The antrum enlarges to fill the entire central region of the follicle and the oocyte sits in a protrusion of follicle cells called the **cumulus oophorus** . At this stage, the oocyte is mature and referred to as a Graffian follicle. The fluid filling the antrum is called *liquor folliculi* and contains substances that maintain the oocyte in meiotic arrest.



Corpus luteum: Once the oocyte is ovulated, the Graffian follicle secretes progesterone and estrogen which maintain the uterine lining. The follicle is now called a **corpus luteum** and appears collapsed with folded walls and is very large ~2cm in diameter. If pregnancy does not occur, the corpus luteum degenerates and is replaced by scar tissue forming a **small corpus albicans** . If pregnancy does occur, the corpus luteum continues to grow reaching ~ 5cm in diameter in humans. The corpus luteum degenerates after the 9 or 10 th week of pregnancy forming a very **large corpus albicans.**



Oocyte: The oocyte in a primordial follicle is 10-25 um in diameter. Using this as a guide, estimate how large the oocyte is in each of the other types of follicles. Immediately surrounding the oocyte is a clear layer known as the **zona pellucida** . Traversing the zona pellucida are fine, filamentous structures called cytoplasmic bridges . Nutritive substances pass from the follicle cells to the oocyte through these channels. The layer of follicle cells surrounding the oocyte is called the **cumulus.** Just prior to ovulation, the oocyte completes the first meiotic division and is ovulated as a secondary oocyte. You probably will not be able to find a **secondary oocyte** , but search for a Graffian follicle which has an oocyte with a **small polar body** on its surface. The second meiotic division will begin in the oviduct and will only be completed if the oocyte gets fertilized.

Atretic follicle : Not all maturing follicles become Graffian follicles. In humans, of the 20-50 that begin to mature each cycle, usually only one becomes a Graffian follicle while the others undergo atresia. Atretic follicles have misshapen cells with darkly staining nuclei.

Theca

The **theca** is a layer of cells and connective tissue that surrounds follicles. It consists of two layers, the **theca interna** and the **theca externa** . The theca interna lies closest to the follicle and the theca externa appears more fibrous. The theca provides nutritive secretions to the follicle cells as well as estrogen-intermediary compounds that the follicle cells convert to estrogen.

LAB ACTIVITY #2: Explain the difference between panoistic an meroistic oogenesis.

OOGENESIS IN INSECTS

In insects, each ovary consists of several ovarioles. Several egg chambers (follicles) will be present within each ovariole Within each ovariole, germ cells are aligned chronologically, with the most mature at the end closest to the oviduct. Two major modes of oogenesis are observed in insects. In **panoisti** c oogenesis, gene products required for oocyte maturation are produced in the oocyte itself. In **meroistic** oogenesis, nurse cells supply most of the gene products required for oogenesis.

LAB ACTIVITY #3: Dissect an ovary from a normal female fly and observe the egg chambers found within the ovary.

Oogenesis in the fruit fly, *Drosophila melanogaster*

Oogenesis in *Drosophila* is an example of meroistic oogenesis, in which oocyte growth and development is dependent upon nurse cells. Nurse cells synthesize huge quantities of messenger RNAs, proteins, and ribosomes and then transport these molecules and organelles to the oocyte. The nurse cells are very active transcriptionally and as a result, prophase is shortened in insects that undergo meroistic oogenesis. In Drosophila, the nurse cells are enclosed with the oocyte in the follicle. The smallest, most immature egg chambers are at the anterior tip of the ovary, while large, mature eggs are at the posterior. Fertilization occurs internally in *Drosophila* , before the egg is laid. *Drosophila* females will lay eggs (ovulate) whether or not the eggs have been fertilized. Young females should contain many egg chambers of different stages of oogenesis. Each egg chamber contains 1 oocyte and 15 nurse cells, which are connected to one another by cytoplasmic bridges or ring canals. Surrounding the oocyte and nurse cells are a single layer of follicle cells. Oogenesis has been divided into fourteen stages. In the first half of oogenesis, egg chambers (stages 1-7) grow larger as the nurse cell' cytoplasm expands, and as their nuclei become polypolid. During stages 8-10, vitellogenesis occurs, whereby the oocyte accumulates and stores vitellin in the form of yolk platelets. The oocyte enlarges as it accumulates yolk. In the latter half of oogenesis, the entire contents of the nurse cell cytoplasm is "dumped" (in a process called nurse cell dumping) or transported into the oocyte via the ring canals. In the final stages of oogenesis, all of the nurse cells and follicle cells degenerate and are sloughed off the egg. During development of the egg chamber the oocyte nucleus arrests in metaphase I of meiosis. Meiosis I and II are completed when the egg is laid (ovulated), and completion of meiosis appears to be independent of sperm entry.

