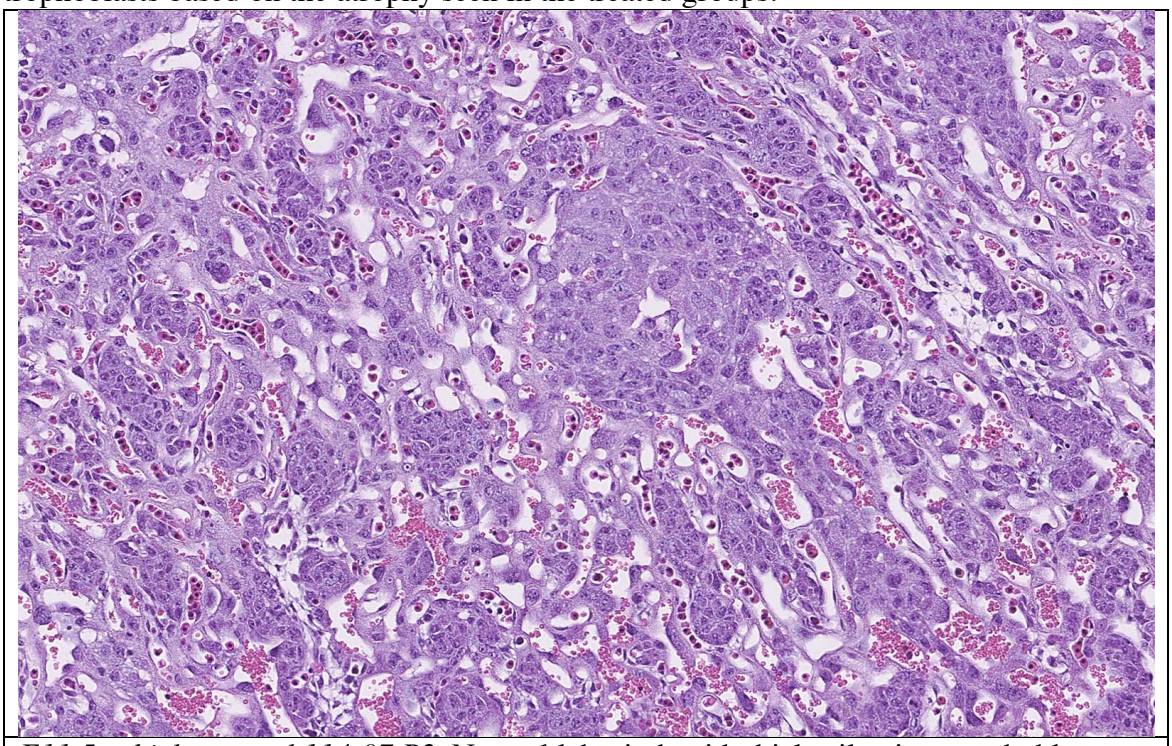
NIEHS Mouse Embryo Phenotyping Core
Group Leader, Susan A. Elmore elmore@niehs.nih.gov 919-541-3474
Support Staff: Brittany Scott, Tina Jones, and Beth Mahler

PFOA/GENX Study Results and Discussion

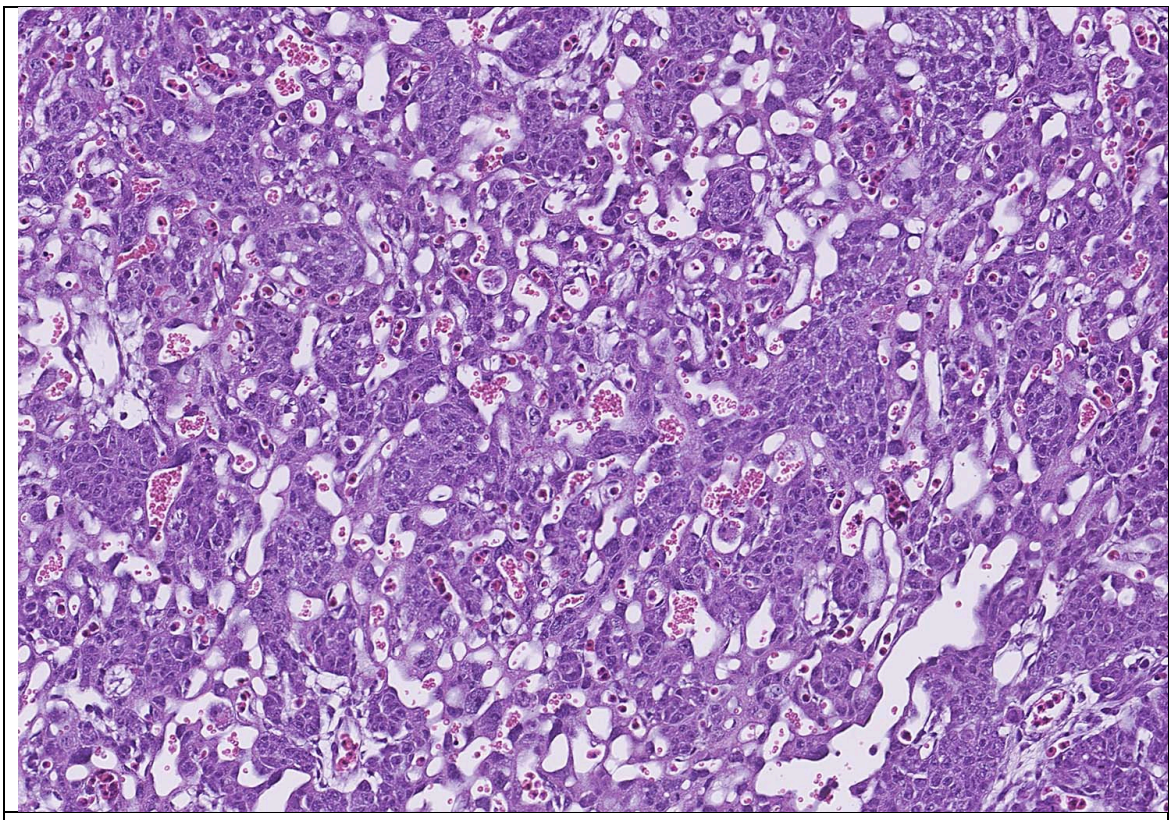
Placentas

In this study placentas from the right uterine horn were taken for histology and the left side was frozen by Fenton lab. Five slides were cut for each cassette (2 sections per slide) and the 1st and last slides stained with H&E. The intervening 3 slides may be used for special stains or immunohistochemistry. R on necropsy notes for "right" is denoted as "P" on histology slides. So R1=P1, R2-P2, etc. All aspects of the placentas were evaluated including the labyrinth, decidua and chorion. The metrial gland and yolk sac were also evaluated when present. If a placenta from the left horn had a gross lesion it was also collected for histopathological evaluation. Many of the lesions seen in this study were dam effects, affecting a majority or all of the embryos, although number of placentas per group are listed below.

Most of the placentas from *E11.5 vehicle controls* were within normal limits. Two embryos shared a placenta. 2 out of 38 were abnormal. The two figures below show the range of normal for the labyrinth from this group of vehicle controls. The focus was on the trilaminar trophoblasts based on the atrophy seen in the treated groups.



