ONPRC Hematoxylin & Eosin (H & E) Ovary Staining Laboratory: Female Reproductive System & Regulation of Ovarian Function

Guiding Question:

How does the female reproductive system work?

Question	Laboratory Questions
What are the important parts of the female reproductive system, and how does the menstrual cycle work?	• How does a scientist obtain ovaries for a study? • How do researchers look at follicle morphology? • How does female reproductive anatomy differ between mammalian species (mice, humans, monkeys, sheep, horses, cats, dogs)? • What can female reproductive anatomy tell us about pregnancy in the different species?

Learning Outcomes:

Identify female reproductive anatomical structures of different species (mice, humans, monkeys, sheep, horses, cats, dogs).

Explain the ovarian cycle (process of follicular development, ovulation, corpus luteum formation).

Explain the menstrual cycle (changes that occur in the uterus under the influence of ovarian hormones).

Define the source and function of hormones involved in the female reproductive system.

Reproductive Vocabulary

Reproductive Science: A branch of science that deals with the mechanism and regulation of reproductive processes and the diagnosis and treatment of reproductive disorders.

Oncofertility: A new discipline that makes connections between oncology and reproductive medicine, providing viable fertility preservation options for people with cancer and other fertility threatening diseases.

Ovarian Follicle Vocabulary

Oocyte: a haploid (possessing half the number of chromosomes found in other cells in the body) female reproductive cell or gamete; also called an egg

Ovary: female reproductive organ that produces oocytes and the female sex hormones

Ovarian Follicle: functional unit of the ovary, contains an oocyte surrounded by granulosa cells and an outer layer of theca cells

Granulosa Cells: estrogen-secreting somatic cells of cuboidal shape on the inside of an antral follicle or surrounding the oocyte in a preantral follicle

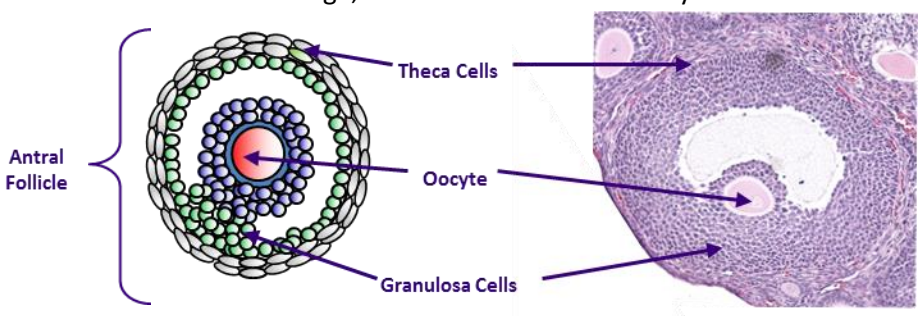
Theca Cells: an enveloping sheath of somatic cells of flattened shape surrounding the granulosa cells of the follicle; a site of androgen substrate production in females which are used in the granulosa cells to produce estrogen

Pre-antral follicle: a follicle of one or many layers of granulosa cells surrounding a developing oocyte

Antrum: fluid-filled space inside a growing follicle

Antral follicle: a fluid-filled follicle within the ovary that houses a developing oocyte

Dominant or Graafian follicle: a large, mature follicle that is ready to ovulate



Drawing and Photo: Oncofertility Consortium

Histology Vocabulary

Morphology: The form and structure of an organism or any of its parts.

Histology: The study of cells and tissues at the microscopic level, branch of anatomy dealing with tissue structure.

Fixation: The preservation of a tissue sample to retain the structure it had in the body. Generally uses a chemical fixative, like formaldehyde.

Embedding: The process of placing a tissue into a preserving material (such as paraffin wax) that provides a support matrix for further manipulation of the tissue, such as sectioning.

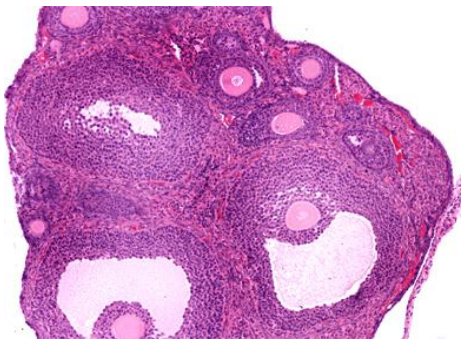
Microtome: A machine designed for cutting uniform (and often very thin) sections of a specimen, such as a paraffin-embedded tissue like an ovary.

