Pitfalls in the Interpretation of Findings in Endocrine Toxicity Studies

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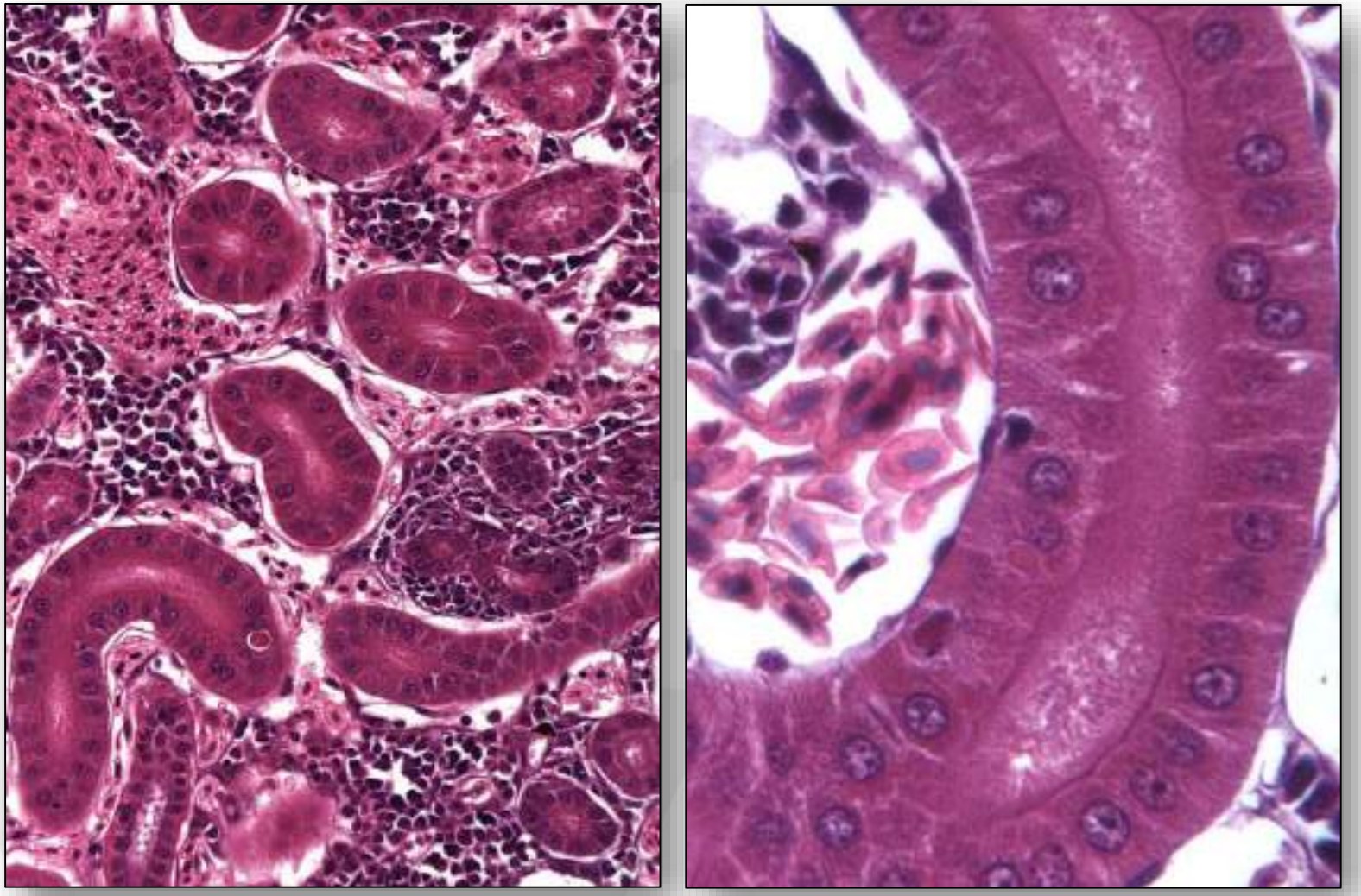
Fish – Tolerance Studies (Diclofenac)

Environ Toxicol Chem. 2013 Feb;32(2):442-52. doi: 10.1002/etc.2085.
Diclofenac: New data on chronic toxicity and bioconcentration in fish.
Memmert U[1], Peither A, Burri R, Weber K, Schmidt T, Sumpter JP, Hartmann A.

'…This leads us to a conclusion that DCF has, with high probability, no adverse effect on both fish species up to 320 µg/L….'

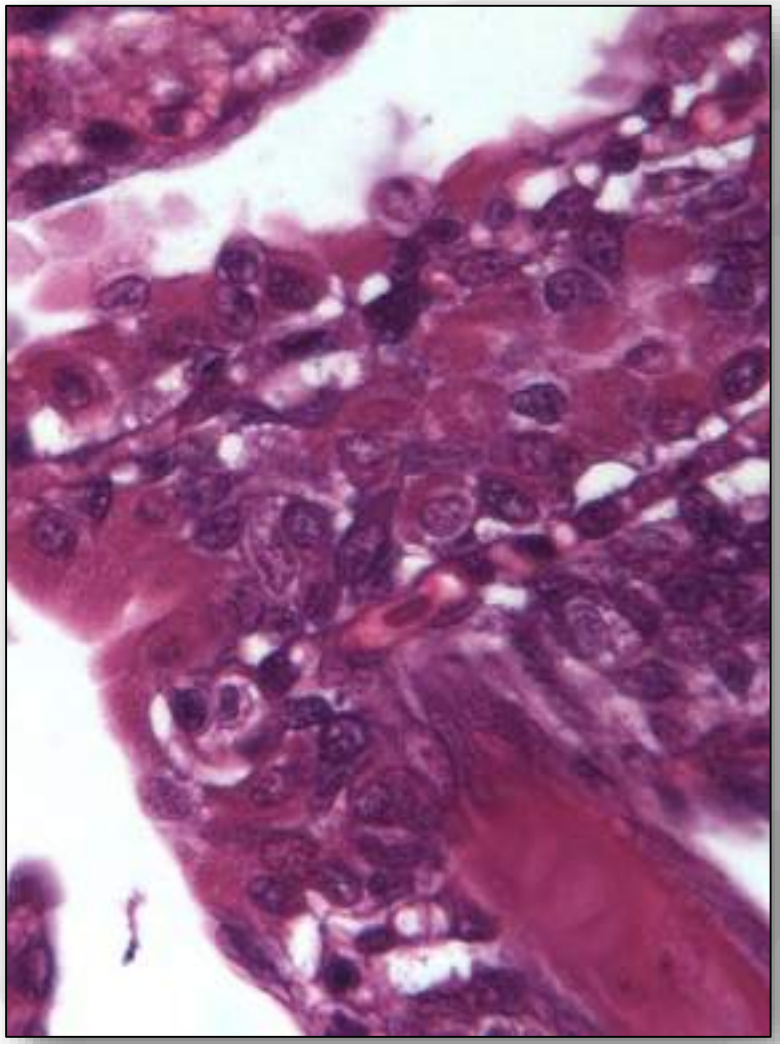
Aquat Toxicol. 2014 Jan;146:127-36. doi: 10.1016/j.aquatox.2013.10.033. Epub 2013 Nov 7.
Pathology working group review of histopathologic specimens from three laboratory studies of diclofenac in trout.
Wolf JC[1], Ruehl-Fehlert C[2], Segner HE[3], Weber K[4], Hardisty JF[5].

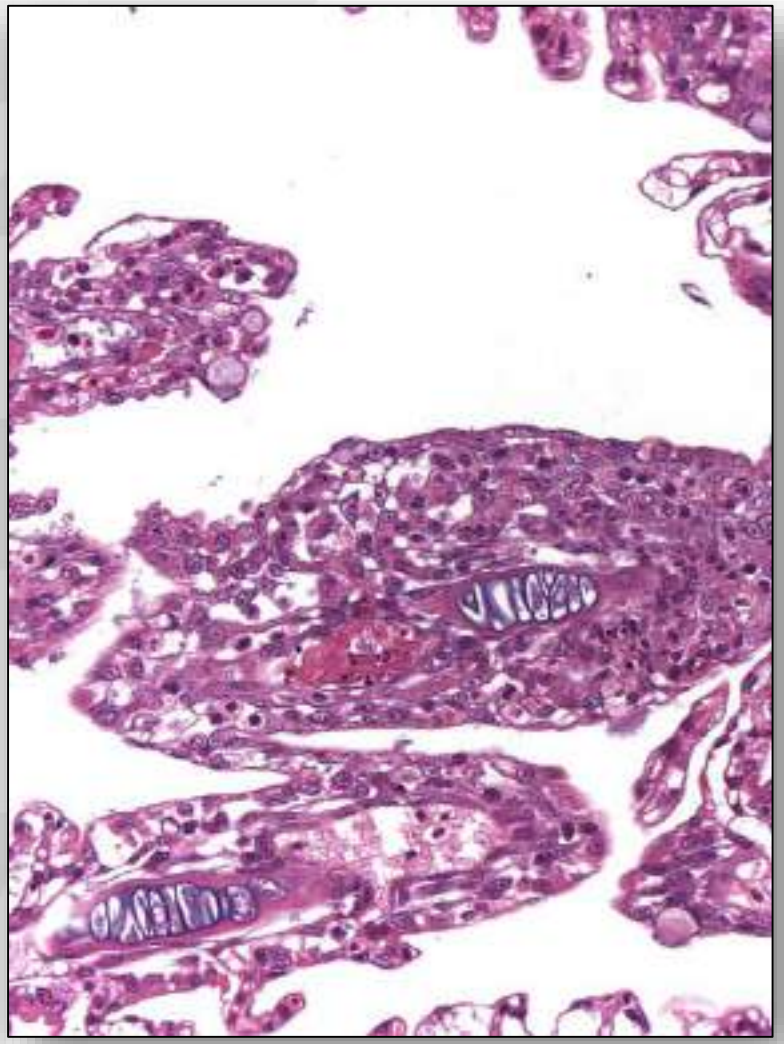
Fish – Tolerance Studies (Diclofenac)



Kidneys: single cell necrosis. All groups

Fish – Tolerance Studies (Diclofenac)





Gills: Clubbing

Wolf JC1, Ruehl-Fehlert C2, Segner HE3, Weber K4, Hardisty JF5. (2014)

Based on the results of this review, findings related to diclofenac exposure included minimal to slightly increased thickening of the gill filament tips in fish exposed to the highest concentration tested (**1000 µg/L**), plus a previously undiagnosed finding, decreased hepatic glycogen, which also occurred at the 1000 µg/L dose level. The panel found little evidence to support other reported effects of diclofenac in trout, and thus the overall NOEC was determined to be >320 µg/L. By consensus, the PWG panel was able to identify diagnostic inconsistencies among and within the three prior studies; therefore this exercise demonstrated the value of the pathology peer review/PWG approach for assessing the reliability of histopathology results that may be used by regulatory agencies for risk assessment.

Too many wrong reported findings in literature!

Toxicol Pathol. 2015 Apr;43(3):297-325. doi: 10.1177/0192623314540229. Epub 2014 Aug 11.

Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: a guide for investigators, authors, reviewers, and readers.

Wolf JC[1], Baumgartner WA[2], Blazer VS[3], Camus AC[4], Engelhardt JA[5], Fournie JW[6], Frasca S Jr[7], Groman DB[8], Kent ML[9], Khoo LH[10], Law JM[11], Lombardini ED[12], Ruehl-Fehlert C[13], Segner HE[14], Smith SA[15], Spitsbergen JM[16], Weber K[17], Wolfe MJ[18].

The Problem of Differentiation

<u>Differentiated gonorchists</u>

PGC differentiate directly into oocytes or into spermatogonia:

Medaka, Fathead Minnow

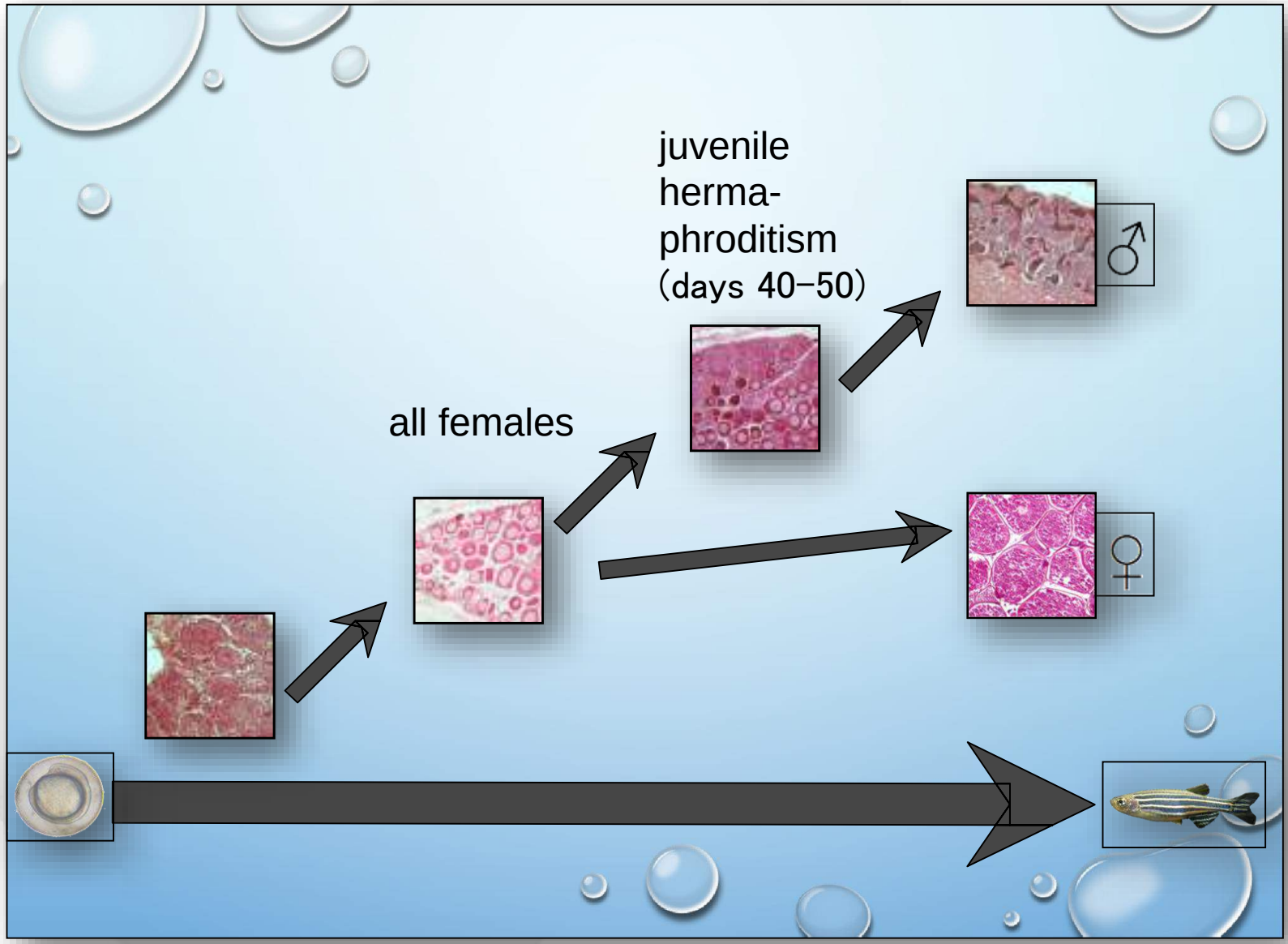
The Problem of Differentiation

<u>Undifferentiated gonorchists:</u>

PGC appear to differentiate at first into ovary-like gonad and thereafter in approx. 50% of animals into testes):

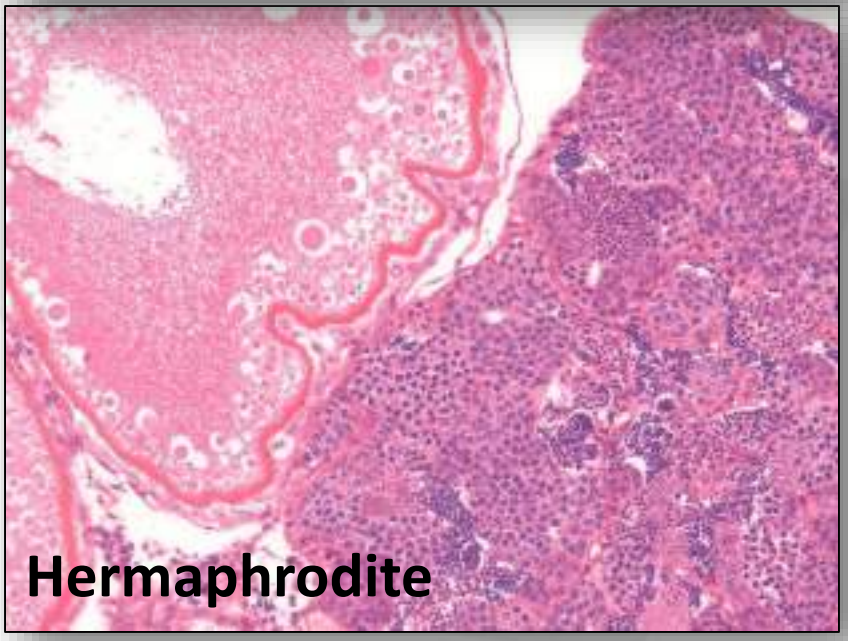
Guppy, Zebrafish

The Problem of Differentiation



LISA BAUMANN, UNIVERSITY OF HEIDELBERG, AQUATIC ECOLOGY AND TOXICOLOGY, LISA.BAUMANN@UNI-HEIDELBERG.DE

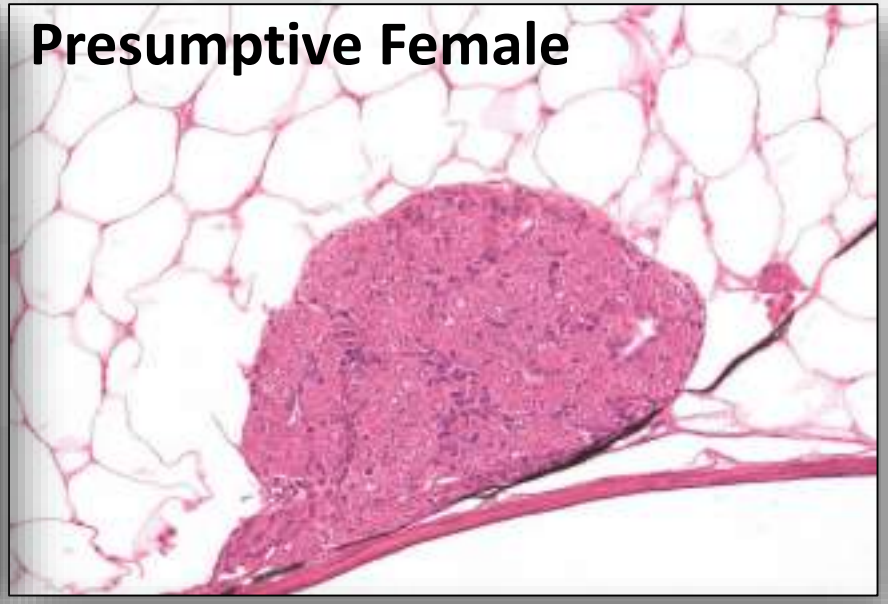
The Problem of Differentiation

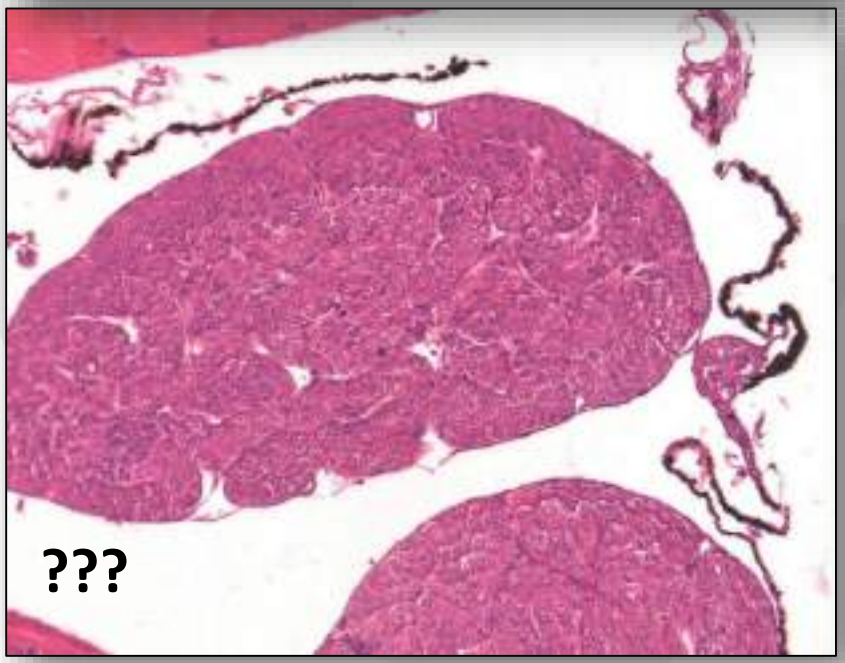


Ovariotestes vs maturation

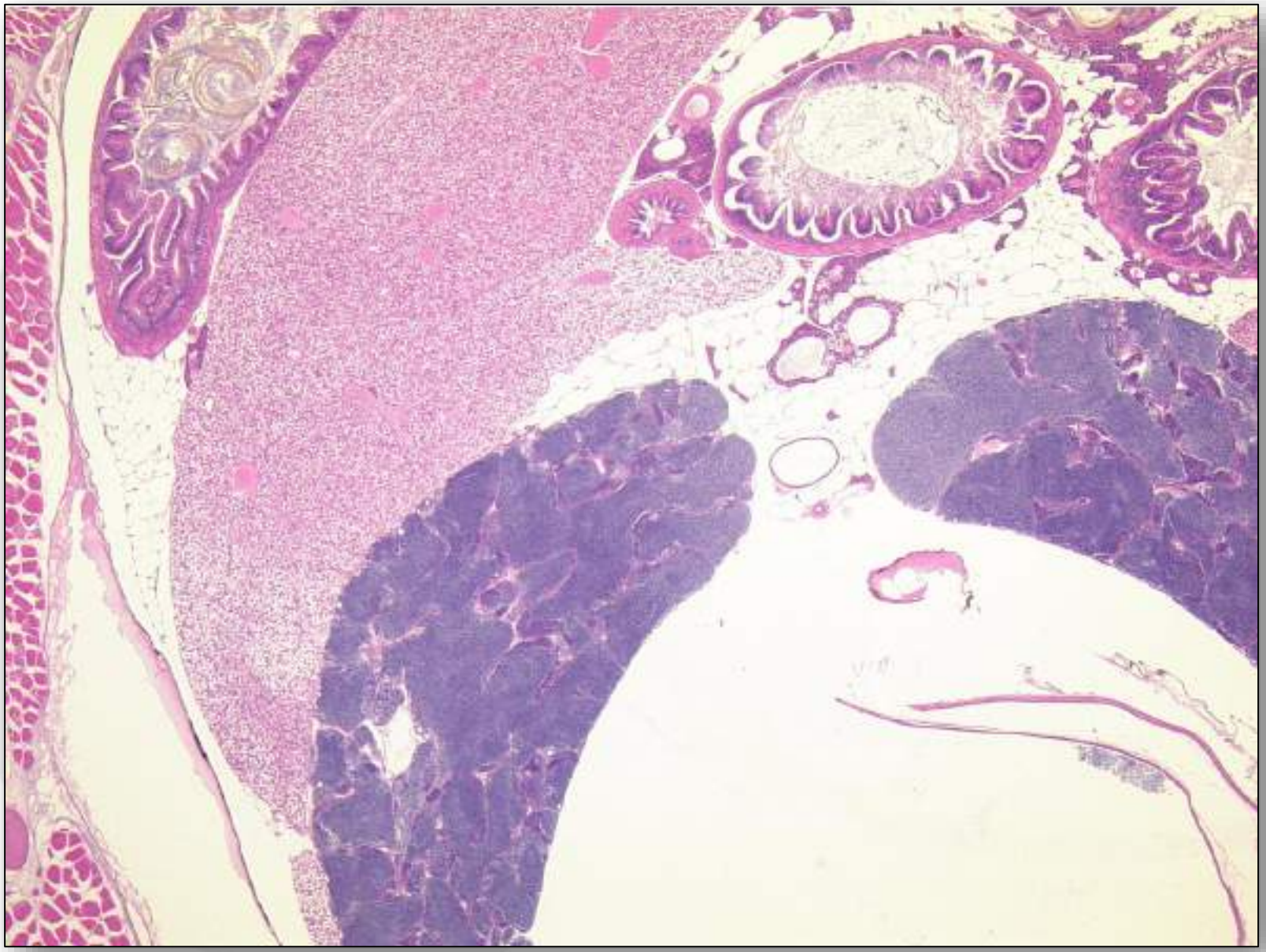
Immature Fish: Indifferent: Mesenteric Attachements