<u>Dissecting the Ovary from the Adult Female Fruitfly,</u>

Equipment and Supplies Needed for Ovary Dissection:

Ether and etherizer, adult female fruitflies, 2 pairs of forceps with fine tips, Microscope slides and cover glasses ,Dissecting and compound microscope, Insect Saline

1. Before dissection, the flies will have been anesthetized with ether.

2. Take anesthetized females and lay the animal on its back. Place a drop of Insect Saline onto the animal.

3. The abdomen of the female will be swollen due to the presence of eggs.

4. Grasp the fly around its "waist" or at the upper half of its abdomen with one pair of forceps.

5. Take a needle/second pair of forceps and squeeze out the abdominal contents.

6. A pair of ovaries will be attached to the gut and yellowish colored Malphighian tubules (excretory organs). Examine the intact ovary, referring to the diagram. The paired ovaries are attached to the single common oviduct and uterus.

7. Clear away the gut and the malphighian tubules.

8. Use a needle or the fine point of the forceps and tease the ovaries, so that the ovarioles are somewhat separated.

9. Put a drop of Methylene Blue and let the preparation stain for 5 minutes.

10. Place a coverslip on the slide and observe under the microscope.

11. Observe under low power, locate the ovary, then move to a higher magnification. <u>At Low Power:</u> Notice that the ovarioles contains egg chambers at various stages of development. Try to distinguish different staged egg chambers within the ovary As you look at the egg chambers, note in particular the structure of a mature, yolk-filled egg.<u>At Higher Magnification:</u> Find how many nuclei you can observe in a single egg chamber. There are 15 nurse cells and a single oocyte nucleus in one chamber. In the egg chamber, many individual nurse cells can be seen in its anterior half, while the single oocyte fills the posterior half. At this time, follicle cells may be observed around the oocyte portion of the chamber.

LAB ACTIVITY #4: Dissect an ovary from a cricket and observe the egg chambers found within the ovary the cricket

Oogenesis in the cricket is an example of panoistic oogenesis, in which gene products to be stored in the oocyte and those required for oocyte metabolism are produced by the oocyte itself. In panoistic oogenesis, oogenesis is often accompanied by the formation of lampbrush chromosomes.

<u>Dissection of the Cricket</u>

Before dissection, the crickets will be anesthetized on ice.

1. Obtain female cricket (it has the ovipositor), place on slide, cut off head first, then cut off legs.

2. Cut open ventral side with scissors, cutting straight down the center.

3. Eggs may "tumble" from the cricket. These are mature eggs, possibly fertilized.

4. Using forceps, isolate the ovaries and place them in a drop of Insect Saline on a slide.

5. Clear away the mature eggs, and look for the less mature part of the ovariole. You are looking for whitish strands coming off smaller sized eggs. The eggs look like rice.

6. Separate eggs and place stringy material on a separate slide. Put one drop of Methylene Blue on the slide and let the preparation stain for 5 minutes.

7. Place a coverslip on the slide and observe under the microscope.

8. Observe under low power, locate the ovary, then move to a higher magnification. How many nuclei can you see in a single egg chamber? There should be a single nucleus, in contrast to the fruit-fly egg chamber.

<u>Finding sperm in the female abdomen</u>

1. In the cricket abdomen, try to locate a "whitish" looking spherical blob. This is the spermatheca, the organ used to store sperm in a female.

