A 7 STAGE SCALE FOR POF's HISTOLOGY CLASSIFICATION IN *Scomber scombrus*.

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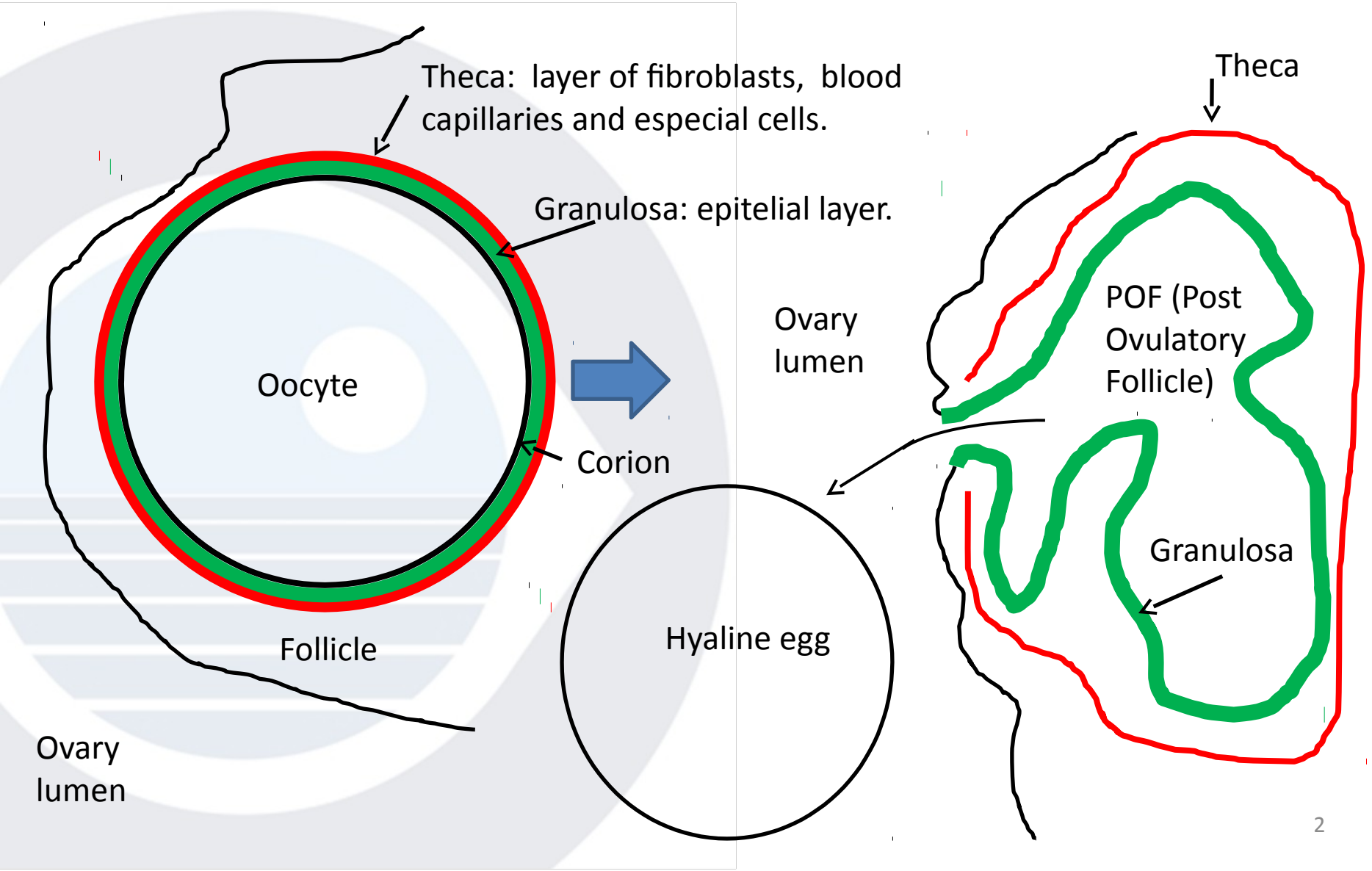
What is a POF?


Theca: layer of fibroblasts, blood capillaries and especial cells.
Granulosa: epitelial layer.
Oocyte
Corion
Follicle
Ovary lumen
Hyaline egg
Ovary lumen
Theca
POF (Post Ovulatory Follicle)
Granulosa

The POF as indicator of the spawning moment.

INITIAL IDEAS.

- The presence of POFs indicates the existence of a spawning.
- The reabsorption of POF begins when the hyaline egg is released to lumen.
- The histological aspect of the POF depends on time that reabsorption has been active.

CONDITION.

If the reabsorption speed of the POF is known...

PROBLEMS.

- Reabsorption depends on fish metabolism.
- Metabolim depends on fish temperature.
- Temperature depends on:
  - ♦ specifics factors.
  - ♦ individual factors.
  - ♦ environmental factors.
- It is very complicate to know reabsorption speed.

SECUNDARY IDEA.

The histological aspect of POF can indicate how much time ago was the spawning.

CONCLUSION.

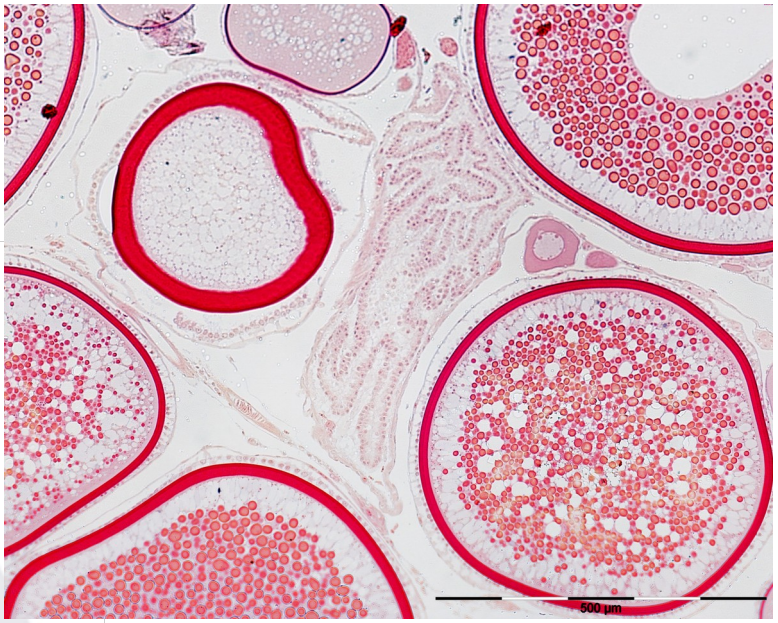
It is possible to know the time of spawning.

REABSORPTION.

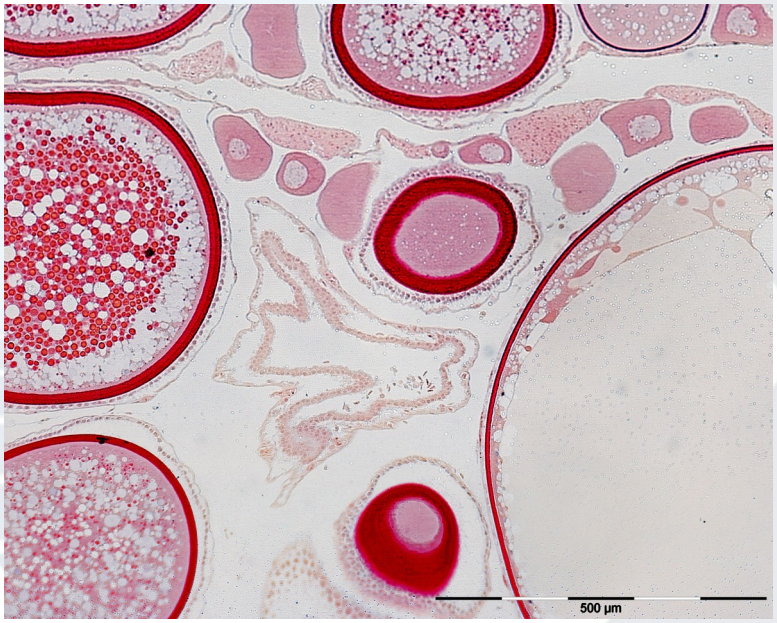
We can considerer 3 steps:

1- Disappearance of lumen.