The Problem of Differentiation: Immature





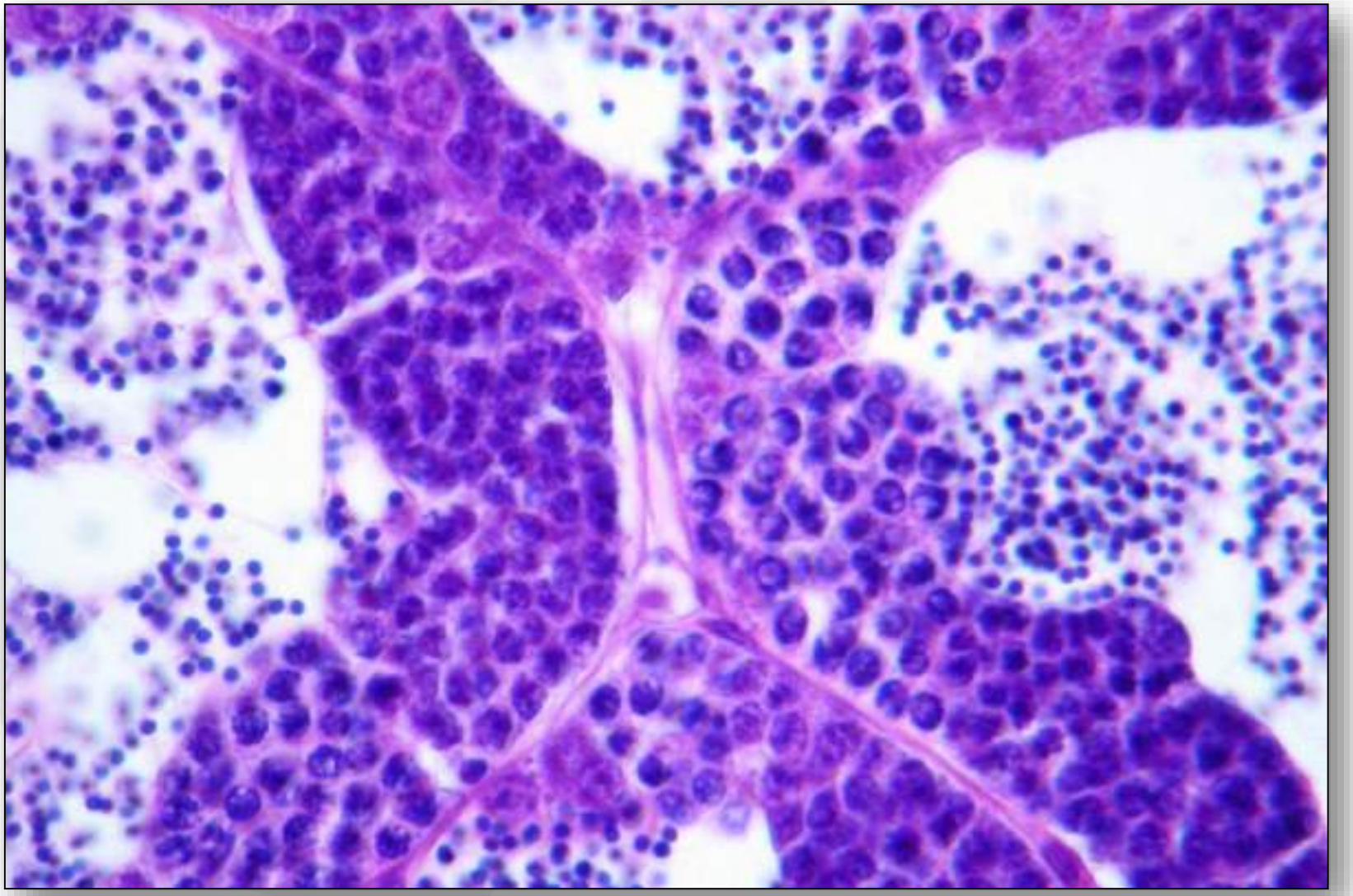
Normal Histology – Males (Fathaed Minnow)



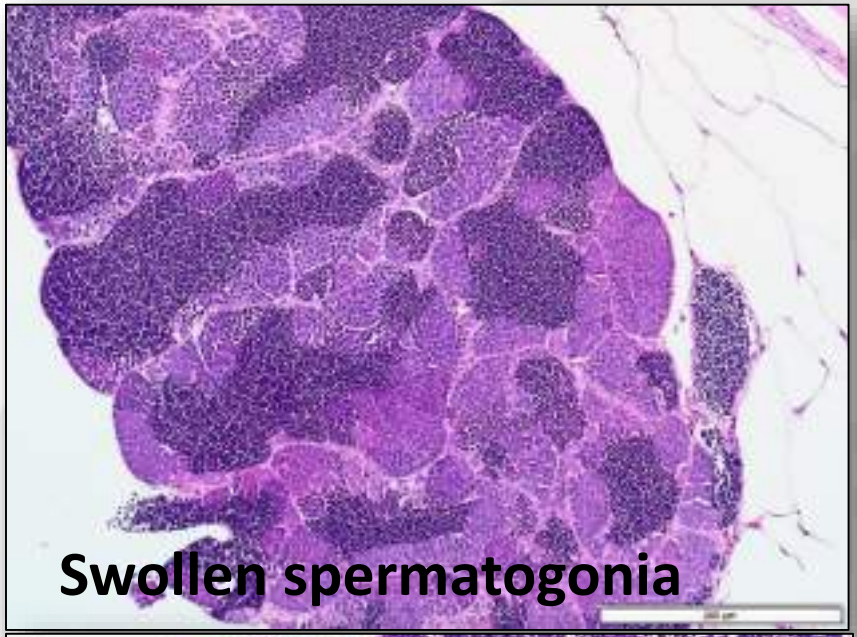
Normal Histology – Females (Fathead Minnow)

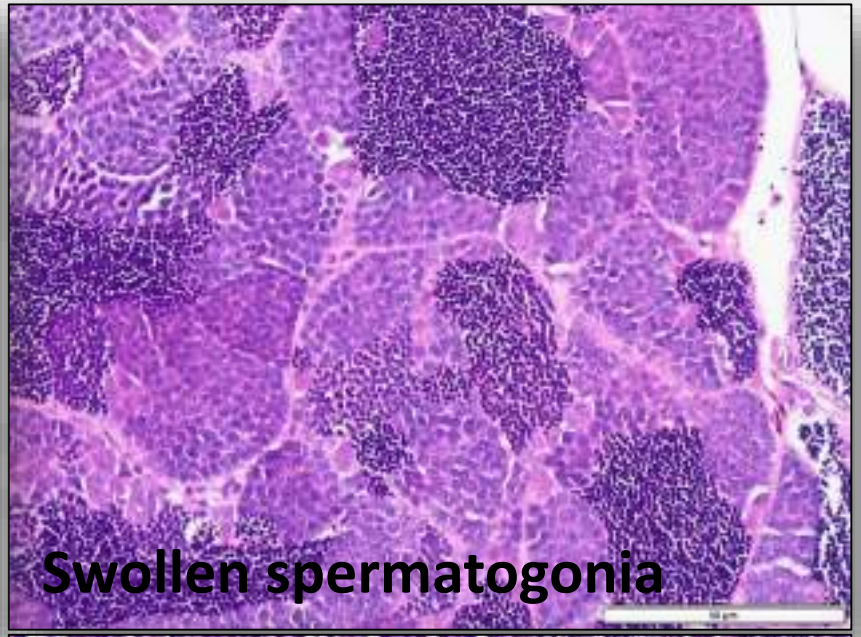
Histology – Males (Fathead Minnow)

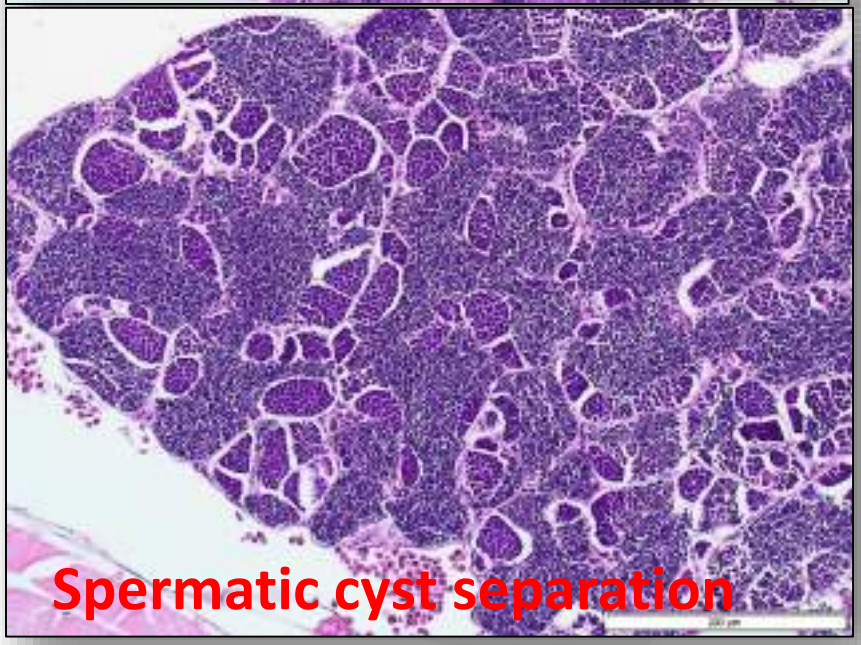
Control: stage 3.
Interstitial cells rare or not visible

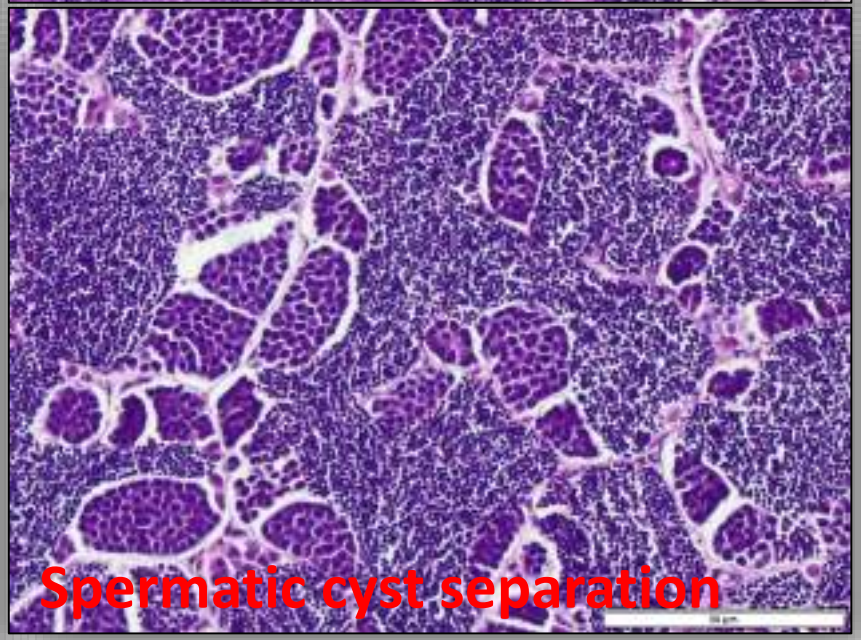


Wrong Fixation. Formalin.







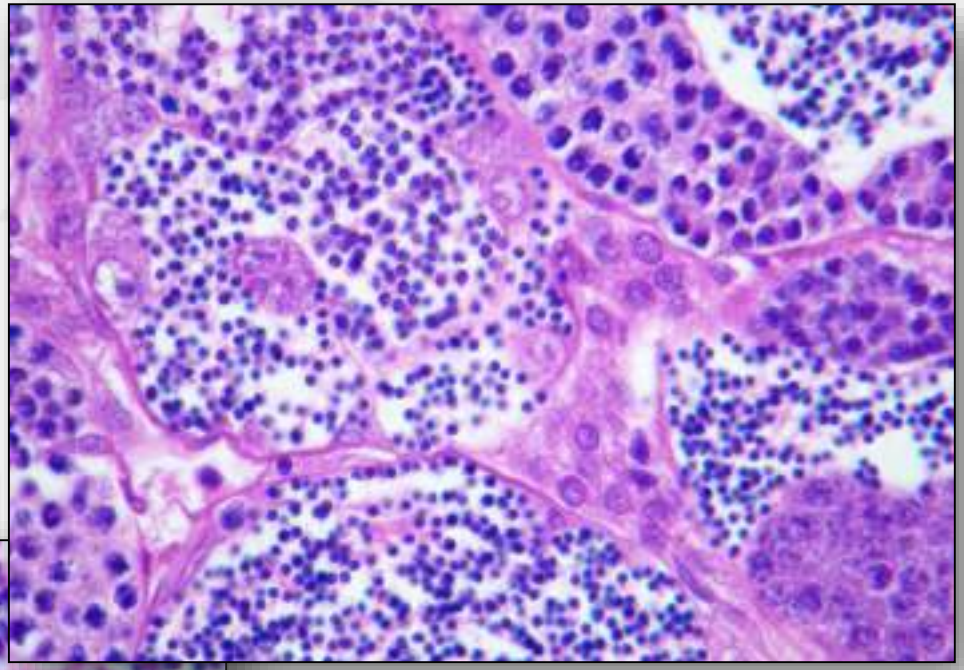


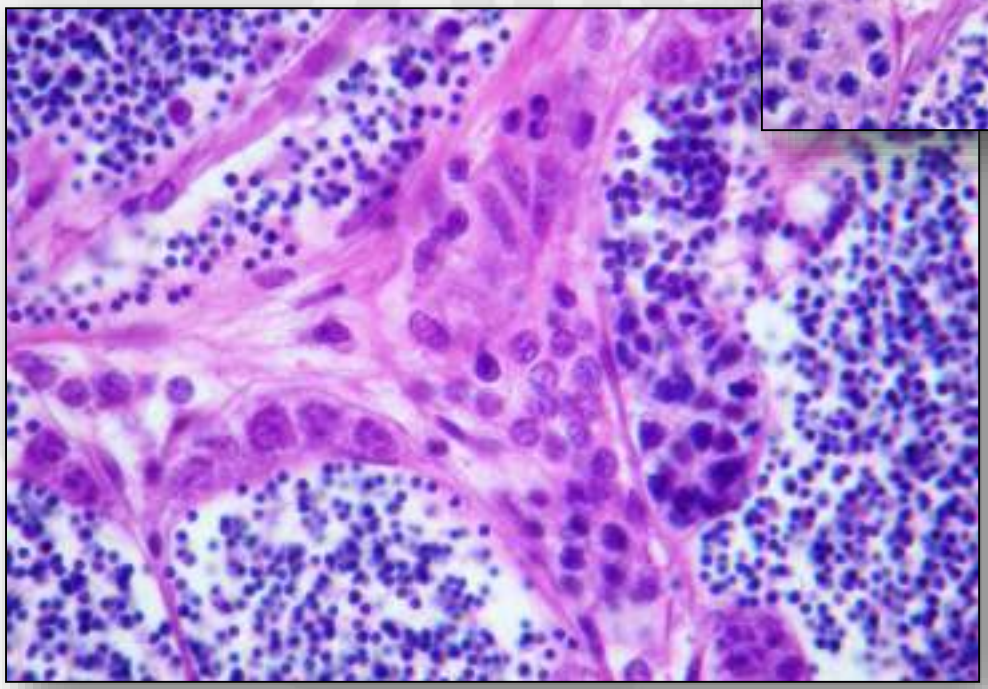
Reproductive Organs: Induced.

Histology – Males. Aromatase-Inhibitors

Prochloraz: 300 μg/L, stage 3.

Multifocal interstitial cell hyperplasia in several animals





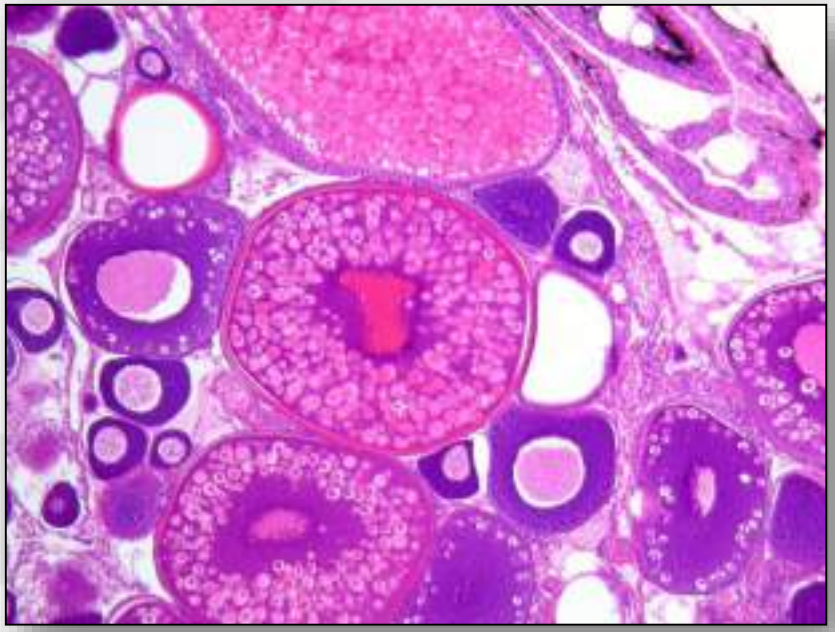
Fadrazole: 100 μg/L, stage 2.

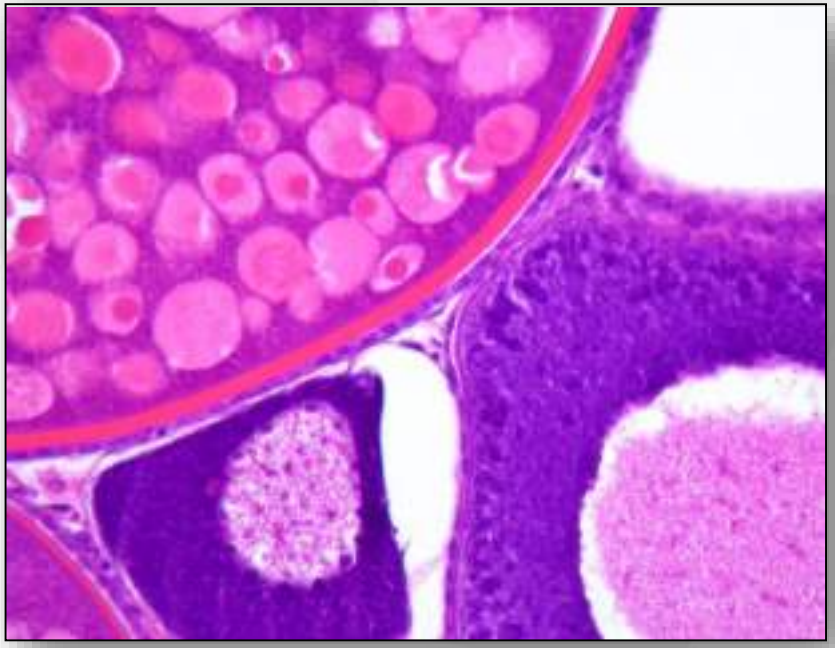
Multifocal interstitial cell hyperplasia/hypertrophy in several animals

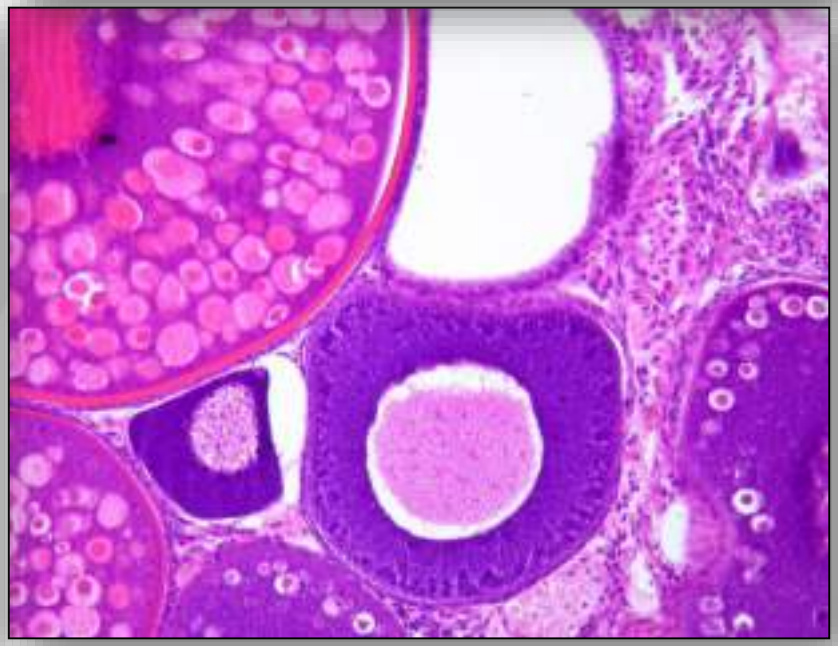
Histology – Females (Fathead Minnow)