2. Place it in a drop of saline (on a slide), tease it with a needle, and mount with a coverslip.

3. Under low magnification, you should see a coiled structure. Look carefully at the edges of this structure. You should see tiny sperm.

4. Move to higher magnification to see the motile sperm better.

Amphibian Development

Introduction to Early Development in Amphibians

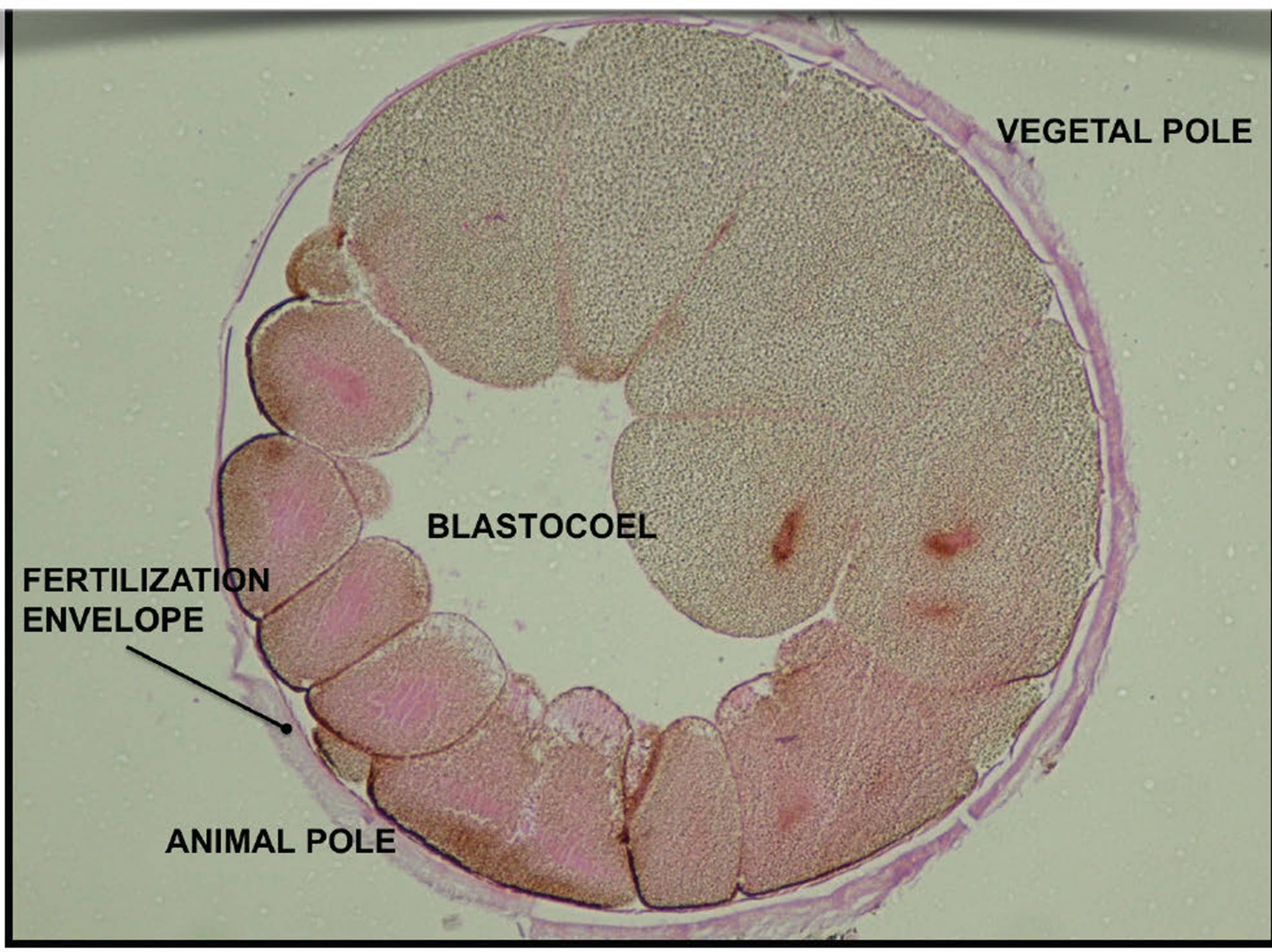
Cleavage is radial and holoblastic in amphibians. The presence yolk impacts the cleavage pattern in these mesolecithal eggs. The first cleavage is meridonal and begins at the animal pole. As the cleavage furrow nears the vegetal pole it is slowed down significantly by the presence of yolk. The second cleavage is meridional and at right angles to the first cleavage. The second cleavage begins before the first cleavage furrow has completely bisected the vegetal pole. The third cleavage is equatorial, but unequal and divides the embryo into 4 micromeres at the animal pole and 4 large macromeres at the vegetal pole. Because cleavage occurs more rapidly at the yolk-free animal region, cells will divide more rapidly here than at the yolk rich vegetal pole. So after time, there will be many small cells at the animal pole and fewer, larger cells at the vegetal pole. As the embryo divides, a fluid-filled cavity called the blastocoel develops near the animal pole.

