

Pathological Alterations in Non-Target Tissues in AMA or FSTRA Studies Might Impact the Study Outcome

Klaus Weber, PhD, DVM, MSBiol, DJSTP¹⁾

Jeffrey C. Wolf, PhD, DVM, ACVP²⁾

¹⁾AnaPath GmbH, Switzerland

²⁾ Experimental Pathology Laboratories, Inc.,
VA, USA

Standard Histopathology

AMA - Thyroid glands

- **General size:** thyroid hypertrophy, thyroid atrophy
- **Follicular cell:** follicular hypertrophy, follicular hyperplasia, follicular lumen area increase/decrease
- **3 severity degrees plus 0**

FSTR – List of findings

Primary Diagnoses		
	For males:	For females:
1	Increased proportion of spermatogonia	Increased oocyte atresia
2	Presence of testis-ova (testicular oocytes)	Perifollicular cell hyperplasia/hypertrophy
3	Increased testicular degeneration	Decreased yolk formation
4	Interstitial (Leydig) cell hyperplasia / hypertrophy	Changes in gonadal staging
Secondary Diagnoses		
	For males:	For females:
1	Decreased proportion of spermatogonia	Interstitial fibrosis
2	Increased vascular or interstitial proteinaceous fluid	Egg debris in the oviduct
3	Asynchronous gonad development	Granulomatous inflammation
4	Altered proportions of spermatozoa or spermatocytes	Decreased post-ovulatory follicles
5	Gonadal staging	
6	Granulomatous inflammation	

There is more in a fish or frog! Non-target tissues on same sections could answer possible ED issues

- Cellular disturbances resulting from systemic toxicity, e.g. apoptotic changes in kidneys
- Off-target toxicity, e.g., glomerulonephritis
- Stress due to induced toxicity in other organs, e.g., inflammation/ulceration in gills,
- Background infectious diseases, e.g., tuberculosis, viral infections
- Parasites, e.g., myxoplasma (cnidaria), protozoa (heterokonta), helminths (mainly cestoda, akanthocephala, nematoda)
- Neoplasms
- Single replica or whole study might be affected.
- **Expect a huge impact on the study outcome!**

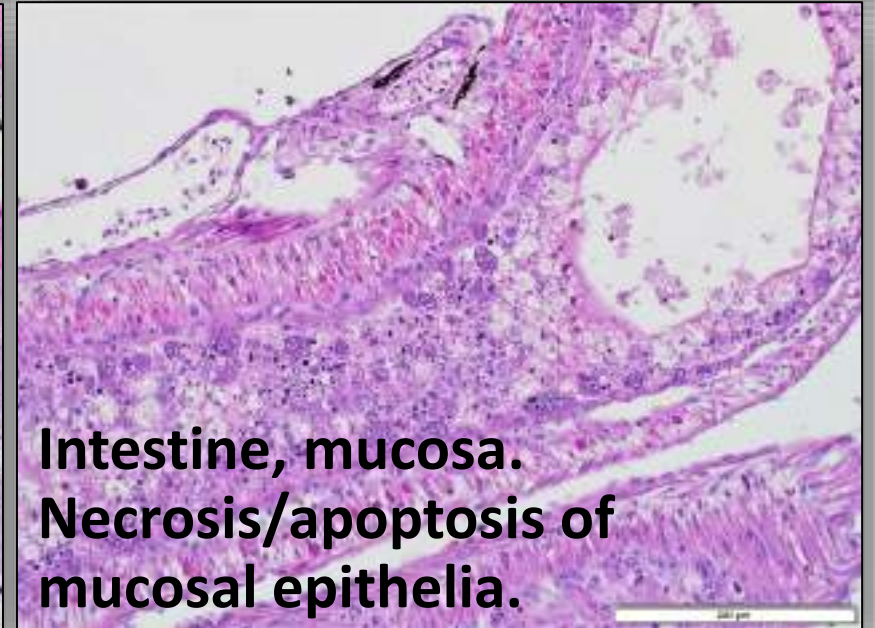
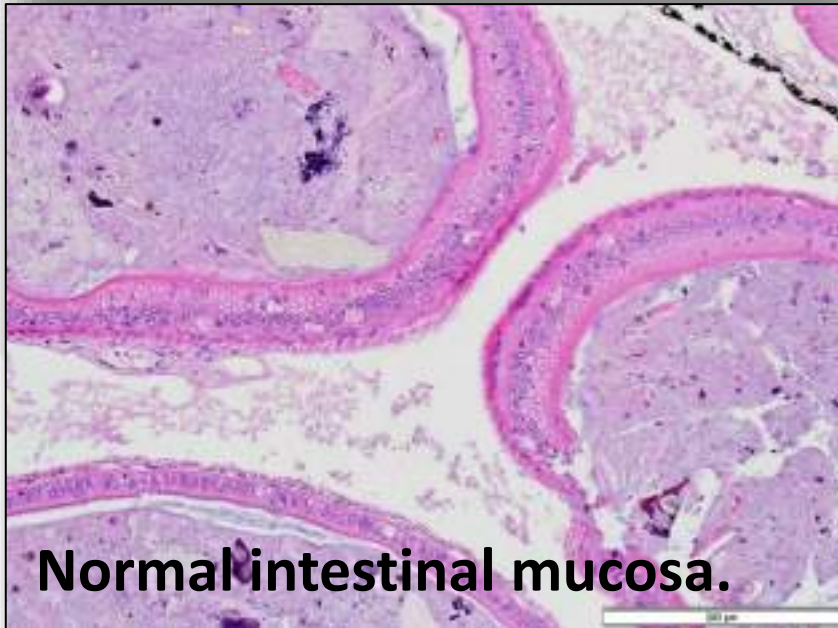
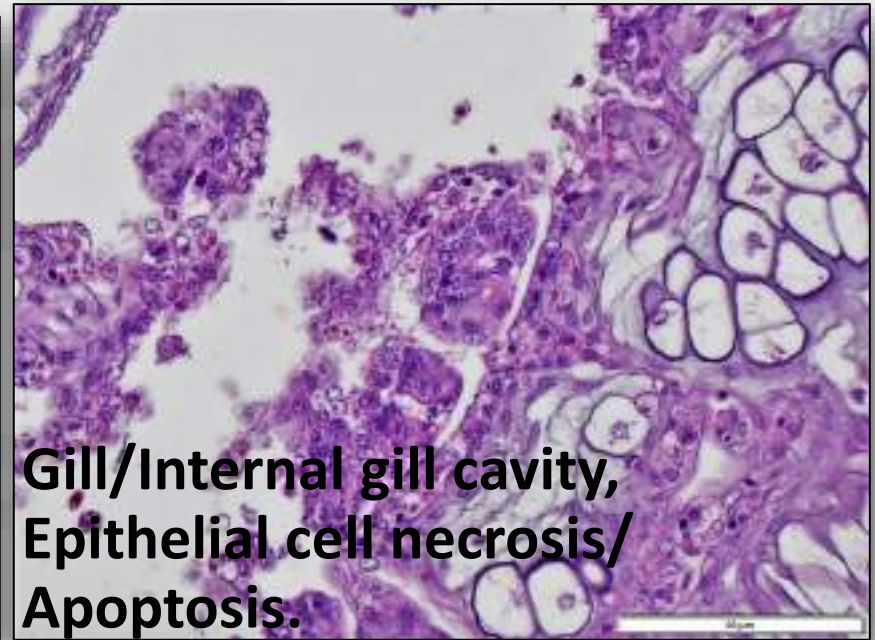
Examples: AMA



