LABORATORY 5
PLACENTA & MAMMARY GLAND

OBJECTIVES:

At the end of this lab, you should be able to:

1. recognize and describe the structure and functions(s) of:
   - chorionic plate (fetal side of the placenta)
   - decidua basalis (maternal side of placenta)
   - chorionic villi (primary, secondary & tertiary)
   - stem villi
   - anchoring villi
   - syncytiotrophoblast
   - cytotrophoblast
   - Hofbauer cells
   - decidual cells
   - maternal blood space
   - amnion

2. distinguish between an early and a late placenta

3. recognize the umbilical cord, umbilical arteries & umbilical vein

4. describe the general histology of the mammary gland

5. distinguish between the inactive mammary gland of the non-pregnant female, the proliferating gland of pregnancy, and lactating gland

6. describe the modes of secretion of the alveolar cells during lactation

LABORATORY:

Study the following slides:

I. PLACENTA (See diagram at the end of this section)
   Slide 98: Placenta
   The placenta has a fetal component called the chorion frondosum and a maternal component called the decidua basalis. This slide shows only chorion frondosum, which consists of the chorionic plate and the chorionic villi that extend from it. The chorionic plate can be distinguished from the decidua basalis by several criteria including:

1. The pale appearance of the chorionic plate, which reflects the highly hydrated nature of the ground substance in fetal tissue.

2. Larger blood vessels (which are main branches of the umbilical arteries and vein) may be visible in the chorionic plate; most of the maternal blood vessels in the decidua basalis tend to be much smaller.

3. Early in development the fetal red blood cells are nucleated. Later in development they will be anucleate. Red blood cells in the maternal blood vessels of the decidua basalis are not nucleated at any time. Therefore if you see nucleated erythrocytes in a blood vessel, you know you are looking at the fetal portion of an early placenta.
The chorionic villi are initially composed of a core of cytotrophoblast cells covered by the syncytiotrophoblast. Such villi are called primary villi. Soon mesenchyme extends into the villi to form their cores, and the villi are then called secondary villi. Finally fetal blood vessels grow into the mesenchyme, and the villi are then called tertiary villi. Primary and secondary villi are present only very early in development, so the great majority of the villi on these slides are tertiary. Within the mesenchymal core of some villi it is possible to identify Hofbauer cells. These are usually the only spherical cells in the mesenchyme. They have a spherical nucleus, which is usually centrally located, and a pale-staining lacy cytoplasm. They are macrophages, and may also play a role in angiogenesis within the villi.

The chorionic villi project from the chorionic plate into the intervillous space (maternal blood space). Both the chorionic plate and the villi are lined by cytotrophoblast and syncytiotrophoblast, with the syncytiotrophoblast being in direct contact with the maternal blood in the intervillous space. Maternal blood enters the intervillous space via the spiral arteries of the uterine endometrium. Exchange between fetal and maternal blood occurs across the syncytiotrophoblast, cytotrophoblast, mesenchyme of the villus, and endothelium of fetal capillary.

Slide 98A: Placenta, 2 Months
Slide 99: Placenta, 4½ Months
Slide 100 (HU Box): Placenta, Human

(NOTE: Slide 99 shows a somewhat earlier stage of development than Slides 98A or 100HU. Also, some versions of Slide 99 have two serial sections of the same placenta side by side. Do not be confused and think that one is the fetal side and the other the maternal side. Both are fetal side).

All these slides show later placentas than Slide 98. The features that allow you to conclude this include:

1. The villi are more highly branched.
2. Fewer fetal red blood cells are nucleated.
3. The layer of cytotrophoblast cells has become discontinuous in places. This is due to the fact that cytotrophoblast cells are fusing with the syncytiotrophoblast faster than they are replicating to produce more cytotrophoblast. Eventually virtually all the cytotrophoblast cells will fuse with the syncytiotrophoblast.

Slide 100: Placenta, and
Slide 100A: Placenta, Late

Both of these slides are of late placenta, which differs from the midterm placenta in the following ways:

1. The villi are even more extensively branched and more closely packed within the maternal blood spaces.
2. The cytotrophoblast is almost completely gone.
3. “All” fetal red cells are non-nucleated. The nuclei you may see within fetal vessels belong to fetal leukocytes.
4. Most fetal blood vessels have moved closer to the periphery of the villi so that they are located just beneath the syncytiotrophoblast. This has the advantage of decreasing the diffusion distance that nutrients & wastes must travel between the fetal & maternal circulation.

5. In many places the nuclei of the syncytiotrophoblast cluster together to form syncytial knots (trophoblastic knots). This leaves long stretches of syncytiotrophoblast where only a thin layer of syncytiotrophoblast cytoplasm separates the fetal blood vessels from the maternal blood space. This is thus another mechanism for minimizing the thickness of the maternal-fetal blood barrier. Syncytial knots frequently pinch off from the villi and enter the maternal circulation. Some of these become emboli in the maternal blood and lodge in the maternal lung. They seem to cause relatively few clinical problems.

