Biology 441 LABORATORY #1 EGG MATURATION, FOLLICLE, CORPUS LUTEUM

PREPARATION AND MATERIALS -THIS INVESTIGATION REQUIRES:
SLIDES: ovary cross sections and longitudinal sections. DIAGRAMS; use of the microscope

REFERENCES: Your textbook, [1]chapter 1. Go buy it now if you haven't already. Other textbooks from the library for photographs and labels of things to be seen. Use only one
at a time and trade with others, to see more than one diagram.[2], [3]

PRELAB OBJECTIVES: to observe the stages in the maturation of the follicle and egg and changes in the ovary following ovulation. Review meiosis using *Ascaris* slides.

REQUIRED TASKS: **make drawings or pictures with labels** for the following stages of follicle development USING THE UNDERLINED STRUCTURES BELOW:
a) primary follicle, b) secondary follicle, c) Graffian follicle, d) corpus luteum. Be sure to look at more than one slide so that you will be prepared to recognize the structures with different stains or types of sections.

HYPOTHESIS TO BE TESTED: there are set steps in development of follicles in response to hormonal stimulation, which ultimately cause ovulation and corpus luteum production. Structure changes as does function.

STUDENTS TO WORK INDIVIDUALLY AND TAKE QUIZ AT THE END OF THE LAB BY LABELING DIAGRAM.

PROCEDURES: In this section you will determine the relationship between structure and function of follicles, and egg maturation and ovulation.

THE VARIABLES OF THE INVESTIGATION ARE: numbers of follicle cells per egg; size and shape of follicle, size of egg, associations of follicle cells with the egg, formation of secretory function of follicle and corpus luteum.

COLLECT DATA: RECORD IT IN A TABLE, Label A set of pictures OR DRAW IT FROM THE SLIDE.. You must keep some sort of looseleaf notebook with information you find out from each lab and hand in at end of lab and then put in the notebook.

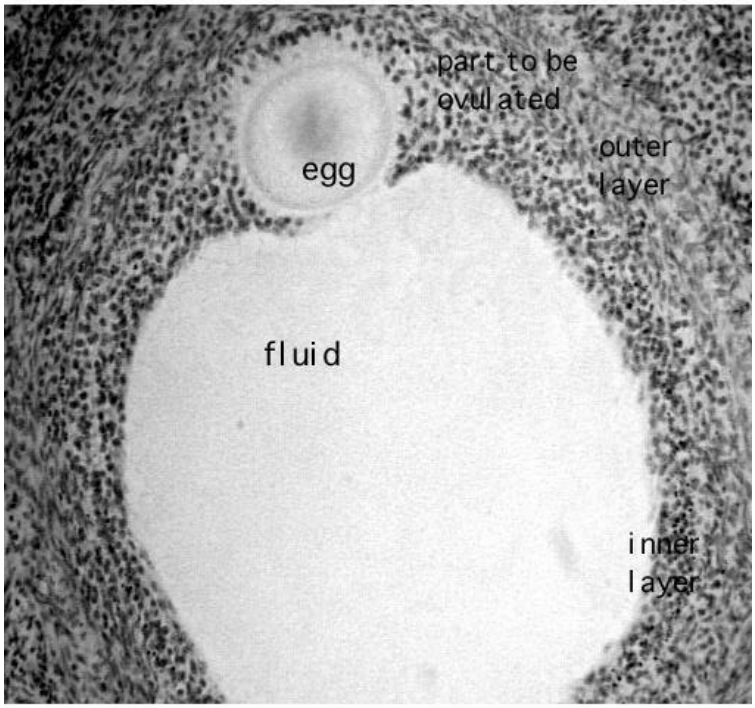
If you are unfamiliar with the microscope, let the instructor help you. There are several kinds, so you may not get the same one each time.

DIRECTIONS FOR FINDING STRUCTURES TO BE LABELED AND MEMORIZED. Observe the slide of cat or rat or human ovary first with the unaided eye holding it up to the light. Don't be satisfied with looking at pictures of the ovary or other things we will do today. The slides hold a lot more information since you can move them around to see relationships, you can up the power. You might find your digital camera handy for taking pictures of good examples you find instead of drawing them. Your wireless laptop can also be brought to class so you can search the internet for answers to questions. To use it on campus you have to get on the internet with your regular account and tell the mainframe your computer's address and your email, so you can use it anywhere on campus.