Note size thyroid effects related to slow development!

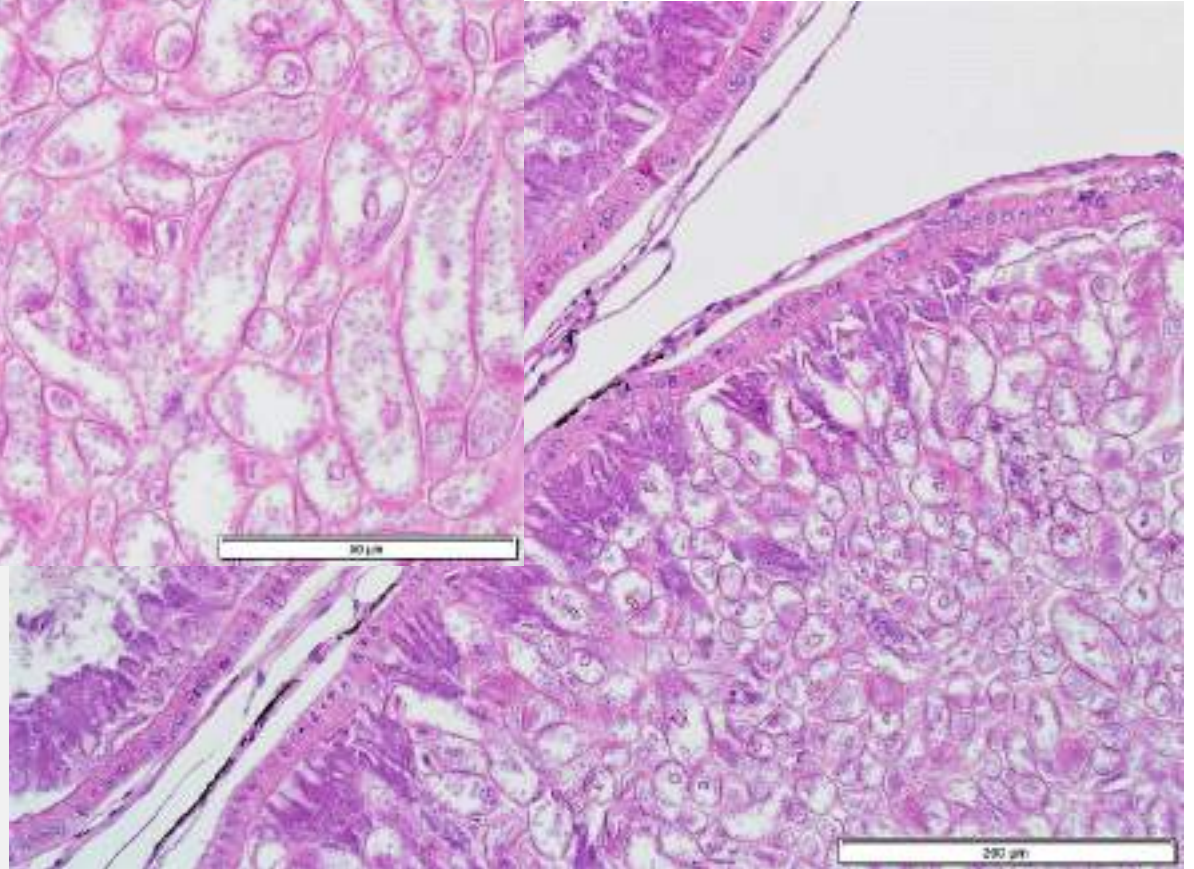
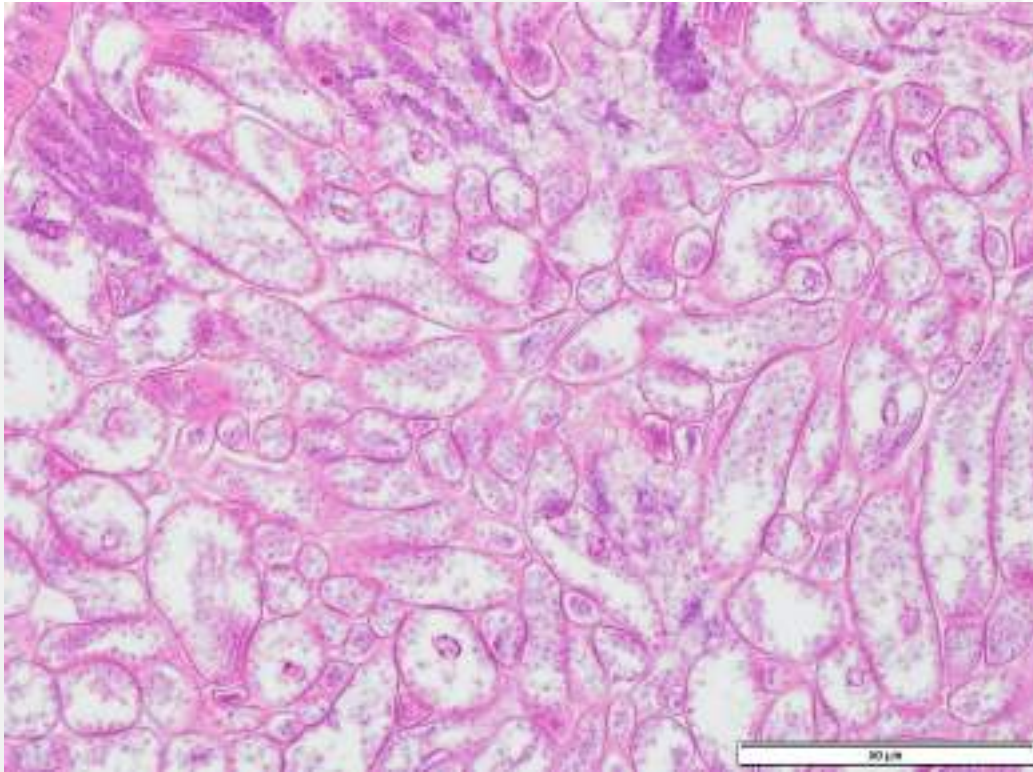


Reason: Induced irritative effects in gills/intestine.