6. There is an increase in the amount of a very eosinophilic material called fibrinoid in the extracellular space of the chorionic plate, the villi and especially the maternal side of the placenta.

Slide 100HU and most versions of Slide 100 show both sides (maternal and fetal) of the placenta. The maternal component of the placenta is the decidua basalis, which is the portion of the uterine wall to which the anchoring villi of the fetal component attach. In this slide distinguish the maternal side of the placenta (decidua basalis) from the fetal side (chorionic plate), using the following criteria:

1. The paler appearance of the fetal side due to its highly hydrated ground substance.
2. The large blood vessels in the chorionic plate.
3. The presence of decidual cells on the maternal but not the fetal side of the placenta.
4. The amnion lies immediately adjacent to the fetal side in the late placenta. It is a simple low cuboidal epithelium, and is visible on some versions of Slide 100. It is located on the opposite side of the chorionic plate from the maternal blood space.
5. The larger amount of fibrinoid associated with the maternal side.

Decidual cells are evident in the decidua basalis on Slide 100. They are large, pale, rounded cells with a relatively large amount of cytoplasm. They are derived from stromal cells of the uterus (i.e., maternal connective tissue). By LM they cannot be distinguished from peripheral cytotrophoblast cells, which are also present in the decidua basalis. Peripheral cytotrophoblast cells are derived from the cytotrophoblast of anchoring villi. They eventually form a continuous layer (the outer cytotrophoblast shell). This makes up the interface with the maternal connective tissue (in the endometrial layer of the uterus).

Any large villus that is directly continuous with the chorionic plate is a stem villus. Any villus that is directly continuous with the decidua basalis is an anchoring villus. If you could follow an anchoring villus from the decidua basalis all the way back to the chorionic plate, you would find that it was also a stem villus.
II. UMBILICAL CORD

Slide 34 (HU Box): Umbilical Cord, Human, c.s.

This is a cross section through the umbilical cord of a full term fetus. Note the mucoid connective tissue (Wharton’s jelly) making up the core of the cord. The amnion forms the outer epithelial covering of the umbilical cord. Distinguish between the two umbilical arteries and one umbilical vein. The arteries have more orderly walls in which you can more easily see the difference between the smooth muscle layer (the tunica media) and the outer layer (the tunica adventitia), which contains connective tissue. The umbilical arteries carry deoxygenated blood from the fetus to the placenta. Each develops from one of the two dorsal aortas of the fetus. The umbilical vein carries oxygen-rich blood from the placenta to the fetus. It is called a vein because it returns blood to the fetal heart.

III. MAMMARY GLAND

The mammary glands of a reproductive age female may be classified as:
1. inactive or resting
2. proliferating during pregnancy
3. lactating
Inactive or resting:
The lobules in an inactive breast consist of a relatively small number of ducts with relatively few alveolar buds. The alveoli cannot be readily distinguished from cross sectioned ducts. The ducts and alveoli within a lobule are surrounded by loose intralobular connective tissue, while the lobules are separated from one another by considerable amounts of dense interlobular connective tissue. The inactive glands of a mature female resemble the glands of a prepubertal female except that in the mature individual there is more adipose tissue and the ducts have more branches.

Pregnancy:
During pregnancy there is rapid growth and branching of the ducts, and development of alveoli made up of secretory cells. In comparison with the inactive breast, each lobule now appears larger and more crowded with cross sections of ducts and alveoli. The amount of connective tissue (especially fat) between lobules decreases, thus forming thinner interlobular septa. It is still difficult to distinguish ducts from secretory alveoli unless the ducts are cut in longitudinal section, revealing their tubular rather than spherical shape. Plasma cells become more numerous in the intralobular connective tissue, since they are producing the antibodies that are secreted into the colostrum and then the milk.

Lactating:
In lactating mammary glands there is an extreme proliferation of secretory alveoli. This causes the lobules of the gland to increase so much in size that they replace most of the interlobular connective tissue, making it difficult to see the septa between lobules. The alveoli become greatly distended by eosinophilic secretory material (colostrum and then milk; colostrum actually begins to accumulate in late pregnancy so that there is a ready supply of food for the infant immediately after birth). It is not unusual for the appearance of neighboring alveoli to vary considerably; some may have a cuboidal epithelium and a relatively small lumen while adjacent alveoli have a more squamous epithelium and a much wider lumen.

Please study as many of the following slides of the mammary gland as you can, and determine whether they represent the inactive, pregnant or lactating condition. Do not rely on the labels on the slides, since “active” mammary gland may refer to either the pregnant or lactating condition, and some of the slides simply appear to be mislabeled. Also, not all versions of the same slide number show mammary glands at the same stage, making it impossible for us to give you a list of which slide number shows what.