Cryostat: A cold temperature chamber containing a microtome used to prepare thin sections from frozen tissues. The tissues are often maintained at -20 degrees Celsius.

Sectioning: Using a microtome or cryostat to make thin sections of tissue (~ 5 microns) that are placed on microscope slides for further analysis

Hematoxylin & Eosin (H&E) Staining: A commonly used morphologic stain for tissue sections; It is the most widely used stain in medical diagnosis; when a pathologist looks at a biopsy of a suspected cancer, the histological sectioni s likely to be stained with H&E.

Hematoxylin colors basophilic structures with blue-purple hue. The basophilic structures are usually the ones containing nucleic acids, such as the cell nucleus.
Eosin Y colors eosinophilic structures bright pink. The eosinophilic structures are generally composed of intracellular or extracellular protein; most of the cytoplasm is eosinophilic; red blood cells are stained intensely red.
The cell structures do not have to be acidic or basic to be called basophilic and eosinophilic. The terminology is based on the attraction of cell structures to the dyes.



H&E Stained Section of a Mouse Ovary

Photo: Onfertility Consortium

the Oncofertility™ Consortium

How do researchers look at follicle morphology?

Histology Procedure:

1. Dehydration

The processor removes water from tissue incrementally by increasing ethanol concentrations and replacing ethanol with paraffin wax.

2. Embedding

Wax-filled tissue is placed in a mold with paraffin and allowed to harden.

3. Sectioning

Molded paraffin blocks are sliced into 5 micrometer (µm) sections using a microtome, floated on a water bath, and placed on glass microscope slides.

4. Staining

Tissue slides are deparaffinized and re-hydrated, then dipped into nuclear and cytoplasmic stains to show mophology.

Photos: ONPRC

Questions to Think About:

Why would someone need to look at the morphology of a follicle?

Students Notes or Questions:

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How can we identify follicles in the ovary?

Purpose: To identify follicles in paraffin sections of monkey ovarian tissue by staining with hematoxylin and eosin

Experiment :

Each student should select one slide containing paraffin sections of ovaries (the slide will have multiple sections incubated).

Each pair of students, place your slides into a slide chamber and follow instructions for H&E lab on the next page.

Then, cover slip your slides (Mary will demo).

Place slide with the tissue sections facing up.

Using an applicator stick, smear a generous amount of mounting medium (DMX) in a line horizontally across the bottom of the slide. Don't worry about putting on too much or about smearing, we can clean this off later.

Place a large cover slip on the bottom of the slide on top of the mounting medium and drop gently onto the slide (you should see the mounting medium flow underneath the entire cover slip.

Using a forceps, CAREFULLY move bubbles to the edge of the cover slip (bubbles will dry out your sections and by separating the cover slip from the section).

Leave slides (under the hood) to dry until our next class.

Use clear nail polish to make a seal around the outside edge of the coverslip.

Dip a chemwipe into Citra and run along edge of coverslip to remove excess mounting medium. Clean entire slide with Windex.

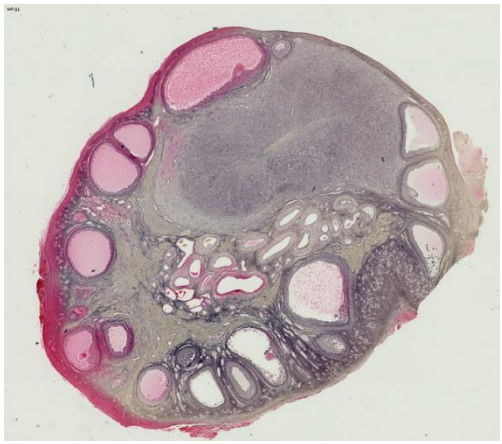
Admire your fabulous work under the microscope ☺

BE SAFE:

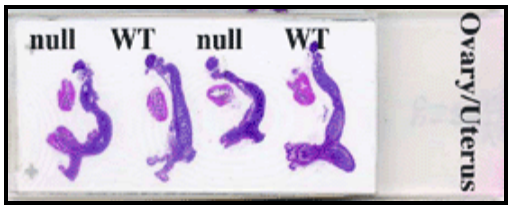
Take care with the glass coverslips, they are very fragile

When working with Citra and DMX, wear gloves and work under the hood

Activity: Place slide under the microscope and look at 4X, 10X and 20X. Identify each class of follicle.