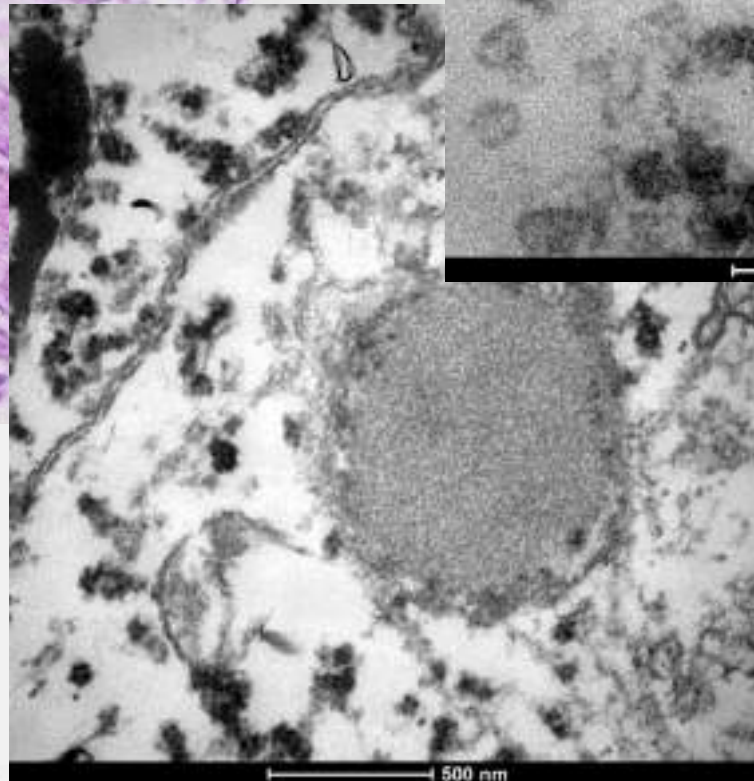
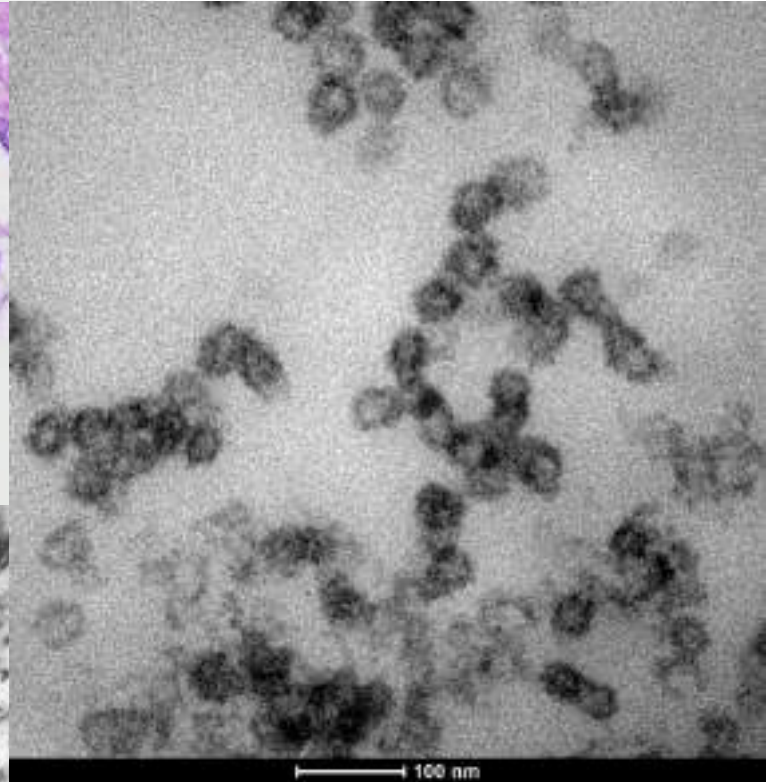
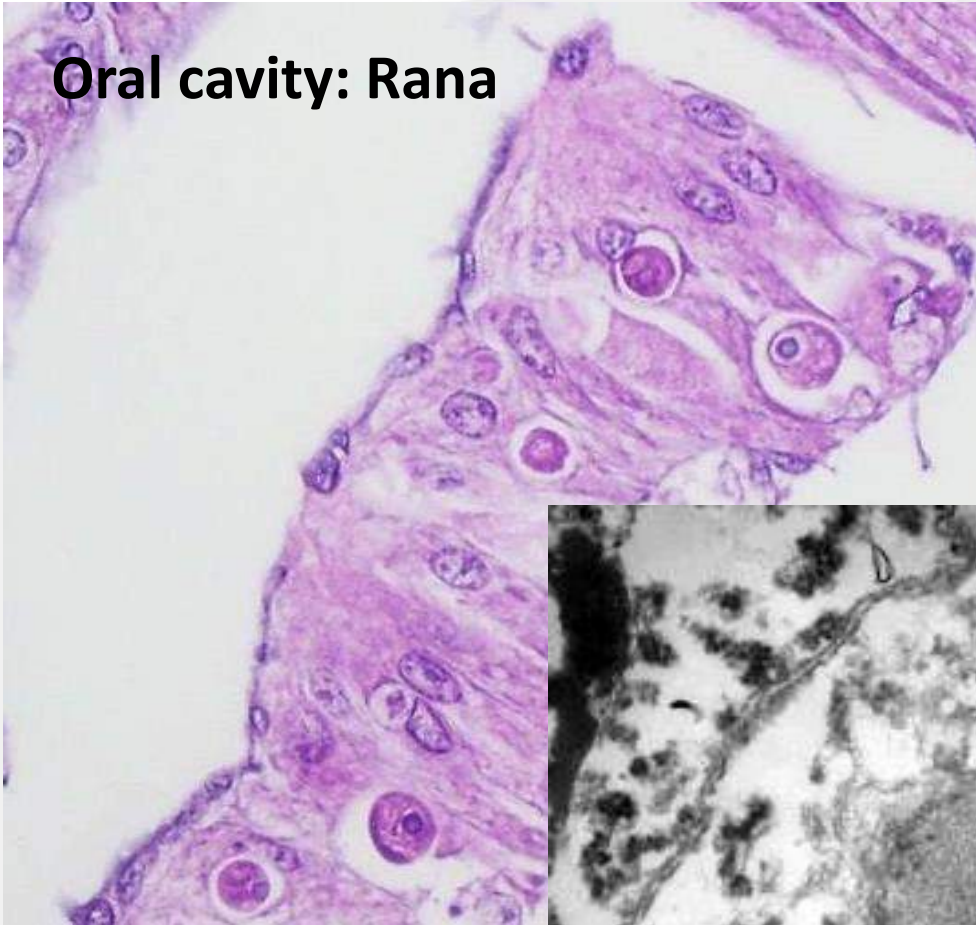
Reason: parasites.