Slide 20 (HU Box): Mammary Gland, Inactive, Human
Slide 95A: Only the version labeled “Mammary Gland, Human H9.351”
Slide 96: Breast, Bouins, H&E
Slide 96A: Mammary Gland, Human
Slide 97 (Two Versions): Mammary Gland or Mammary Gland during Pregnancy
Slide 95: Active Breast
Slide 95A: Only those labeled “Mammary Gland Form. H&E Homo 28 Yrs”
Slide 21 (HU Box): Mammary Gland, Active
Slide 96B: Mammary Gland, Human, Active
IV. ELECTRON MICROSCOPY (RHODIN)

PLACENTA

Study the structure of a tertiary villus (Figs. 34-57 to 34-59). Identify syncytiotrophoblast, cytotrophoblast cells, fetal blood vessels, and maternal blood space. Realize that the syncytiotrophoblast is a true syncytium (i.e., a multinucleate cell formed by the fusion of uninucleate cells). In Fig. 34-58 observe that the cytotrophoblast layer has already become discontinuous in places. Part of a Hofbauer cell (identifiable because of the lysosomes and phagosomes in its cytoplasm) is visible in this figure. Review the layers of the placental barrier (maternal-fetal blood barrier) shown in Fig. 34-59, and understand that later in pregnancy this barrier would be even thinner due to the absence of cytotrophoblast. In Figs. 34-57 & 34-58, distinguish between fetal and maternal red blood cells.

MAMMARY GLAND

Compare the light micrographs of resting (Fig. 34-63), proliferating (Fig. 34-64) and lactating (Fig. 34-65) mammary glands at low mag.

Study the alveoli of the lactating gland (Figs. 34-67 & 34-68). Notice that the milk in the lumen has two different morphologically recognizable components: large lipid droplets and small dark-staining protein particles (mainly casein). The protein particles are contained within membrane-bound vacuoles in the cytoplasm (Fig. 34-70) and are released by merocrine secretion. In contrast, the lipid droplets in the cytoplasm have no limiting membrane, and are released by apocrine secretion (Fig. 4-24). As a part of this secretory process the lipid droplet acquires a membrane by budding through the plasma membrane. Also notice in Fig. 34-68 that the secretory alveoli are surrounded by myoepithelial cells. Myoepithelial cells can be identified by EM because of their content of actin filaments, which are involved in contractility. These cells are often difficult to identify by LM.
## LABORATORY 5 CHECKLIST
PLACENTA & MAMMARY GLAND

### LIGHT MICROSCOPY

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<thead>
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<th>Structure</th>
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<tr>
<td>chorionic plate</td>
<td>decidua basalis</td>
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<tr>
<td>amnion</td>
<td>decidual cells</td>
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<tr>
<td>tertiary villus</td>
<td>anchoring villus</td>
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<td>stem villus</td>
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<td>cytotrophoblast</td>
<td>umbilical artery</td>
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<td>umbilical vein</td>
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<td>Wharton’s jelly</td>
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<td>Hofbauer cells</td>
<td>inactive mammary gland</td>
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<td>syncytial knots</td>
<td>proliferating mammary gland of pregnancy</td>
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<td>maternal blood space</td>
<td>lactating mammary gland</td>
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<td>early vs. late placenta</td>
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### ELECTRON MICROGRAPHS

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<tr>
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<td>apocrine secretion of milk lipids</td>
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<tr>
<td>cytotrophoblast</td>
<td>merocrine secretion of milk proteins</td>
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<td>maternal blood space</td>
<td>myoepithelial cells of mammary gland</td>
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<tr>
<td>fetal capillaries in placenta</td>
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**NOTE:** These checklists include MOST of the structures that you should be able to identify. Exams may include structures not on these lists.
FOCUS QUESTIONS
LAB 5: PLACENTA & MAMMARY GLAND

See whether you can answer the following questions. The correct answers are posted on the course website (http://neurobio.drexelmed.edu/education/ifm/microanatomy) under “Lab Focus Questions”.

1. When syncytial knots pinch off of placental villi and enter the maternal circulation, why do they preferentially end up in the maternal lung rather than in some other organ?
2. Are all anchoring villi part of a stem villus?
3. Through what vessel(s) does fetal blood enter the placenta? Trace its pathway within the placenta. Through what vessel(s) does it leave, and where does it go?
4. How does maternal blood enter and leave the placenta? What is its pathway within the placenta?
5. Within the placenta, maternal blood is always in contact with and confined by what cell layer?
6. As the placenta matures during pregnancy, what structural changes occur that result in increased efficiency of oxygen exchange between fetal and maternal blood?
7. Name some structural features that allow you to distinguish the maternal side of the placenta from the fetal.
8. What is a placental cotyledon?
9. What is the difference between a primary, secondary and tertiary placental villus?
10. Does a late placenta have primary and secondary villi as well as tertiary?
11. During pregnancy, why do plasma cells become more numerous in the intralobular connective tissue of the mammary gland?
12. Why can’t all the components of milk (lipid and proteins) be released by merocrine secretion?
13. Myoepithelial cells contract to help move secretory product into or along the duct system of a gland. In the lactating mammary gland, are the myoepithelial cells stimulated to secrete by direct innervation or by a hormonal signal?