- It is associated with the beginning of the degradation of the granulosa.



***Scomber scombrus*. Resin. 100x. Pas-Mallory&Trichrome**



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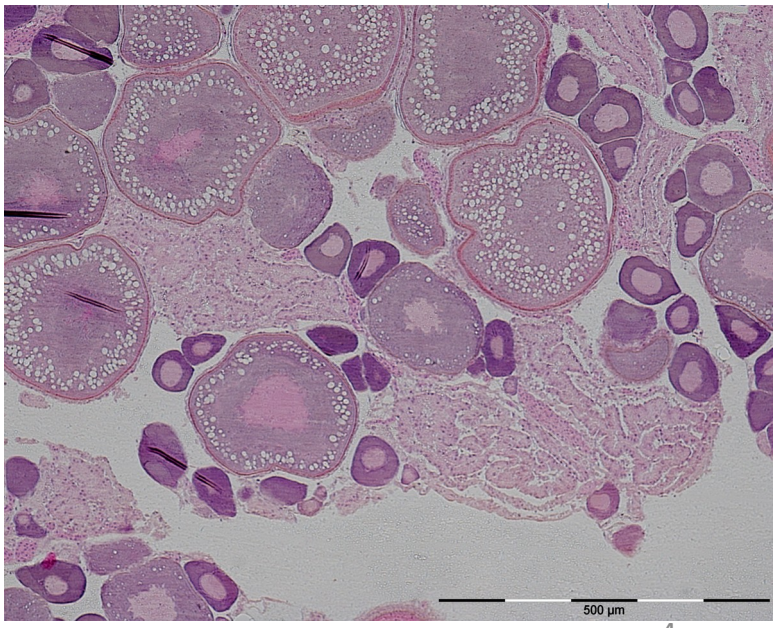
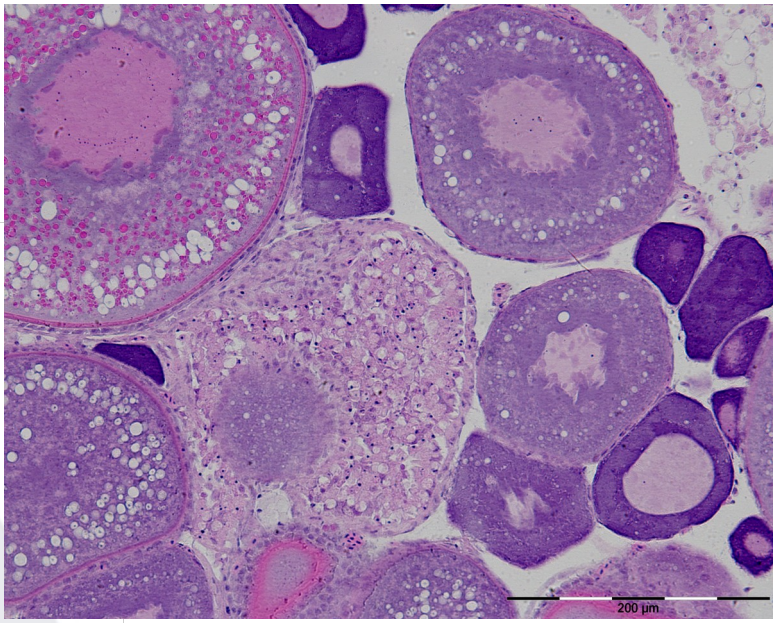


***Sardina pilchardus*. Resin. 100x.H&E.**

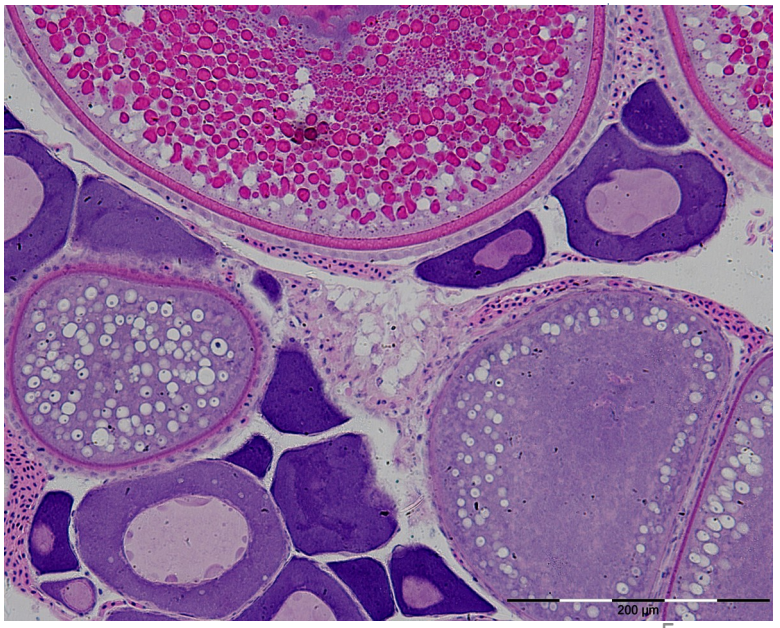
2- Reabsorption of the granulosa.

- With intense degradation of the granulosa followed by its reabsorption. There are a important reduction of the POF size.



***Sardina pilchardus*. Resin. 200x.H&E.**

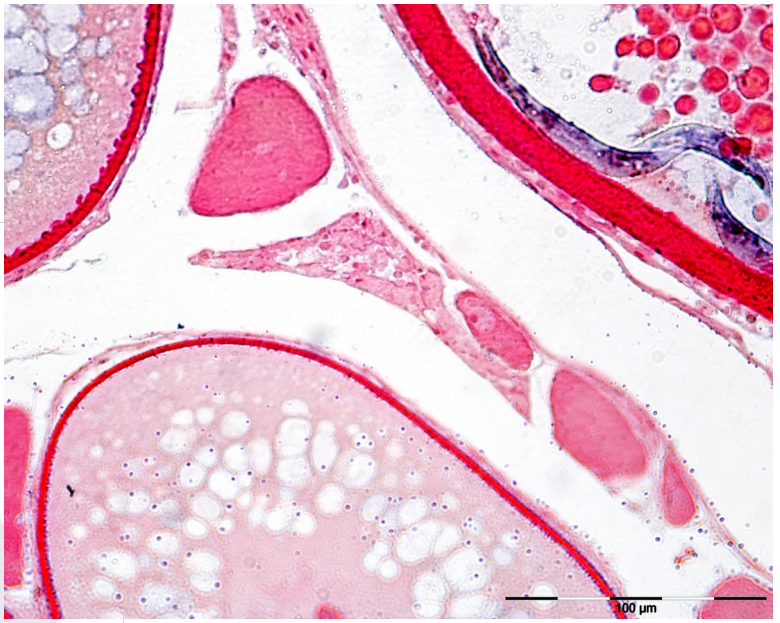
***Scomber scombrus.* Resin. 200x.Pas-Mallory&Trichrome**



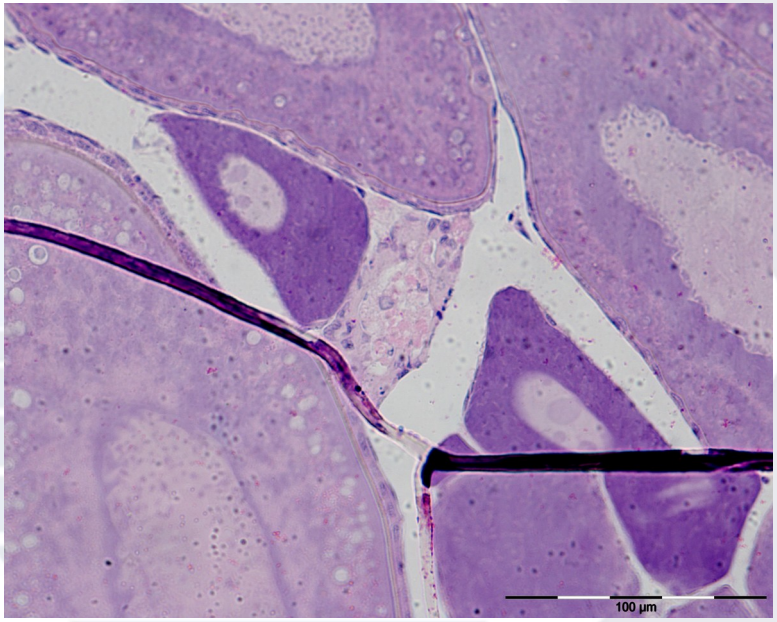
***Sardina pilchardus*. Resin. 200x.H&E.**

3- Disolution of the theca.