**Intestine filled with organisms.
Paraopalina spec.**

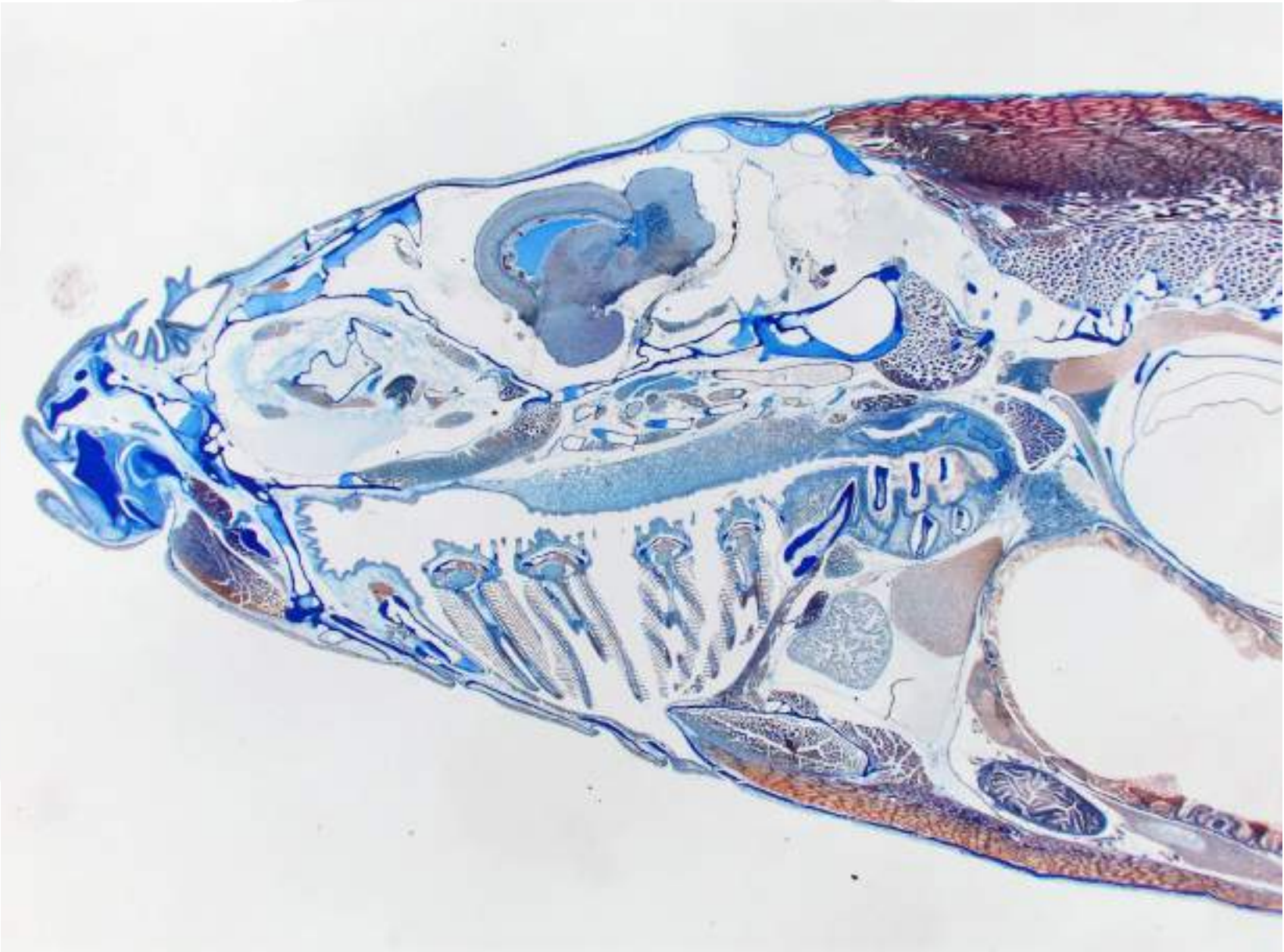


Reason: virus