Control: stage 2

Half of follicles are early or mid-vitellogenic

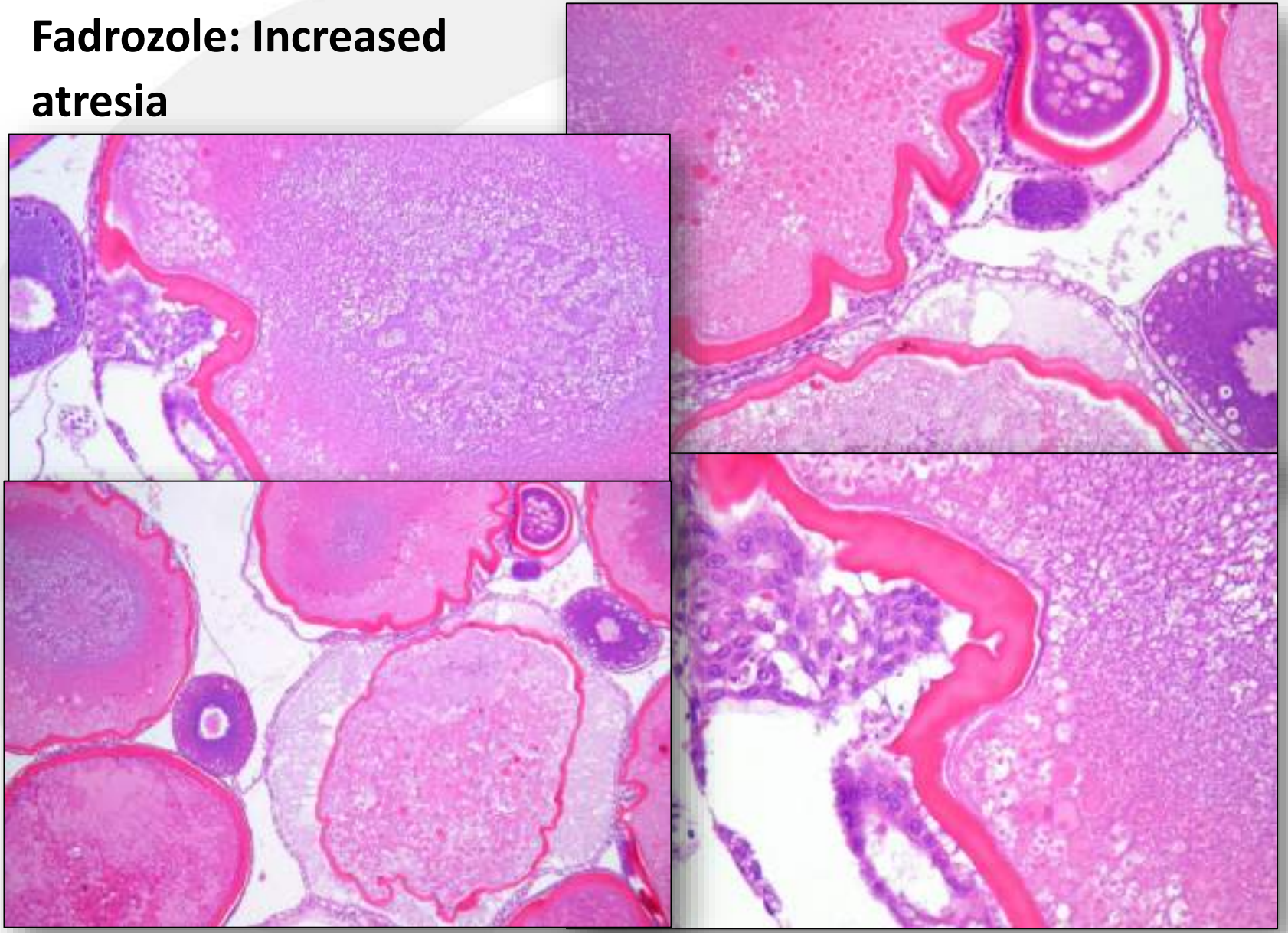




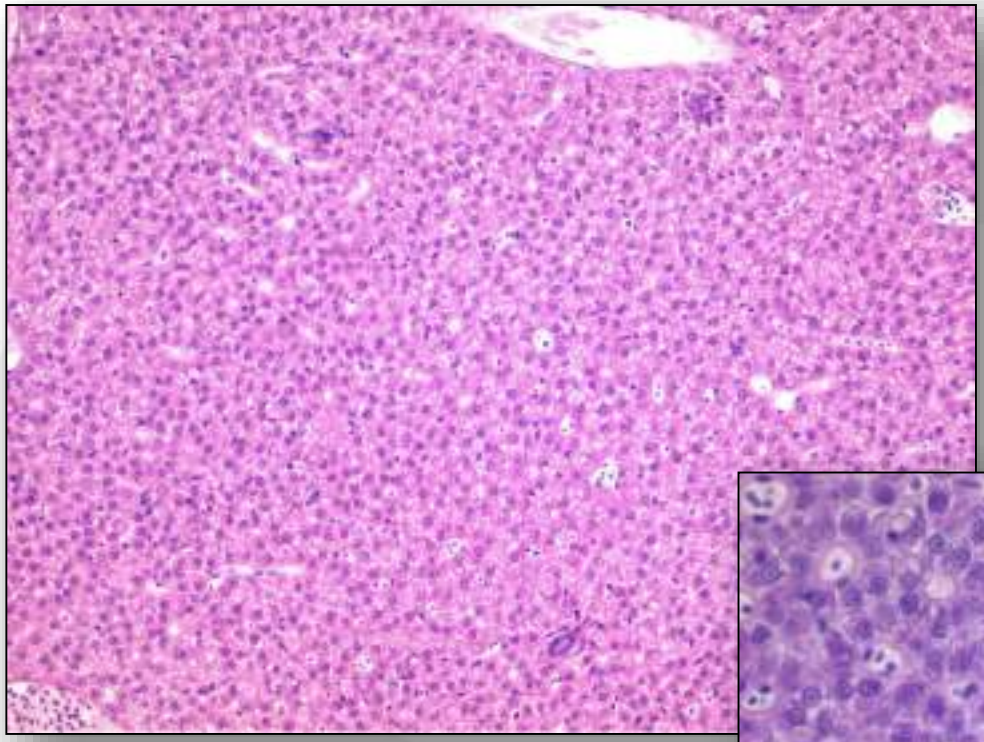


Histology – Females. Aromatase-Inhibitors

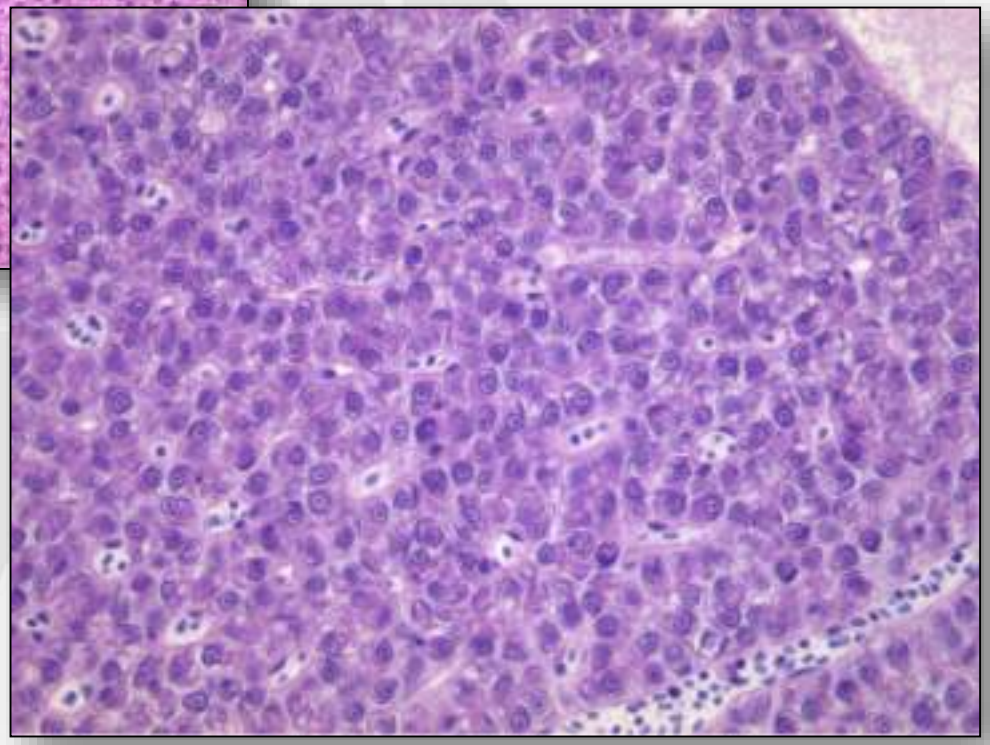
Fadrozole: Increased atresia



Effects by Estrogen in Other Organs

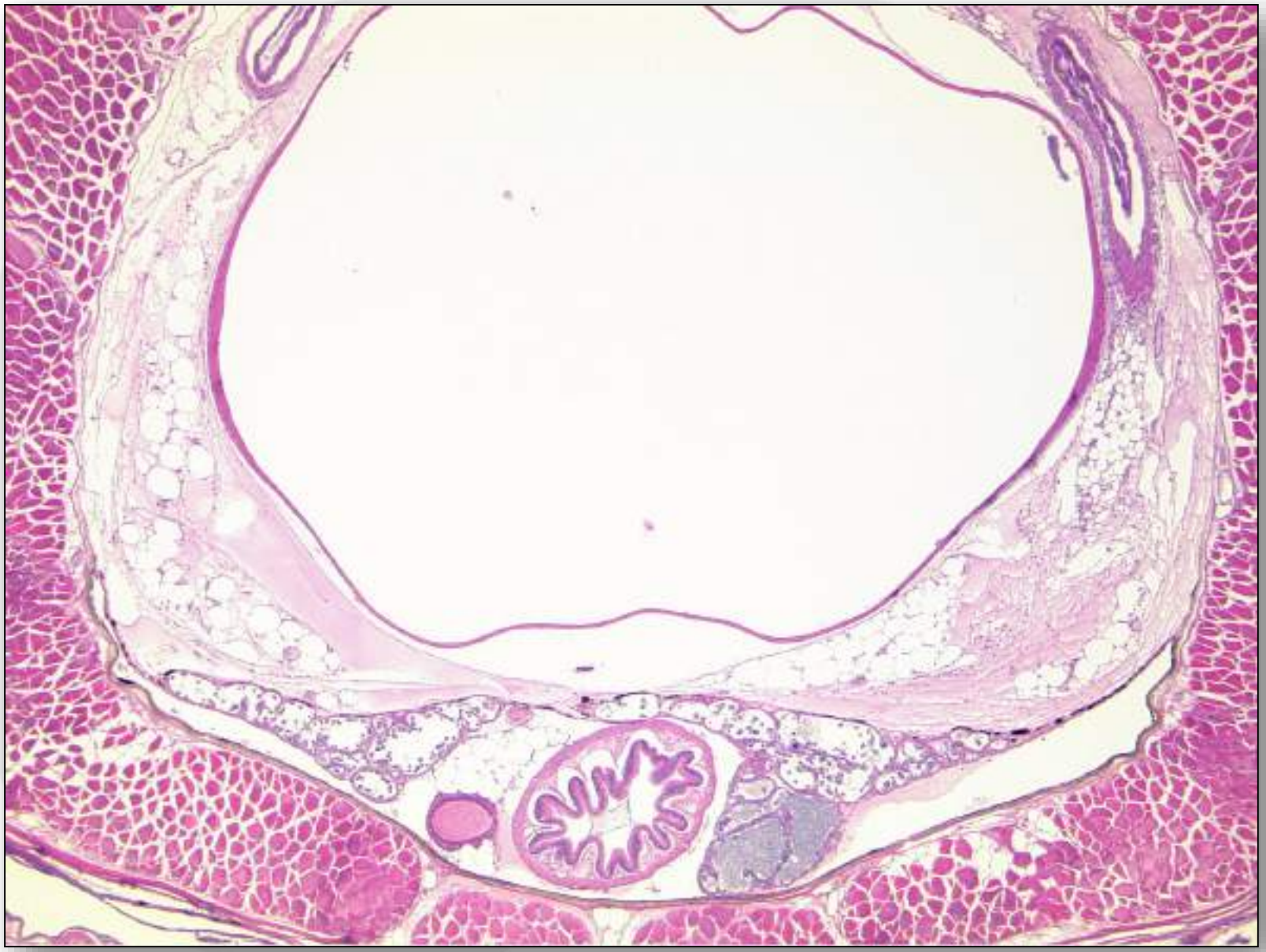


Liver: Control.
Female



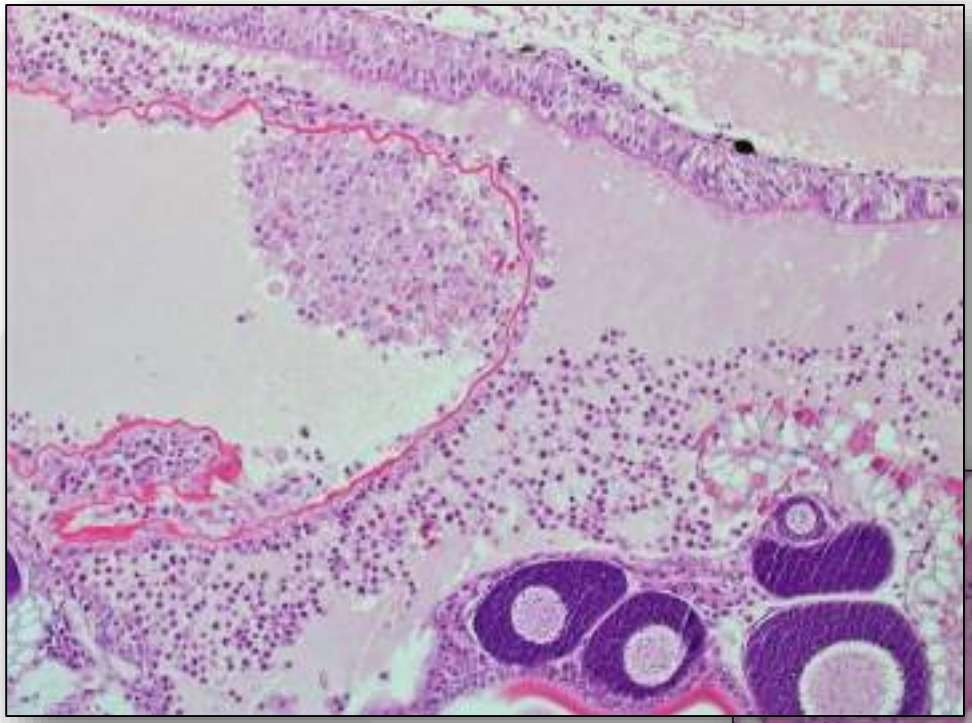
Liver: Increased basophilia (increased Metabolism)

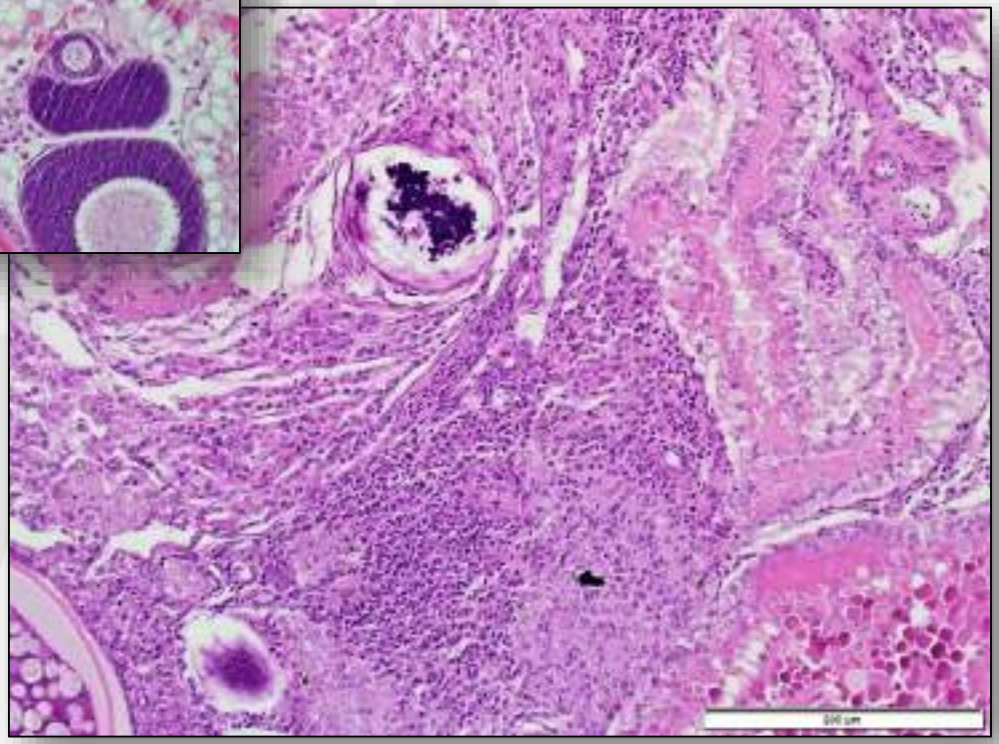
Effect by Anti-Estrogenic Compound



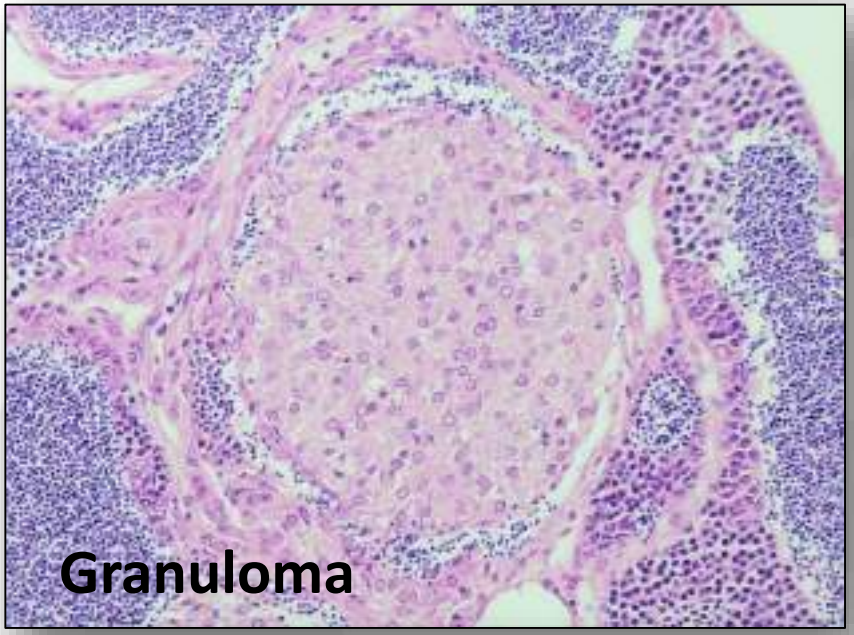
Background Alterations of Minor Significance.