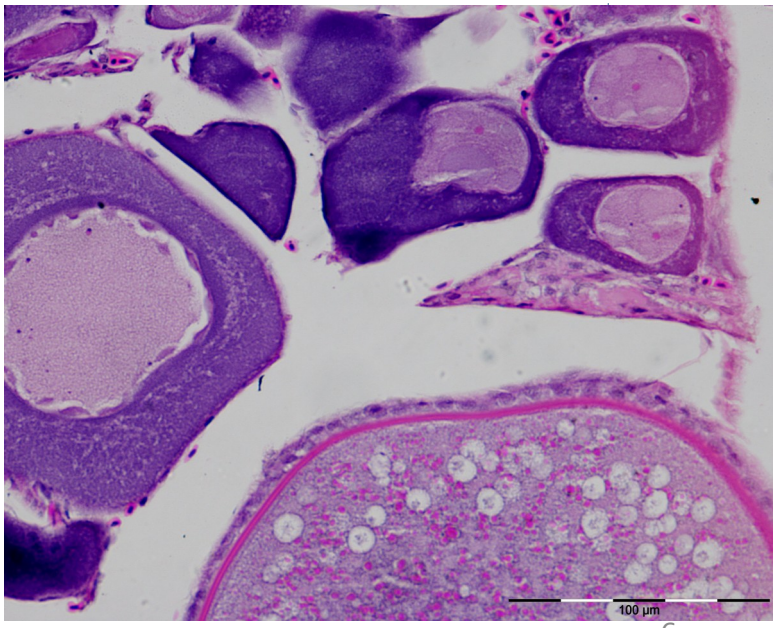
- The POF, basically theca, reduces its size until dissapearing in the stroma.



***Scomber scombrus*. Resin. 400x.Pas-Mallory&Trichrome**

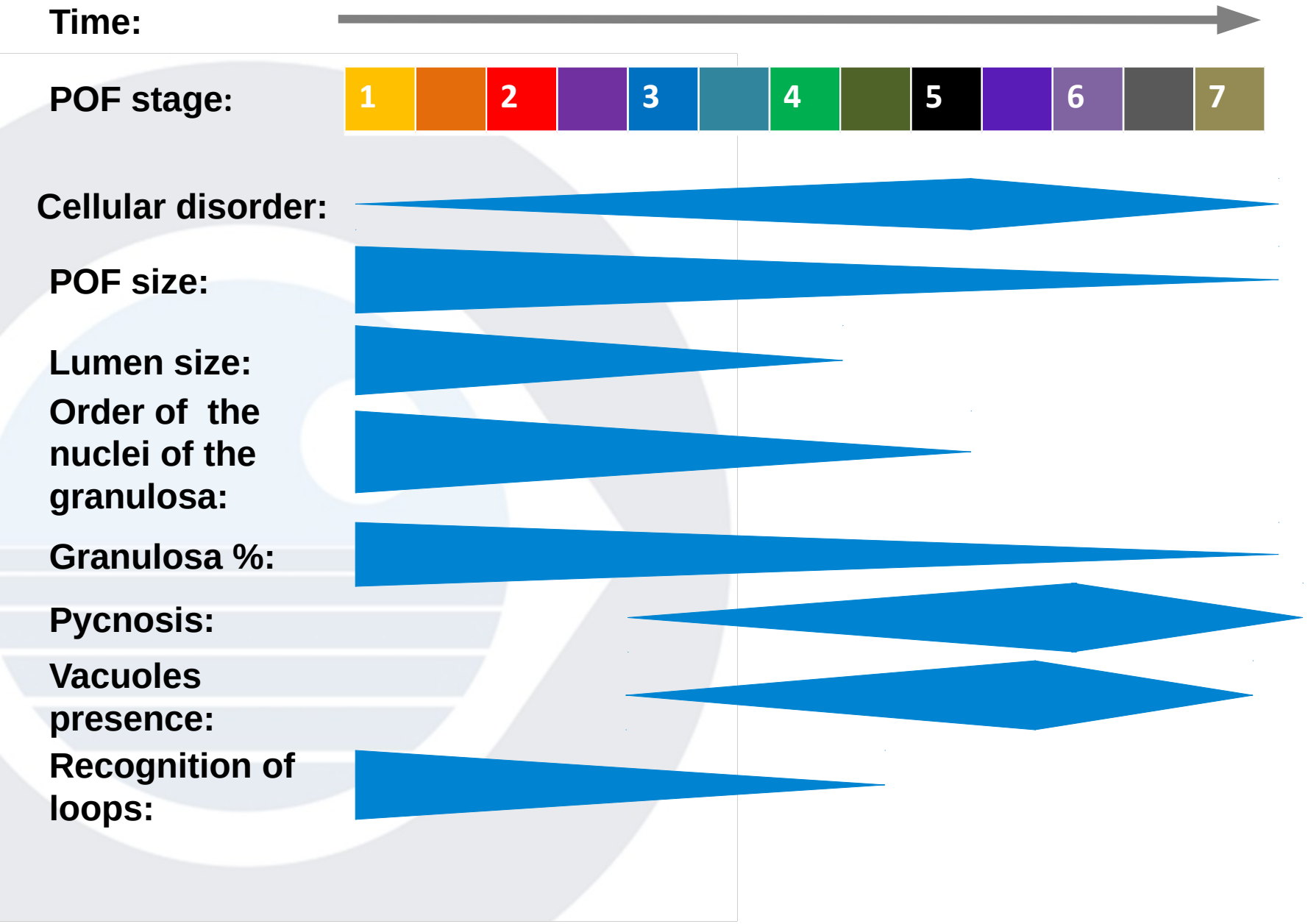


***Engraulis encrasicolus*. Resin. 400x.H&E.**



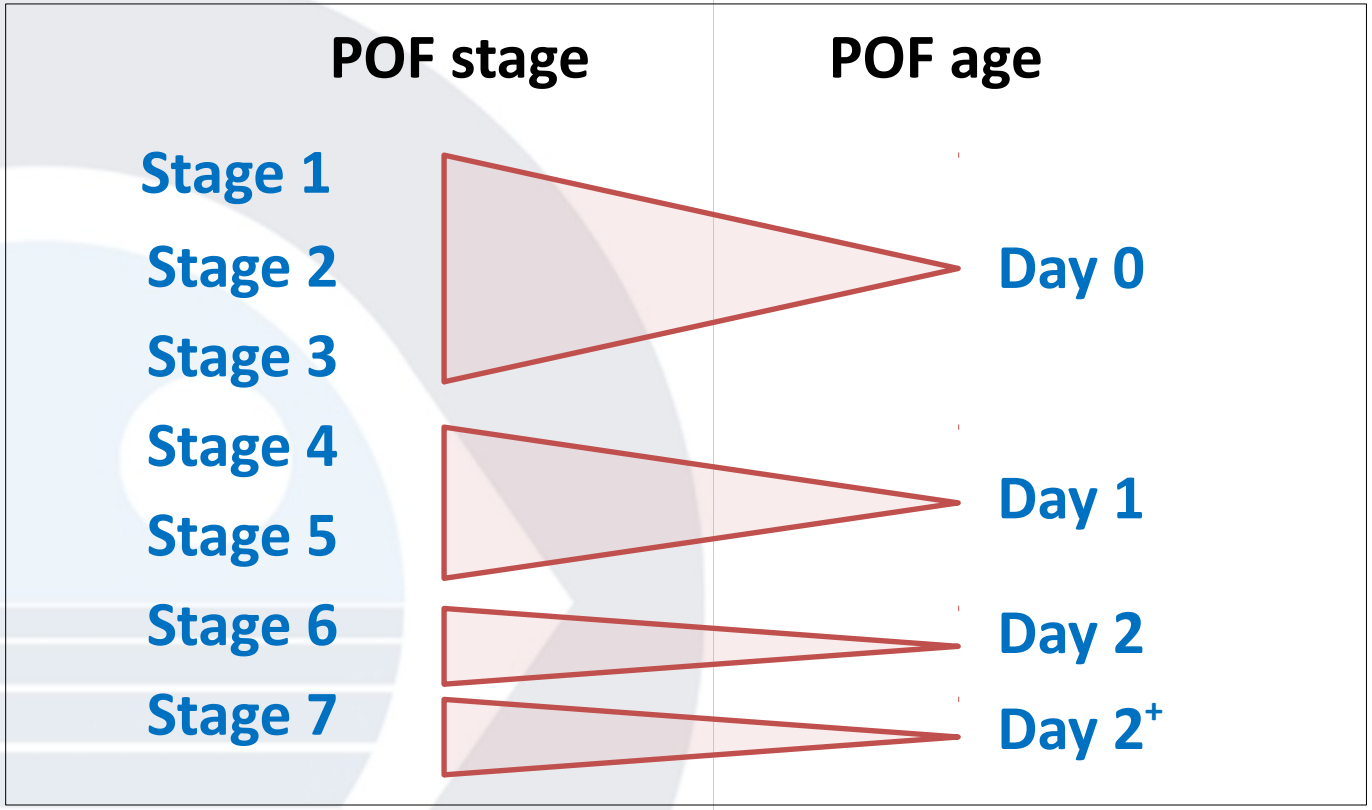
***Sardina pilchardus*. Resin. 400x.H&E.**

