Teacher's Guide for Hematoxylin and Eosin Lab

Equipment:

1 staining rack with:
- 5 staining buckets of Citra (removes paraffin from tissue)
- 4 staining buckets of 100% ethanol (dehydrates)
- 3 staining buckets of 95% ethanol (dehydrates)
- 2 staining buckets of 75% ethanol dehydrates)
- 1 staining bucket of Hematoxylin (stains nucleic acids in tissues – as well as skin, clothes, books, etc.)
- 1 staining bucket of Eosin Y (stains intracellular and extracellular protein so stains cytoplasm – as well as skin, clothes, books, etc.)
- 1 staining bucket of acid alcohol (dehydrates and prepares tissue for taking in Eosin Y)
- 1 staining bucket of lithium carbonate (neutralized the acid)

4 buckets for water set on lab bench (rinses)
Per student group, 1 slide chamber dipper to hold slides to be immersed in staining buckets
Slide per student containing paraffin section of tissue
Coverslip per student
Permount
Small diameter wooden application stick for applying Permount
Clear nail polish

The staining buckets and rack(s) can be found at Market Lab http://www.marketlab.com/coplin-jars-stain-trays-tissue-dye/c/stains-kits-accessories/ Many high school teachers find the Stain Train Jars and Organizer easier to use than the Compact Staining Bank option. The jars hook together so no rack is needed and the volume of the jars is less than that of the Compact Staining Banks buckets.

Citra (Citra Clearing Solvent), Hematoxylin, Eosin Y, and Permount can be ordered through Fisher Scientific.
https://www.fishersci.com/us/en/catalog/search/products?keyword=citrus+clearing+solvent&nav

Ethanol, lithium carbonate, slides, cover slips, and wooden applicator sticks can be ordered from Carolina Biological Supply
http://www.carolina.com/chemistry/specialty-chemicals/specialty-chemicals-d-l/10176.ct?N=3985816713&intid=srchredit_ethanol

To make the lithium carbonate solution, add 0.80 grams of lithium carbonate to 250 ml of distilled water.
To make the acid alcohol solution, add 10 ml of 12M HCl to 1000 ml of 70% ethanol.

Answers to questions:

Page 4
Someone would need to look at the morphology of a follicle to see the stage of development of the follicle. In particular, one is looking to see how close to ovulation the follicle is.

Page 7
C= Cortex
F= Follicle
O= Oocyte
CL= Corpus luteum
M= Medulla
BV= Blood vessel
L= Ligament
Classes of follicles in C = primordial and primary follicles

Page 8
-For drawings, see Female Reproduction-ONPRC Module 1-Student Handout.
-There are around 500 follicles in a mouse ovary and 500,000 in a human ovary.
-As the woman ages, she loses ovarian function by the time she reaches menopause. By that time, there are very few functioning follicles since most have greatly decreased in size.
-A researcher would want to grow a follicle by itself (*in vitro*) to study how they grow. When ovarian tissue is cryopreserved (frozen), the unthawed follicles will need to grow to the antral follicle stage in order to ovulate and release the oocyte.

Page 9

-The mouse reproductive tract has 2 uterine tracts while the human has one so the mouse can have multiple babies form in the uterus while humans typically have only one. The mouse uterine (fallopian) tube is very short while the human tube is much longer. The bladder is in located approximately in the same place in both.

-Researchers use mice for research since they are a small mammal, produce many litters of babies, produce many oocytes and many babies per litter, and have a short lifespan.

-Researchers use monkeys for research since they most closely resemble the human female reproductive system.

Page 10

Answers on the upper photo, left side, top to bottom are:
Intestines
Bladder
And on the upper photo, right side, top to bottom are:
Liver
Spleen

Answers on the lower photo are:
Left – Uterus (also points to upper photo)
Center – Kidney
Right – Ovary (also points to upper photo)