The large clear vesicles are the Graffian follicles, or mature follicles. Each follicle should have an egg in it, but think of a round structure and the possible ways to cut it into sections, and you can see that if the egg is at one side, the section could miss the egg. If there is an egg, you can miss the nucleus or nucleolus in the

same manner, due to the cut, so it will take you some time to find a good example of each stage of follicle development to draw.
Now place a slide on the microscope stage, and observe it with low power by first making sure the diaphragm is adjusted to allow enough light through the slide, position the section of ovary in the light path using the moveable stage knobs. Always find the section by focusing the low power first. This time we will leave it under low power for observation. Most organs such as ovaries (and kidneys, and glands) have an outer cortex

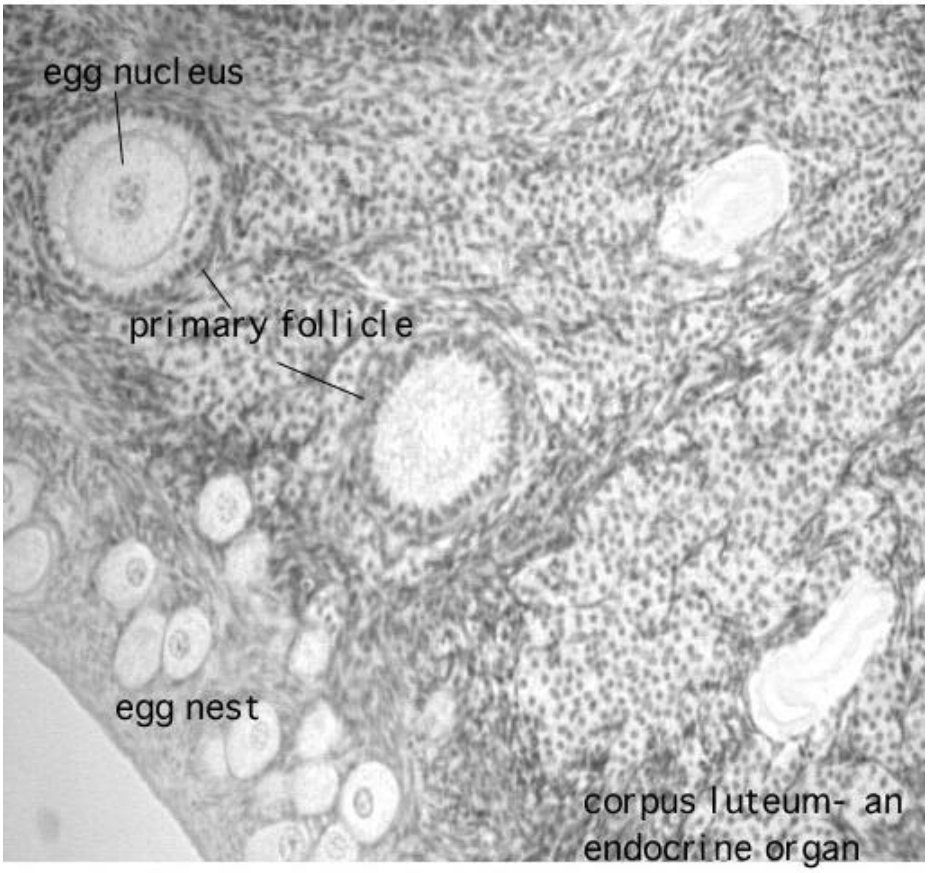
Graffian follicle with fluid



and an inner medulla. The ovary has an enlarged cortical region containing the stroma which surrounds the eggs and follicles. The medulla has connective tissue and blood vessels, but is not prominent. Testes have enlarged medullas. That is one of the differences between ovaries and testes. The outermost layer of the ovary is the germinal epithelium in the fetal female and gives rise to the oogonia before birth. No further production of eggs will occur in the life of the female. The oogonia are advanced to the stage of primary oocytes, also before birth, the first step of oogenesis, meaning that they have entered the first meiotic division. They are arrested and stored at that stage in egg nests and several move from these to follicle development each month, with normally only one follicle in either ovary maturing for

ovulation in any one month in humans, while the rest of the follicles that started to develop degenerate. A new batch has to start.each month, with one winning out for causes as yet