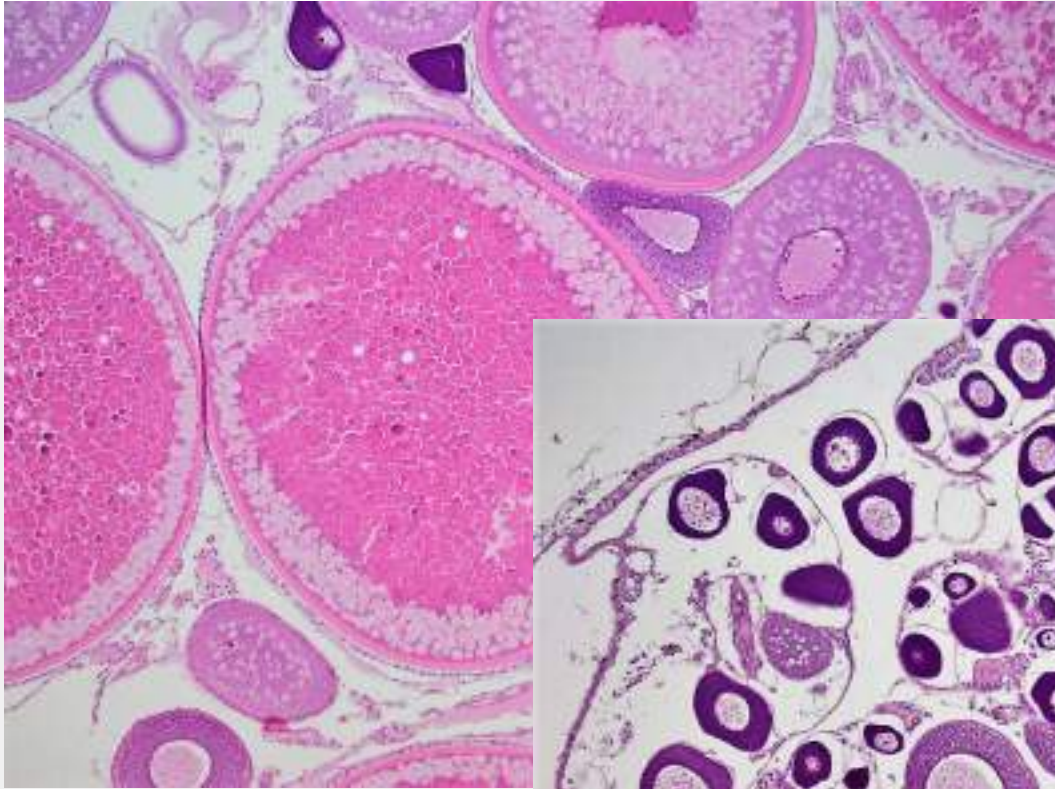
Oral cavity: Rana



Examples: FSTRA

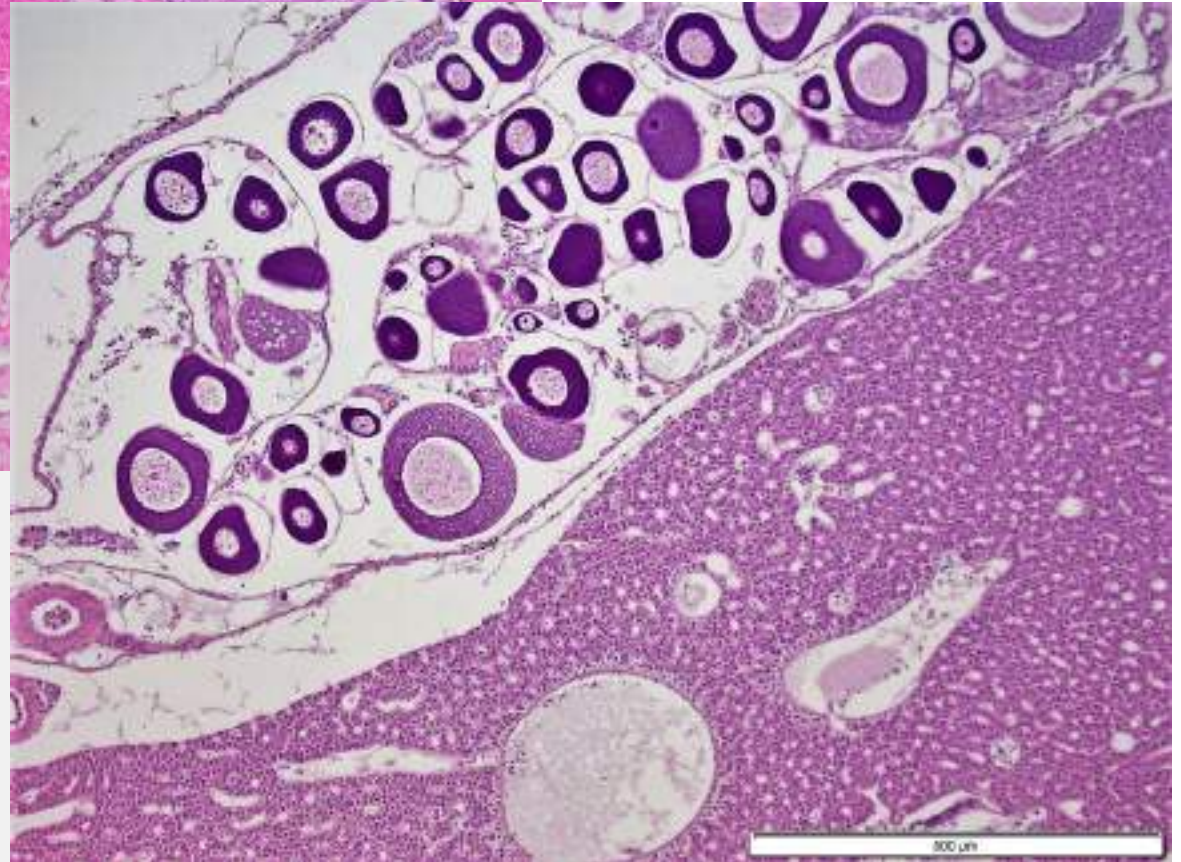


Example: FSTRA: ovaries.