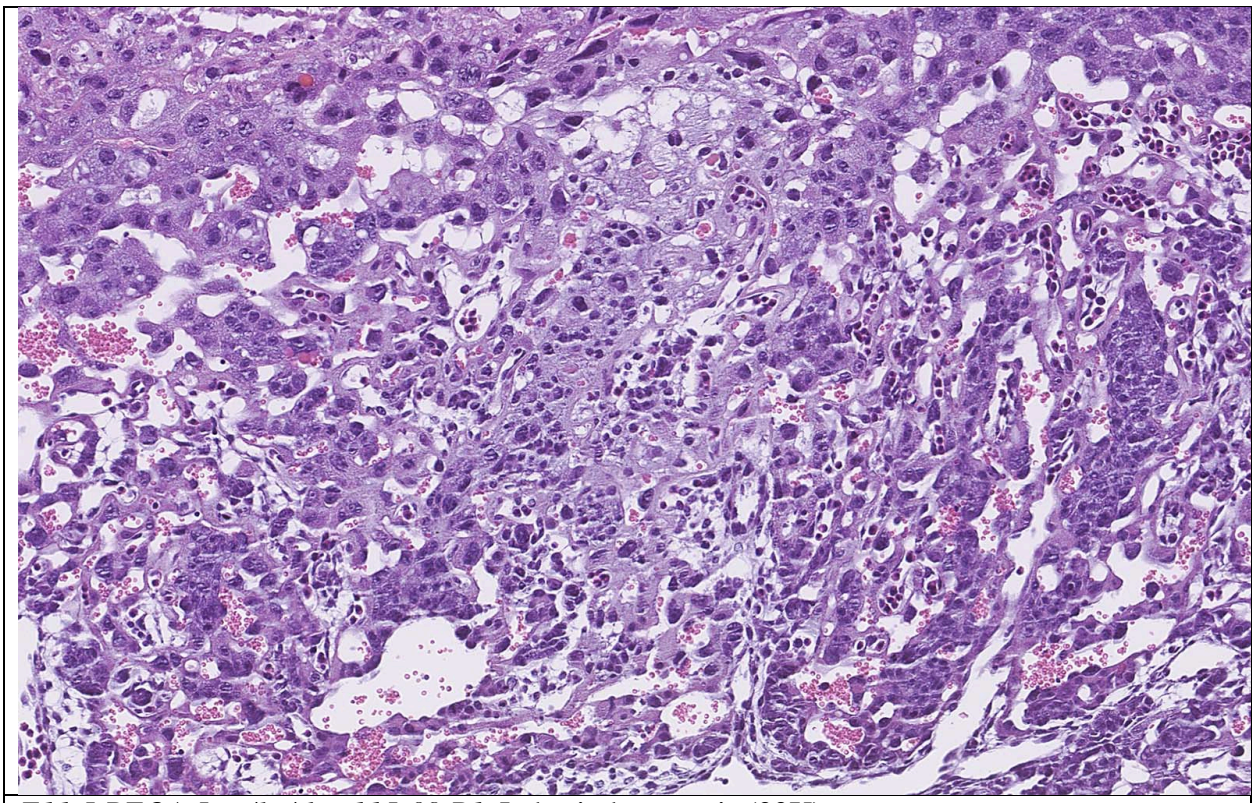
E11.5 vehicle control 114-07 P2. Normal labyrinth with thick trilaminar trophoblast layer (20X).



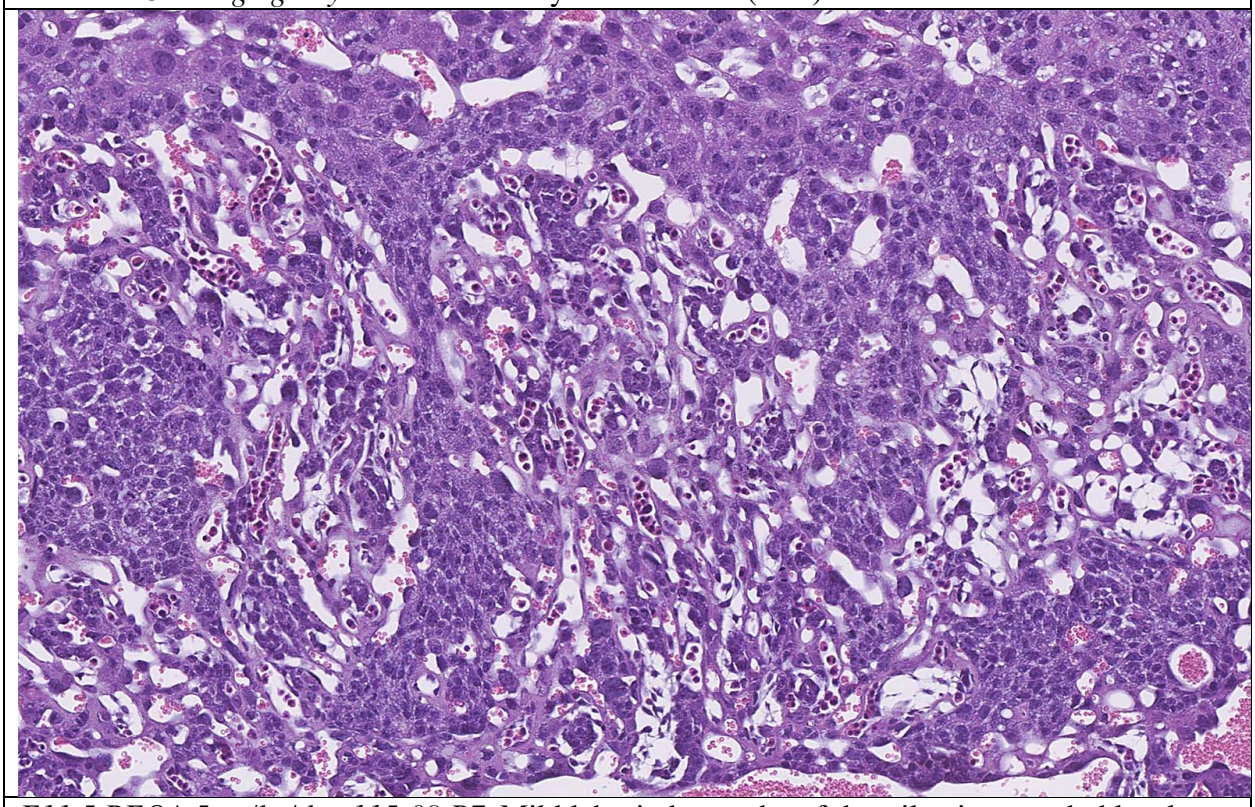
E11.5 vehicle control 114-07 P5. Normal labyrinth (20X).

Most of the placentas from the *GenX 2mg/kg/day* group were within normal limits. 3 out of 43 were abnormal. One placenta from the left horn was abnormal. Most of the placentas from the *Genx 10mg/kg/day* group were within normal limits. Two embryos shared a placenta (twins). 5 out of 34 were abnormal.

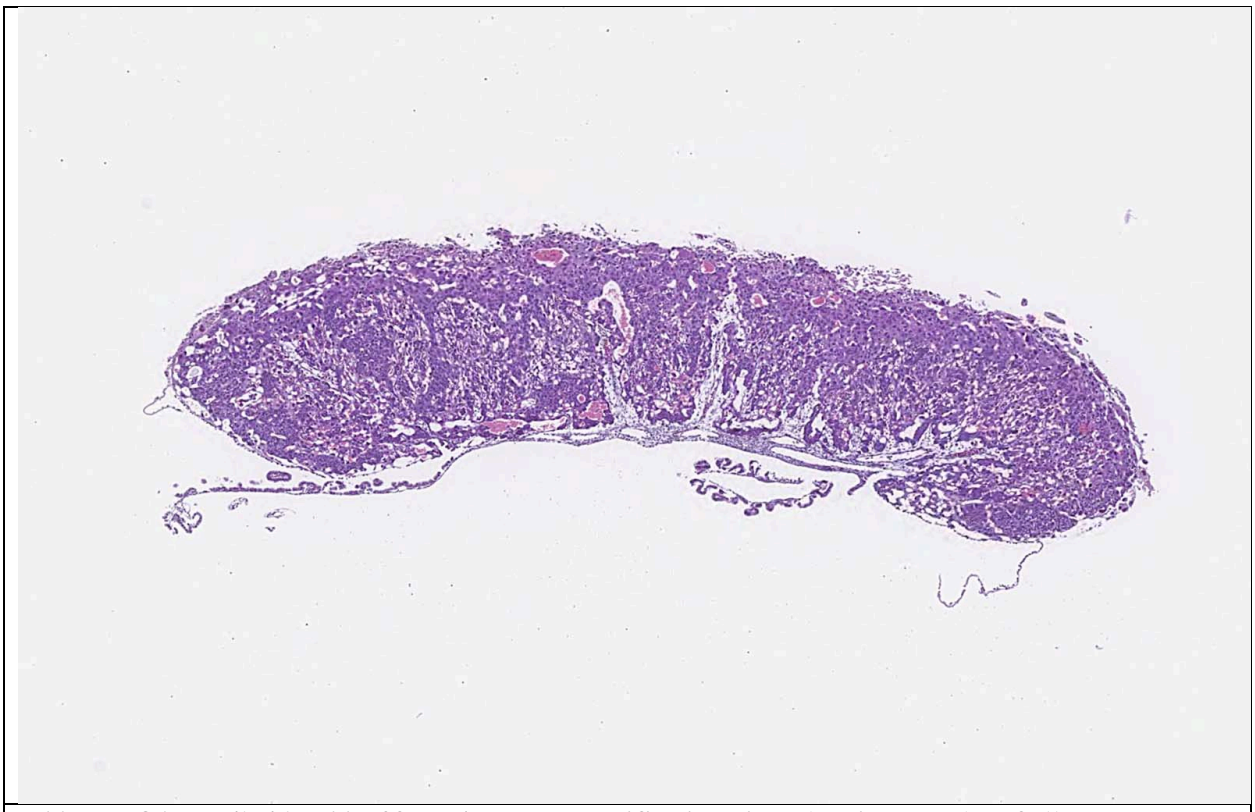
Most of the placentas from the *PFOA 1mg/kg/day* group were normal. 4 out of 36 were abnormal. One placenta from the left horn was consistent with twins. The majority of placentas from the *PFOA 5mg/kg/day* group were normal. 6 out of 39 were abnormal. Two embryos from the right horn shared a placenta (twins). The figures below illustrate labryrinth multifocal moderate necrosis and mild atrophy.