Rat ovary with primary follicle
and old corpus luteum

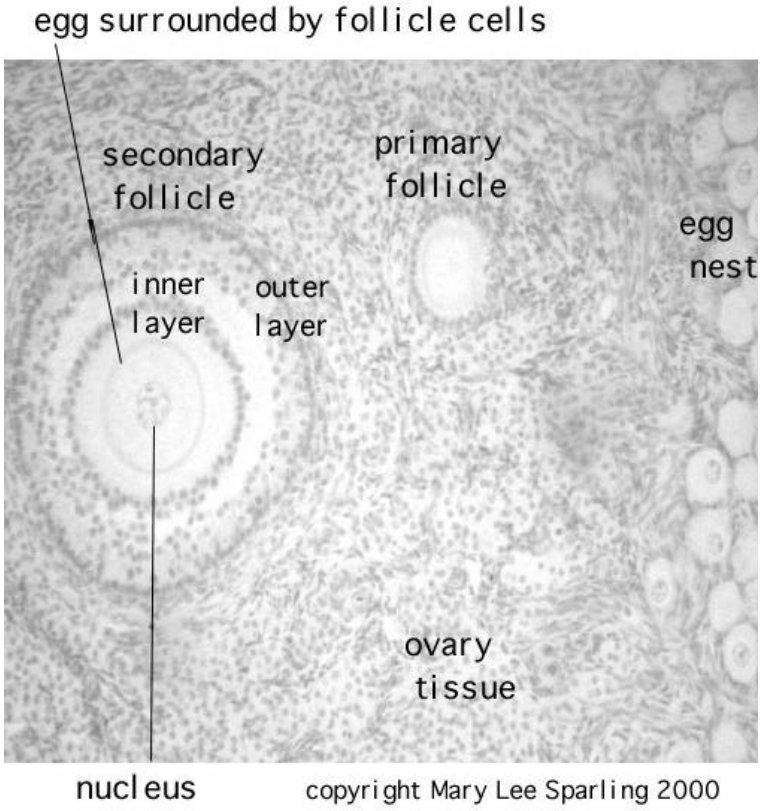


primary follicles have one layer of follicle cells which later divide to make two and then more layers which secrete fluid in Graffian follicle and steroid hormones to go into circulation

unknown.

To find an egg nest, first find the outer ovarian covering of epithelium (cells with one free edge.) Just under that is a layer of connective tissue stroma cells surrounding some groups of pink or lightly stained cells which are the primary oocytes surrounded by

a thin layer of a few follicle cells, each. Each egg with its attached follicle cells is a primary follicle when the layer is one cell thick. Draw an egg nest and a primary follicle, labeling underlined structures, on the sheet to be handed in. Also label nucleus and nucleolus of the oocyte. These follicle cells multiply when the level of FSH goes up, and give rise to a secondary follicle. Find a secondary follicle with 3 or 4 layers of cells and



draw.

As a result of further multiplication, the follicle cells make a thicker layer, and the cells start to.secrete material which causes formation of a cavity in the center of the layer called the antrum, and the oocyte is left at one side of the follicle, connected to the outside layer but surrounded by the inner layer. The space of the antrum is filled with liquor folliculi which has estrogenic hormones in it, and these make their way to the blood stream to circulate throughout the body.

Further growth produces the Graffian follicle. Find a large Graffian follicle and draw. The outer circle of follicle cells differentiate into three layers, granulosa toward the antrum, and two theca layers surrounding that, the theca interna and the theca externa which are cells oriented around the follicle in a swirl, and the interna contains capillaries which appear as little round holes. The follicle cells around the oocyte stick out into the

antrum, are also the granulose type, and will be shed with the egg when it is ovulated, as the cumulus oophorus (or.corona radiata.) The egg increases in size from about 0.035 mm to 0.08 mm during the follicle maturation. Keeping that in mind, make a guess about the size of the follicle at the varius stages of development and enter this in the table in the sheet to be handed in.