EVOLUTION OF DIFFERENT CHARACTERISTICS DURING THE DEGENERATION OF THE POFs.



POFs classification

Proposed equivalence between 7 stages scale and age of POFs.

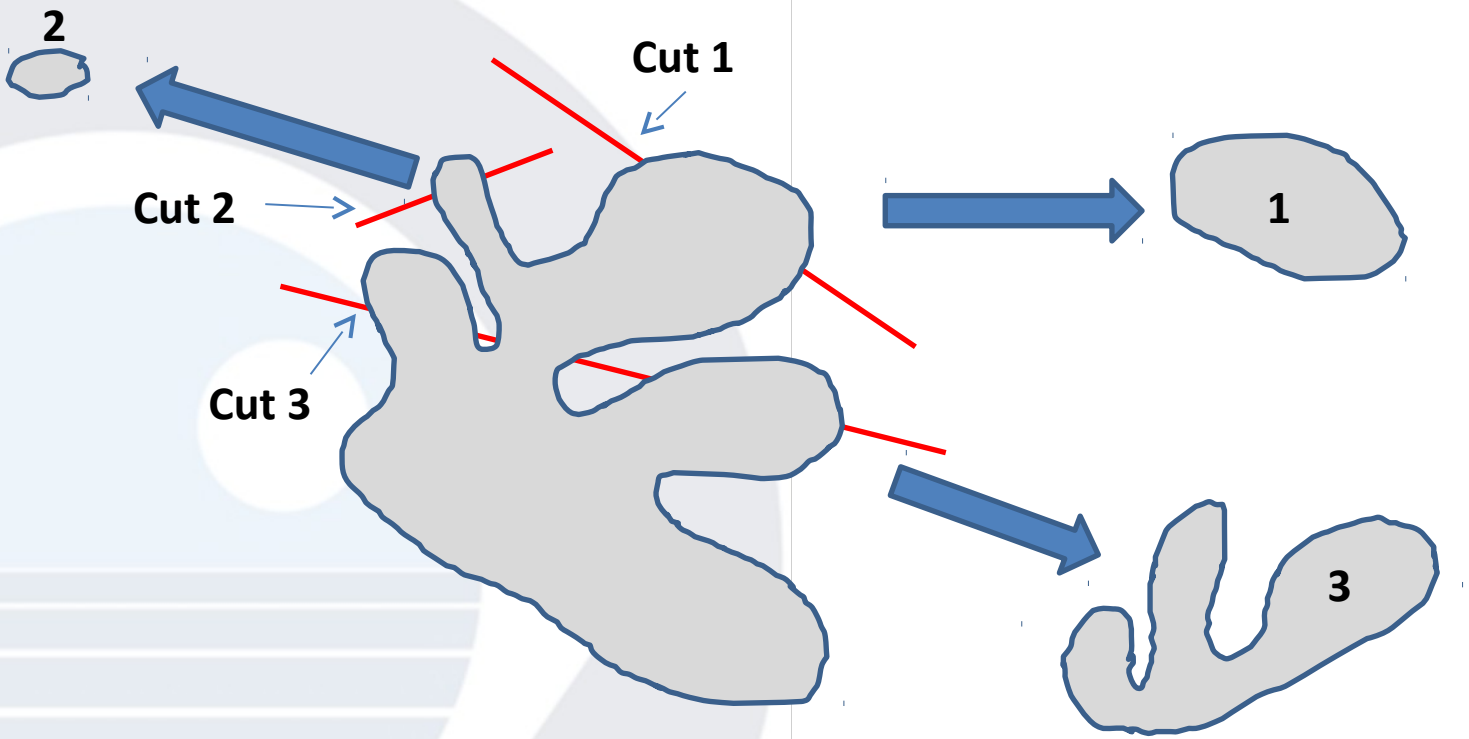


WARNING¡¡

- **This equivalence is not demonstrated in Scomber scombrus. It is an assumption.**
- **This equivalence is temperature dependent.**

POFs in histological slides.

1. The appearance and size of the POF in a slide depends of the cut.



2. Cuts smaller look older.

3. This can produce confussion between near stages.

4. As a result, in general, stage of a sample is determined as the minor POF stage on the slide.

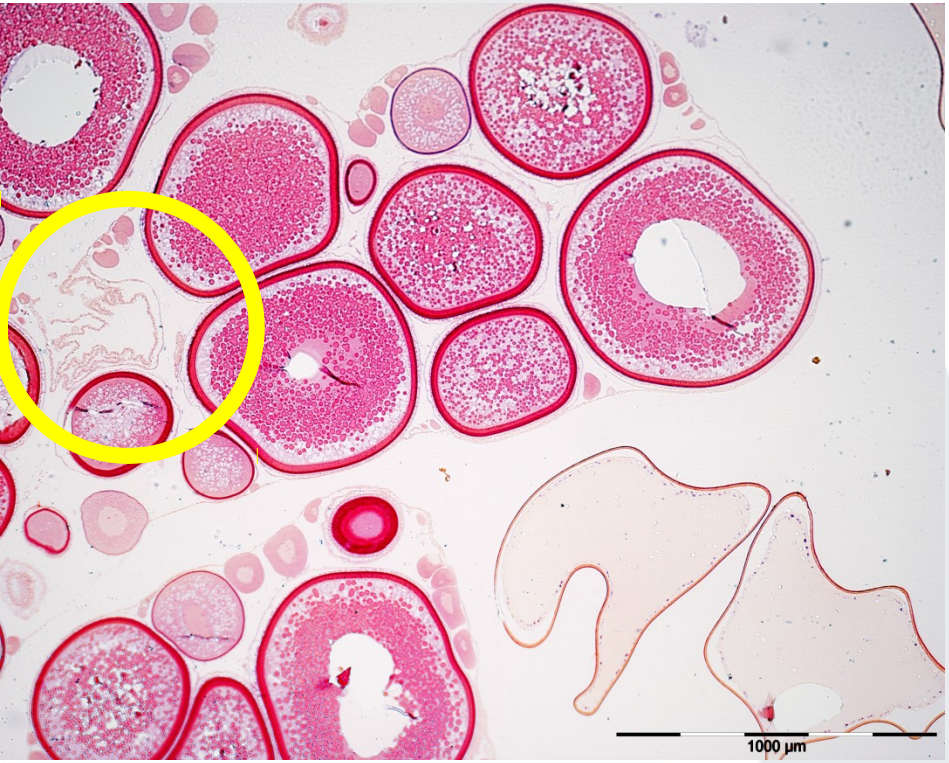
DESCRIPTION OF A 7 STAGES SCALE FOR POFs CLASSIFICATION.