A section of the monkey ovary showing histology.



Microscope slide with mouse ovary/uterus sections

Photos: Dr. Mary Zelinski, PhD, ONPRC

Each student should select one slide containing paraffin sections of ovaries (the slide will have multiple sections). Choose a partner and a timer.

Place your slides into a slide chamber, frosted end up, and place the chamber into the bucket in the rack containing Citra (start on the LEFT), let sit for 5 min.

Move slide chamber into the next bucket of Citra, let sit 5 min.

100% ethanol, 5 min.

100% ethanol, 3 min.

95% ethanol, 1 min.

70% ethanol, 1 min.

Fill water bucket with water, put on lab bench, place slide chamber in water, 5 min, blot on 3 layers of paper towels. Dump out water.

Place slide chamber into the bucket with hematoxylin, 5 min.

Fill a water bucket with water in sink, place slide chamber inside, run water GENTLY into bucket until the water runs clear, blot on 3 layers of paper towels. Dump water.

Dip chamber into acid alcohol bucket twice (2 dips).

Dip chamber into lithium carbonate bucket until sections turn blue, 15 dips.

Fill water bucket with water in sink, place slide chamber inside, run water GENTLY into bucket until the water runs clear, blot on 3 layers of paper towels. Dump water.

Place chamber into the bucket with eosin, 3 min.

Fill water bucket with water, put on lab bench, place slide chamber in water for 1 min.