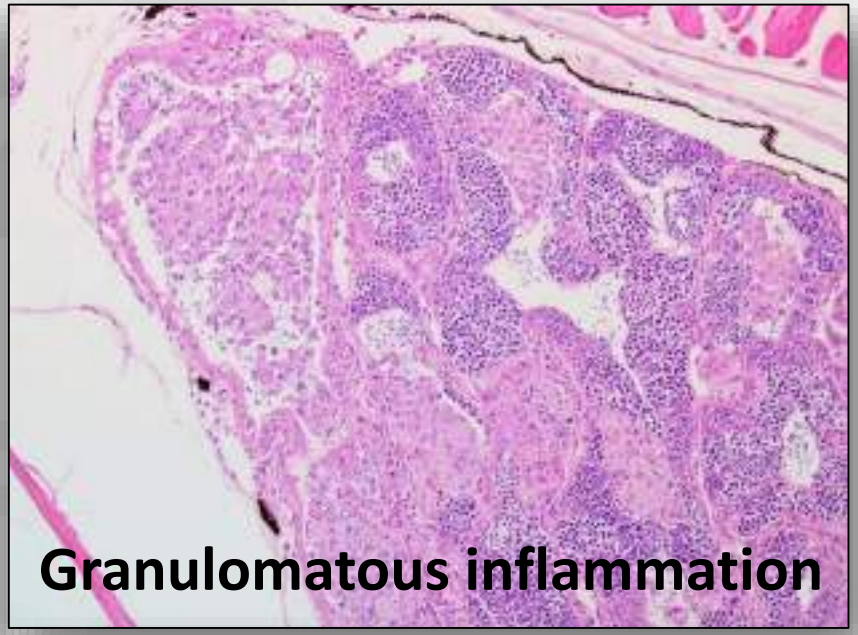
Background. Ovaries. Inflammation

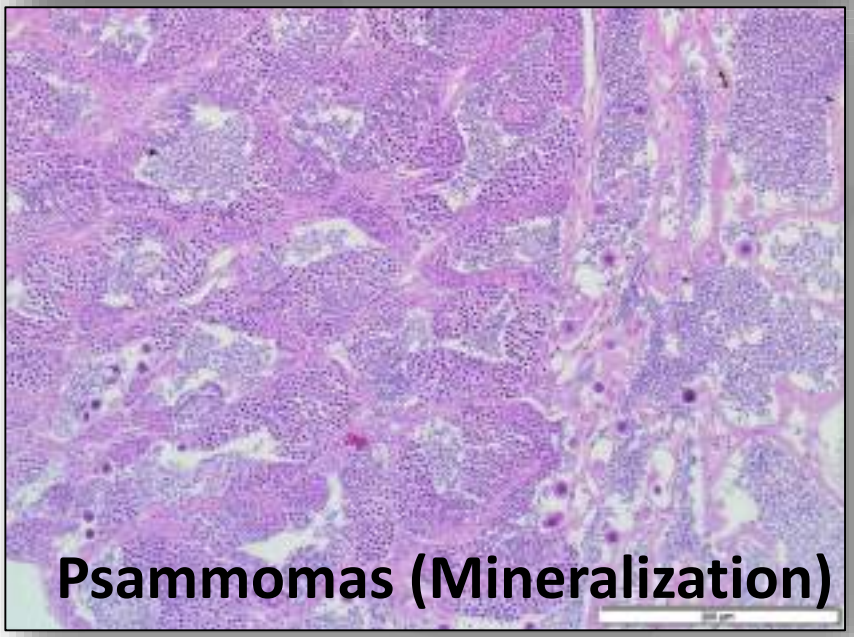


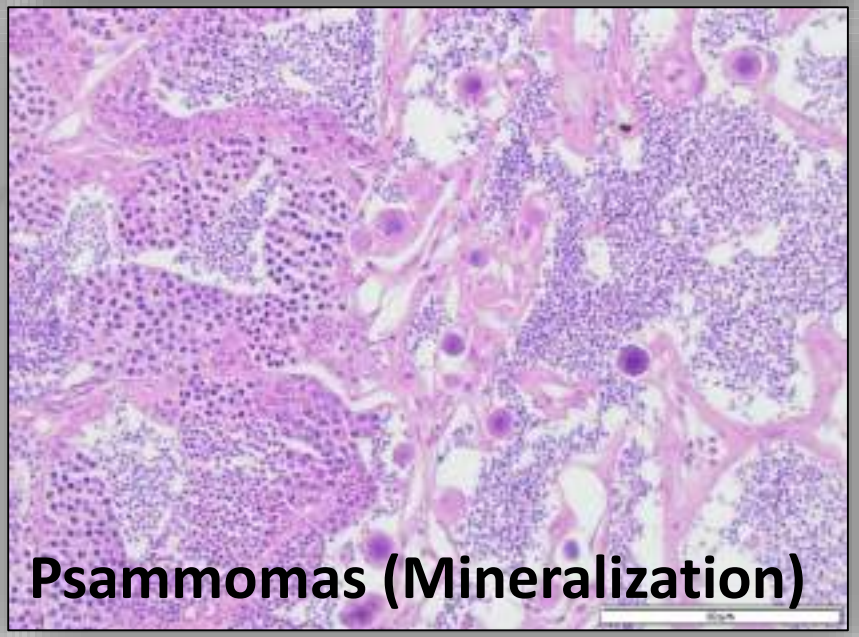


Background. Testes

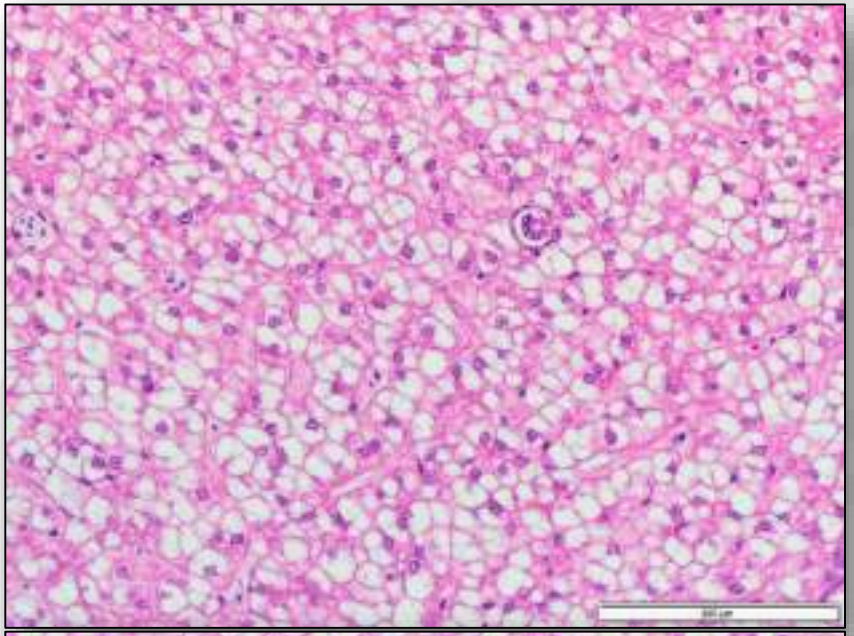






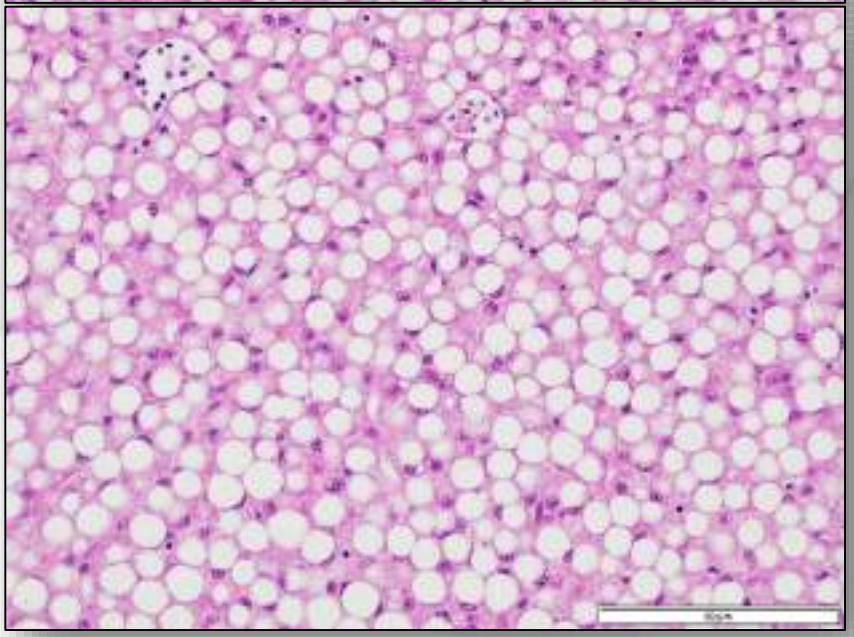


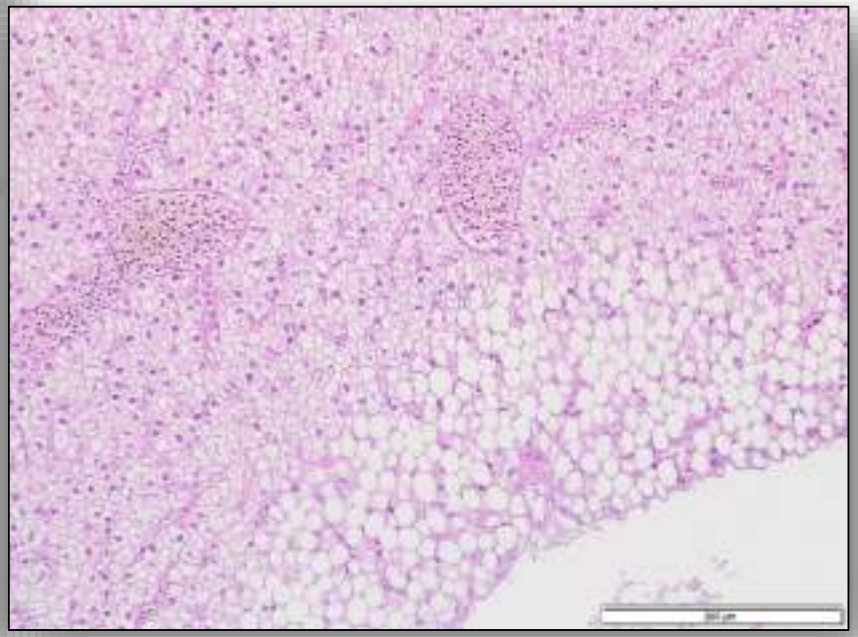
Background. Liver.



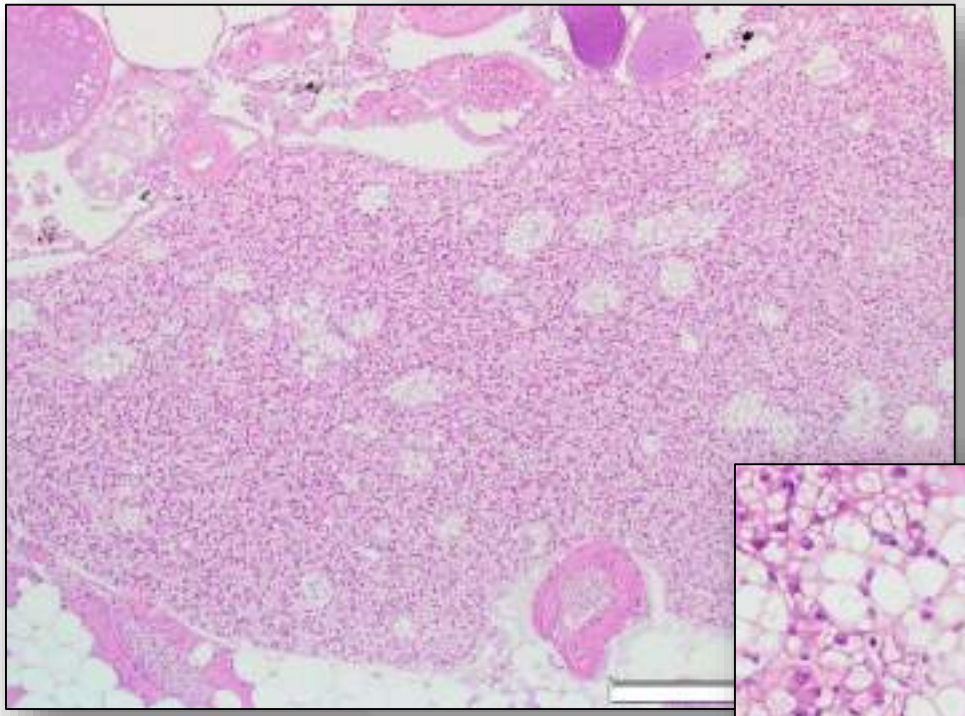
Male: Control.

Vacuolation (fatty change may be diffuse or focally increased.

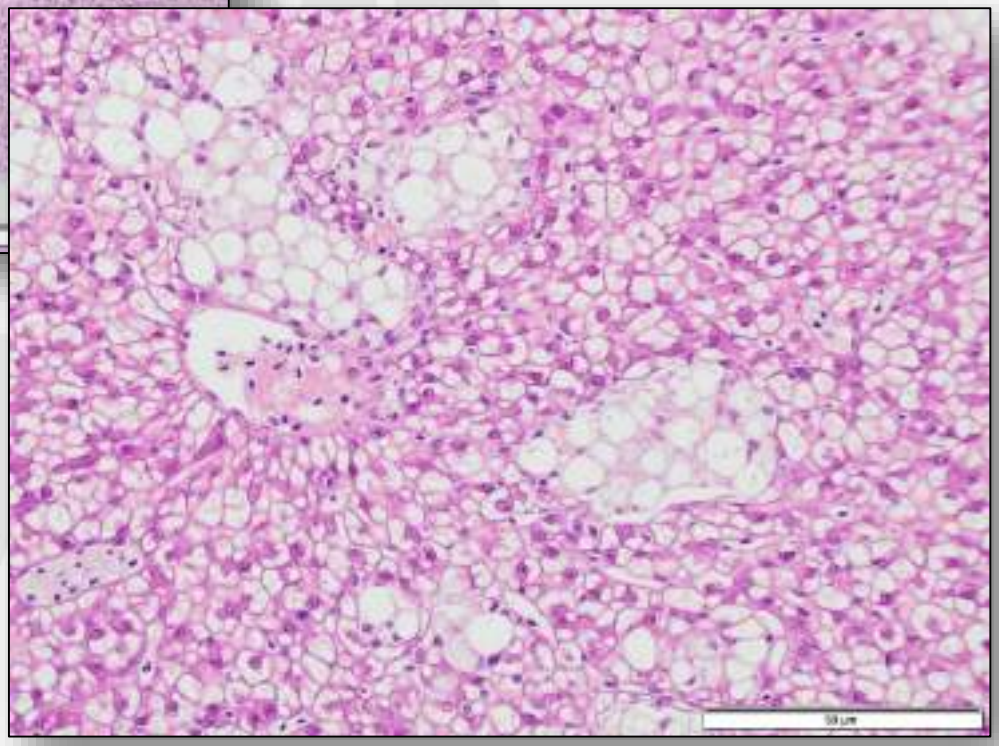




Background: Liver.

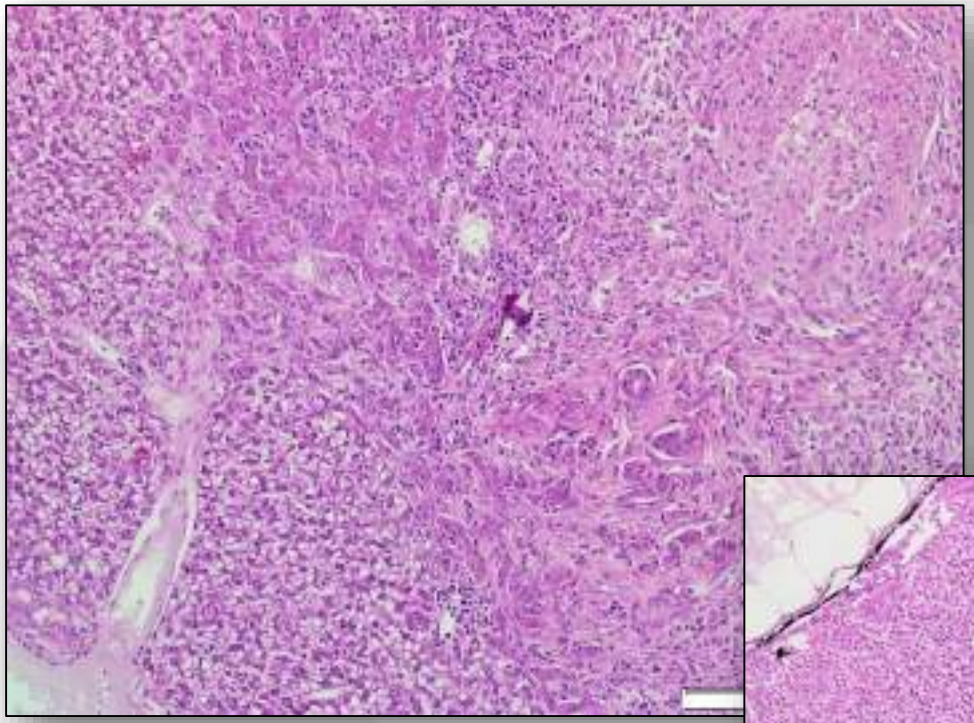


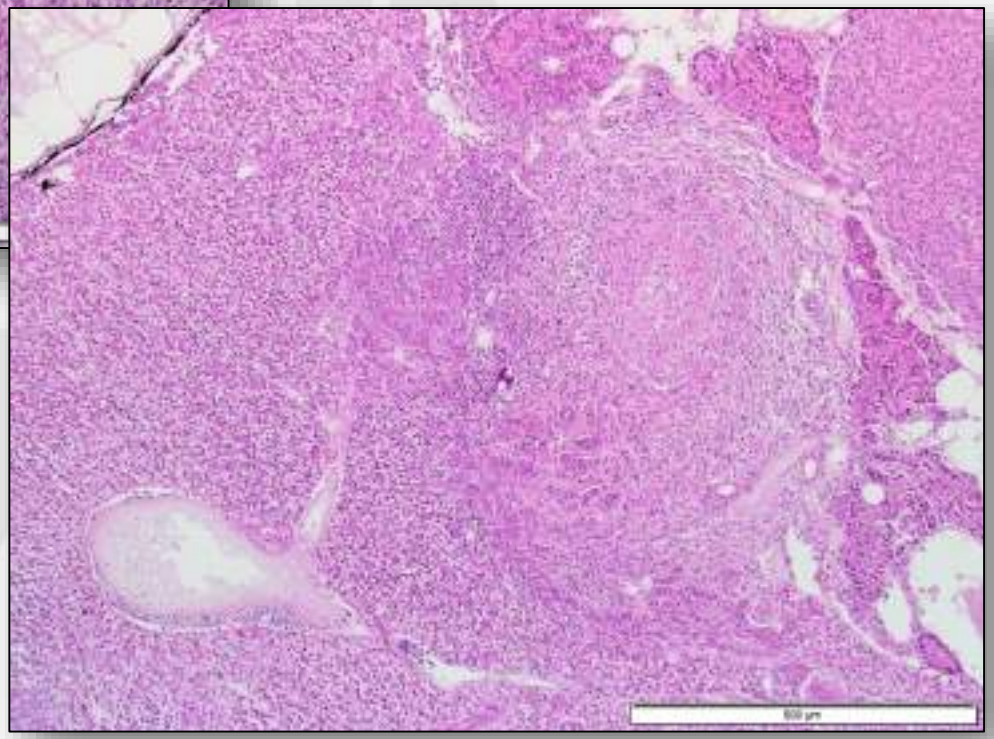
Male:
Multifocal increased vacuolation.



Male:
Formation of granuloma-like lesions.

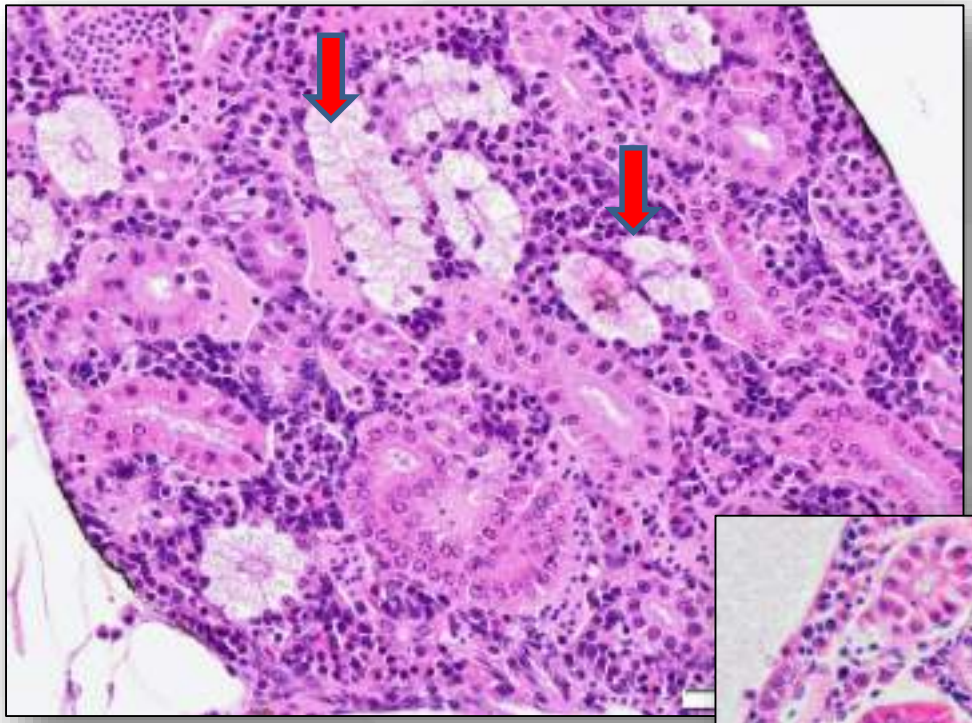
Background. Liver.





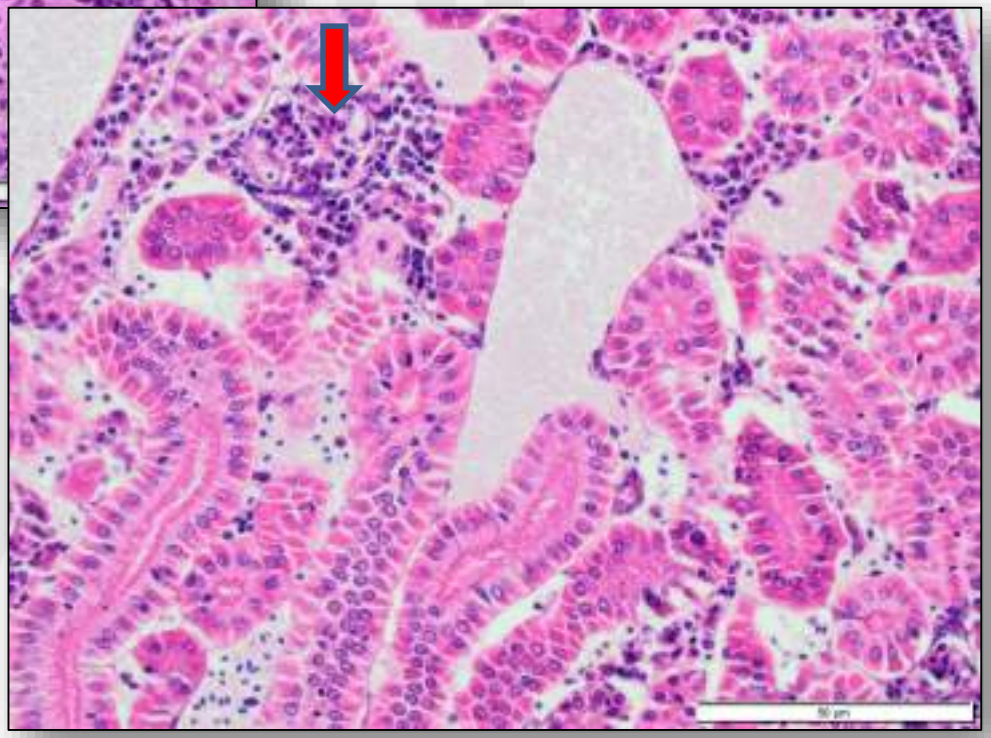
Female:
Chronic inflammation.

Background. Kidney



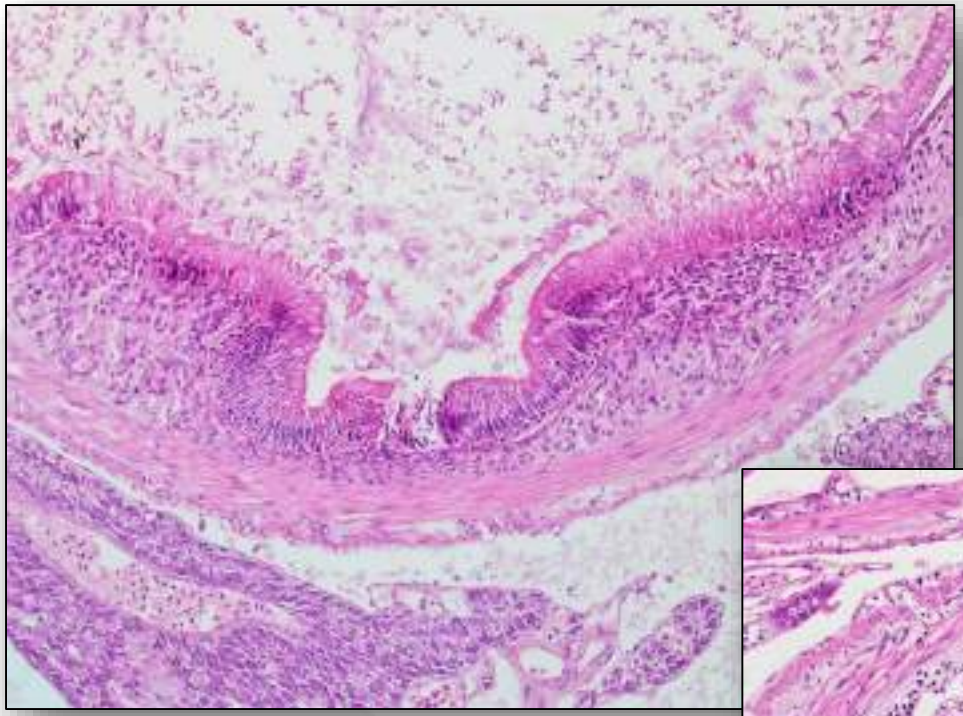
Normal. Transition Head/Trunk kidney.

Note: proximal tubules (arrow).
Hemopoiesis.