STAGE 1

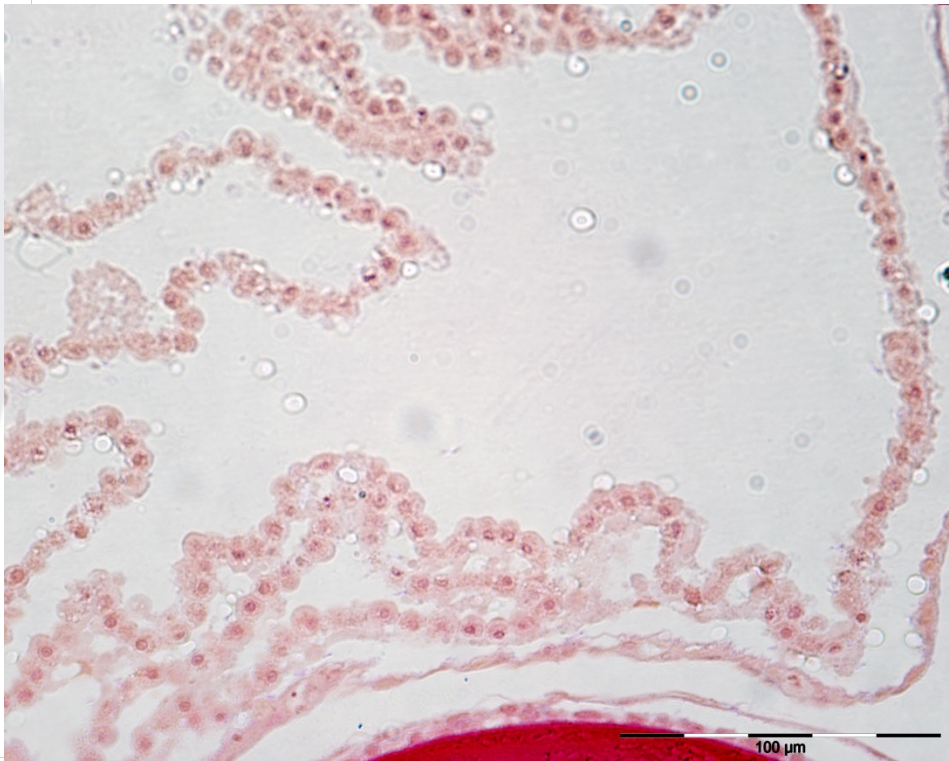
- Newly-formed POF. Often still with hyaline eggs.
- Large POFs. With cord-like structure, extended or folded with large loops. It’s easy to follow the line of cells.
- Large lumen.
- Granulosa cells are arranged in narrow lines, and with many nucleus in an apical position. Cell boundaries are quite clear.
- The theca is still very stretched. Separated from the granulosa, thin and, at first, no very clearly distinguishable, being clearer as the POF advance toward stage 2.
- No signs of degeneration.