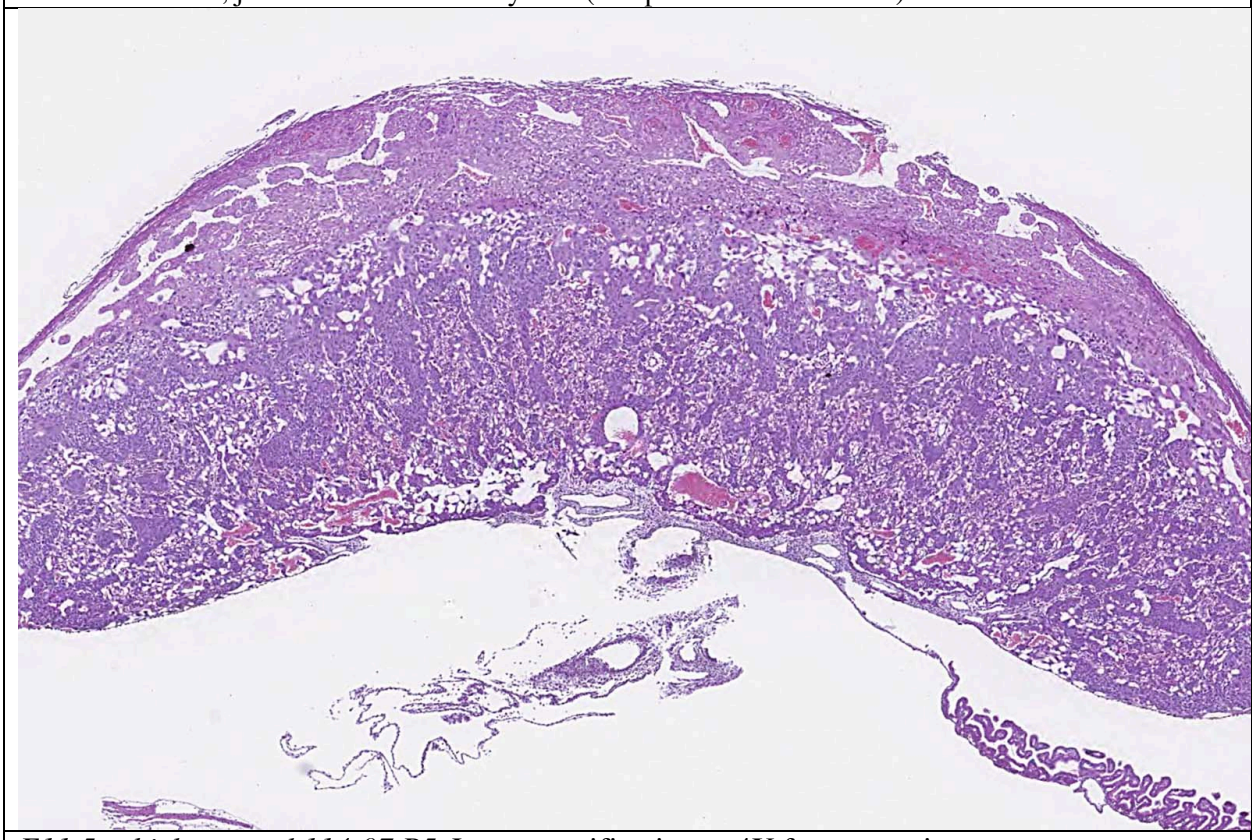
E11.5 PFOA 5mg/kg/day 115-09 P1. Labyrinth necrosis (20X).



E11.5 PFOA 5mg/kg/day 115-09 P7. Mild labyrinth atrophy of the trilaminar trophoblast layer (20X).

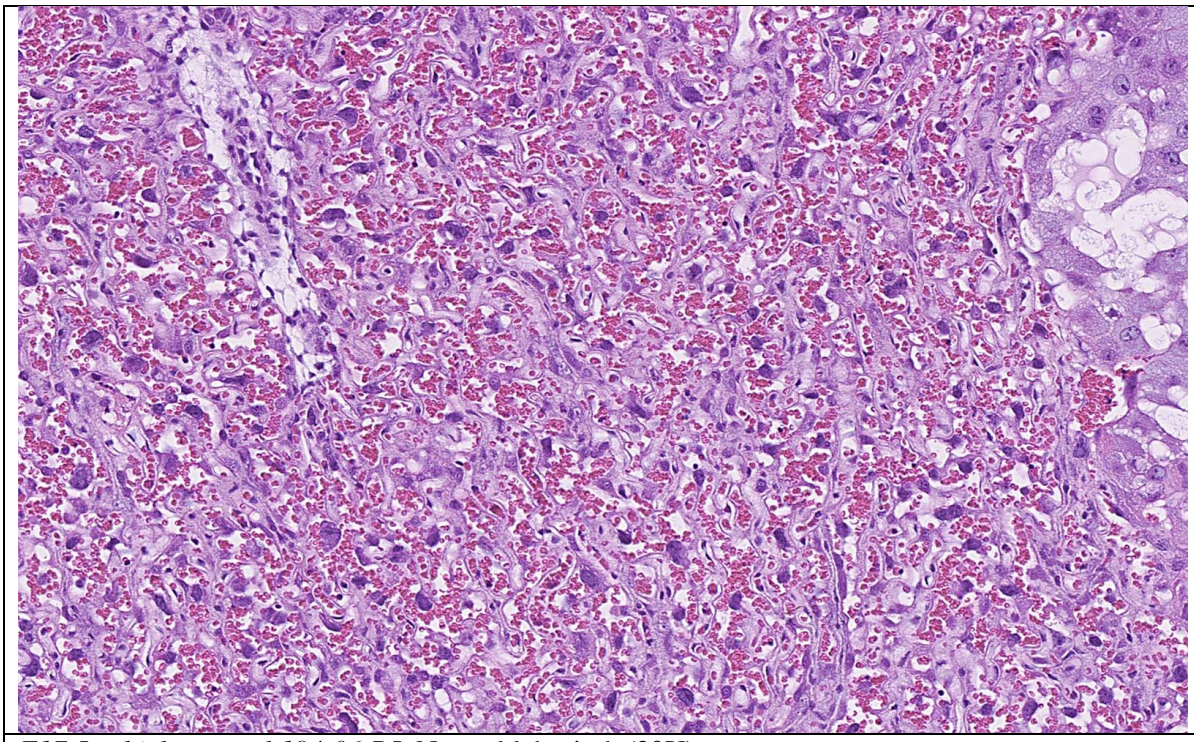


E11.5 PFOA 5mg/kg/day 115-09 P7 4X. Low magnification view showing atrophy of all areas: maternal decidua, junctional zone and labyrinth (compare to 114-07 below).

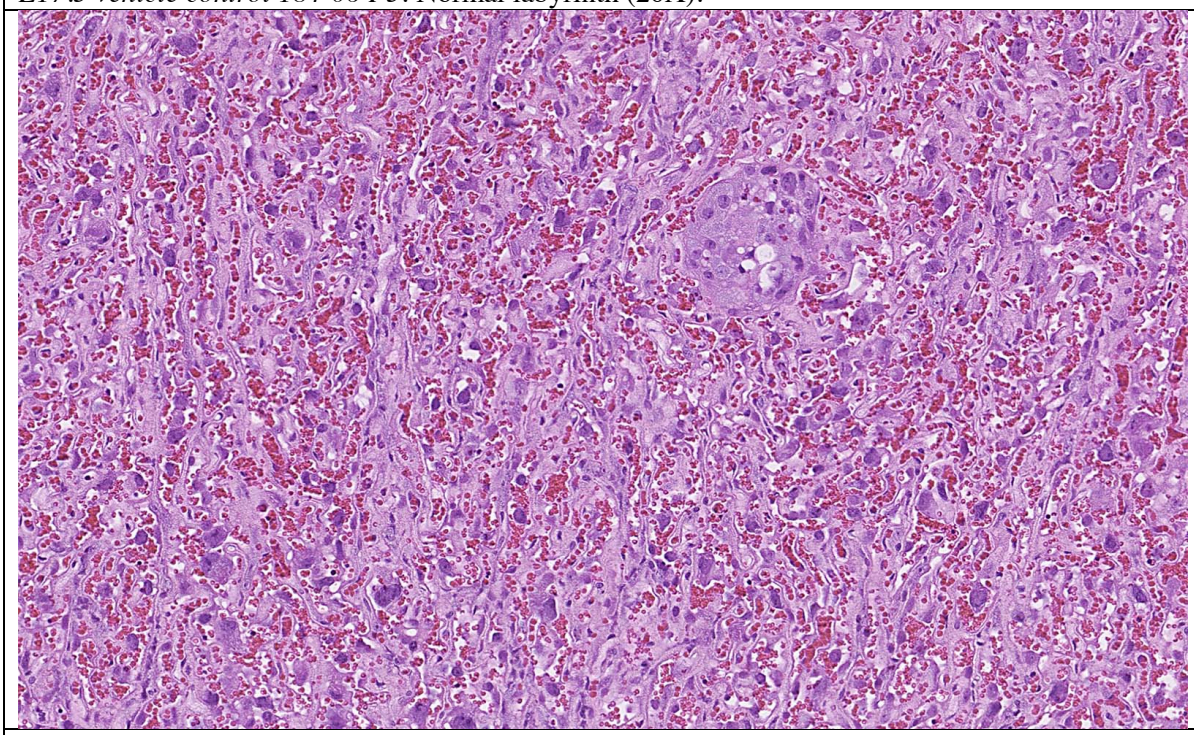


E11.5 vehicle control 114-07 P5. Low magnification at 4X for comparison.