Buckets in rack, now start from the RIGHT end:
70% ethanol, brief dip

95% ethanol, brief dip

95% ethanol, brief dip

100% ethanol, 1 min

100% ethanol, 1 min

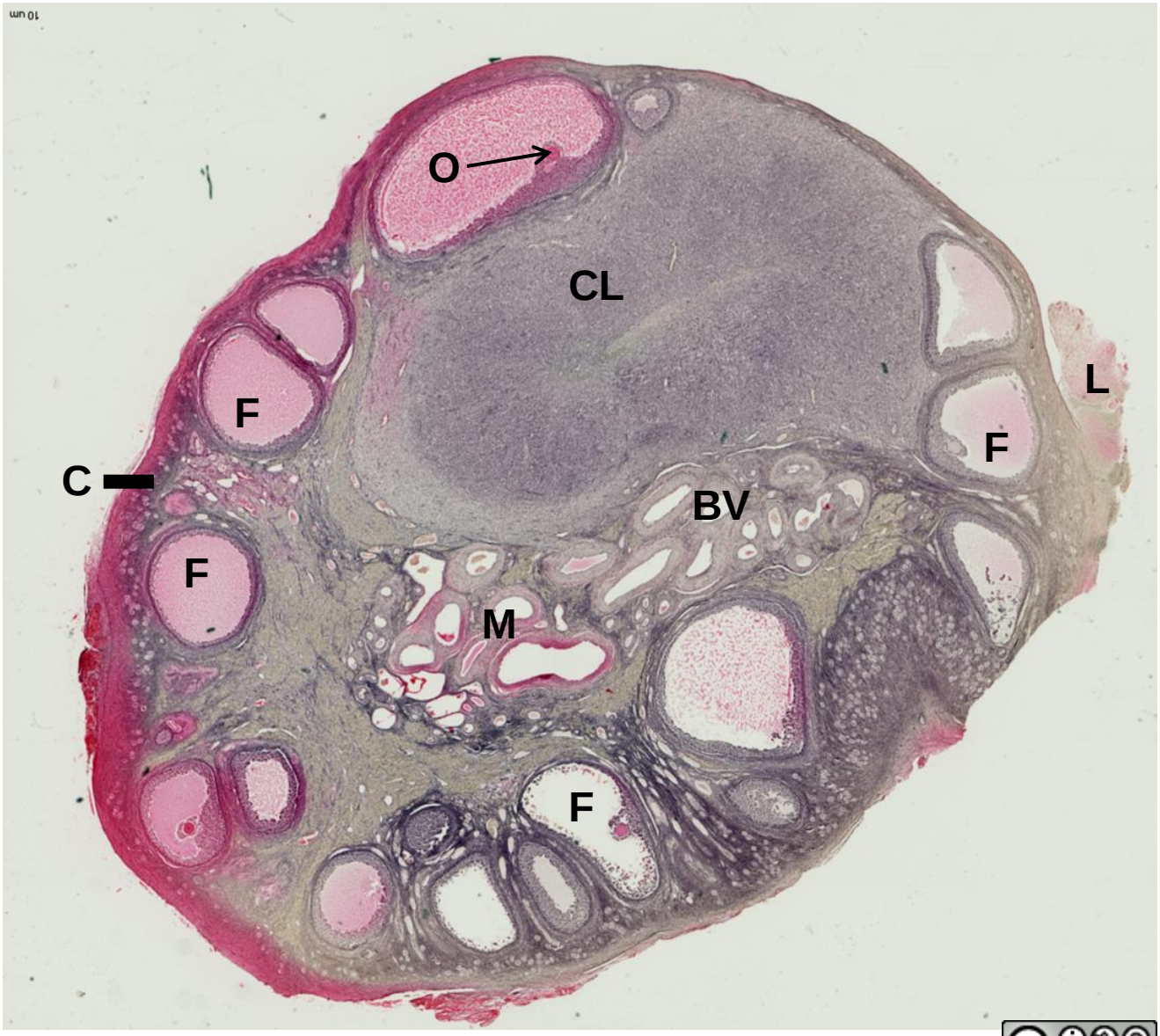
Citra, 1 min

Citra, 1 min

Citra, 1 min **Keep in Citra until ready to cover slip (see demonstration)**

Section through the middle of a rhesus monkey ovary stained with hematoxylin and eosin

Photo: Dr. Mary Zelinski, PhD, ONPRC



C = _____ CL = _____

F = _____ M = _____

O = _____ BV = _____

What classes of follicles would you find in C? L = _____

_____

How do researchers look at follicle morphology?

Draw each type of follicle and label the following cell types:

Oocyte, Granulosa Cells, Theca Cells, Antrum

Primary Follicle
1 cell layer

→

Secondary Follicle
≥2 cell layers

→

Antral Follicle
multiple layers

Questions to Think About:

How many follicles are in a mouse ovary? A human ovary?

How do the number of follicles in the ovary change with age?

Why would a researcher want to grow a follicle by itself?

Students Notes or Questions:

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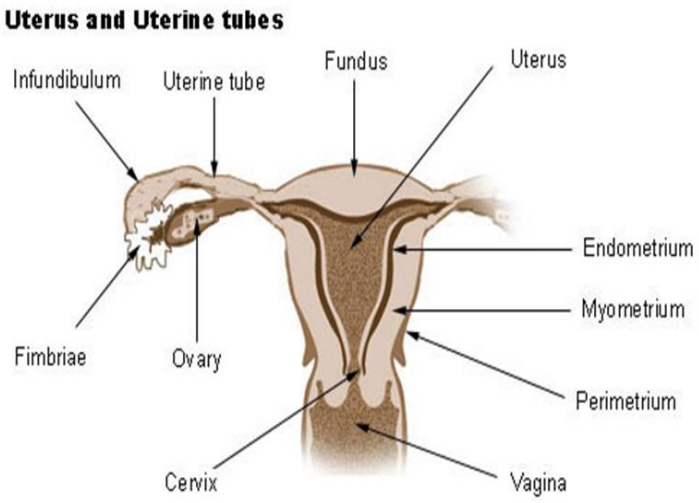
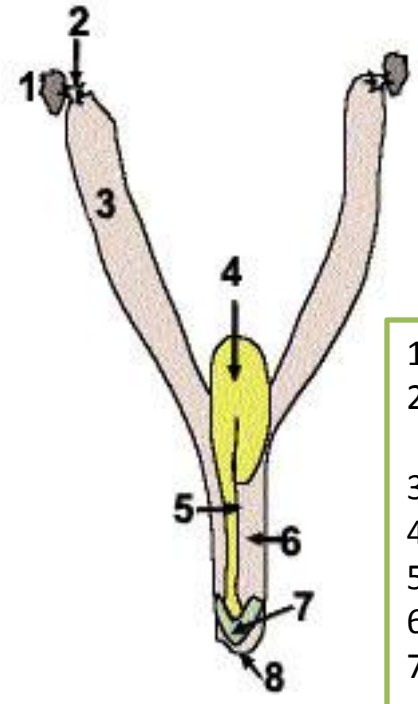
How do researchers perform preliminary studies for human research?