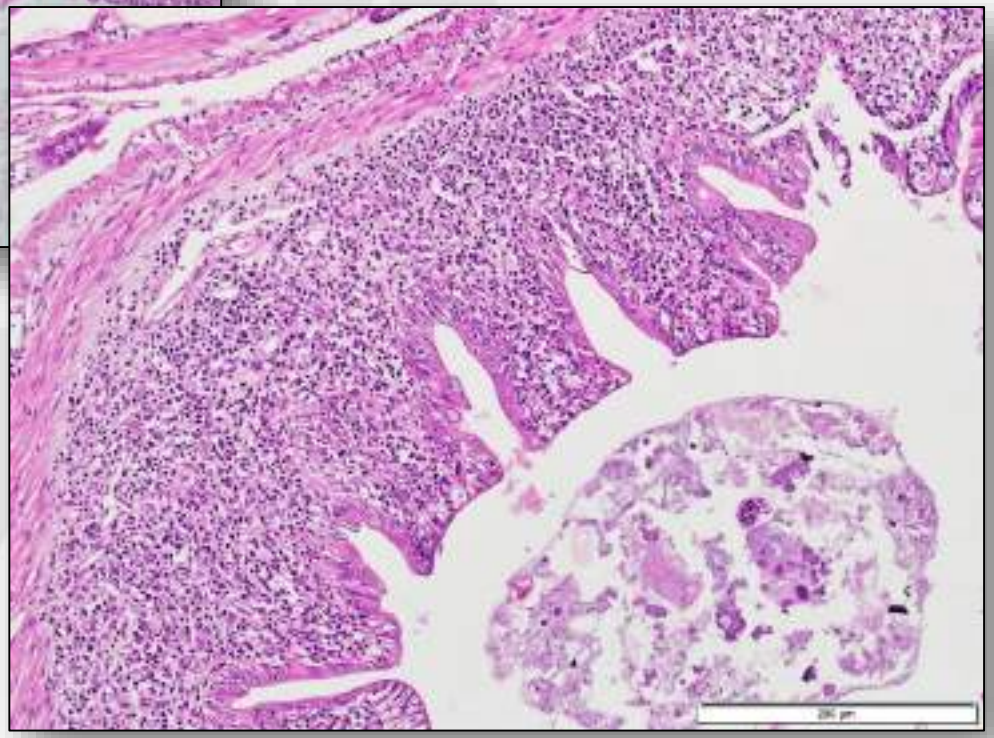


Mononuclear cell focus.

Backgound. Intestine

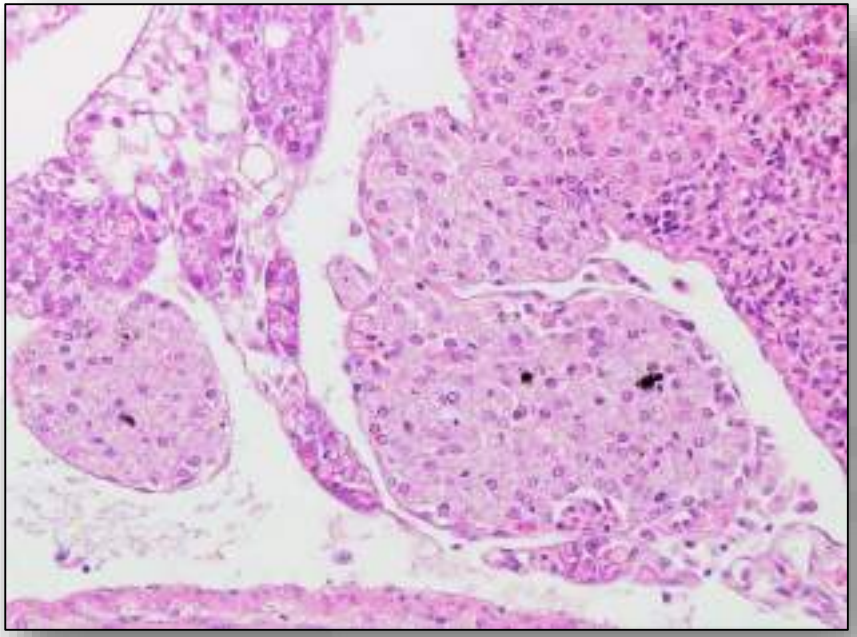


Foregut.
Focal inflammation in submucosa.

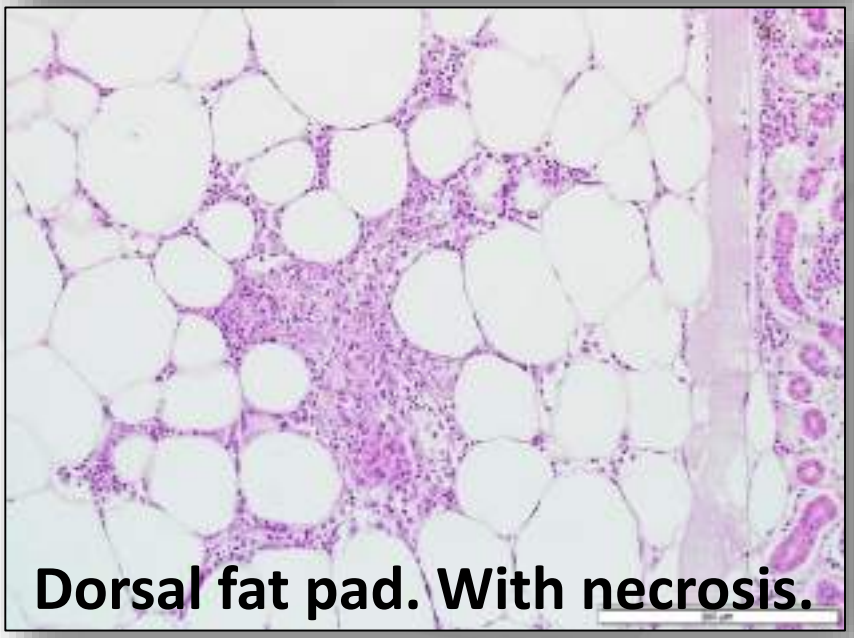


Endgut.
Focal inflammation in submucosa/mucosa.

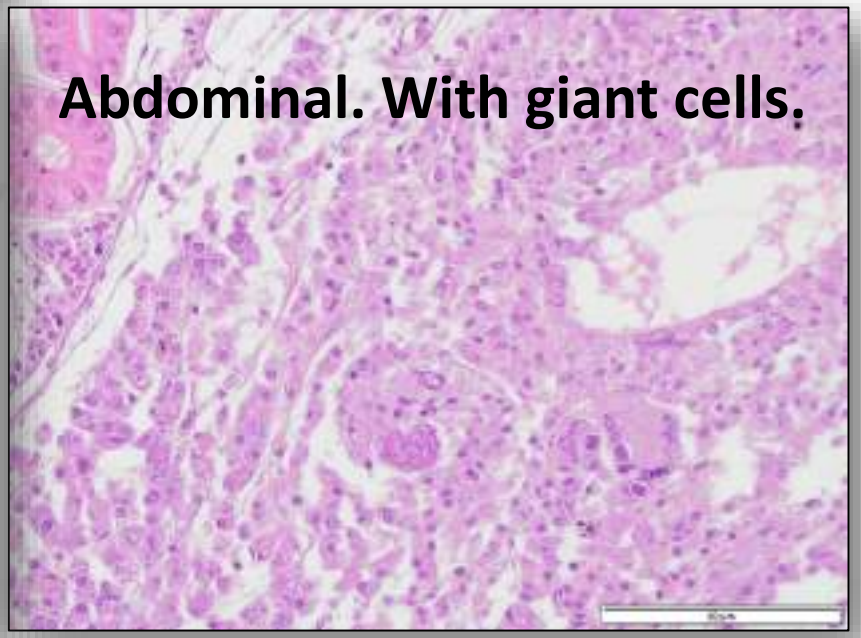
Background. Granulomatous Inflammation



Abdominal tissues.



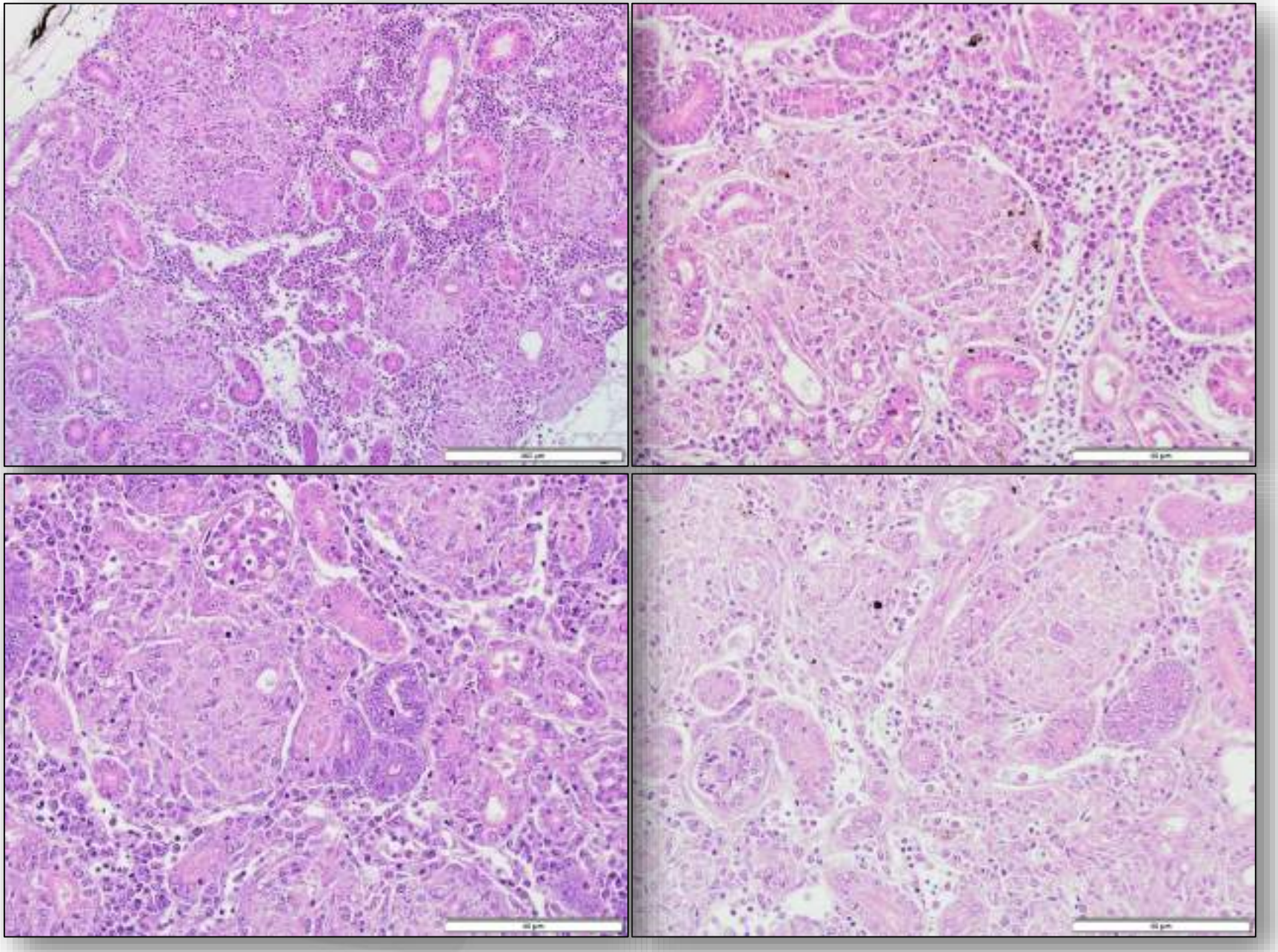
Dorsal fat pad. With necrosis.



Abdominal. With giant cells.

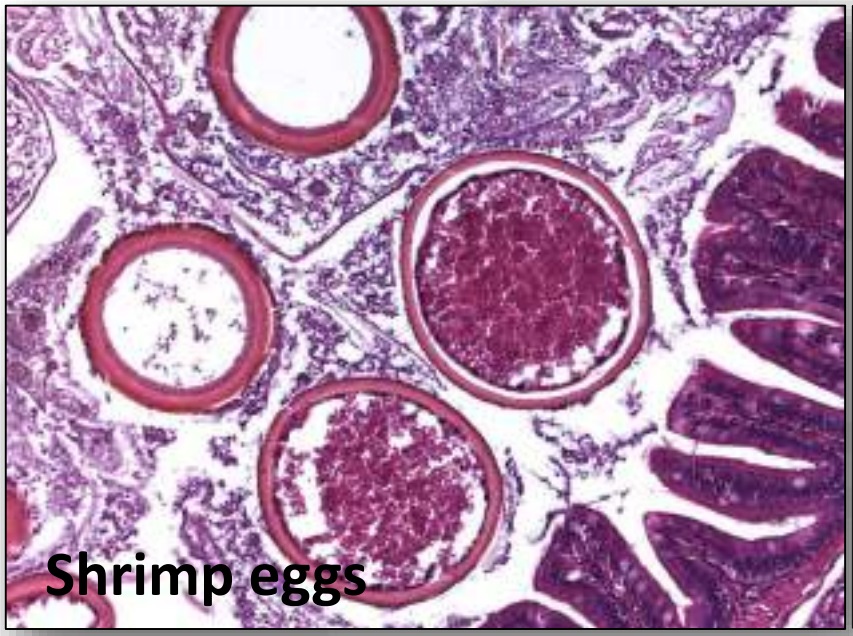
Induced Findings in None Reproductive Organs.

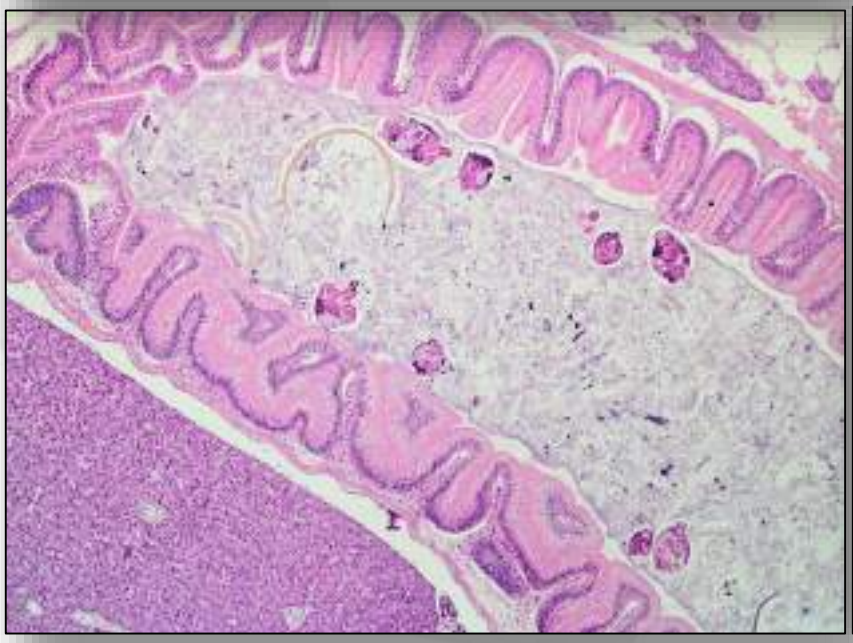
Induced. Kidney. Chronic Inflammation.

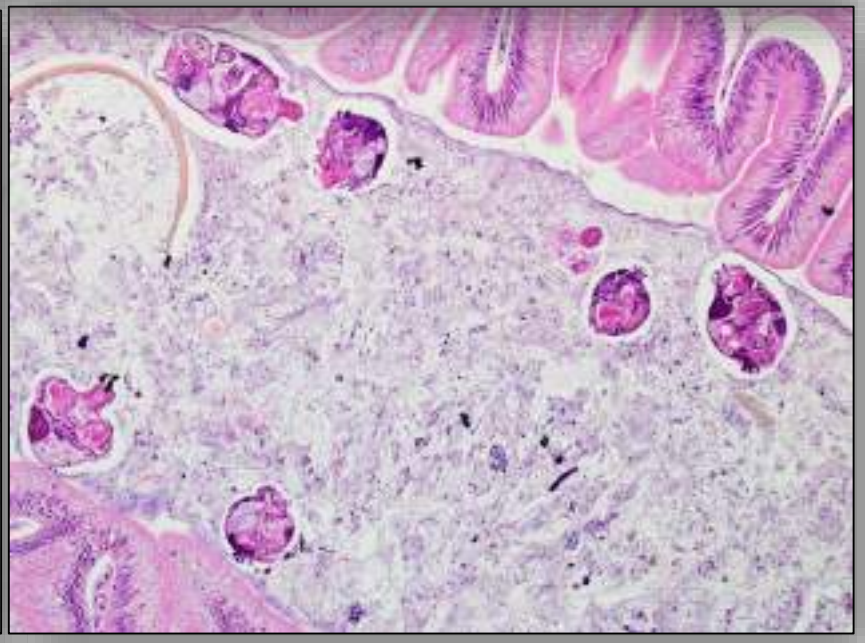


Parasites.

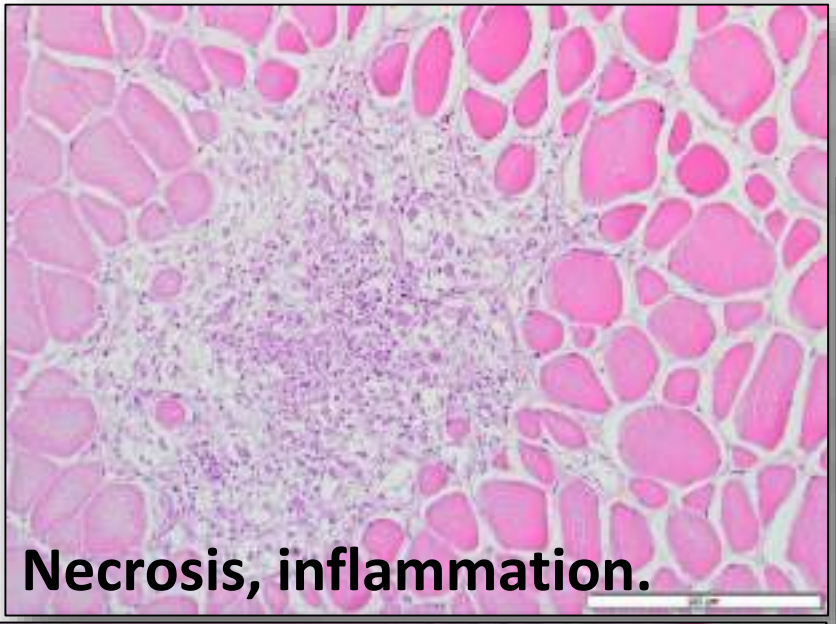
Background. Intestine. No Parasites!







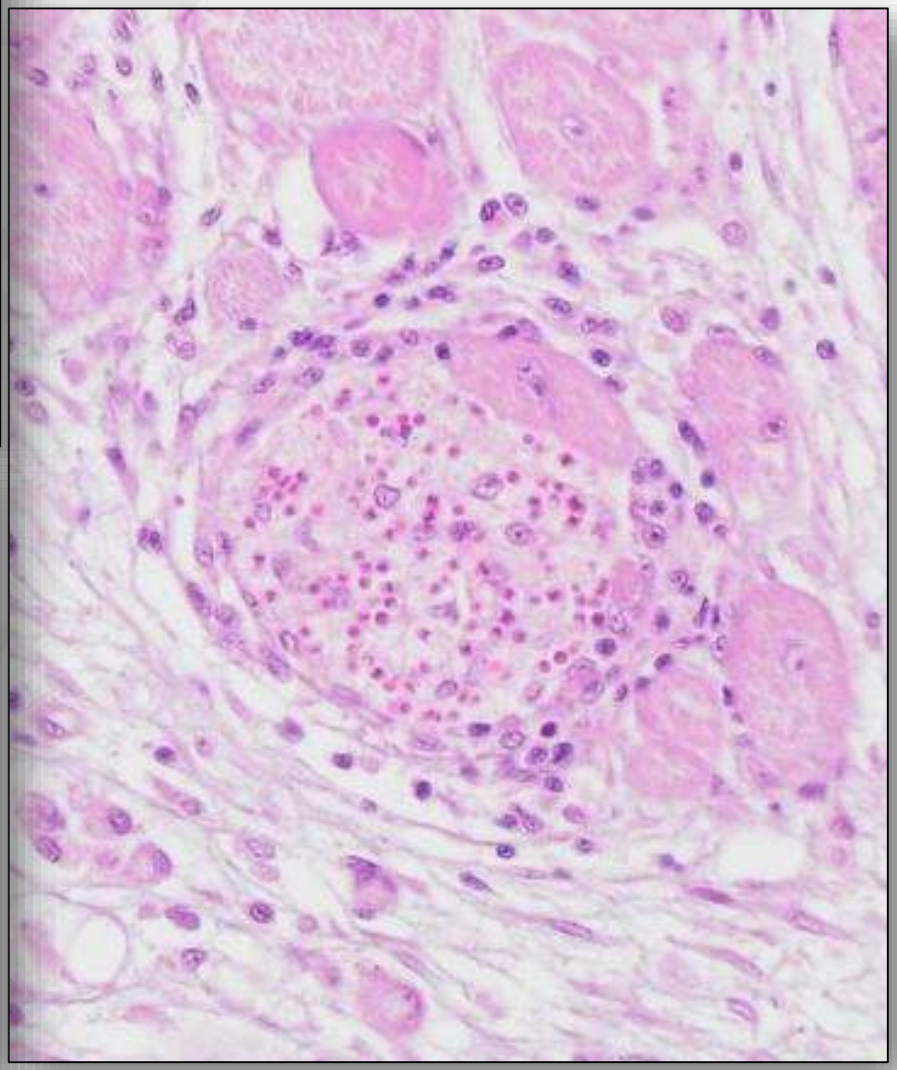
Background. Skeletal Muscle. Microsporidia.



Necrosis, inflammation.

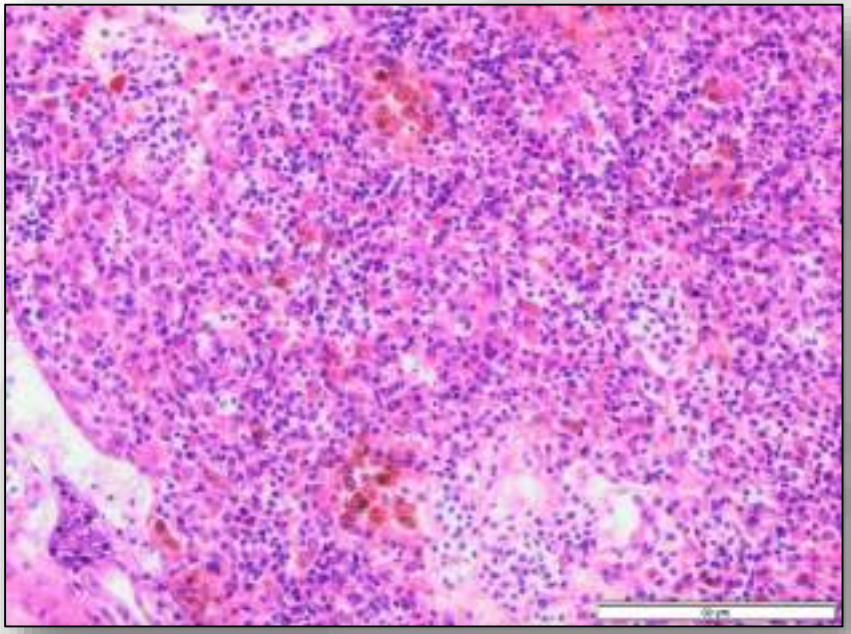
Necrosis, inflammation.

Protozoan in cyst.
Bed-slipper shape.