All 41 placentas from *E17.5 vehicle controls* were within normal limits. The two figures below illustrate the range of normal seen in this group of controls.

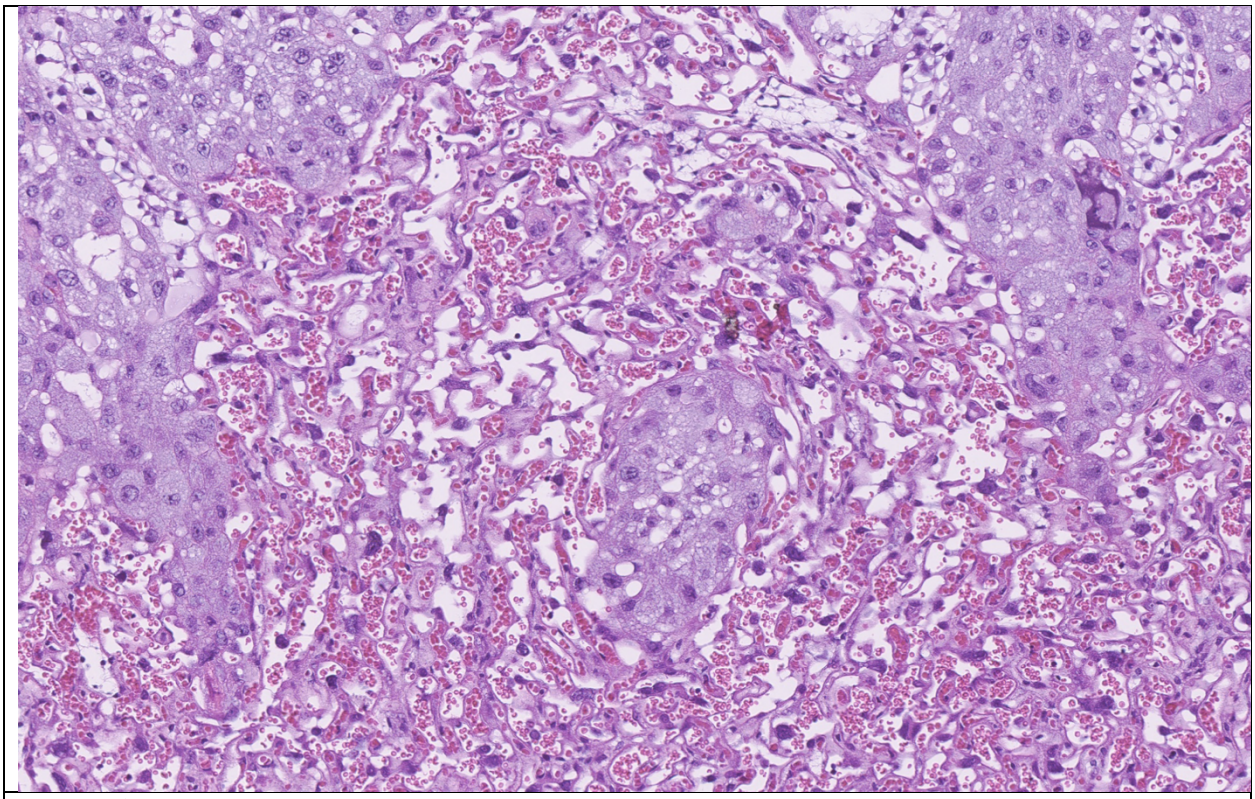


E17.5 vehicle control 184-06 P5. Normal labyrinth (20X).

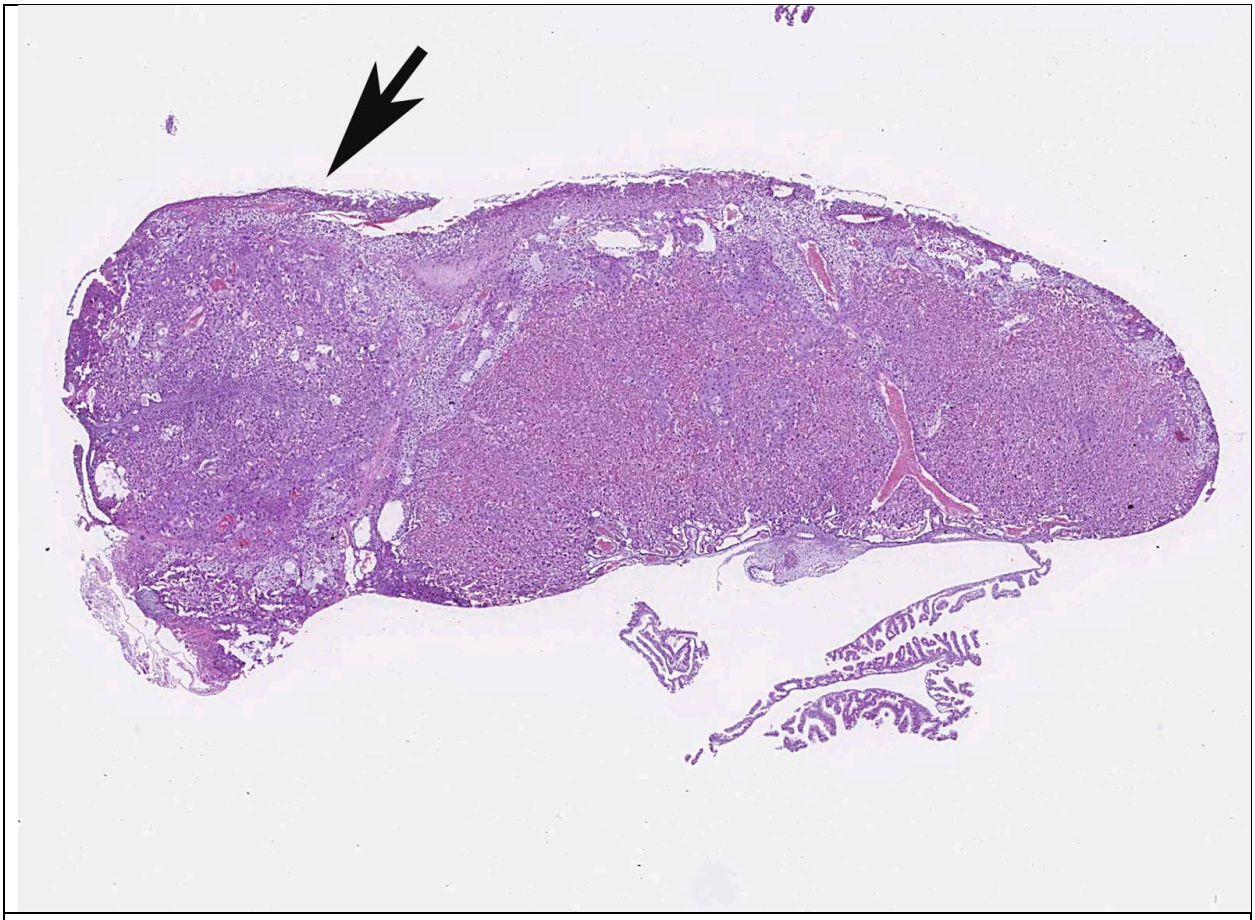


E17.5 vehicle control 187-07 P5. Normal labyrinth with a thicker trilaminar trophoblast layer (20X).