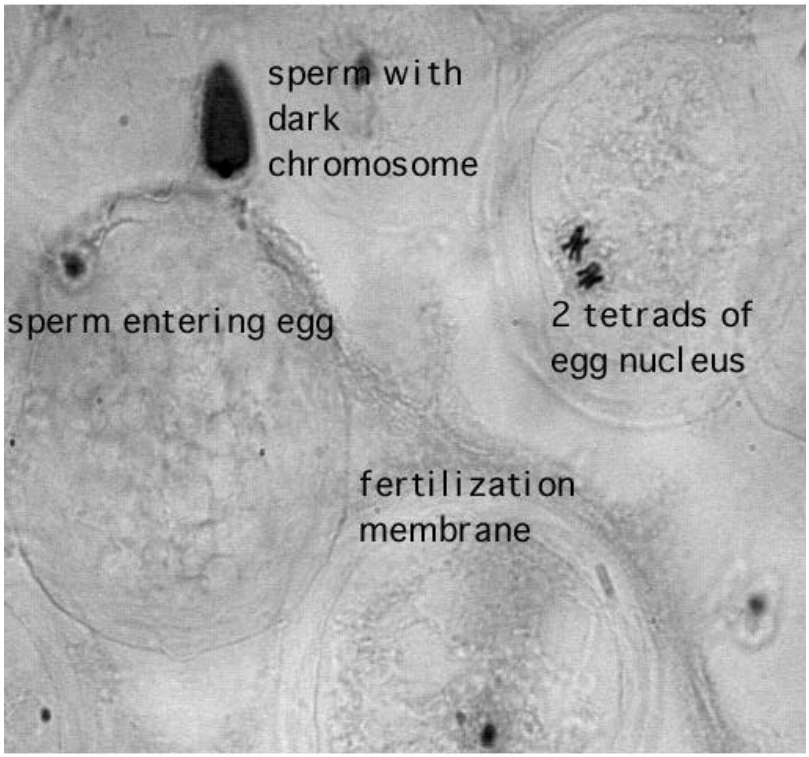
Be sure to see a corpus luteum. Compare its size to that of the follicles.

Read about what kinds of pituitary hormone receptors are present in the various cell types, and which cells produce steroid hormones (p12-13).The follicle continues to grow under FSH stimulation, and then the LH starts to be produced in response to estrogen production by the follicle theca and granulosa cells (with the LH-stimulating factor from the hypothalamus as intermediate.) LH is needed for the final stages of follicle development leading up to ovulation. The follicle reaches a final size of 15 mm. At the same time, in response to the hormones, the egg resumes the first meiotic division and starts to give off a polar body at the time of ovulation. This will not be seen on your slide. The cells of the follicle not only produce hormones, but they also supply nutrient materials to the egg, as the surface of both the oocyte and the follicle cells have microvilli which are interdigitated and allow passage of materials into the egg. The region where there egg and follicle cell proceeses are found passes through a clear area around the egg, as seen under the microscope, the zona pellucida, and through the vitelline membrane, a glycoprotein layer on the outside surface of the egg. The surface of the ovary bulges at the site of follicle, which has moved to the surface and has thinned out the tissue overlying it. The follicle breaks and the fluid and the secondary oocyte surrounded by the cumulus is released. The egg starts its second meiotic division, but is arrested once again in the middle of the division, and will not complete it unless a sperm penetrates it. Sometimes ovulation is accompanied by pain as the ovary surface is broken through by the follicle. The cells which remain behind (the granulosa and theca) give rise to the corpus luteum (yellow body) in response to LH and start to secrete progesterone. Find, measure , and draw a corpus luteum. It looks like a large round solid mass since the antrum has been filled in by the follicle cells and blood vessels are seen in it. If it is not on your slide look for another one. The high levels of estrogen and progesterone cause buildup of the uterine wall and glands and also causes the inhibition of release of FSH and LH stimulating factors from the hypothalamus, so the pituitary stops releasing FSH and LH, and menstruation starts, the corpus luteum degenerates about 9 days after ovulation and becomes a corpus albicans (scar tissue) and the whole ovarian cycle starts again. Can you see a corpus albicans on your slide? When the pill is taken as contraception, the level of estrogen and progesterone is raised artificially, and that prevents the production of FSH and LH needed to get follicle maturation and ovulation. When pregnancy occurs, the corpus luteum is maintained by the chorionic gonadotropin hormone like LH made by the embryo and the pituitary is more or less shut down as far as menstrual cycle hormones.

Ascaris egg maturation and fertilization. There are two slides of the Ascaris ovary. They contain two different segments of the ovary, and the eggs are of different stages following sperm entry. The unfertilized eggs lack a fertilization membrane, and you can see the triangular amoeboid sperm around.them. They contain a nucleus with two large chromosomes. These are primary.oocytes. After sperm entry, you can see the sperm inside the egg, with its dark chromosomes, and you can see the beginning of an elevated

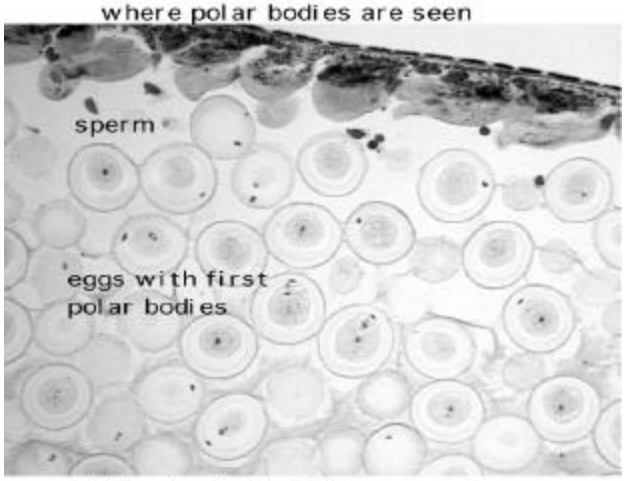
fertilization membrane and the beginning of the meiotic divisions of the egg, with visible tetrads. This is the material which Boveri used to discover meiotic division.