1. Ovary
2. Uterine tube
3. Uterus
4. Bladder
5. Urethra
6. Vagina
7. Clitoral gland
8. Vulva

Human

http://training.seer.cancer.gov/anatomy/reproductive/female/tract.html

Mouse

Drawing: Oncofertility Consortium

the Oncofertility™ Consortium

Questions to Think About:

What differences are there between the mouse and human reproductive tracts?

Why do researchers use mice for research?

Why do researchers use monkeys for research?

Students Notes or Questions:

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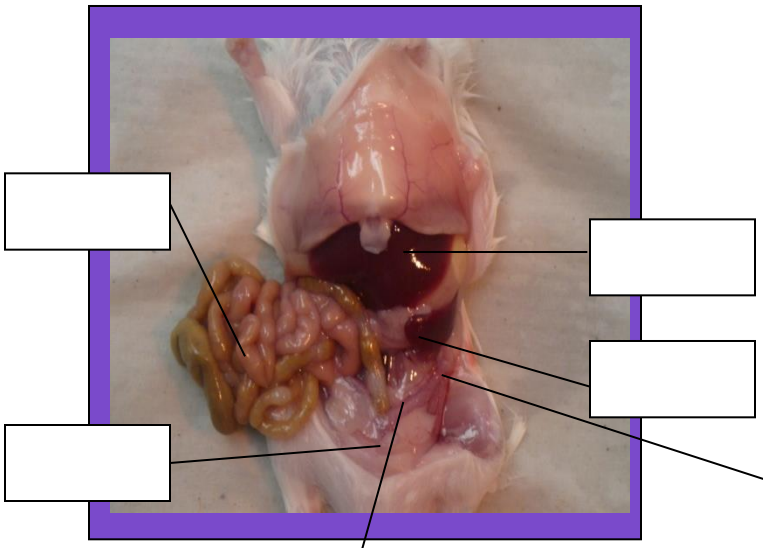
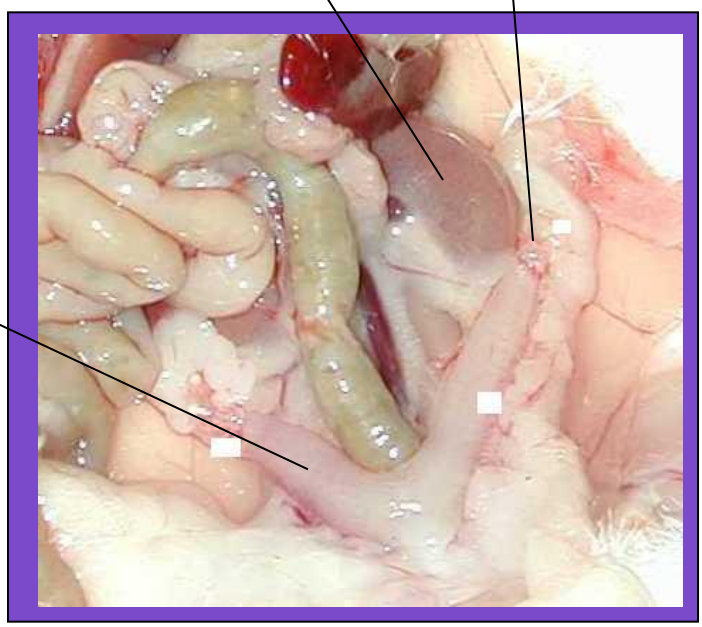
How do researchers perform preliminary studies for human research?

Identify the mouse structures below.



Fill in the blanks with the following words:

Ovary
Liver
Intestines
Kidney
Bladder
Uterus
Spleen



Photos: Oncofertility Consortium