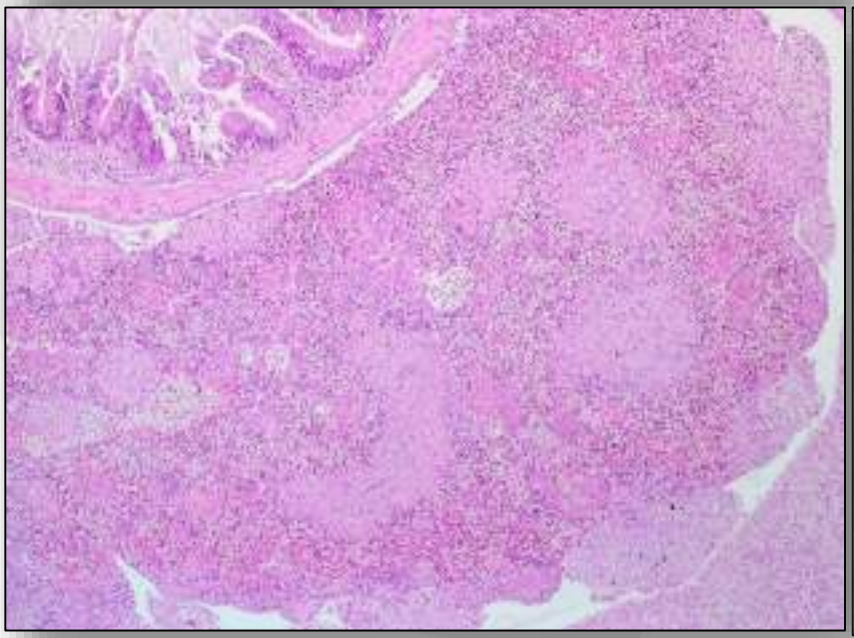
Infection.

Background. Spleen.

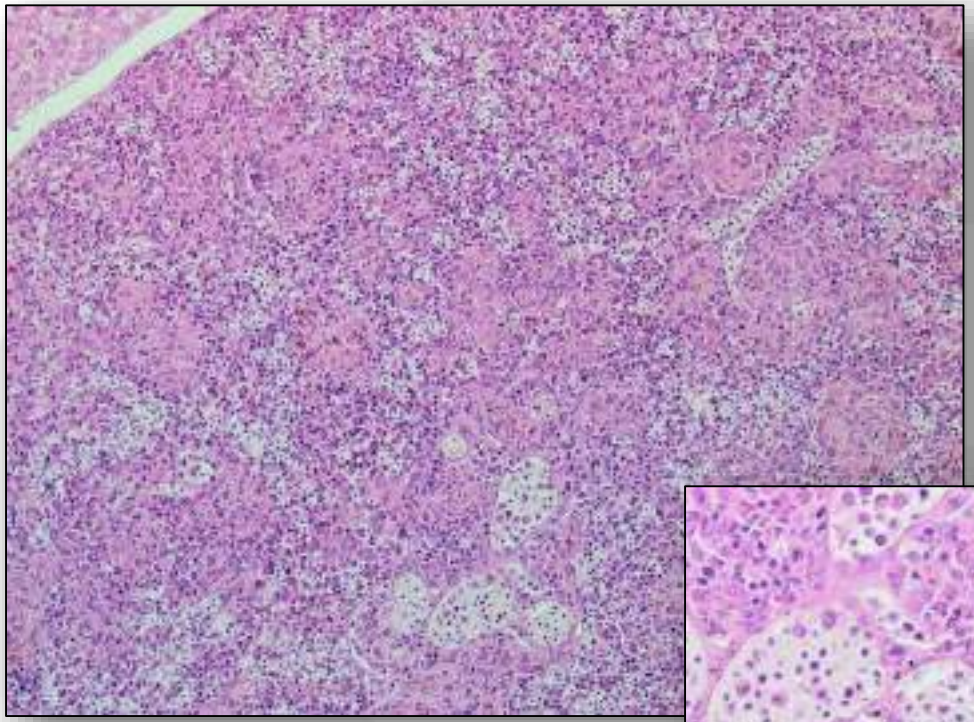


Pigment, hemosiderin.

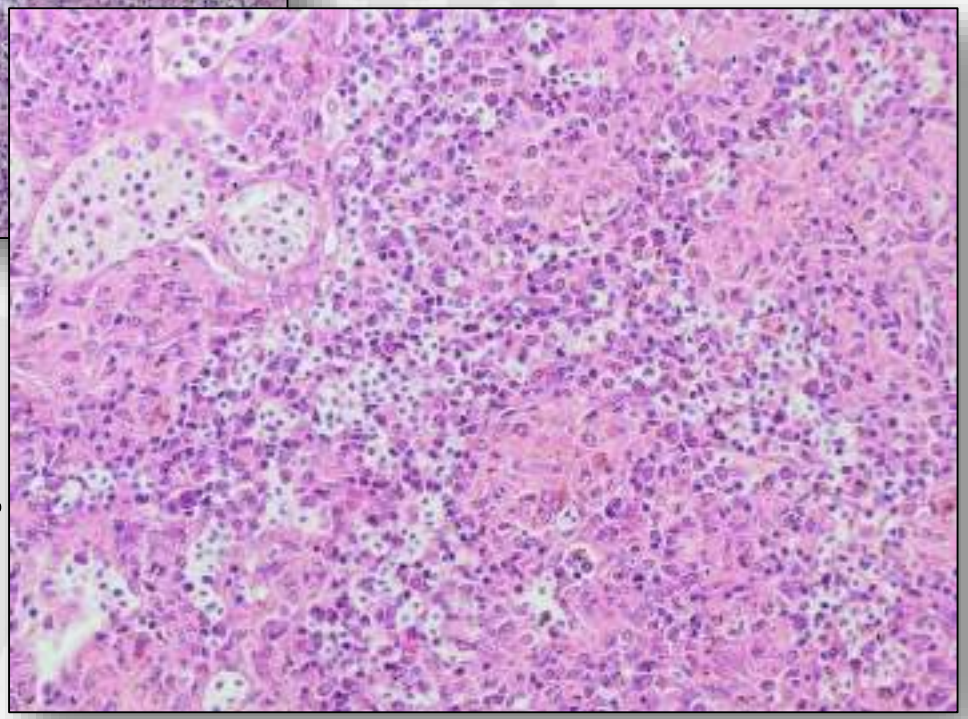
Granulomatous inflammation.



Background. Spleen.

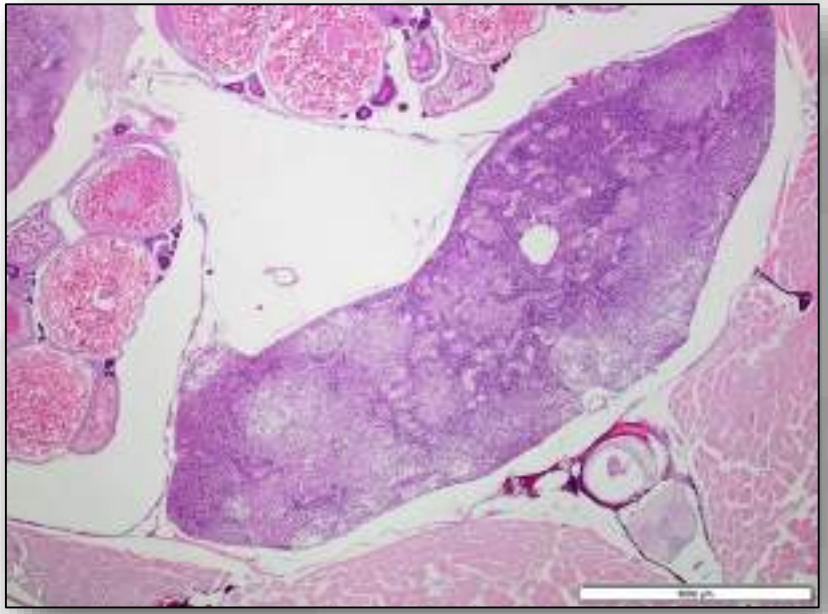


Granulomatous inflammation.

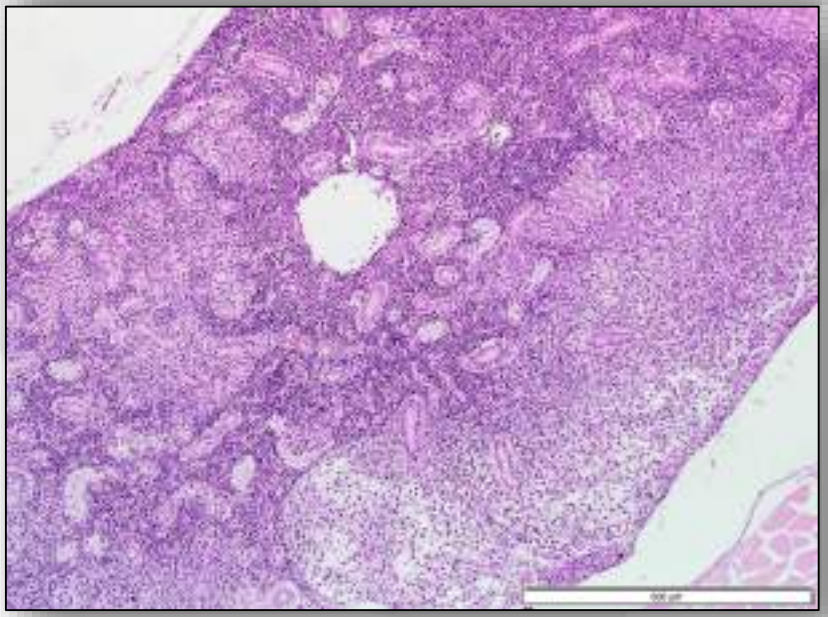


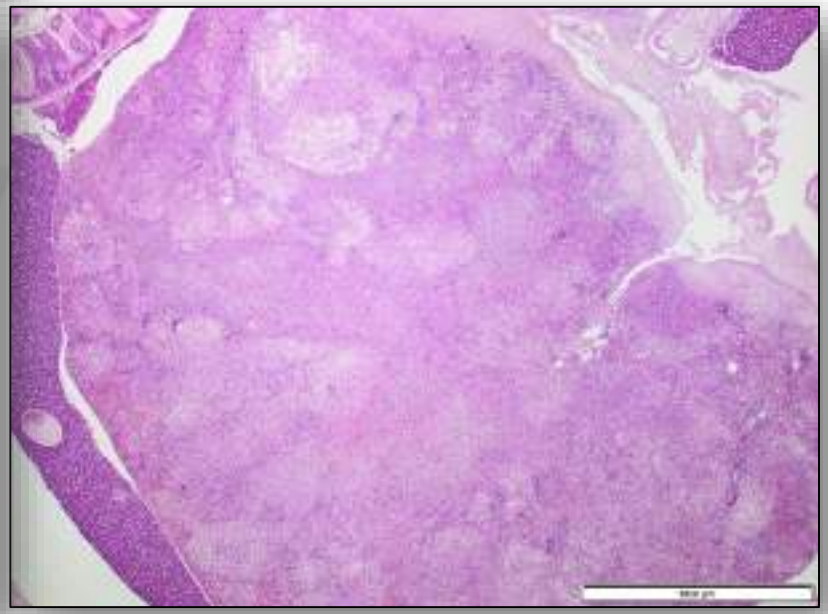
Note: central areas with necrotic cells.
Centrally stored pigment.
Suspective of tuberculosis.

Background. Spleen.

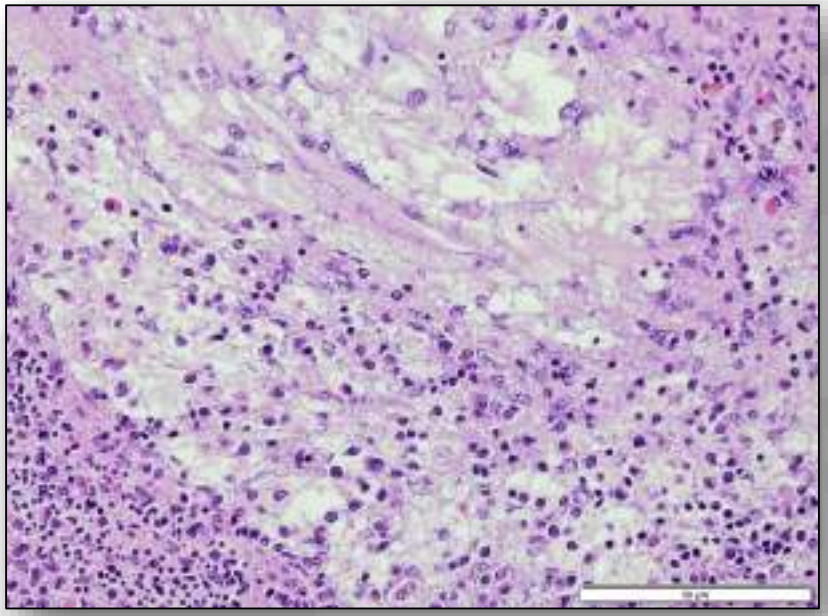


Granulomatous inflammation.



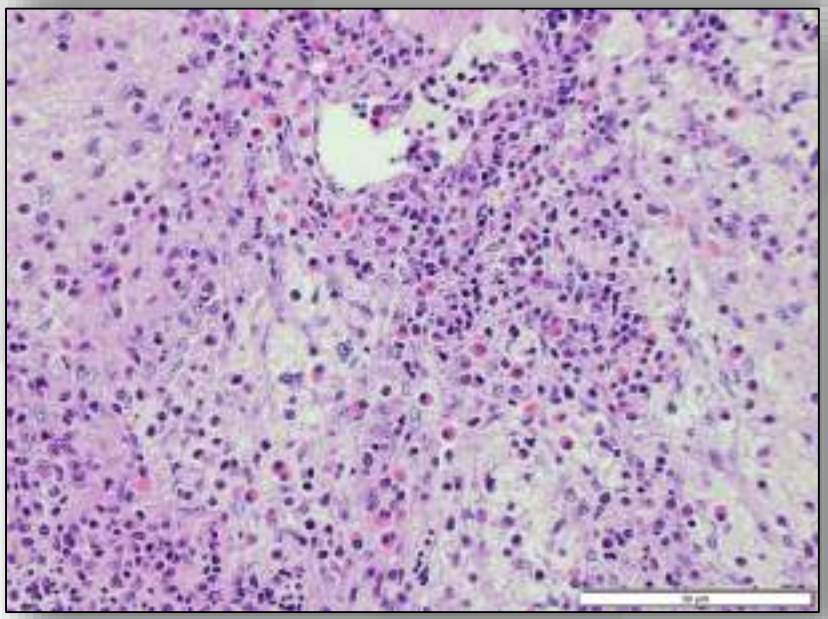


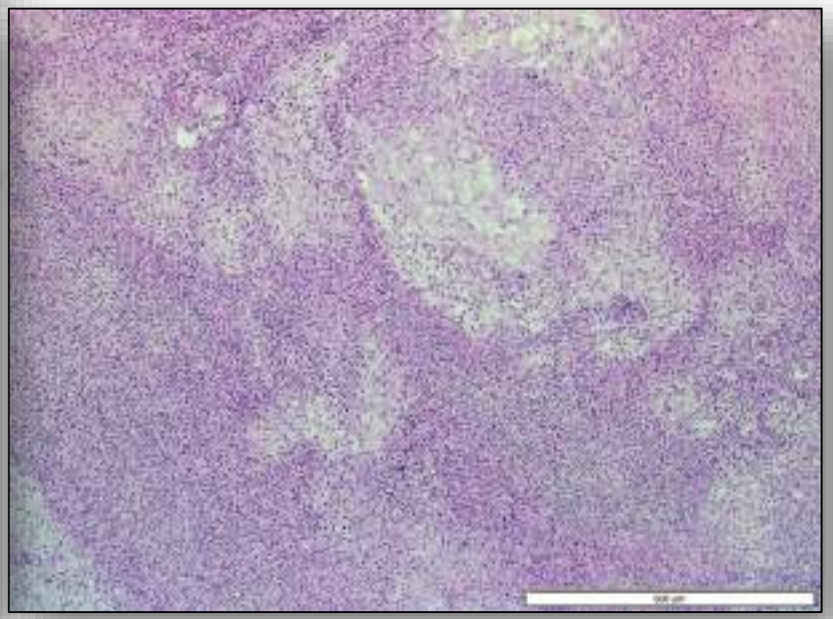
Background. Spleen.



Granulomatous inflammation.

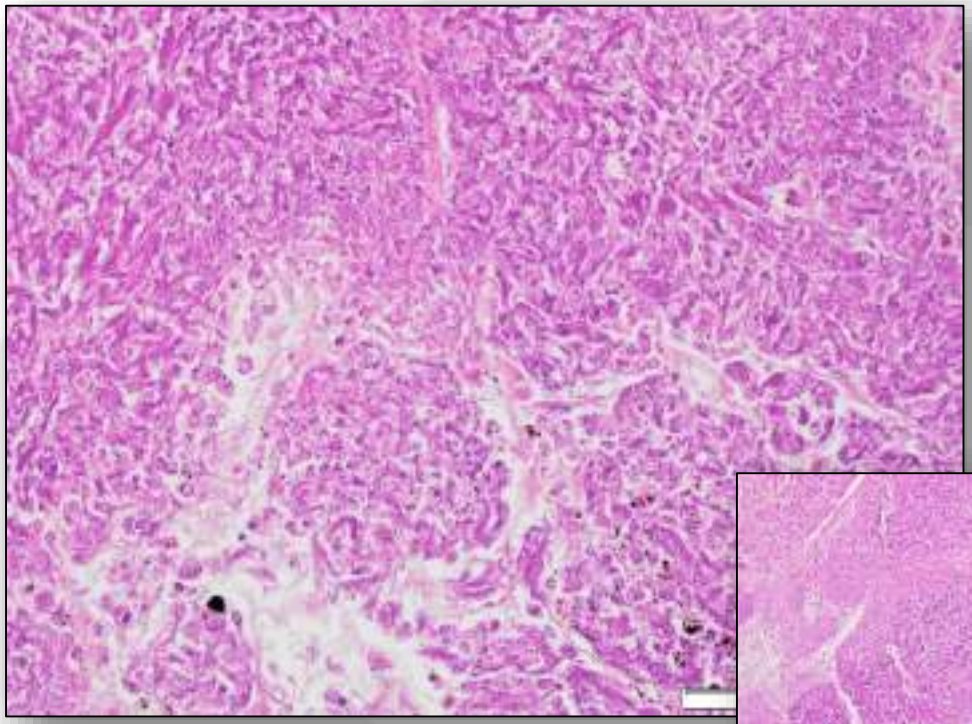
Note central necrosis.
Suspicious of tuberculosis.

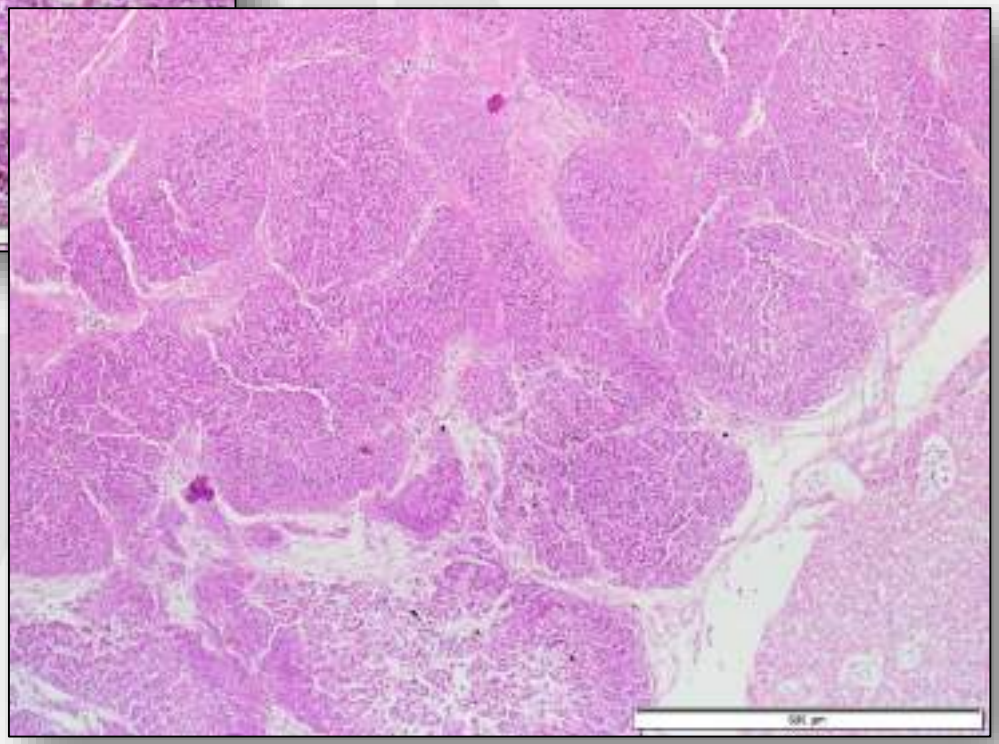




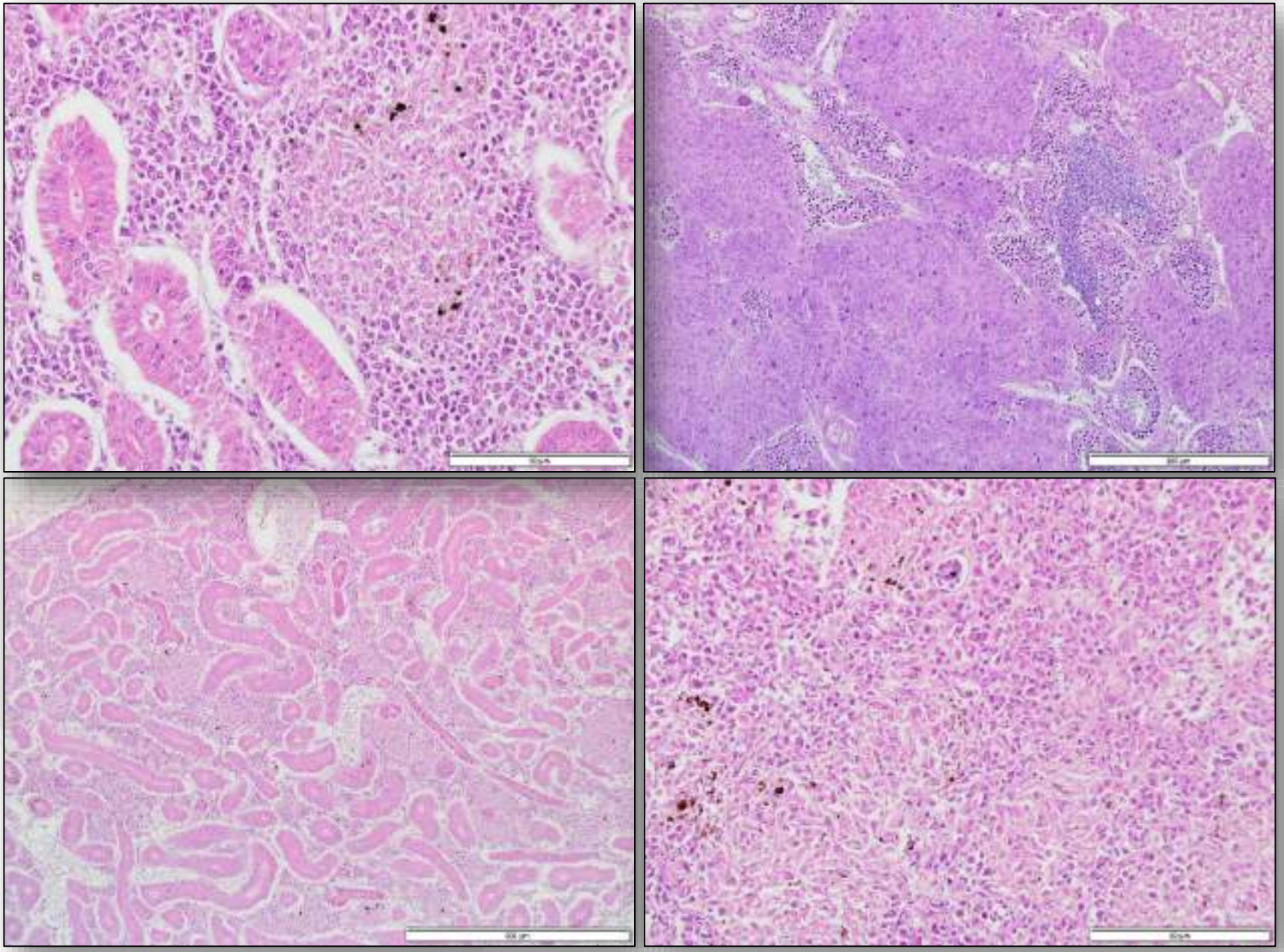
Neoplasia.