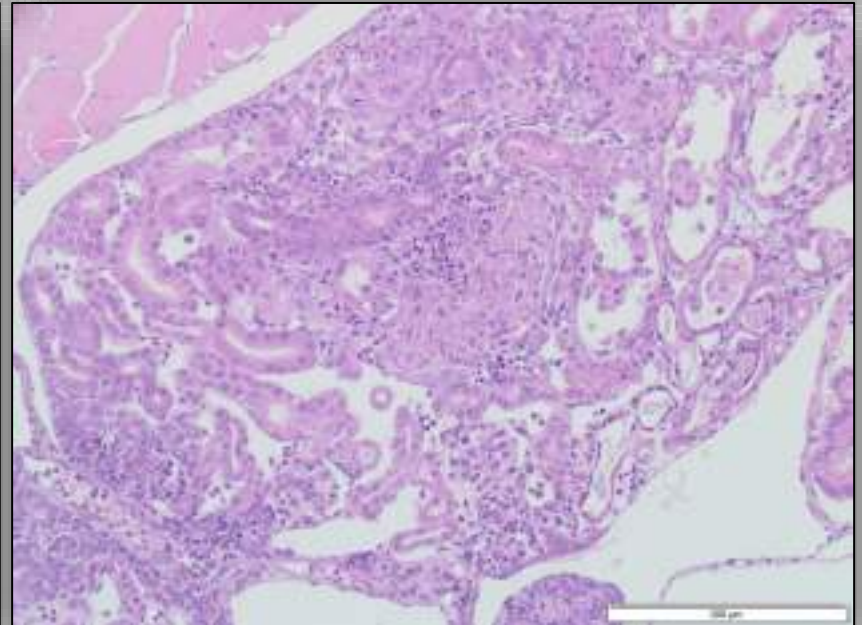
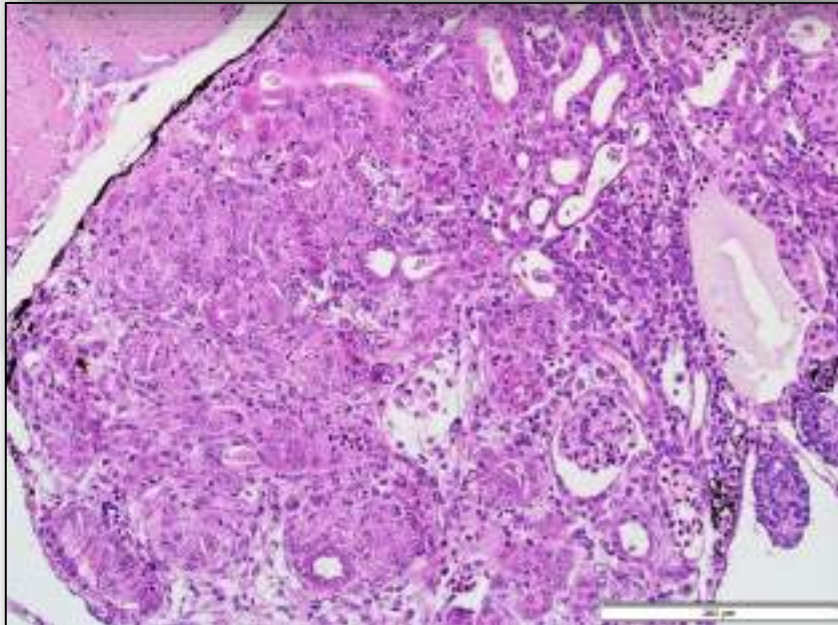
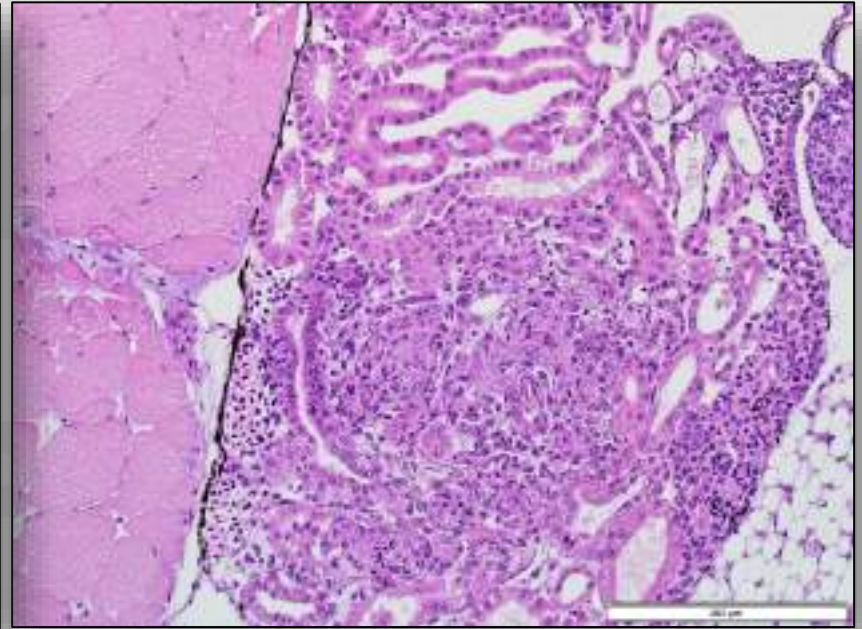
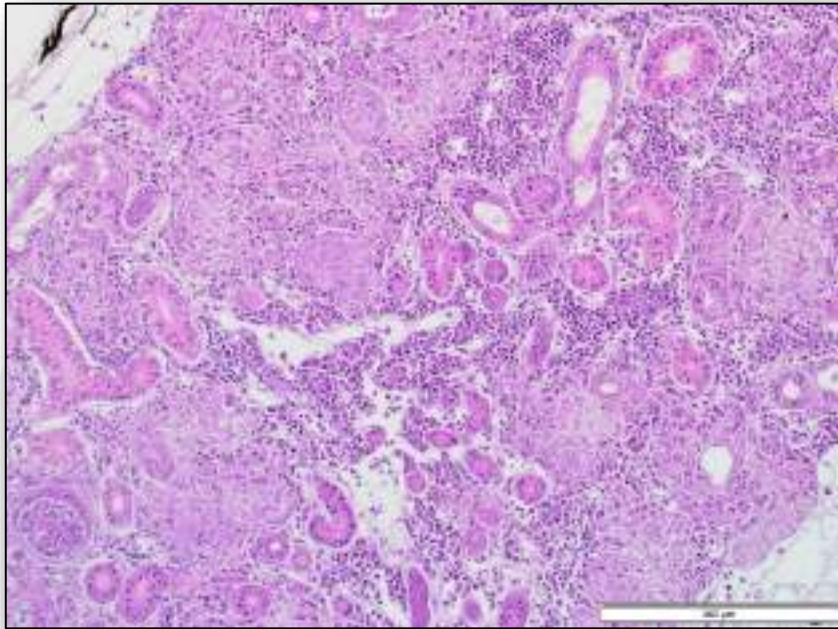