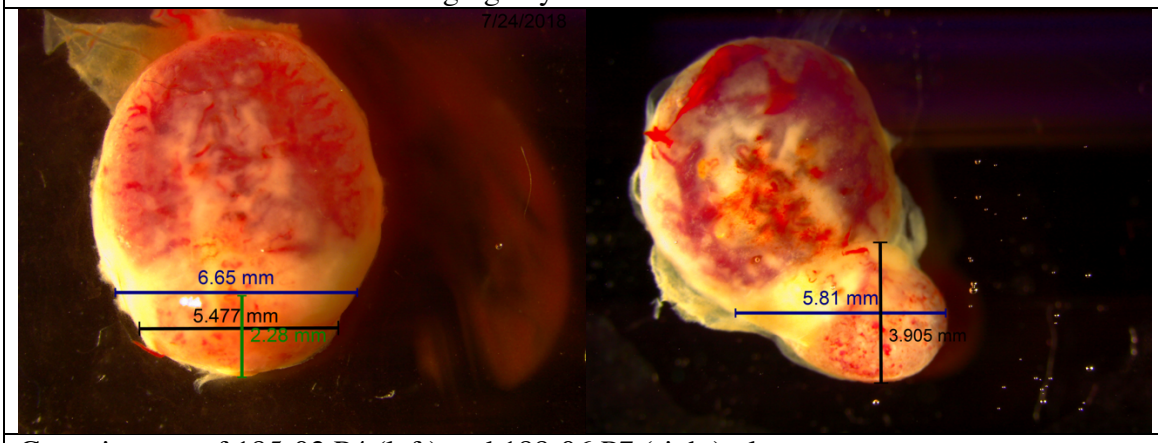
Placentas from the *GenX 2mg/kg/day* group showed a range from normal to moderate labyrinth atrophy (16 out of 29 were abnormal).



E17.5 GenX 2mg/kg/day 184-02 P7. Moderate labyrinth atrophy (20X).

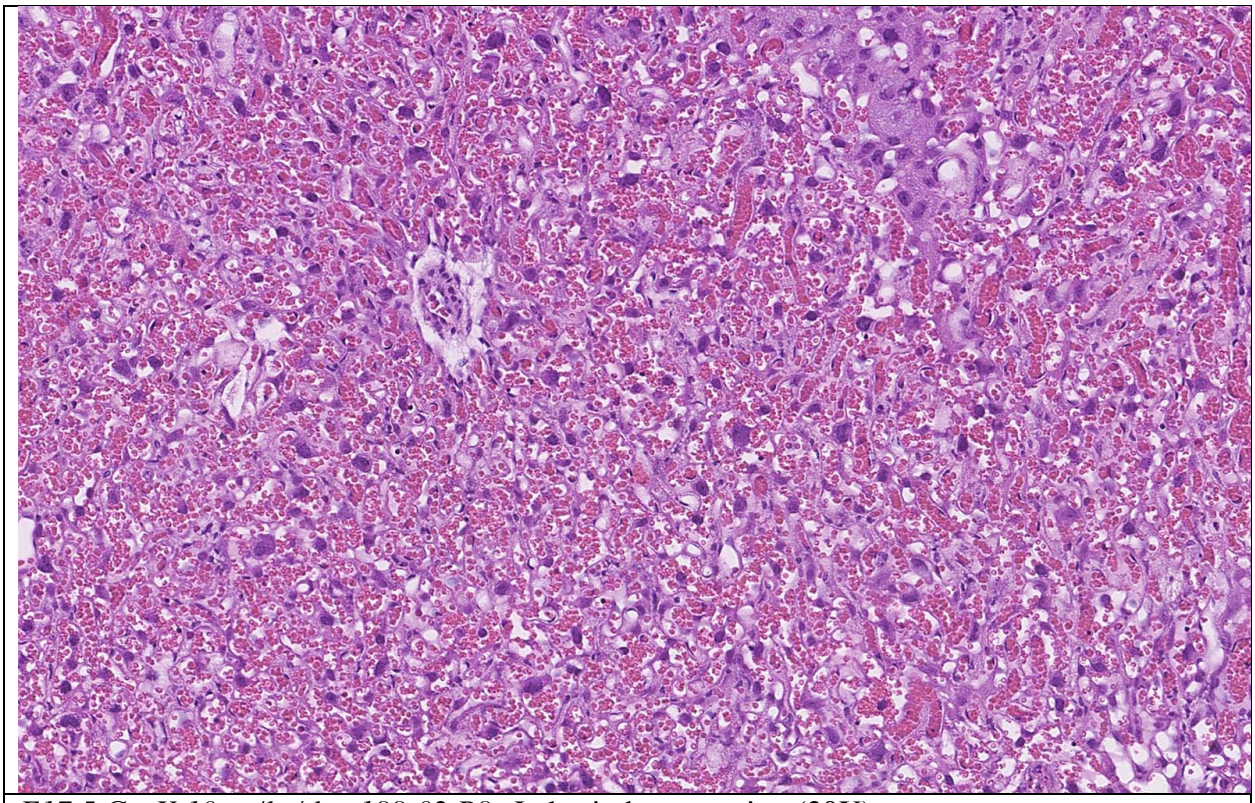


E17.5 GenX 2mg/kg/day 185-02 P4. Nodule of tissue from junctional zone (arrow), most likely developmental malformation. A similar lesion was also seen in 184-05 P4 vehicle control and 188-06 P7 PFOA 1mg/kg/day.

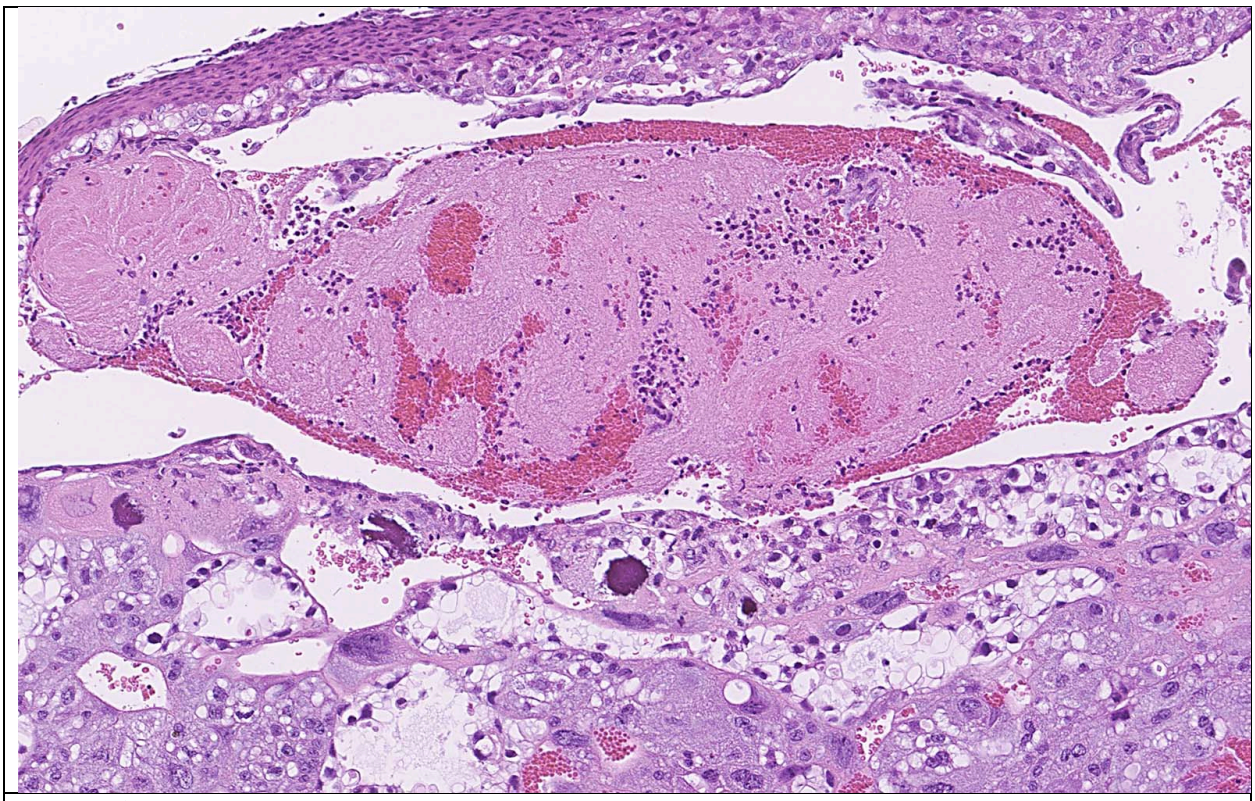


Gross images of 185-02 P4 (left) and 188-06 P7 (right) placentas.

There was also a range in the *GenX 10mg/kg/day* group but the severity of labyrinth lesions generally increased. Some were within normal limits and others showed a range from minimal to moderate labyrinth atrophy. For placentas from the 188-02 dam, all placentas showed diffuse mild labyrinth congestion. Although some degree of decidual clot formation is within normal limits, if there were an unusually high number or the size was larger than normal, then they were diagnosed.