Amphibian gastrulation begins when a group of cells on the future dorsal side of the embryo, just below the equator in the region of the grey crescent. sinks into the embryo. This movement is mediated by cell shape changes. The cells constrict at their apical surface and expand at their basal surface forming a bottle shape, hence these cells are called bottle cells. This results in the formation of a slit-like invagination called blastopore. The initial invagination is called the dorsal lip of the blastopore. Eventually, this initial invagination will spread in circular fashion forming a circular blastopore with dorsal, ventral and lateral lips.

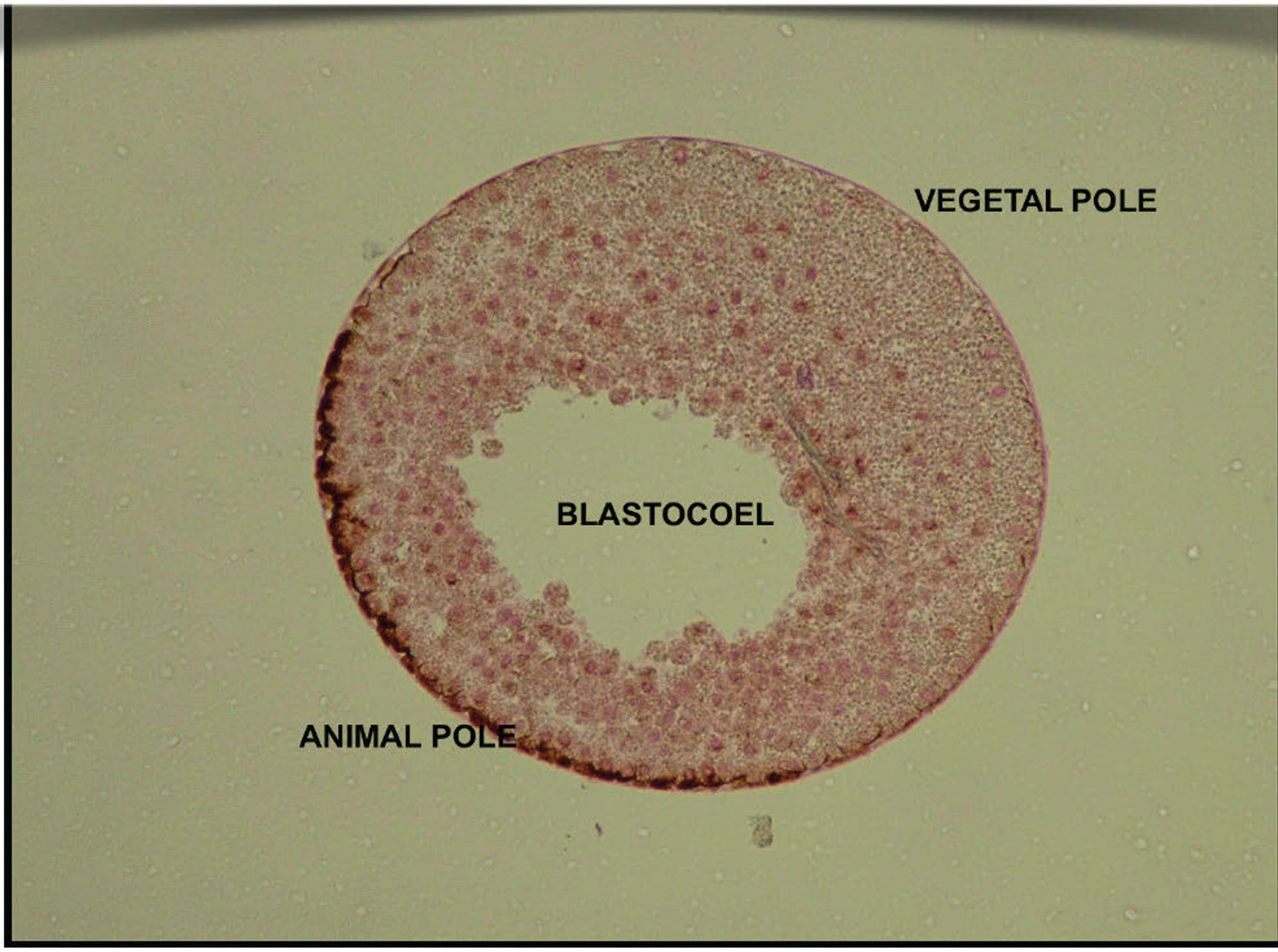
Future mesoderm cells migrate toward the blastopore, when they reach the dorsal lip of the blastopore, they fold back on themselves and continue moving toward the future anterior. As cells involute over the blastopore, the archenteron is formed which displaces the blastocoel. While involution is occuring at the blastopore lips, the ectoderm precursors are spreading over the entire embryo by the process of epiboly.