Stage1

Resin. PAS-Mallory Trichrome. 40x



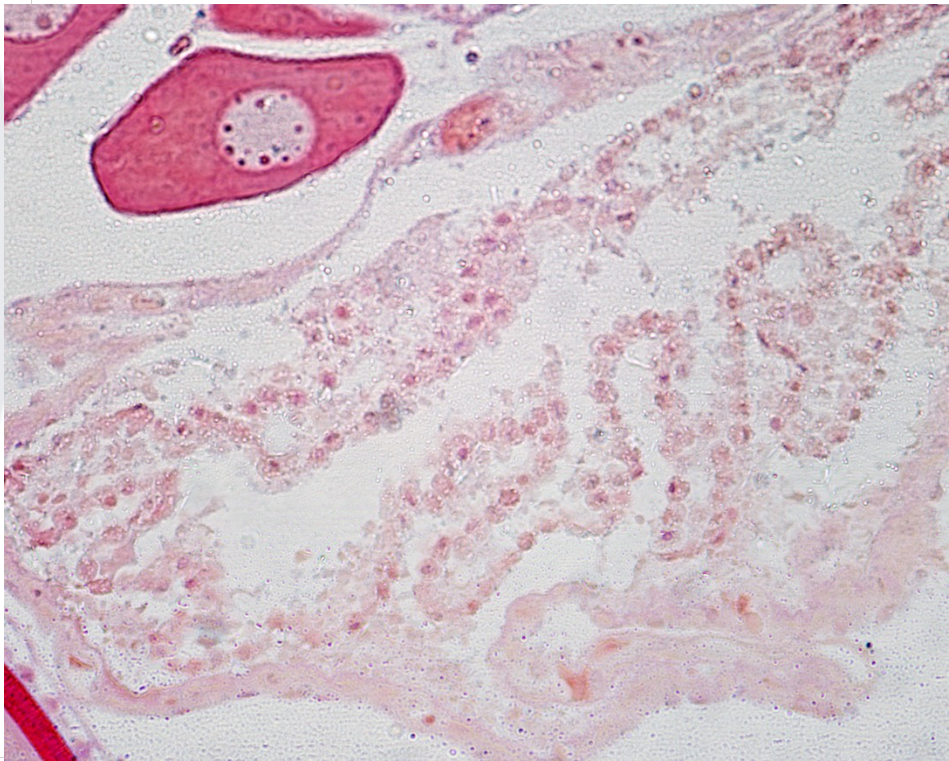
Resin. PAS-Mallory Trichrome. 400x



Stage1

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x

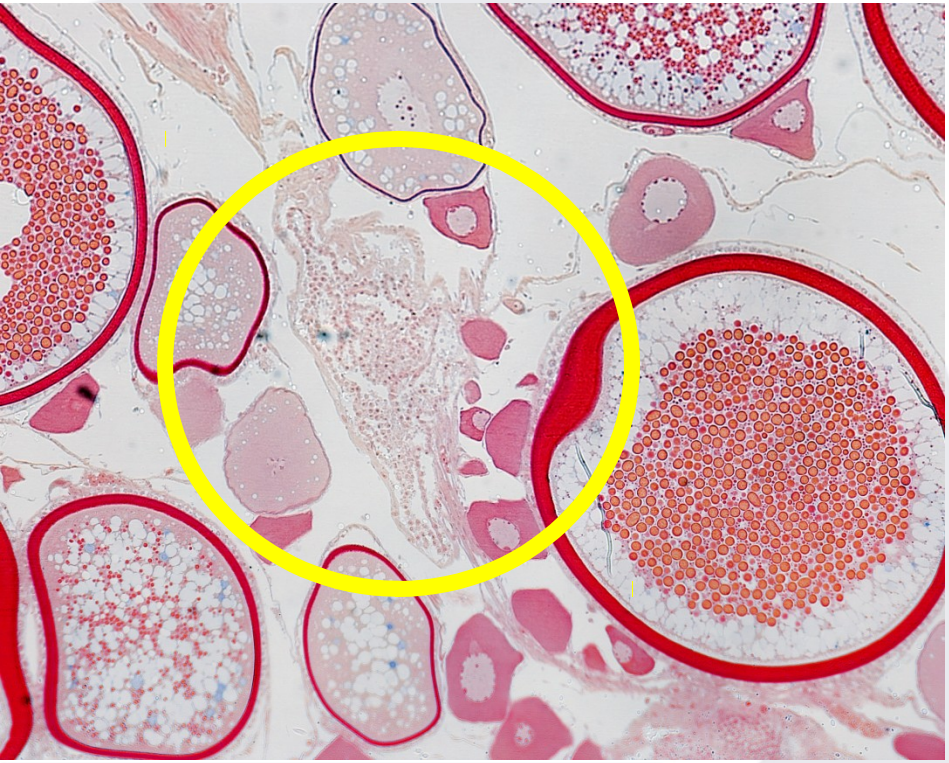


STAGE 2

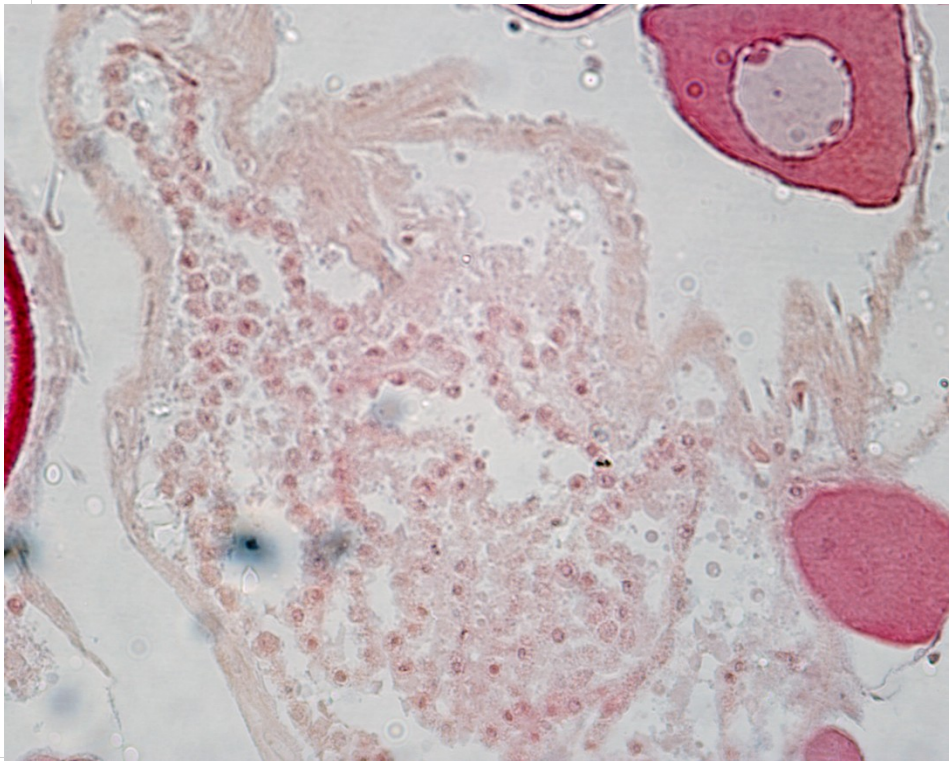
- It’s unusual to observe hyaline eggs.
- Large POF, with evident loops. More folded than stage 1.
- Large lumen.
- Granulosa cells still arranged in lines, but these are wider. More nuclei in a basal position than in stage 1. Slightly hypertrophied cells, with a columnar or cubical appearance. It’s possible that in some part of the POF the line of cells is difficult to follow.
- Theca is clearer.

Stage2

Resin. PAS-Mallory Trichrome. 40x



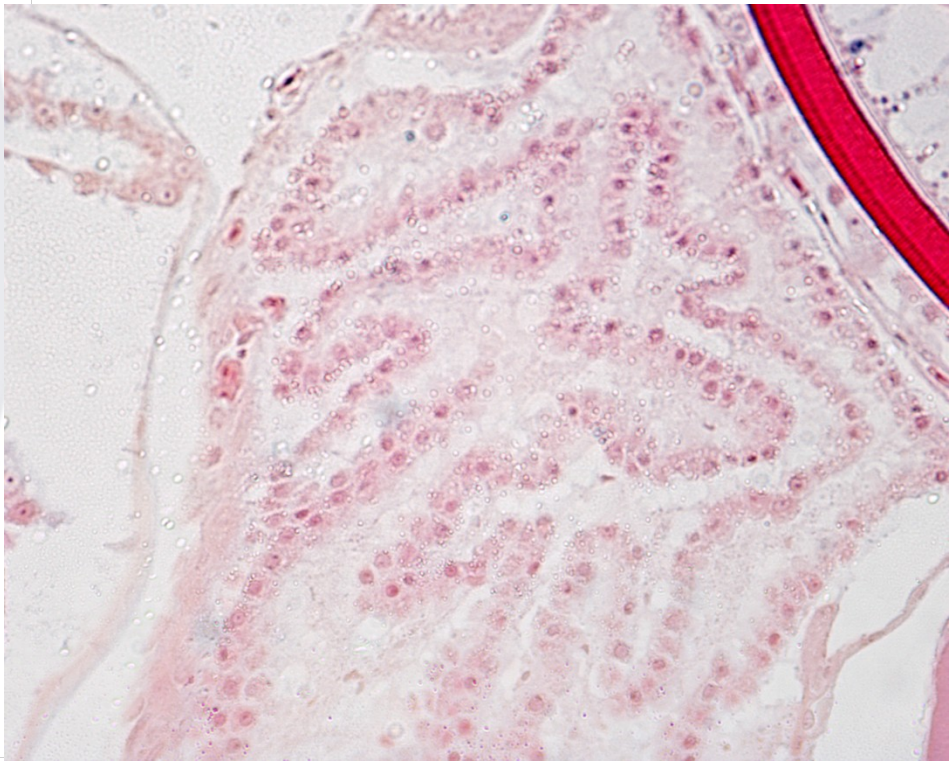
Resin. PAS-Mallory Trichrome. 400x



Stage2

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x



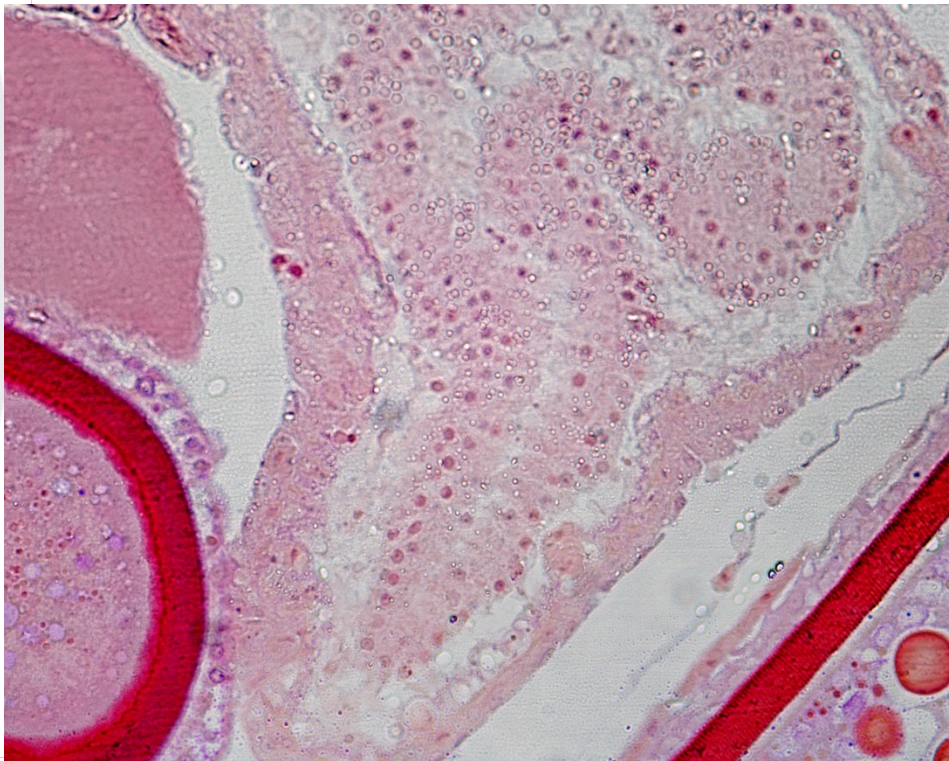
STAGE 3

- Granulosa folds are still clearly recognized.
- Granulosa cells are still aligned but less ordered and in thicker lines. Less obvious cell boundaries.
- Lumen clearly reduced in size with respect to stage 2.
- Evident signs of degeneration:
  - Pycnotic nuclei in the granulosa cells.
  - First vacuoles in granulosa cells.
- Theca is closer to granulosa and frame it.