E17.5 GenX 10mg/kg/day 188-02 P8. Labyrinth congestion (20X).

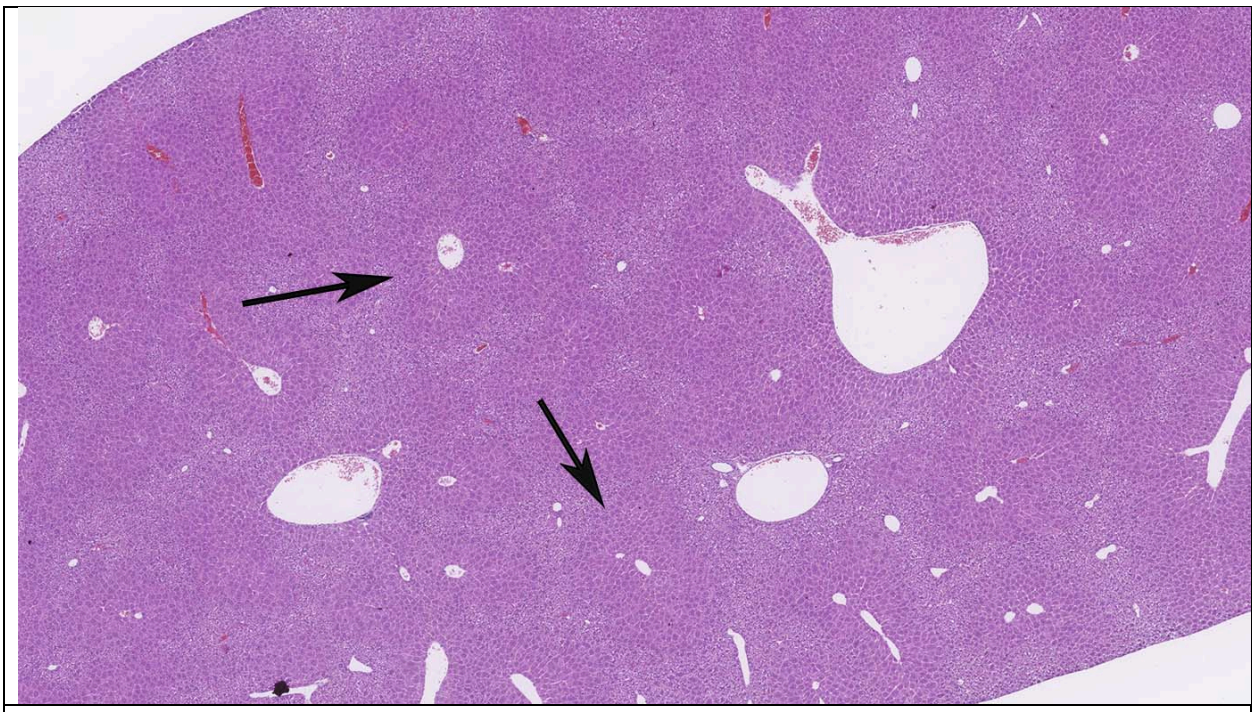


E17.5 GenX 10mg/kg/day 187-02 P1. Early clot formation in decidua with fibrin formation and trapped cells (20X).

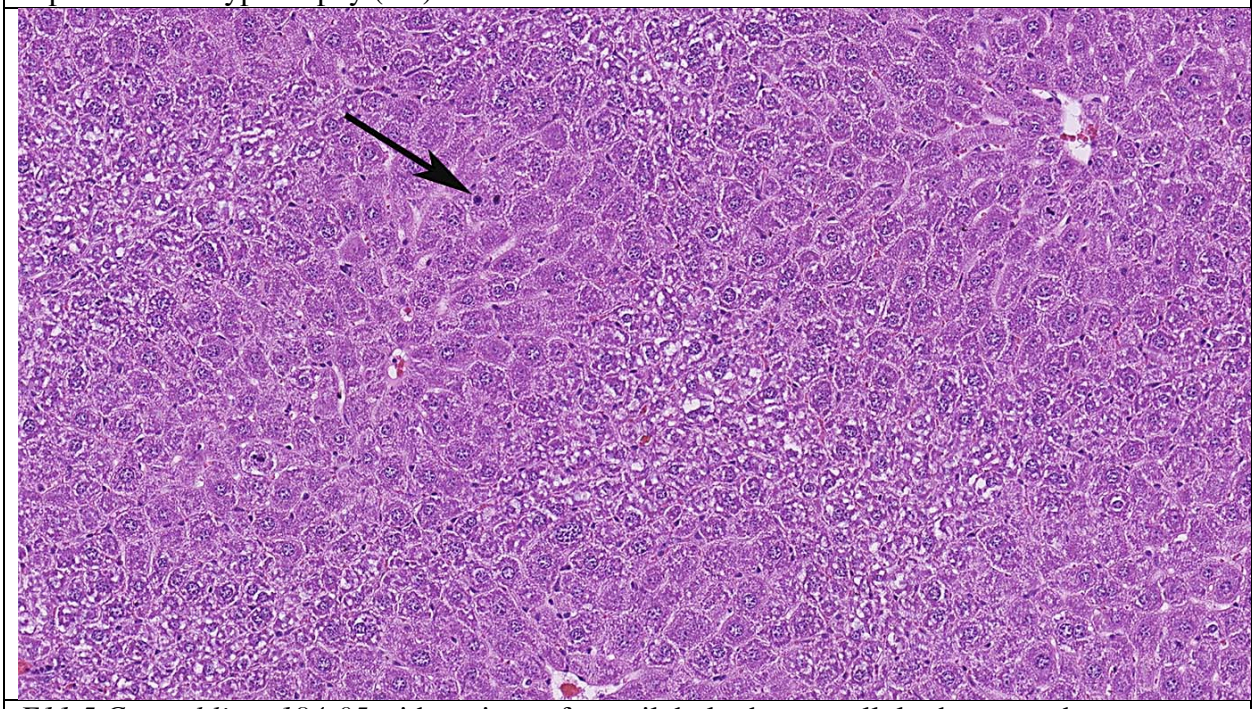
Most of the placentas from the *PFOA 1mg/kg/day* group were within normal limits. Two placentas from the left horn showed minimal and moderate labyrinth atrophy. The placentas from the *PFOA 5mg/kg/day* group showed a range from within normal limits to mild-moderate labyrinth atrophy and congestion. 13 out of 39 were within normal limits and 26 out of 39 were abnormal. Placentas from 3 dams all showed diffuse congestion, from mild to moderate severity.

Livers

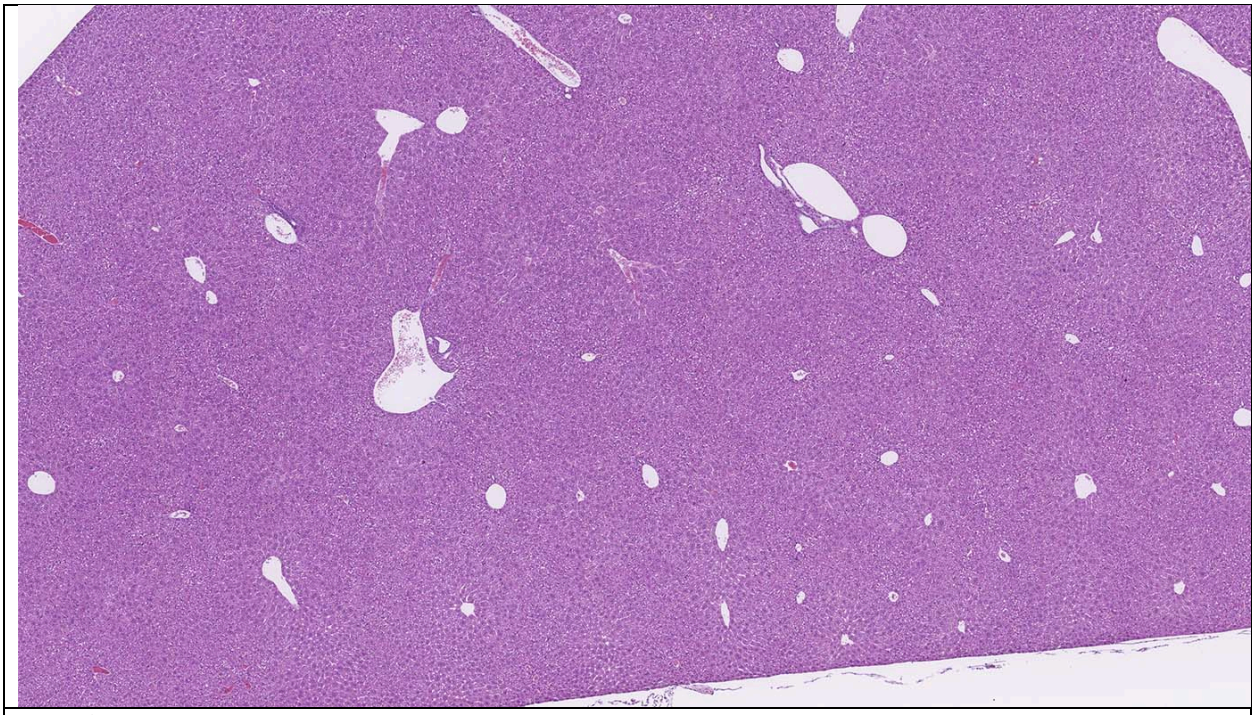
In this study, all livers from pregnant treated dams (gavage) were compared to vehicle control (water) livers from pregnant dams. There were no treatment related changes in the E11.5 vehicle control livers. The changes seen in the 17.5 vehicle control livers were centrilobular hepatocellular hypertrophy with karyomegaly, increased mitotic figures and decreased glycogen as well as increased basophilic granular cytoplasm in all hepatocytes. These changes are considered within normal limits for pregnant dams in this age and group of mice. The liver of the mouse adapts to the demands of pregnancy via a dramatic growth response driven by hepatocyte proliferation and size increase (Dai et al. 2011. Exp Biol Med, 236(11): 1322-1332). This pregnancy-induced hepatomegaly is considered a physiological event of liver growth confirmed in published studies by DNA content increase and detection of hepatocyte hyperplasia (increased mitotic figures) and hypertrophy.