LAB ACTIVITY #1: Examine the available microscopic slides and learn to identify the structures/features listed in **bold** .

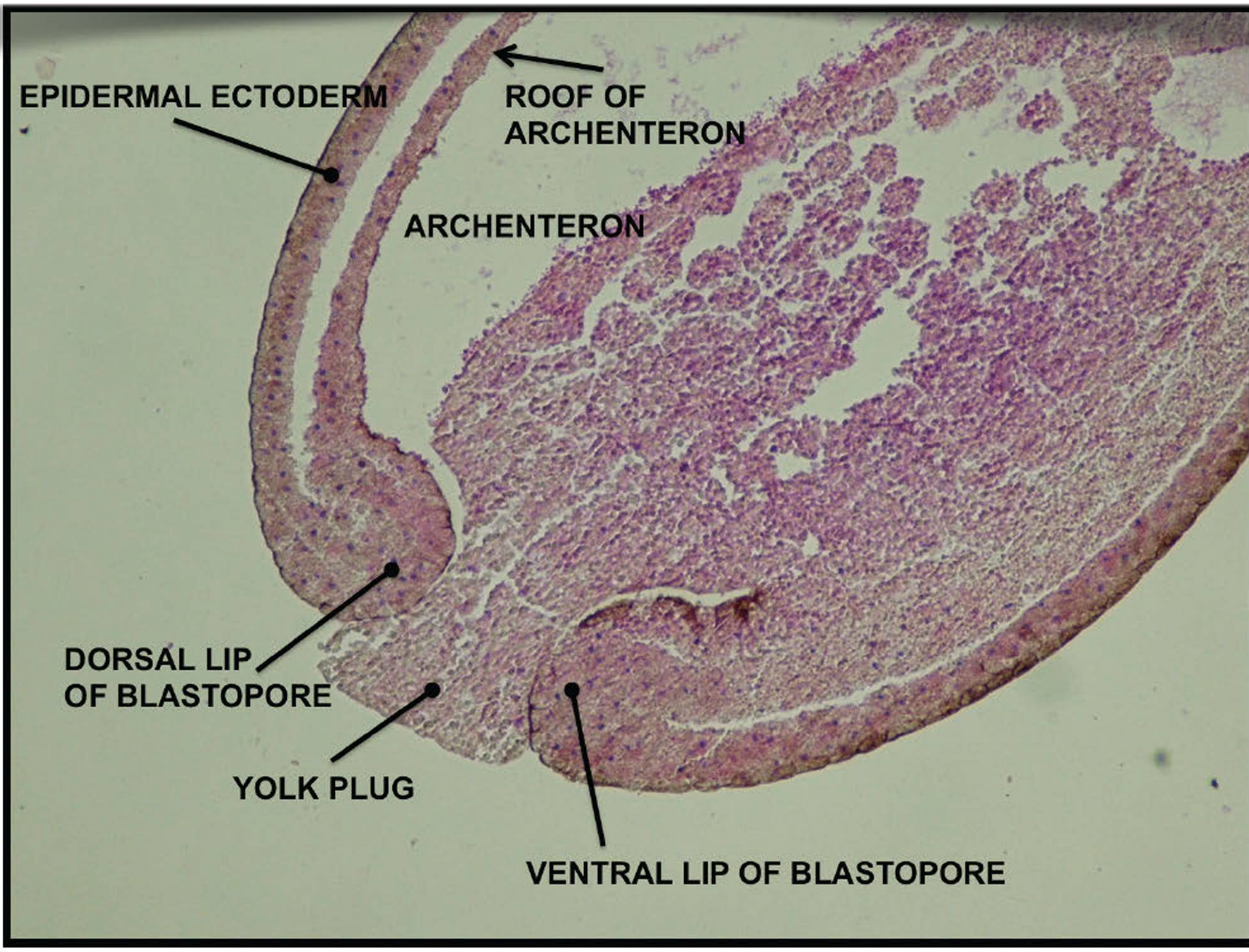
Cleavage Stage: In early cleavage stage amphibian embryos, there are large macromeres at the **vegetal pole** and smaller, more numerous micrometers at the **animal pole**. The cleavage furrows have not completely bisected the macromeres. Because cleavage occurs more rapidly at the yolk-free animal region, cells will divide more rapidly here than at the yolk rich vegetal pole. So after time, there will be many small cells at the animal pole and fewer, larger cells at the vegetal pole. As the embryo divides, a fluid-filled cavity called the **blastocoel** appears. The **fertilization envelope** is still visible.