Background. Testes. Necrosis by Sertoli Tumor.

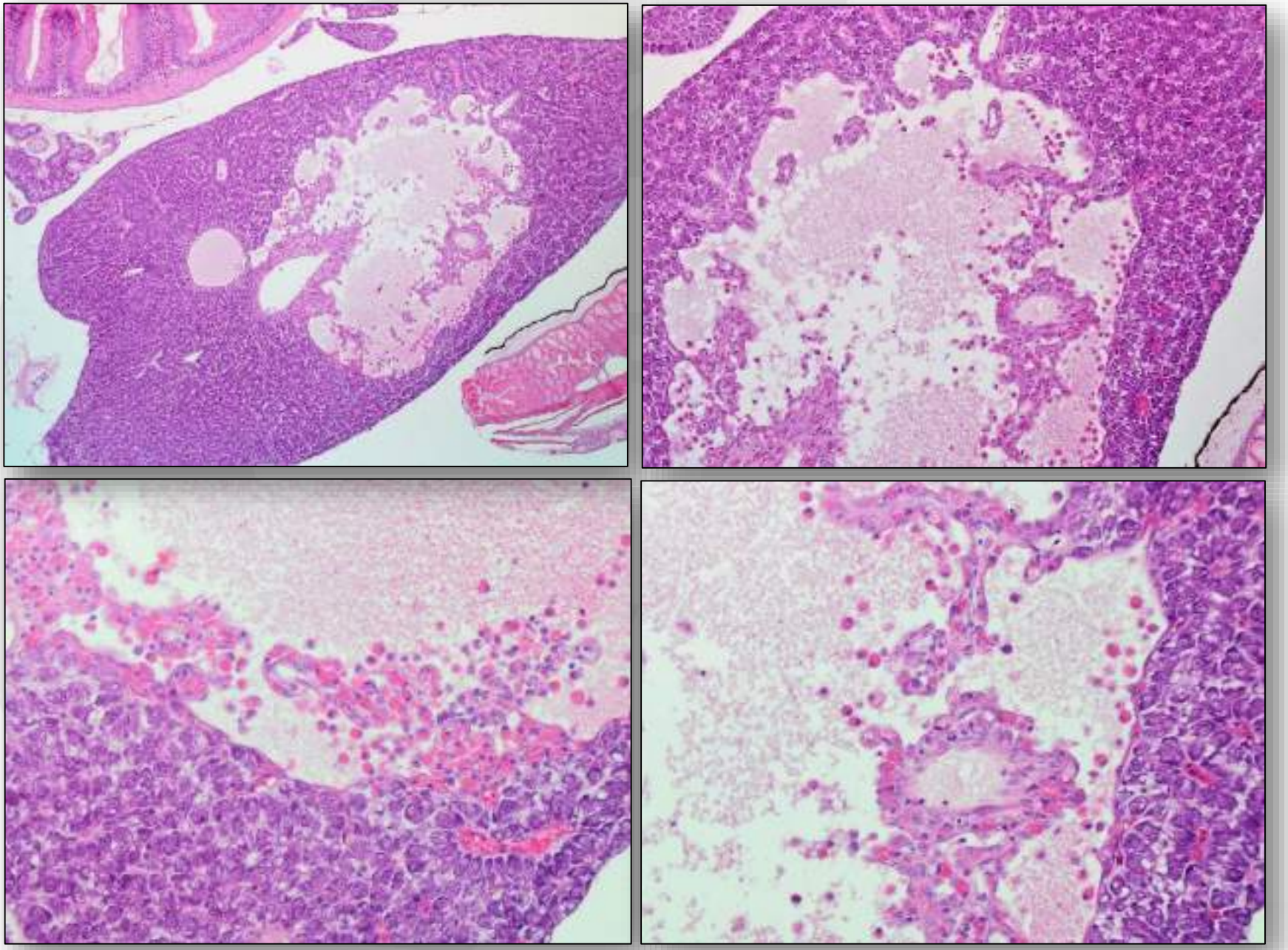




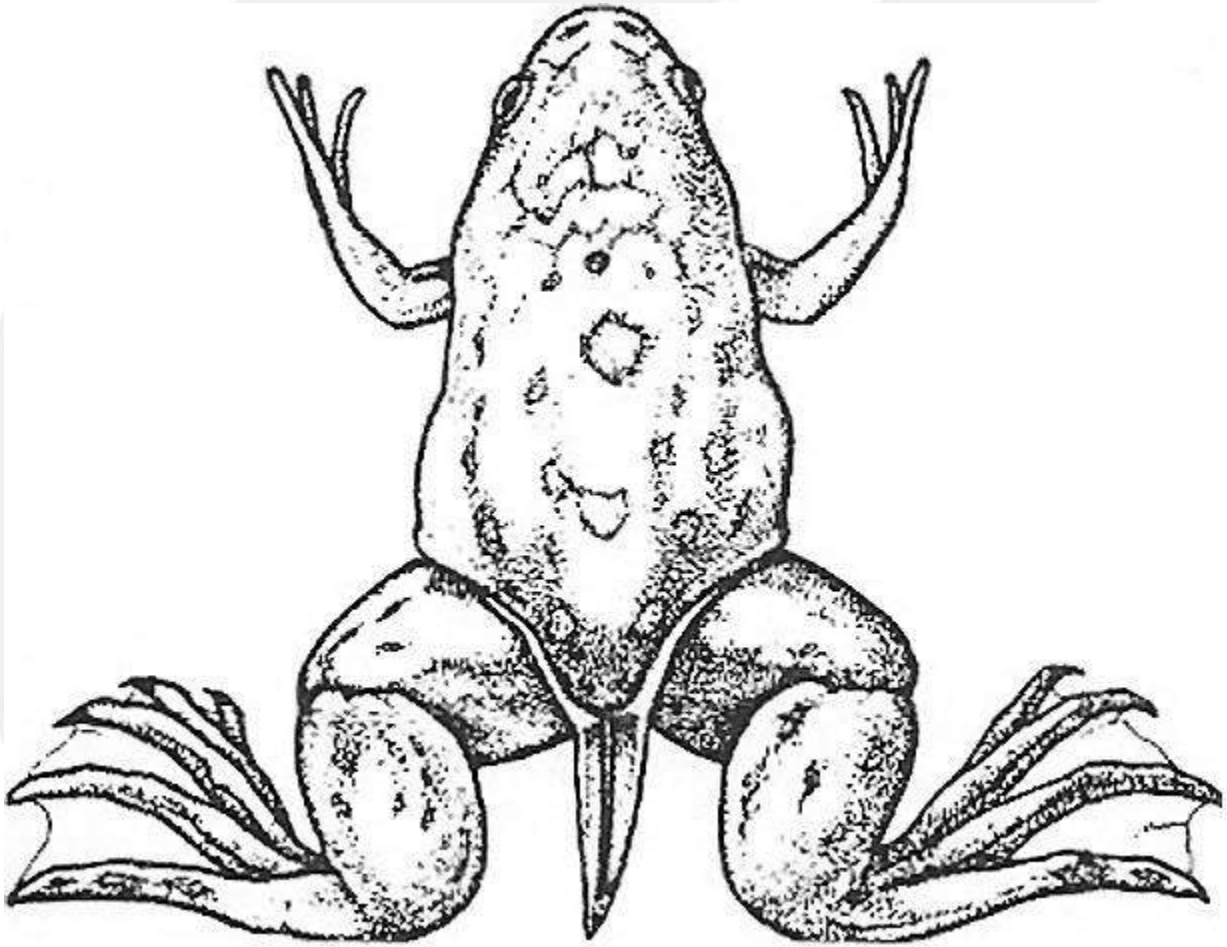
Background. Lymphoma.



Background: Liver. Angioma.

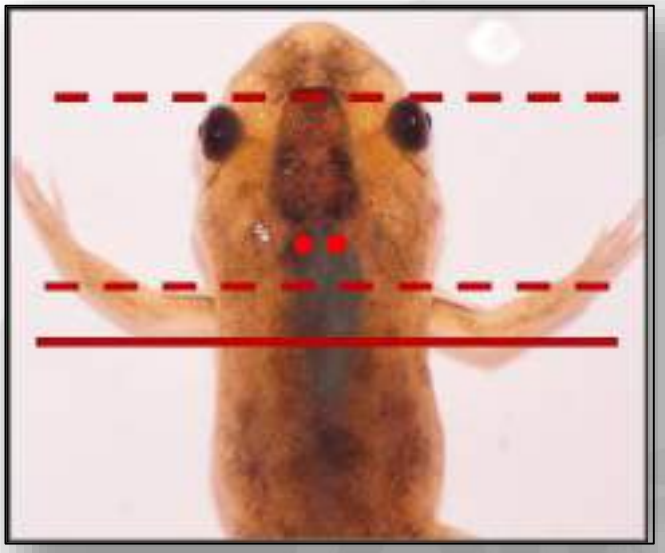


Xenopus

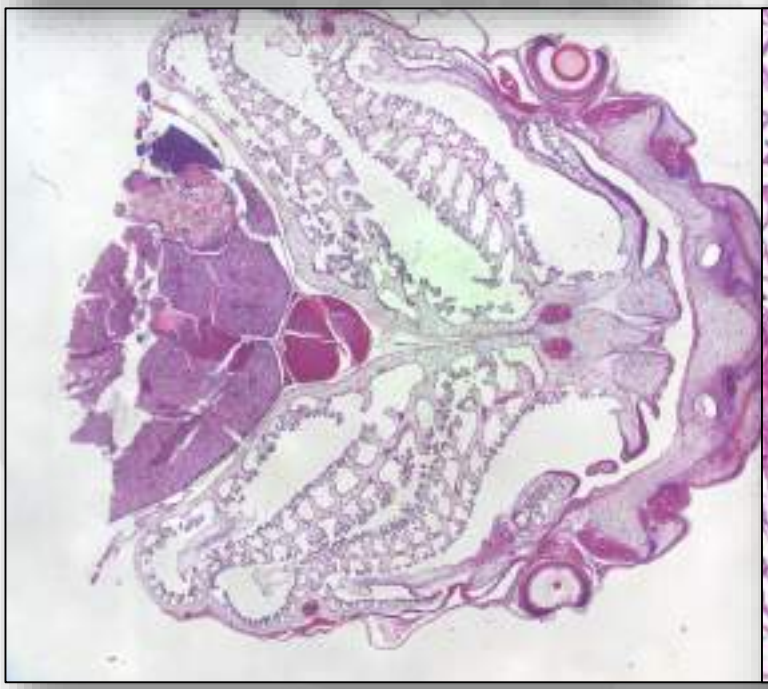


Stage 64, dorsal view
53 days pf @ 23°c

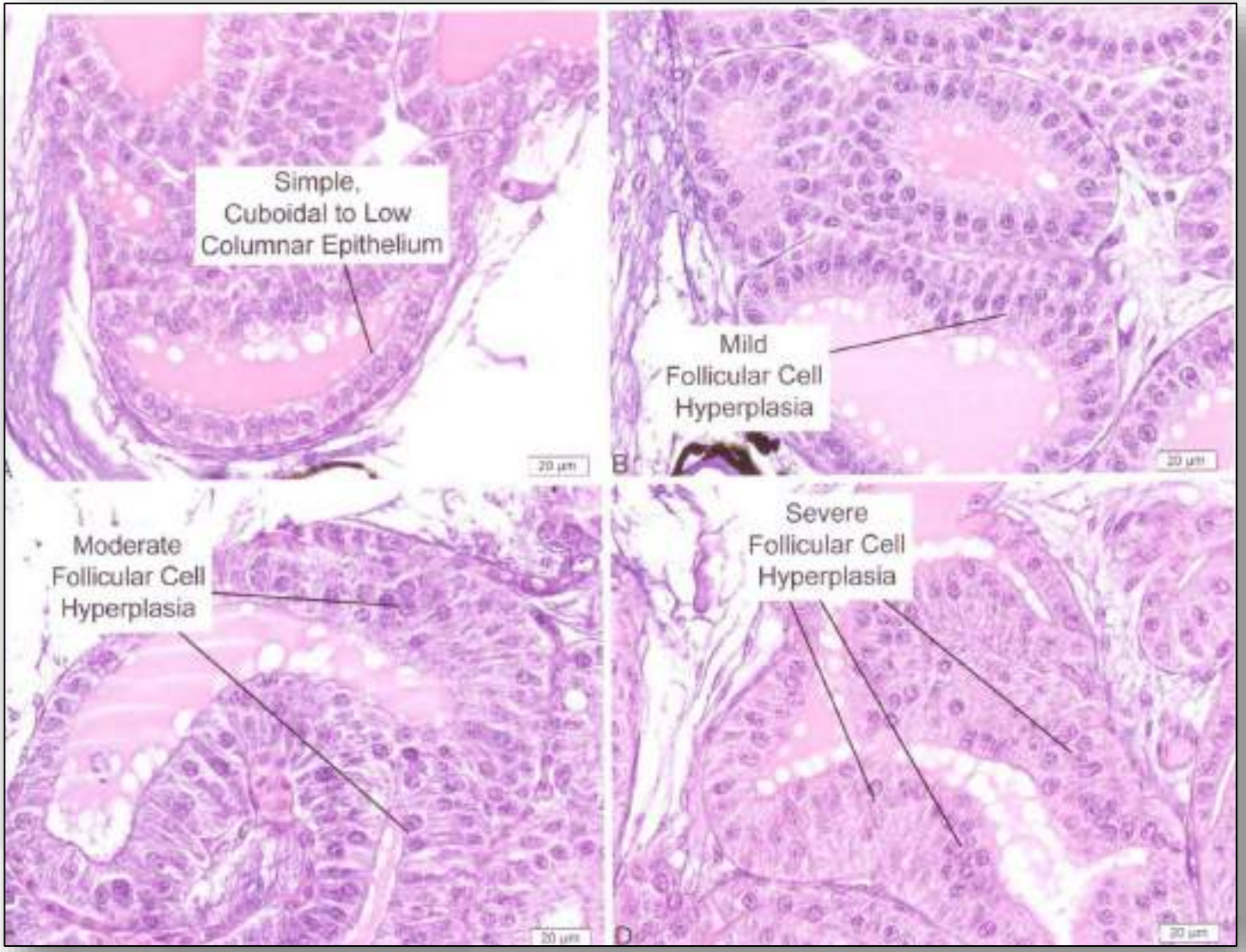
Location of Thyroid Gland in a Tadpole



The Amphibian Metamorphosis Assay



Grim K et. al (200) Thyroid Histopathology Assessments for the Amphibian Metamorphosis Assay to Detect Thyroid-active Substances. Toxicol Pathol



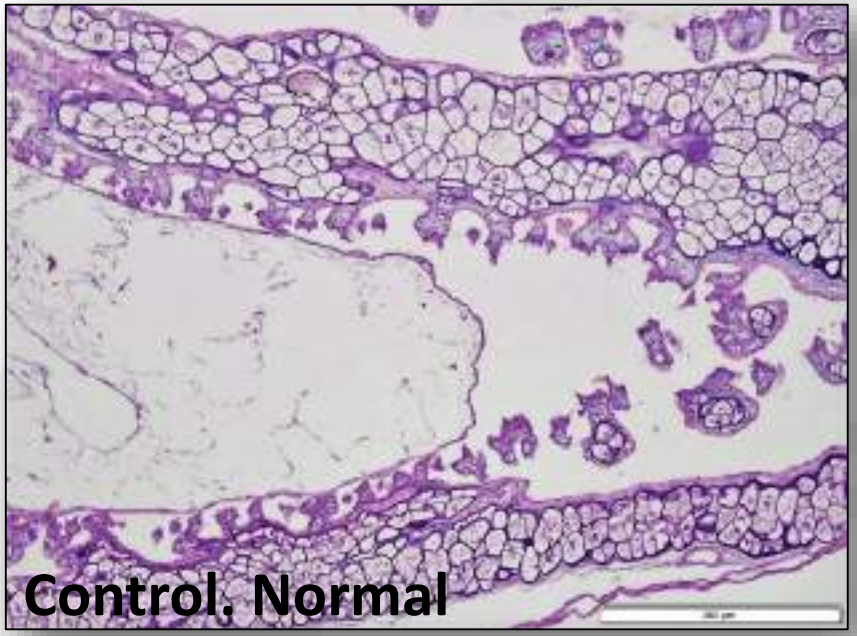
Thyroid Gland: Endocrine Disruptors

Control

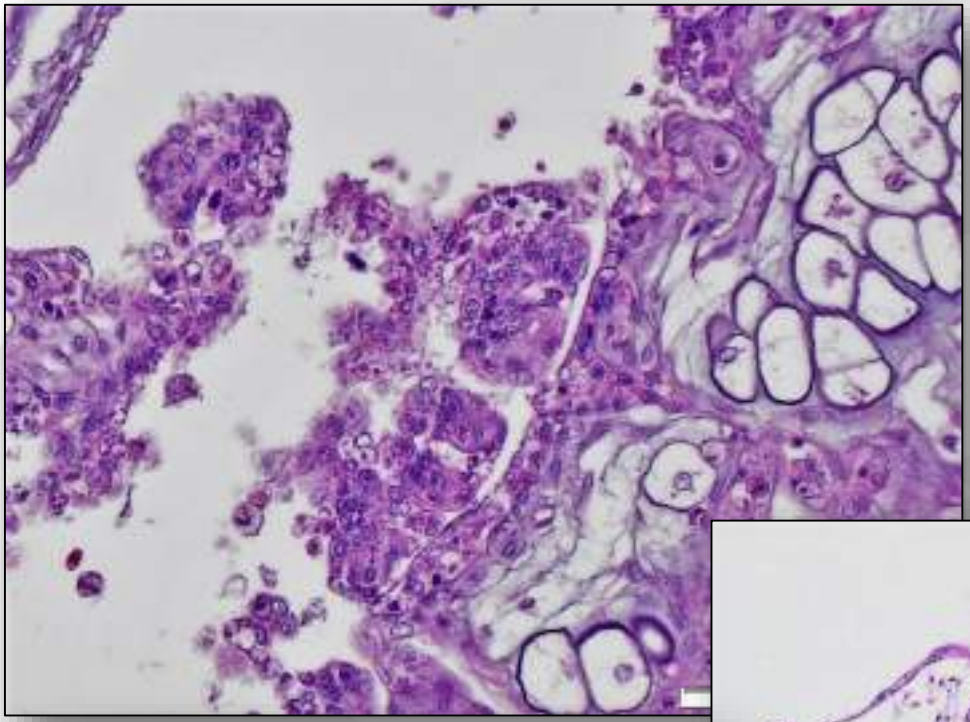
Test Item:
minimal follicular hypertrophy

Induced: No Endocrine Disruption!

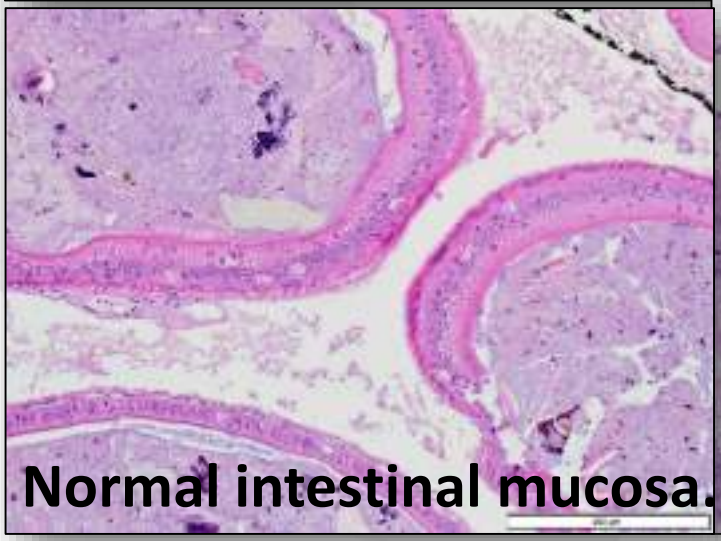
Other Organs. Induced Irritative Effects. Gills.



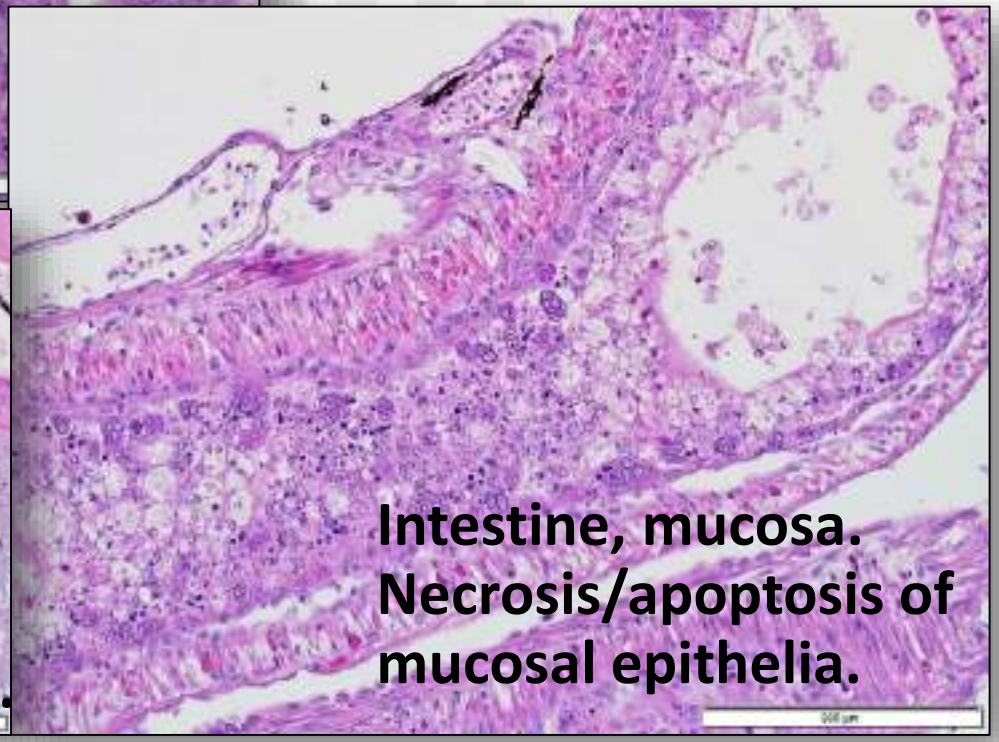
Other Organs. Induced Irritation. Gills/Intestine



Gill/Internal gill cavity, Epithelial cell necrosis/ Apoptosis.