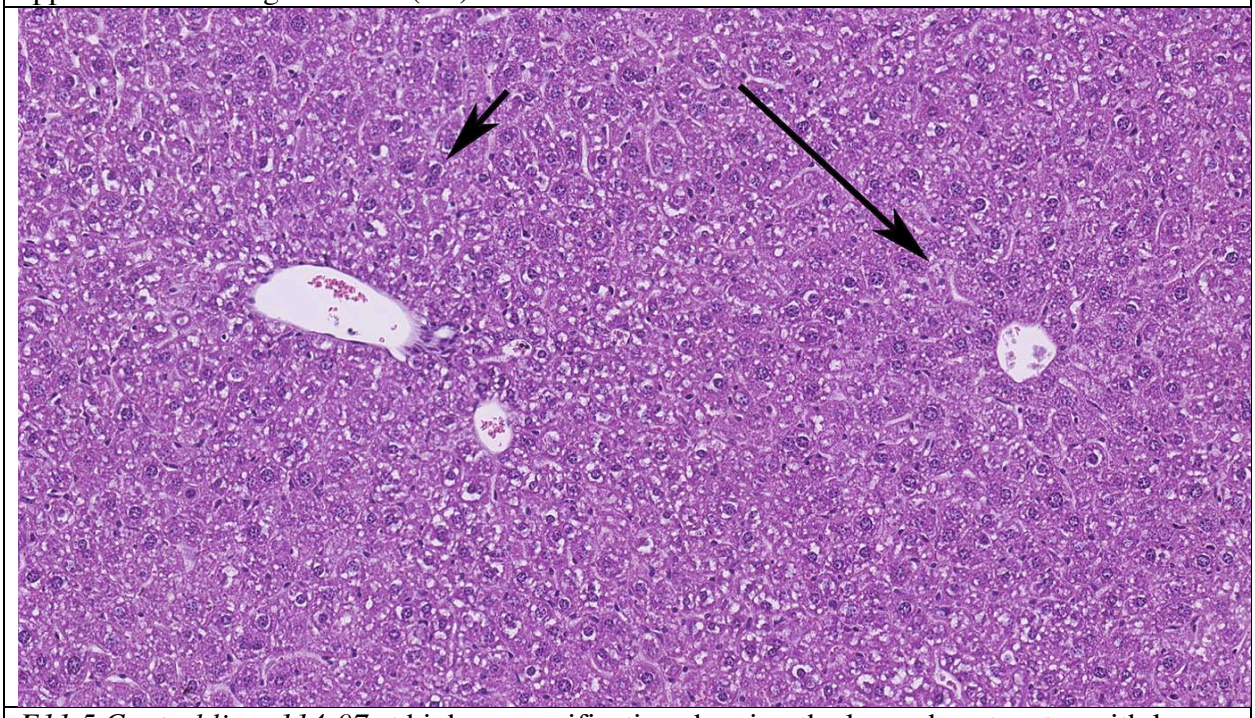
E17.5 Control liver 184-05. Arrows point to just two examples of areas of centrilobular hepatocellular hypertrophy (4X).



E11.5 Control liver 184-05 with regions of centrilobular hepatocellular hypertrophy, karyomegaly and occasional mitotic figures (arrow) (20X).

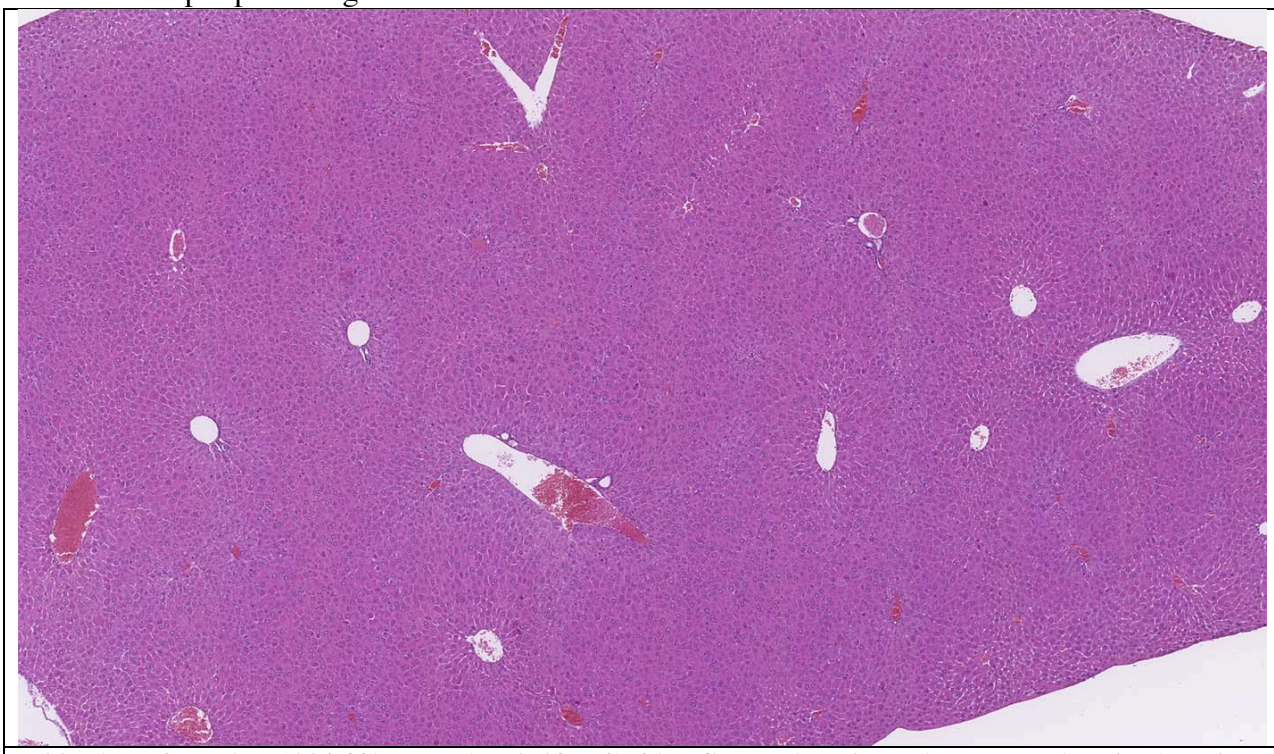


E11.5 Control liver 114-07 with centrilobular hepatocellular hypertrophy that is not as apparent at low magnification (4X).