Stage3

Resin. PAS-Mallory Trichrome. 40x

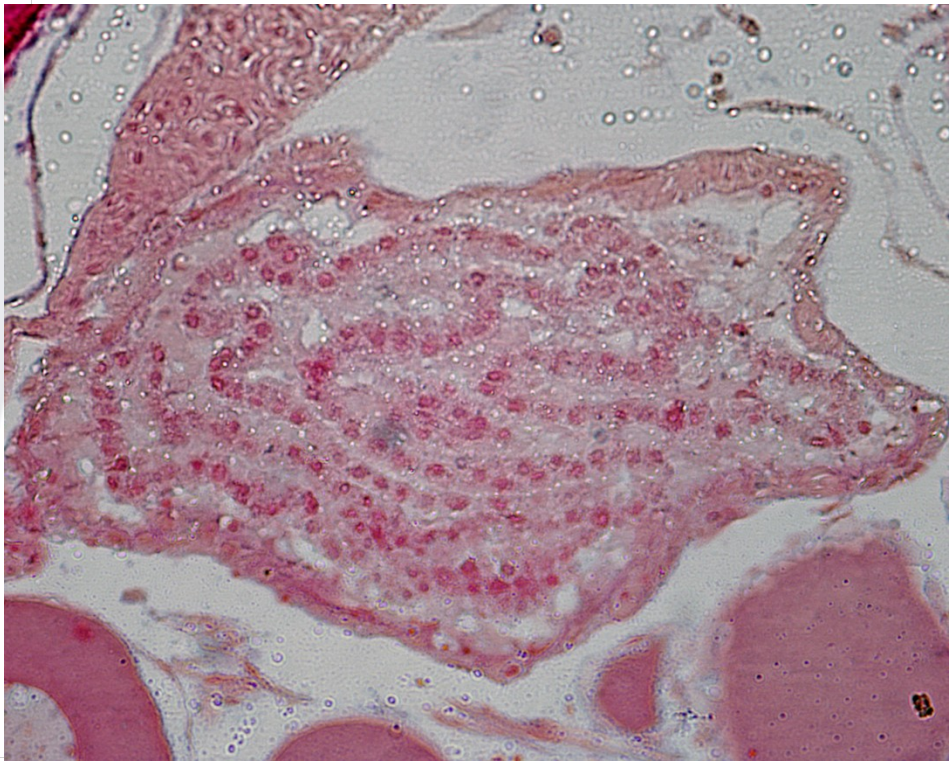
Resin. PAS-Mallory Trichrome. 400x



Stage3

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x

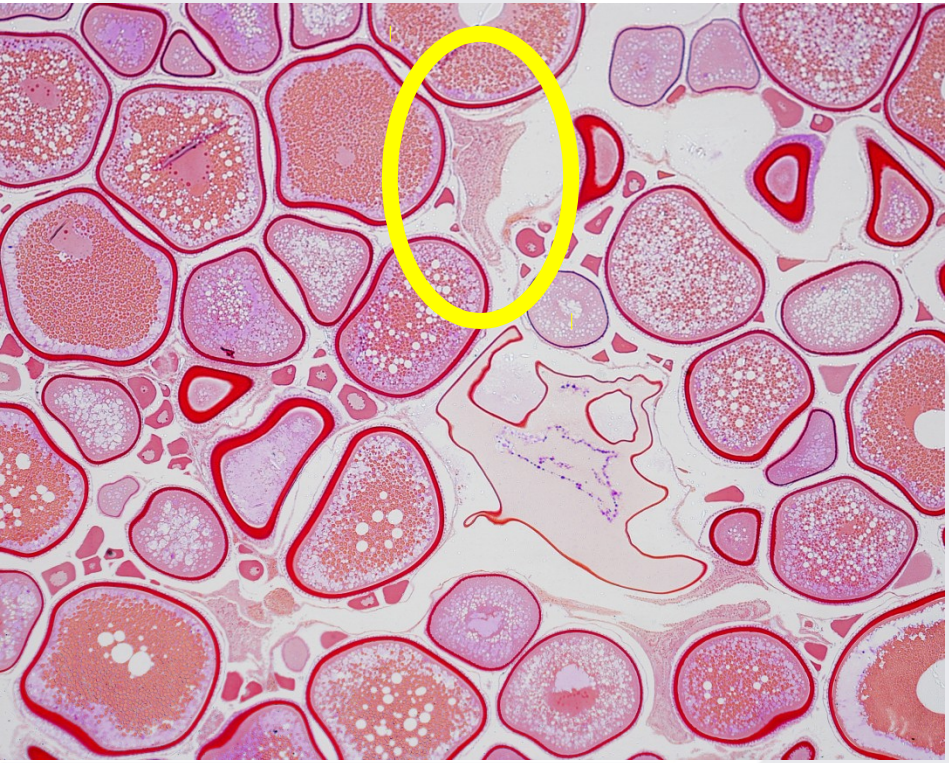


STAGE 4

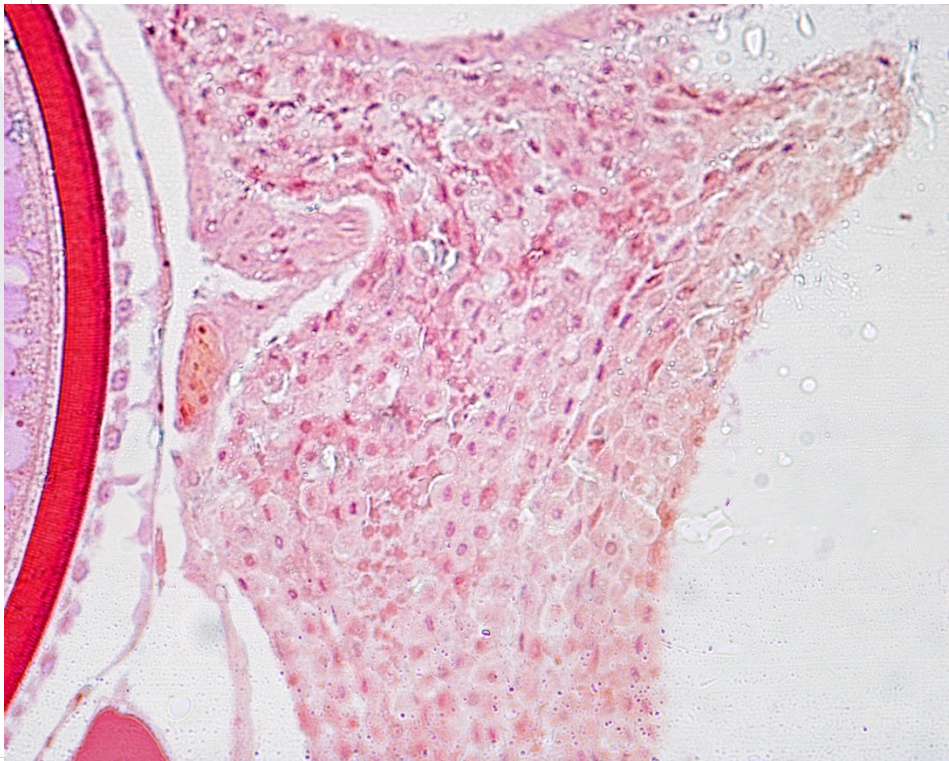
- The POF are still large, but lower than in stage 3.
- Compact POF in which it is strange to distinguish folds in the granulosa.
- The lumen is small and sometimes can’t be seen. If is visible is as white lines, more o less wide, than drawn granulosa folds.
- Granulosa cells are more disordered and their limits are not apparent. Pycnotic nuclei and vacuoles are more frequent.
- Theca is closer to granulosa. It is more difficult to see than in stage 3.