**Control.
Normal ovarian
Stages.**

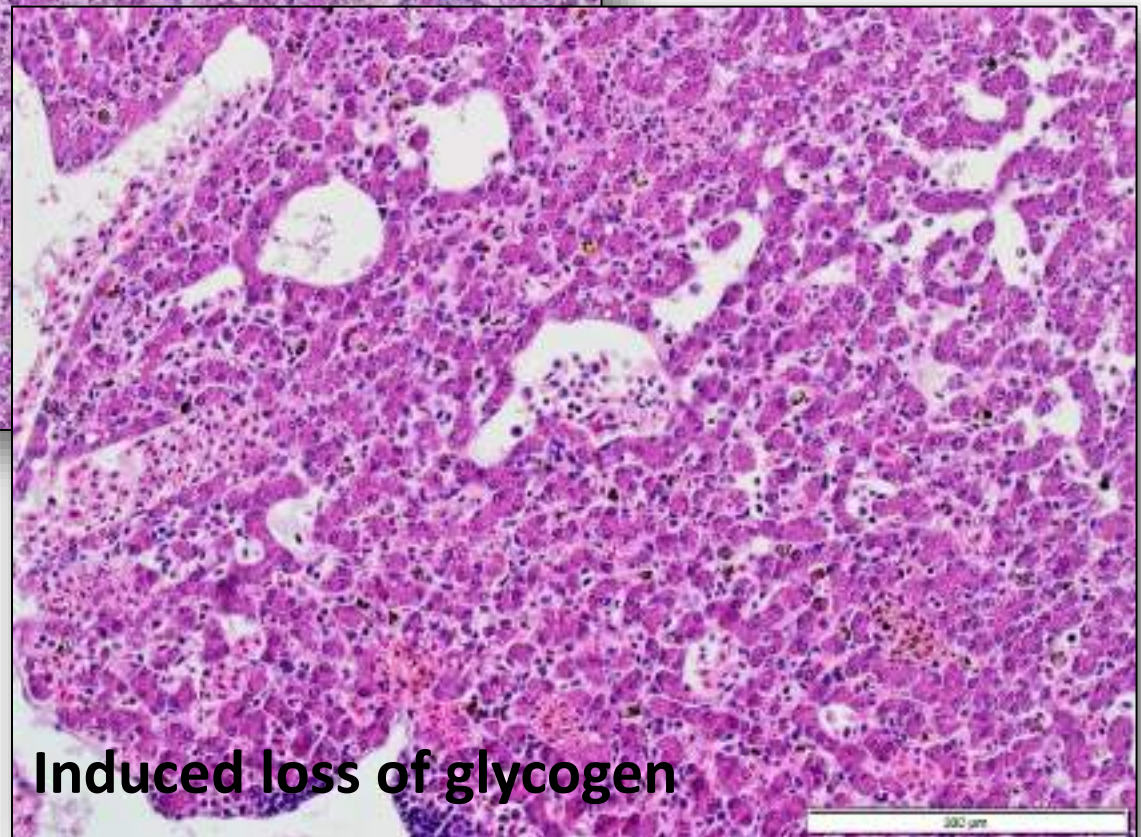
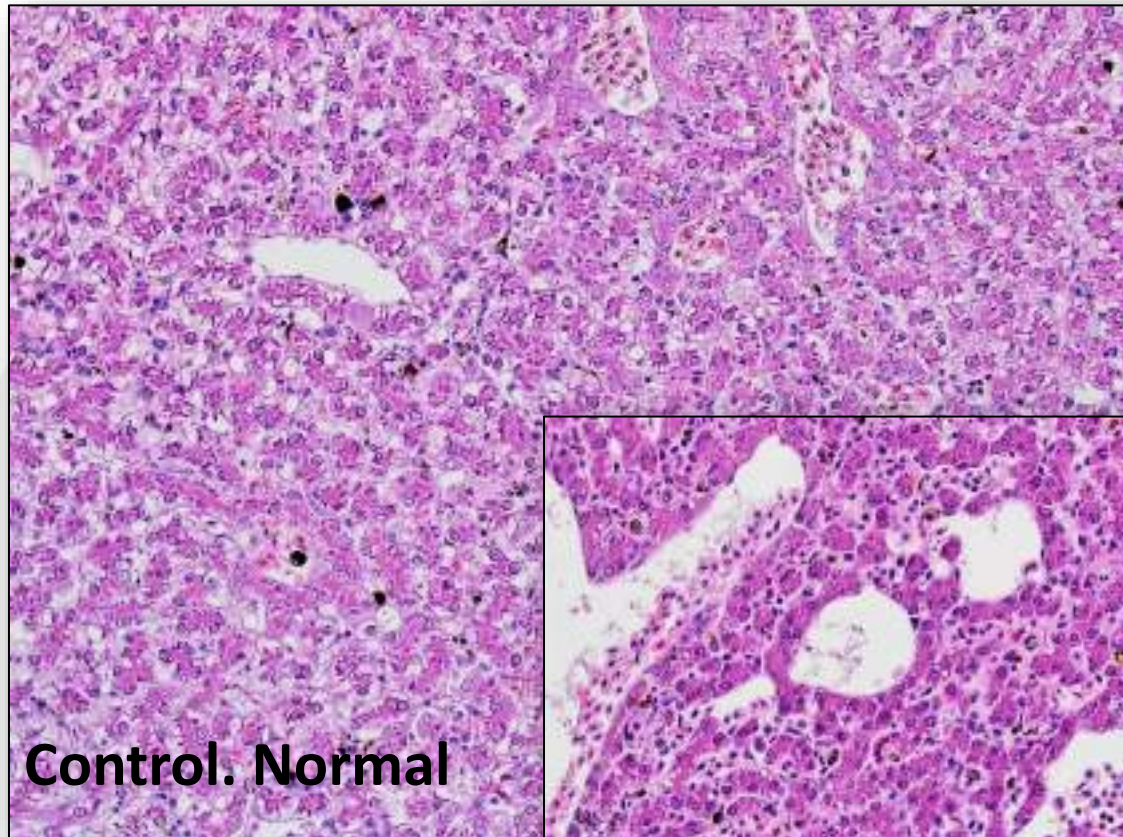
**Test item group.
Lower stages, liver
normal**



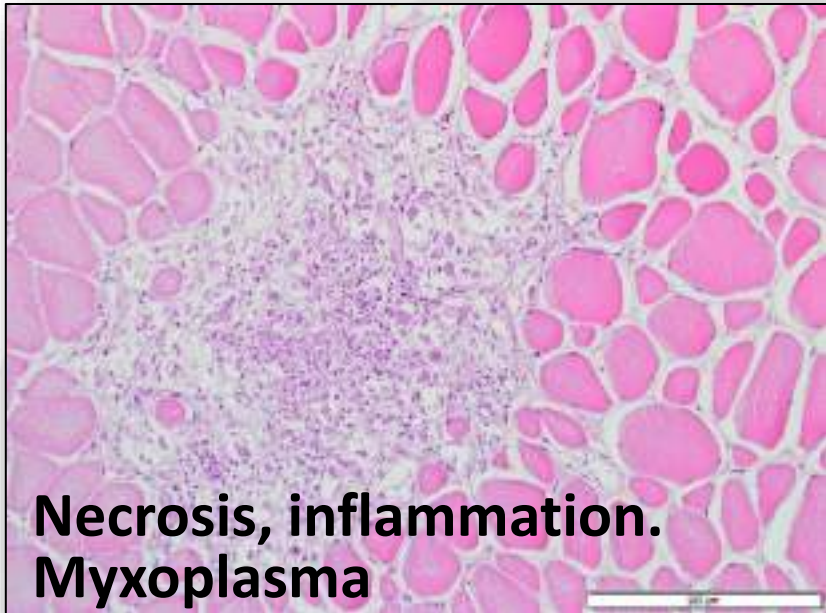
Reason: Induced inflammation in kidneys.