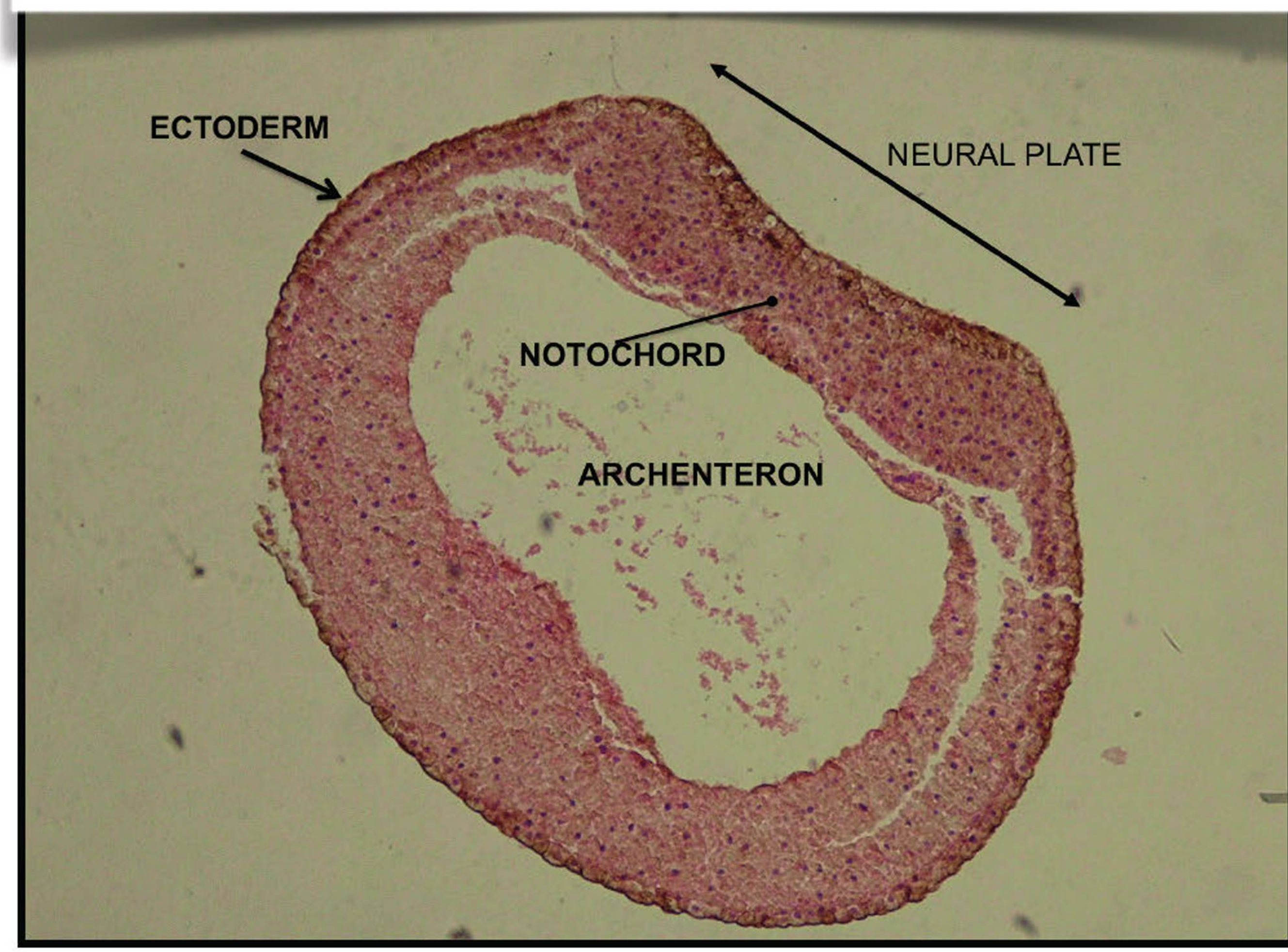
Blastula Stage: The cleavage stage culminates with the formation of a blastula consisting of an outer layer of cells surrounding a fluid-filled **blastocoel**.