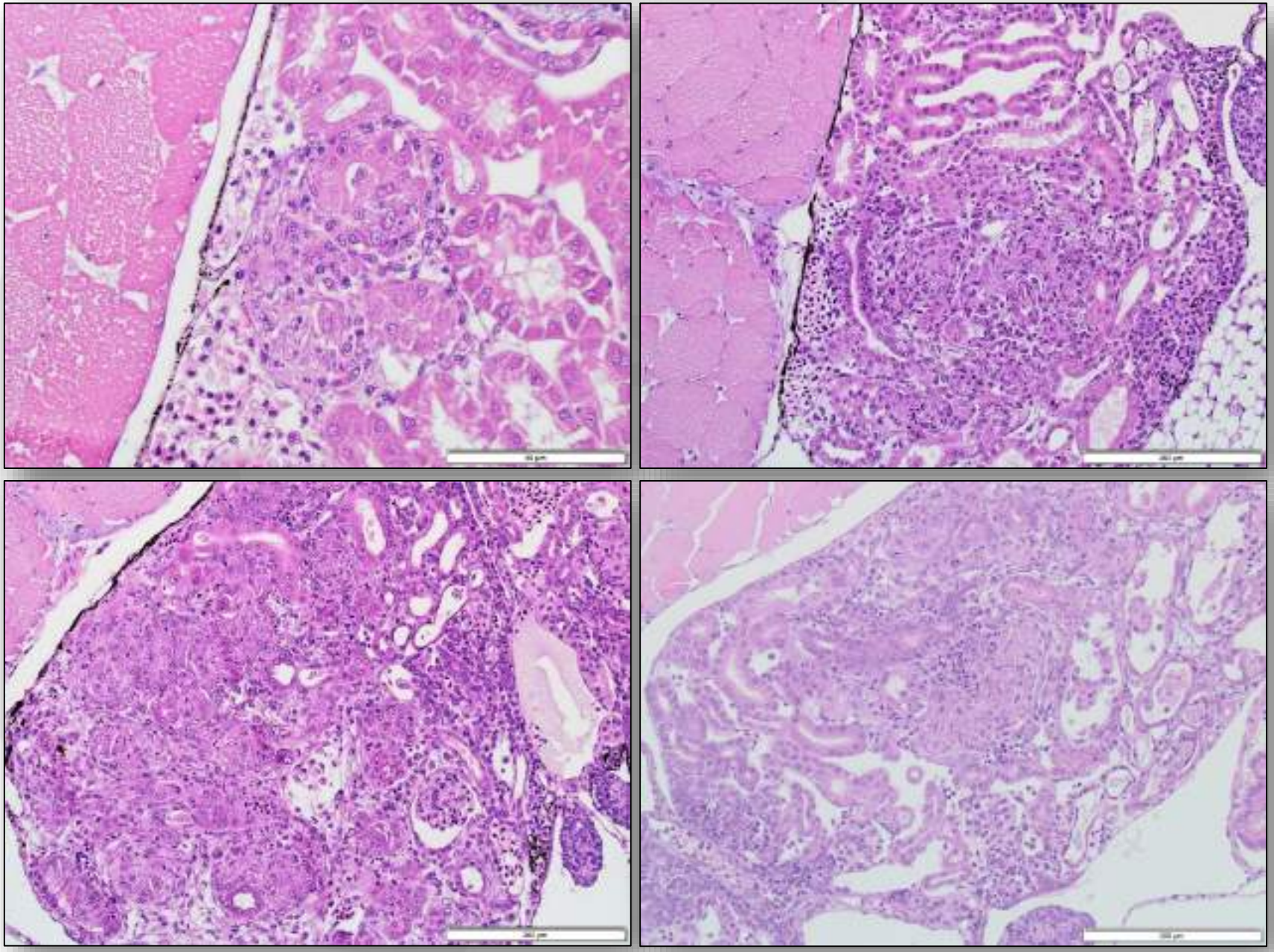


Normal intestinal mucosa.

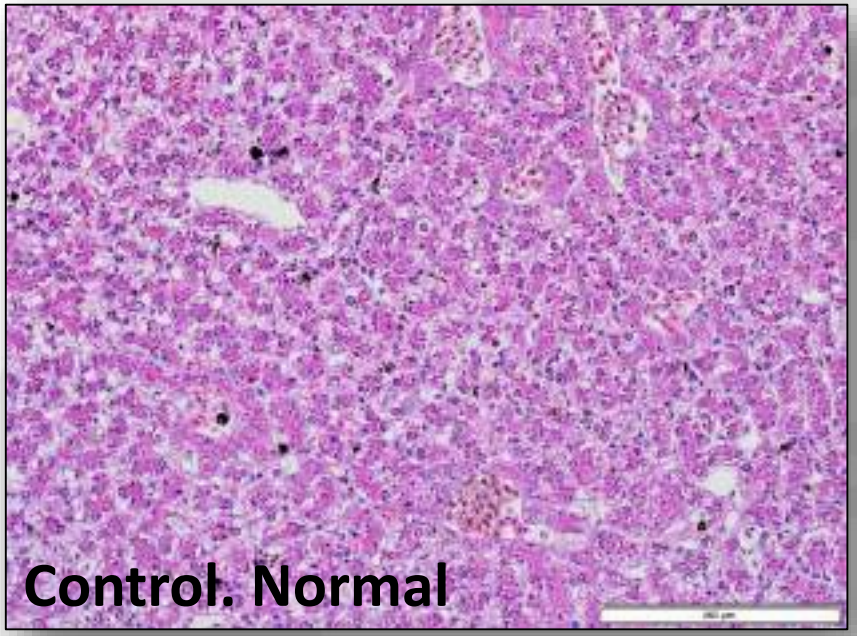


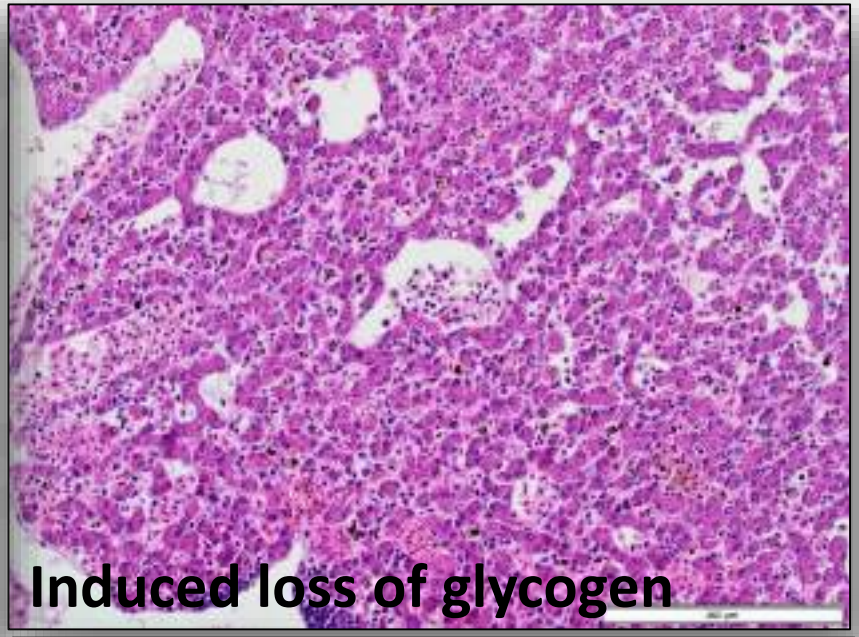
Intestine, mucosa. Necrosis/apoptosis of mucosal epithelia.

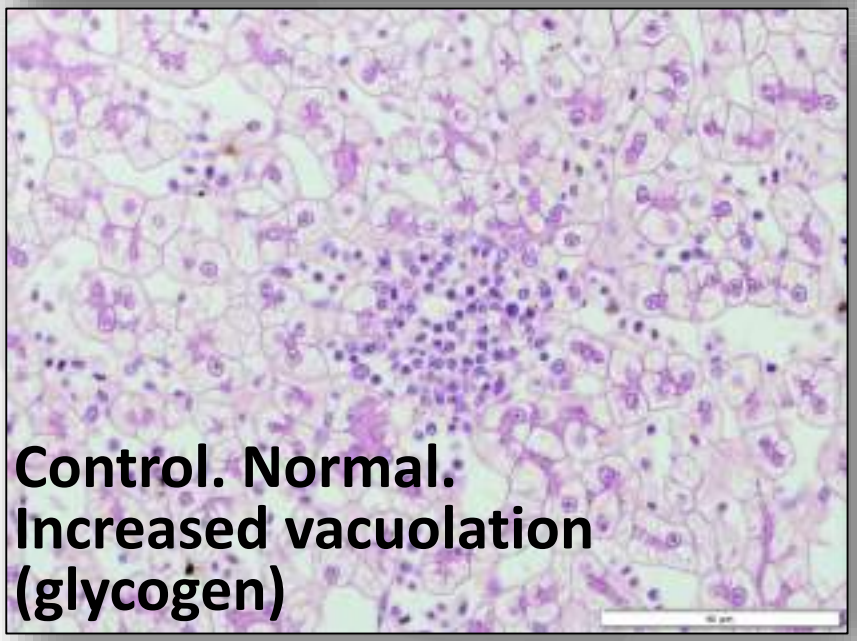
Induced. Renal Inflammation.

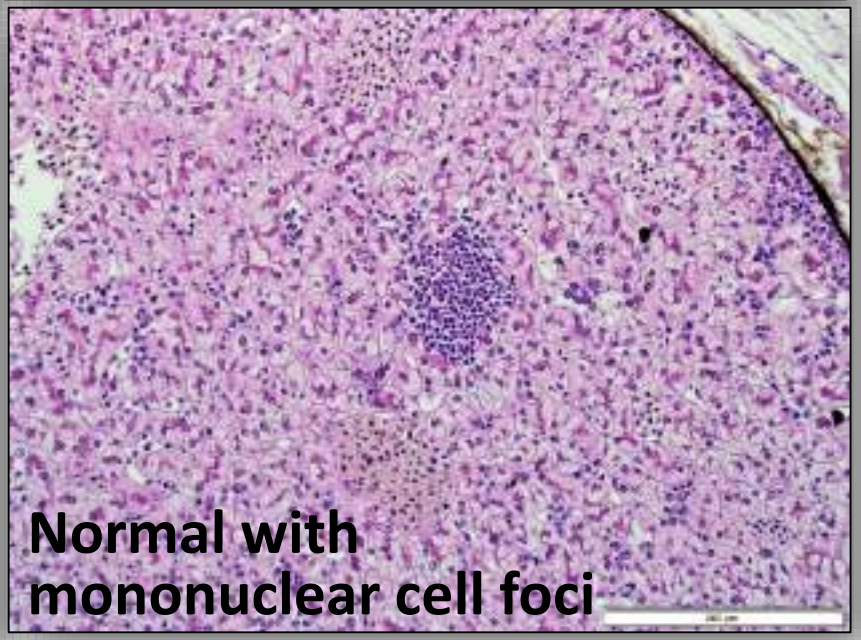


Liver Findings.

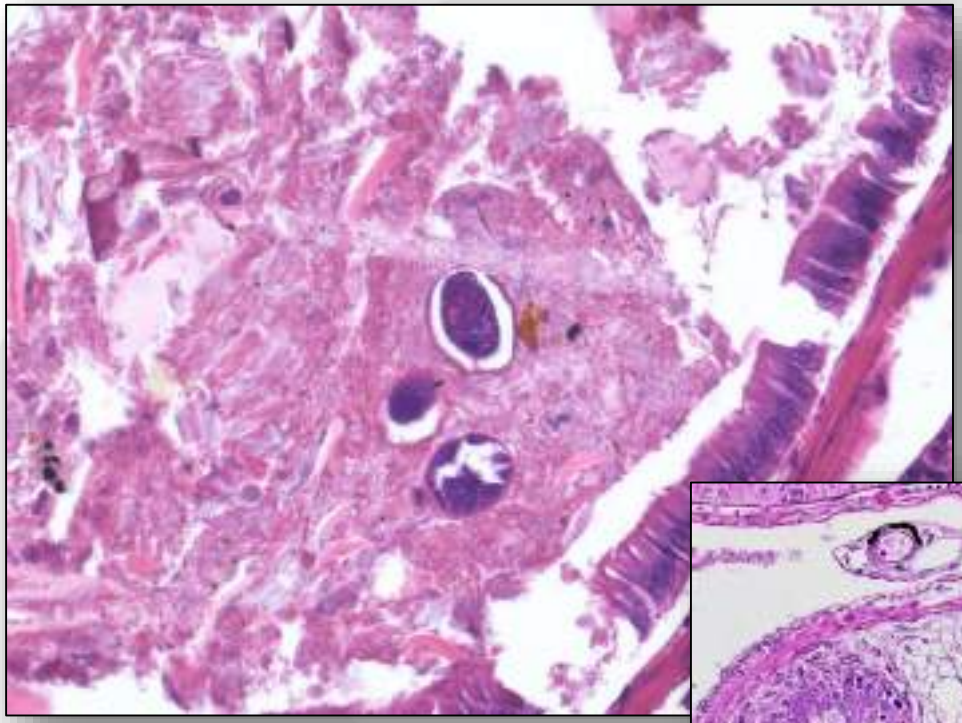








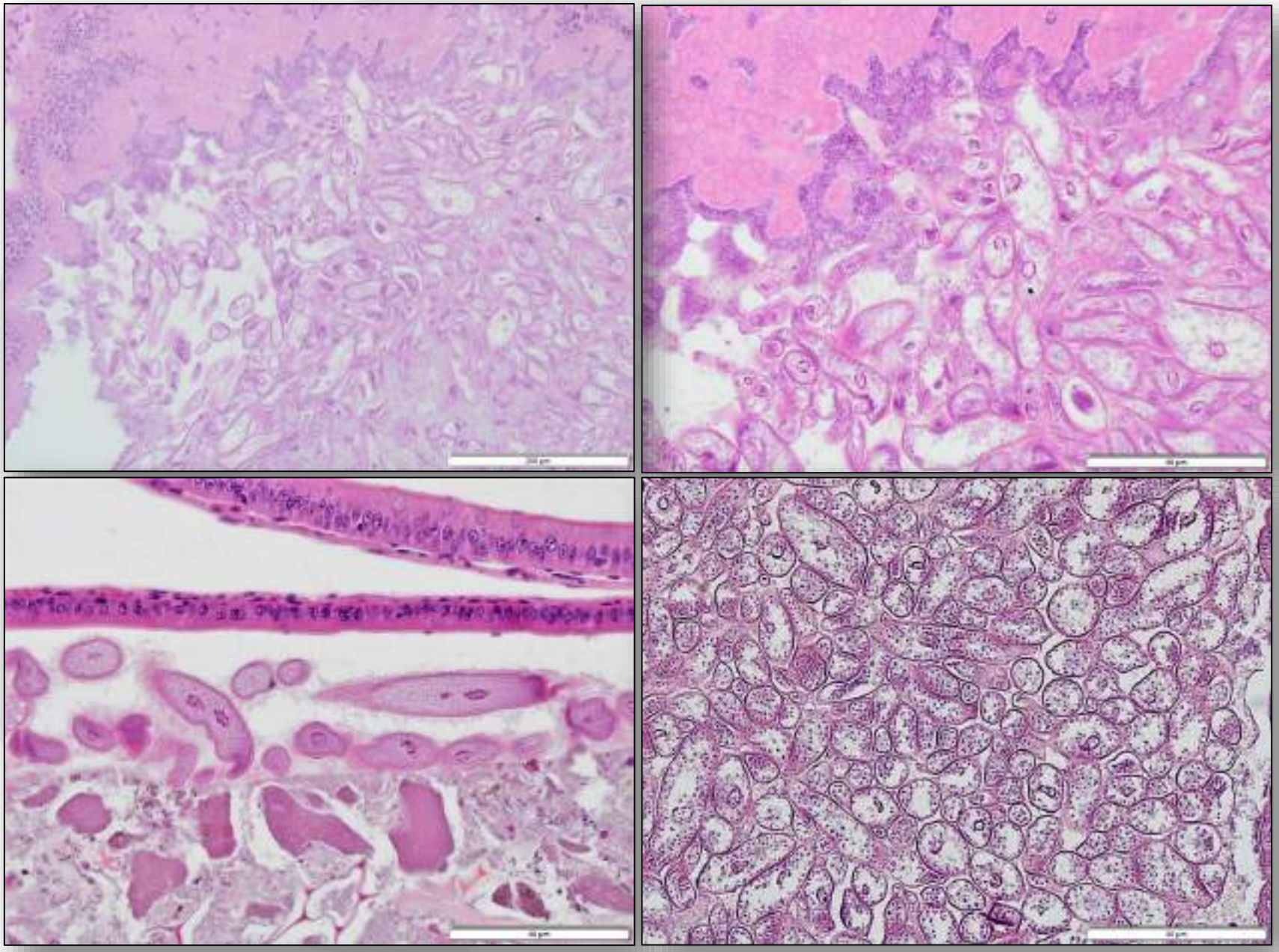
Background. Intestine.



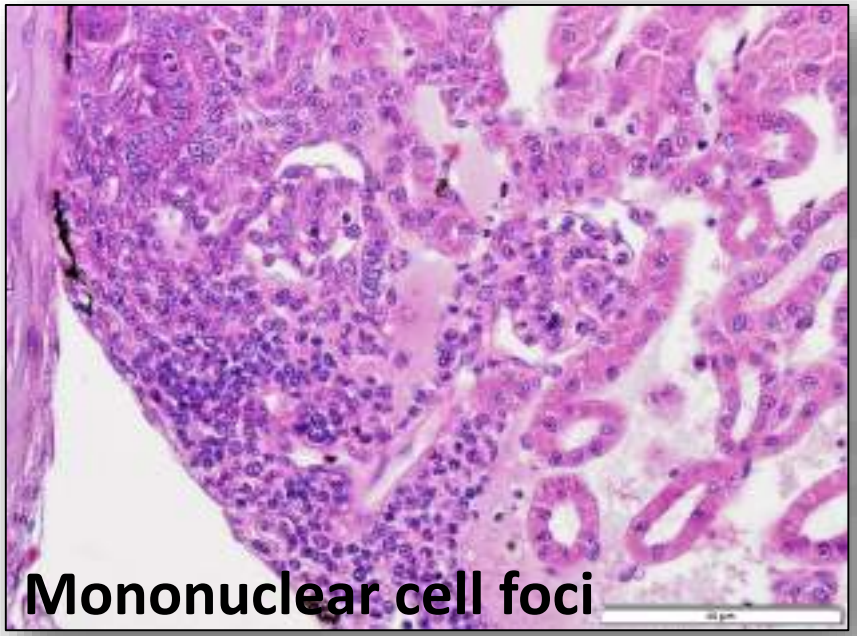
Unknown protozoa.

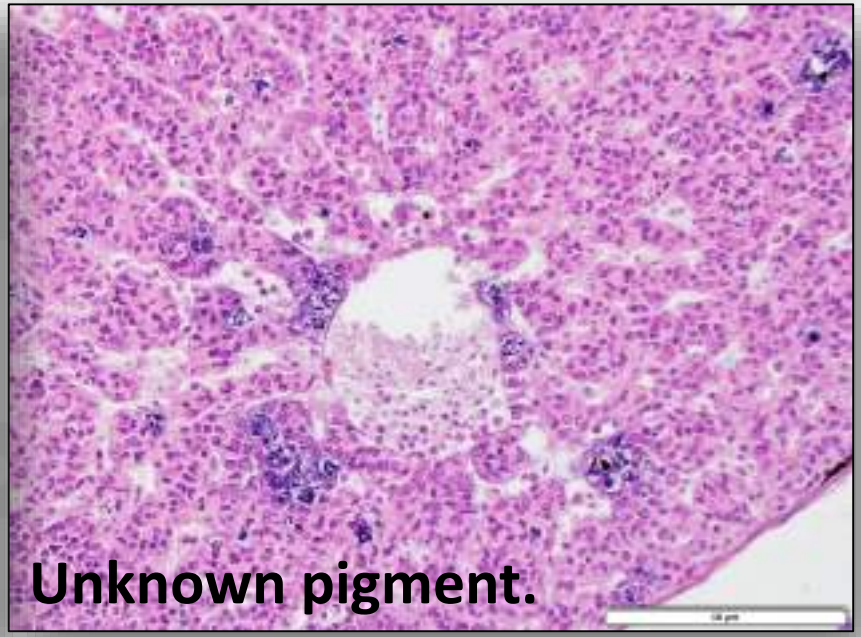
Focal submucosal inflammation.

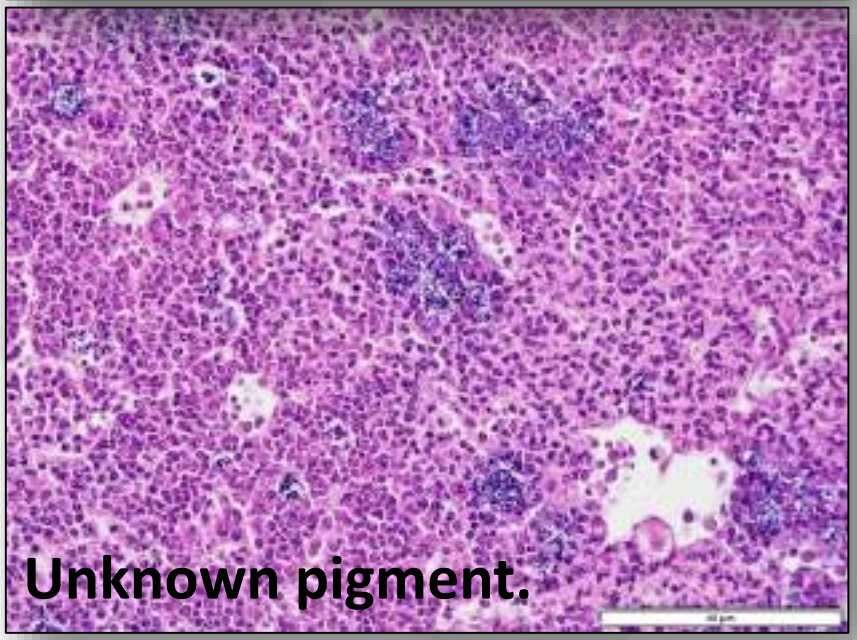
Background. Intestine. Protoopalina spec.



Background. Kidney.







Background. Other Organs.

Granuloma in fat tissue abdminal from cavity

Summary

- Many background lesions to consider
- Artefacts: Attention! Fixation!
- Examination of reproduction organs/thyroid glands only is an artefact!
- Not every finding mimicking ED is indicating ED!
- Still to learn a lot!