Other possible issues (selected), Stress: Liver.



Other possible issues (selected), parasites, infections.



**Necrosis, inflammation.
Myxoplasma**



**Cnidarian in cyst.
Bed-slipper shape.**

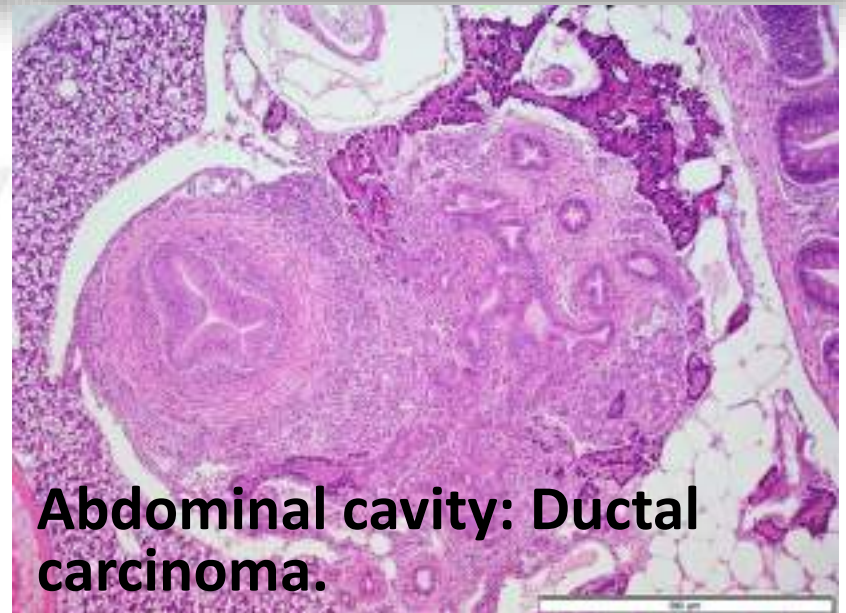
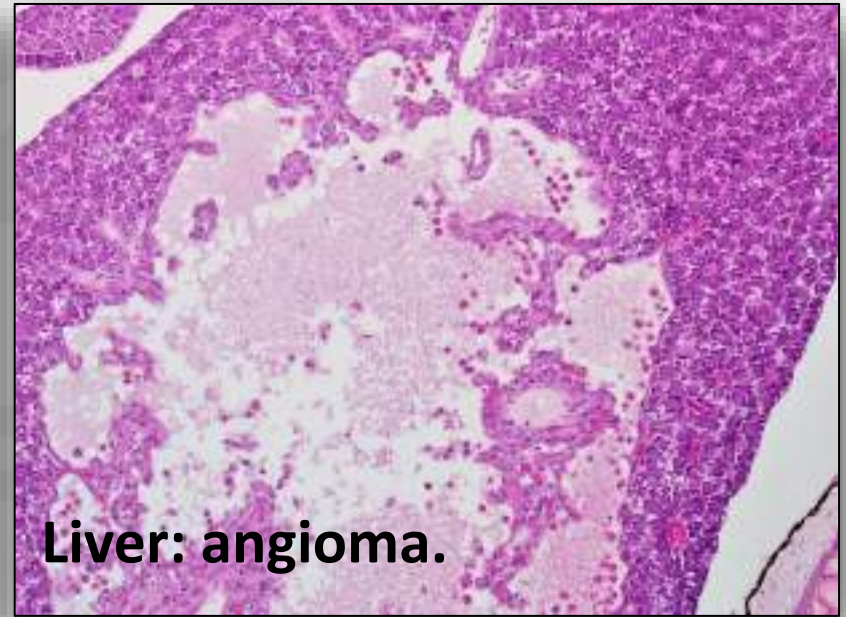


Cestode in intestine.



Cestode: larvae.

Other possible issues (selected), neoplasms.



Guidelines/guidance

OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads

- **Describe: histopathology of fish gonads**
- **No specific mention of what is to evaluate except some comments on kidney and liver**

Histopathology Guidance Document for the Larval Amphibian Growth and Development Assay (LAGDA)

- **Pathologists should specifically evaluate the target tissues identified in the guidelines; however, changes observed in other tissue types may also be recorded. This especially pertains to findings suspected to be treatment-related, or findings that might otherwise impact the study results (e.g., systemic inflammation or neoplasia).**

Guidelines/guidance

Medaka Extended One Generation Reproduction Test (MEOGRT)

..Consequently, liver and kidney histopathology may also be assessed in detail to help better understand any responses in mechanistic endpoints. However, if these detailed evaluations are not performed, gross abnormalities observed incidentally during the histopathological evaluation should still be noted and reported...’.

Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (MEOGRT) - Part 1’,

‘...Pathologists should specifically evaluate the target tissues identified in the guidelines; however, changes observed in other tissue types may also be recorded. This especially pertains to findings suspected to be treatment-related, or findings that might otherwise impact the study results (e.g., systemic inflammation or neoplasia)...’

Summary

Although guidance documents for these studies either obliquely suggest or directly recommend the recording of study-relevant findings observed in non-target tissues, there may be resistance to this out of concern that this practice might add unneeded complexity to the study results.

Given that a major purpose of these particular screening assays is to determine (if possible) whether effects of the test substances are endocrine-mediated, it is imperative that the pathologist obtain as much information as possible from the examined specimens.

Furthermore, the pathologist has an ethical obligation to record any and all observed findings that may be study-relevant. This presentation will provide examples to demonstrate potential impacts on AMA and FSTRA studies of background findings or test article-related changes in non-target organs.

Followed by publication.