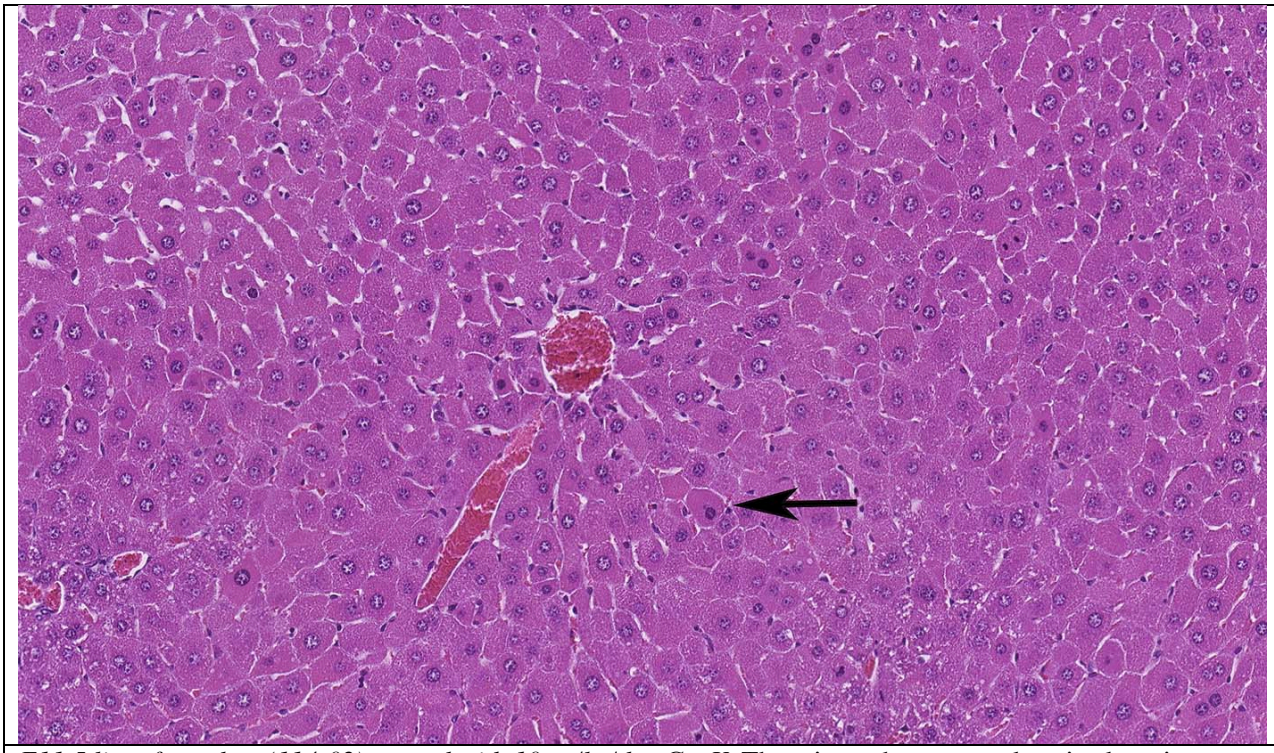


E11.5 Control liver 114-07 at higher magnification showing the larger hepatocytes with larger nuclei, decreased glycogen and increased basophilic granular cytoplasm in the regions surrounding the central veins (long arrow) compared to the hepatocytes surrounding the periportal regions (short arrow) (20X).

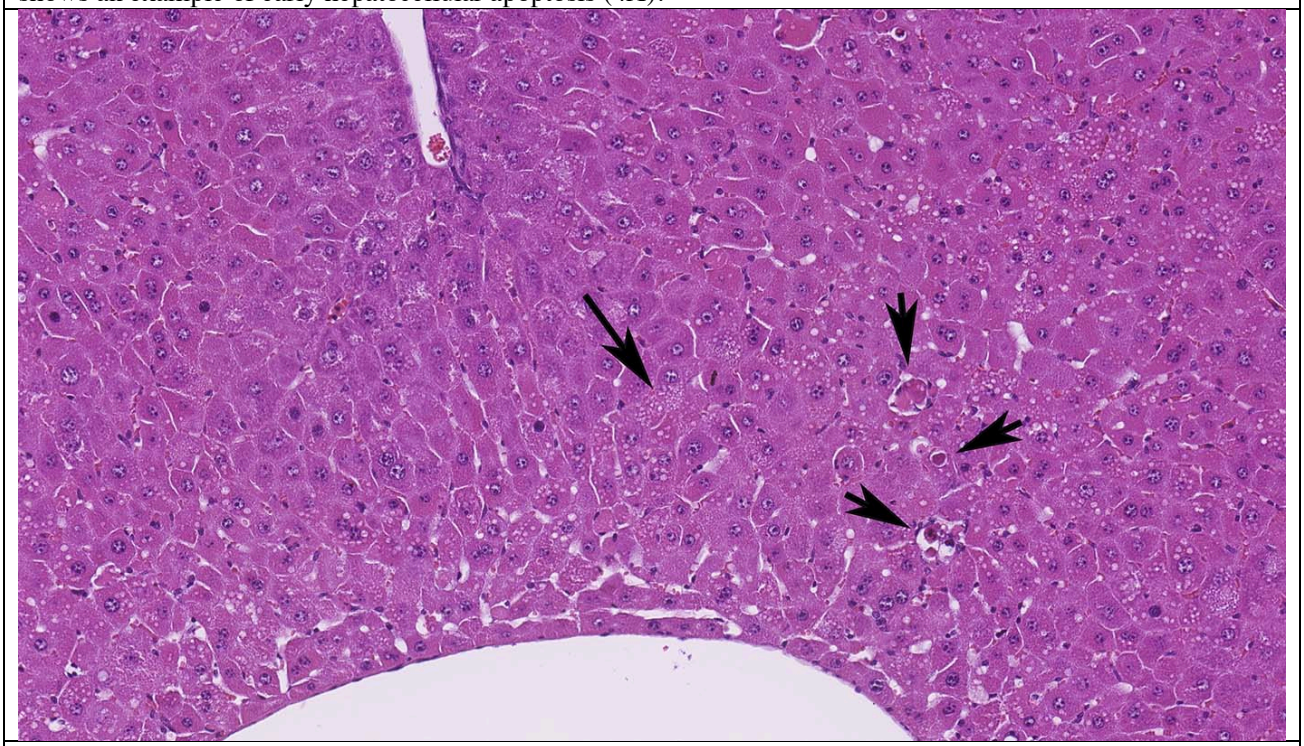
All livers from treated E11.5 and E17.5 dams showed some degree of cytoplasmic alteration. This change was characterized by hepatocellular hypertrophy with decreased glycogen and intensely eosinophilic granular cytoplasm. As the cytoplasmic alteration increased in severity, there was a decrease in mitoses and an increase in cell death (necrosis +/- apoptosis). Also, as the severity increased, there was extension of the cytoplasmic alteration into the midzonal and periportal regions.



E11.5 liver from dam (114-02) treated with 10mg/kg/day GenX. There is moderate cytoplasmic alteration characterized as centrilobular hepatocellular hypertrophy with decreased glycogen. There is extension into the midzonal and periportal regions, and decreased glycogen in periportal regions (4X).



E11.5 liver from dam (114-02) treated with 10mg/kg/day GenX. There is moderate cytoplasmic alteration characterized as centrilobular hepatocellular hypertrophy with decreased glycogen around a central vein. Arrow shows an example of early hepatocellular apoptosis (4X).



E17.5 liver from dam (188-09) treated with PFOA 5mg/kg/day. There is marked cytoplasmic alteration characterized as centrilobular hepatocellular hypertrophy and decreased glycogen in the centrilobular region (around a central vein). There is frequent cell death and degeneration in centrilobular hepatocytes (short arrows) and accumulation of hepatocellular cytoplasmic small vacuoles with distinct borders (long arrow) (20X).

Reports have shown that this change of cytoplasmic alteration is caused by peroxisome proliferation. An increase in microsomal enzymes often occurs with a zonal or specific lobular pattern and commonly occurs following exposure to enzyme inducing xenobiotics. There is enlargement of the hepatocyte cytoplasm secondary to an increase in the cytosolic protein or number of organelles (e.g., smooth endoplasmic reticulum, peroxisomes, mitochondria). Classically, hepatocyte hypertrophy occurs without increase in hepatocyte numbers or DNA (i.e., hyperplasia or polyploidy), however, combinations with increased mitoses do occur (e.g., PPAR-alpha agonists). Studies have led to the hypothesis that persistent proliferation of peroxisomes serves as an endogenous initiator of neoplastic transformation in liver by increasing the intracellular production of H_2O_2 by the peroxisomal oxidase(s).

Tissue processing: Some of the liver sections with moderate and marked hypertrophied centrilobular cells appear to have fracture artifact, most likely due to the fragility of the tissue.

Kidneys

All aspects of the E11.5 and E17.5 kidneys were evaluated and diagnoses were made with no threshold: cortical glomeruli; cortical and medullary tubules; papillary collecting ducts; parenchyma; and vascular tree including renal artery, interlobar artery, interlobular artery, arcuate artery and renal veins. Cross sections (XS) and longitudinal sections (LS) were evaluated. All kidneys from vehicle control and treated animals were within normal limits (WNL). All kidneys from nonpregnant animals were WNL, showing one or more spontaneous background lesions, with the exception of E17.5 animal 188-01 that had multiple dilated cortical tubules and associated lesions.