Ascaris eggs at the primary oocyte stage, starting meiosis I

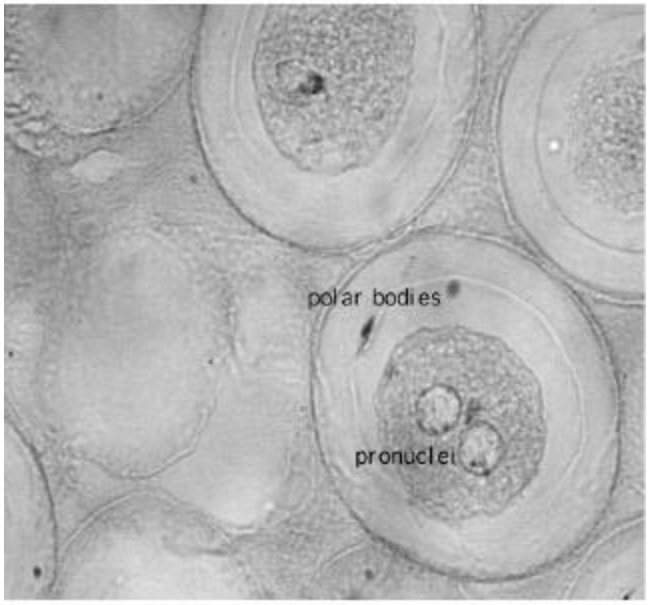


DRAW A PRIMARY OOCYTE AT FIRST MEIOTIC METAPHASE with tetrads and sperm nucleus. This division results in formation of the secondary oocyte and the first polar body which can be seen on the next.section, out under the fertilization membrane which is well lifted off the egg with a perivitelline space, and a shell being deposited on the outside of the egg by the oviduct. **Find the second meiotic division, with two.dyads and a polar body. DRAW.** This division results in the second polar body, and the mature ootid. After the maturation division, there is fusion of pronuclei and return to the diploid chromosome number. DRAWINGS:make drawings of all stages as seen on your slide and label them using your text to discover which is which.

Ascaris second meiotic division shell has been deposited over fertilization membrane, space has formed around egg where polar bodies are seen



Ascaris mature oocyte with 2 polar bodies and male and female pronuclei preparing to fuse



OBSERVE SLIDES OF VAGINA, UTERUS AT VARIOUS STAGES OF MENSTRUAL CYCLE. See pg 20 of text. How can you tell which is the inner side or outer side? What is different about the uterus at various stages of the menstrual cycle?

References: can be found on the computer in the classroom for you to copy.

1. Carlson, B., *Human Embryology and Developmental Biology* 4th ed. 2005, Philadelphia: Mosby.
2. Payne, C. and G. Schatten, *Golgi dynamics during meiosis are distinct from mitosis and are coupled to endoplasmic reticulum dynamics until fertilization.* Developmental Biology, 2003. **264**(1): p. 50-63.
3. Vigneron, C., Perreau C, Dupont J, Uzbekova S, Prigent C, Mermillod P., *Several signaling pathways are involved in the control of cattle oocyte maturation.* Mol Reprod Dev., 2004. **69**(4): p. 466-74.

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ANSWER SHEET FOR INVESTIGATION #1 EGG MATURATION, FOLLICLE,

CONCLUSIONS : What kind of meaningful statement can you make about the Comparison of the sizes of the various follicles?

What kinds of factors cause the changes?

Are there different functions of the different cell types?

NAME SOME FACTORS IMPORTANT IN REGULATING EGG MATURATION AND OVULATION and tell their functions.

What is the difference in the stage of maturation of the egg at the time of sperm penetration in humans, sea urchins, and Ascaris?.What factors trigger meiosis in each case?

How do hormones or sperm cause these resulting changes?

Make a diagram of kinases, phosphatases, phospholipases, calcium channels , that control meiosis and their associations with pituitary hormone receptors.

Make a diagram of chain of events resulting from steroid hormone stimulation in a cell.