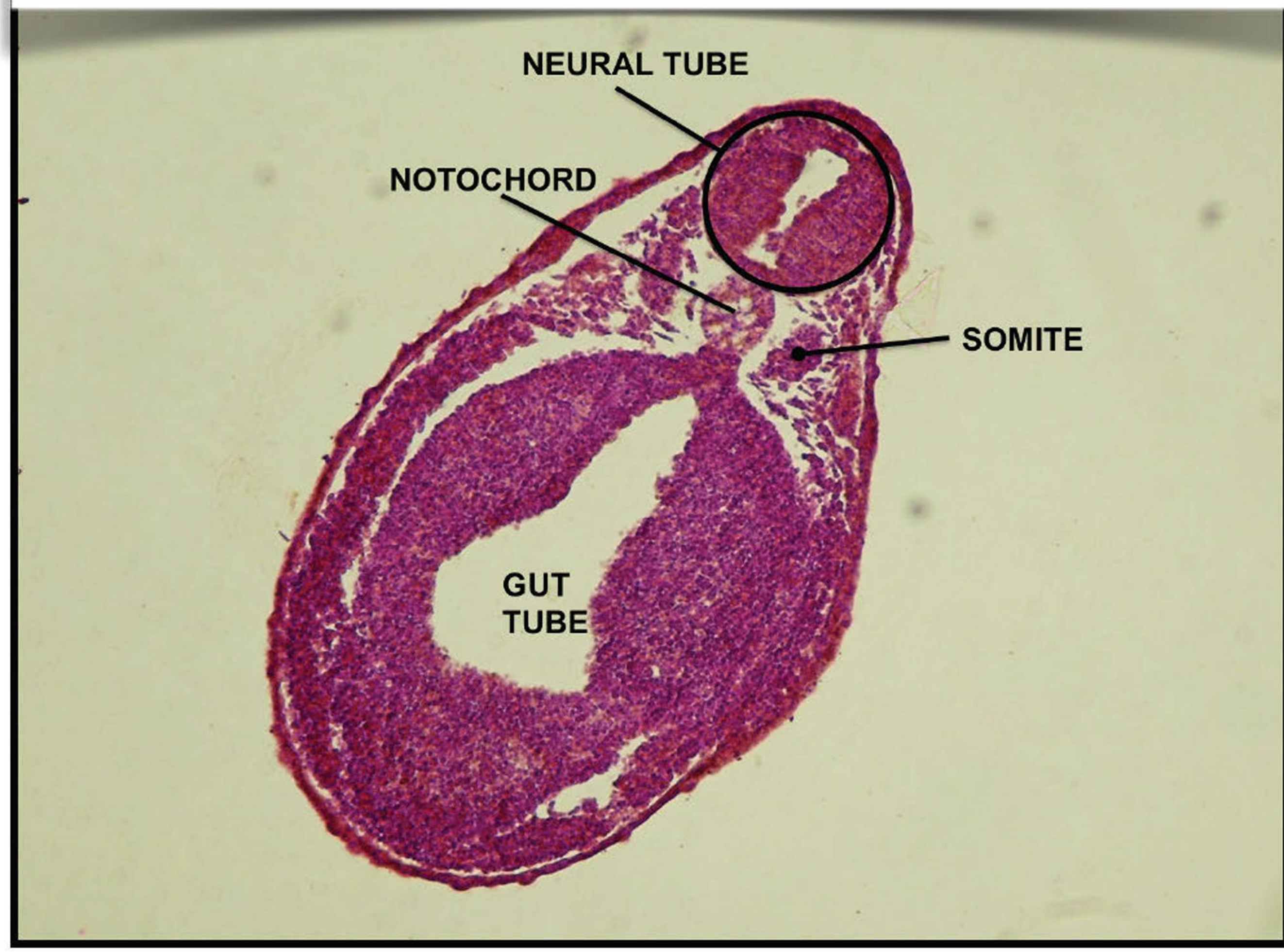
Yolk-plug stage: Gastrulation begins when a group of cells on the future dorsal side of the embryo sinks into the embryo forming the **dorsal lip** of the blastopore. Cells migrate toward the blastopore and when they reach the dorsal lip of the blastopore, they fold back on themselves and continue moving inward. As cells move inward, a new pouch is formed called the **archenteron,** the future gut. As gastrulation progresses, lateral and **ventral lips** are formed over which additional cells involute. This forms a ring around the large, yolky endoderm cells creating the **yolk plug**.



Neural-plate stage: Neurulation is a sequence of morphogenetic events culminating in the formation of the neural tube. Neural ectoderm cells reside on the dorsal surface of a late gastrula. After gastrulation, these cells begin to move toward the dorsal midline and the future anterior. Simultaneously, the cells elongate and form a raised plate called the **neural plate** on the dorsal side of the embryo. A depression called the neural groove develops along the midline of the neural plate and ridges of cells called neural folds arise along the boundary between the neural plate and the surrounding epidermis. The anterior portion of the neural plate will give rise to the brain and the posterior part the spinal cord. The remaining **ectoderm** cells assume a squamous or flat shape and will form epidermis.



Serial cross sections of 4mm embryos: Serial sections represent a series of successive, parallel sections cut through the embryo from anterior to posterior. Different structures will be visible depending on what part of the embryo the section cam from. In 4mm amphibian embryos, the **neural tube** is visible on the dorsal side of the embryo as a round tube lying over the **notochord**, a supportive element of mesodermal origin. On either side of the notochord are chunks of mesoderm called **somites**. Ventral to the notochord is the **gut tube**. Note that not all of these structures will be present in every section. Examine your sections and note which sections the neural tube, notochord, somites and gut tube are present in.



LAB ACTIVITY #2: Preserved embryos: examine the available stages of preserved amphibian embryos.

You should be able to identify the following structures: **Cleavage furrow, animal and vegetal pole, blastomeres, blastopore, yolk plug, keyhole stage, neural plate neurula, neural fold neurula, neural tube neurula, developing eye, stomodeum**