Stage 4

Resin. PAS-Mallory Trichrome. 40x



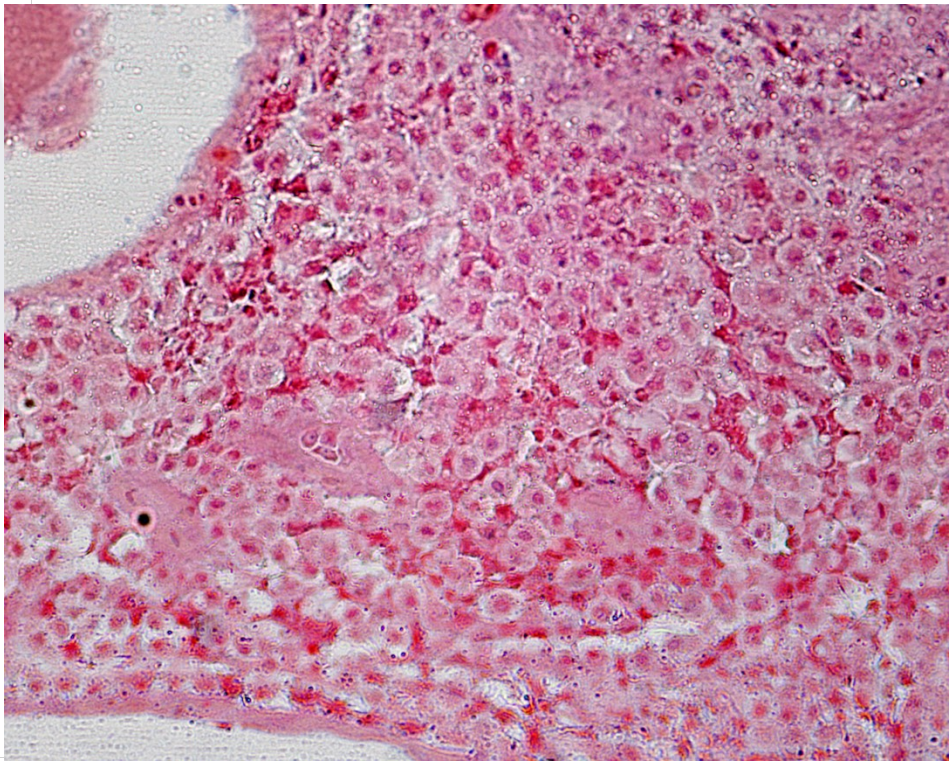
Resin. PAS-Mallory Trichrome. 400x



Stage 4

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x



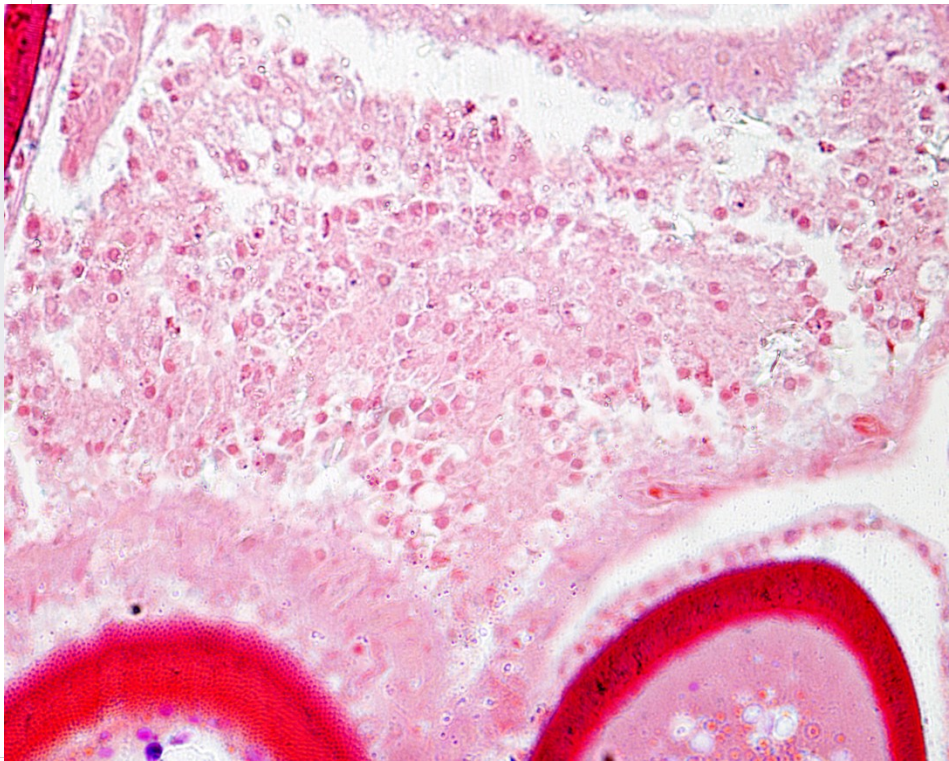
STAGE 5

- Strong decrease in size with respect to the previous stage.
- POF without ordering patterns except some short alignment of nuclei. Folds aren't visible and POF looks quite degenerate, with a more regular shape than previous stages.
- Lumen isn't visible.
- Granulosa presents numerous pyknotic nuclei and vacuoles. Only few cells are intact. It's possible to see large white areas of vacuoles that can lead to confusion with the lumen.
- Theca layer is made wider.

Stage5

Resin. PAS-Mallory Trichrome. 40x

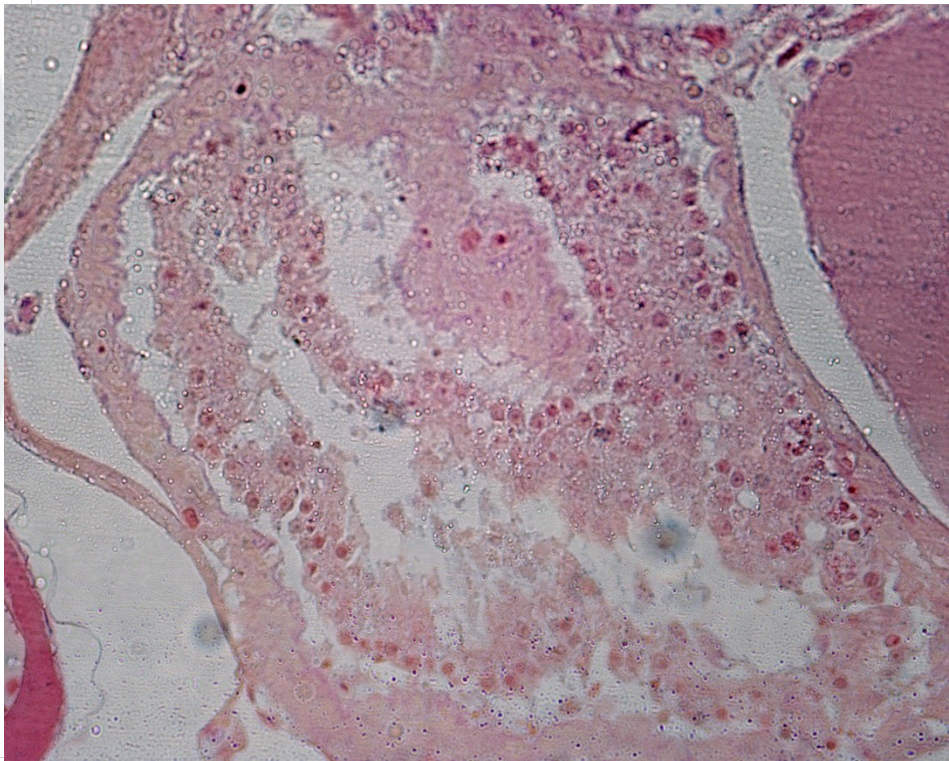
Resin. PAS-Mallory Trichrome. 400x



Stage5

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x



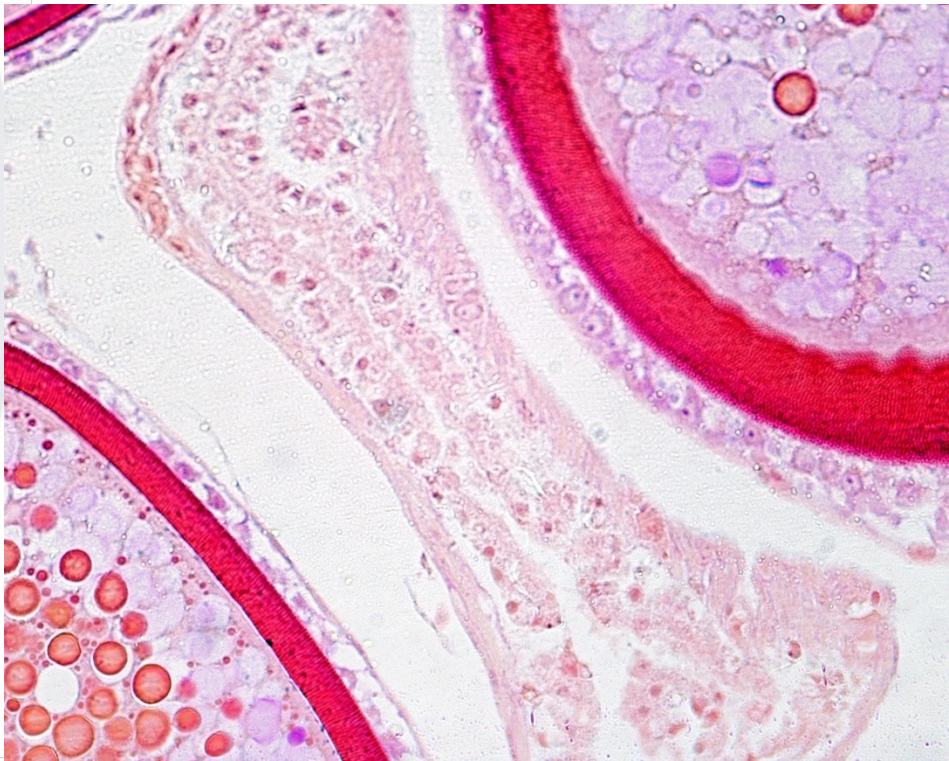
STAGE 6

- POF of reduced size that shows a polyedric shape, frequently triangular.
- The lumen is absent.
- Granulosa is reduced to a few remaining cells, normally with pycnotic nuclei and some vacuoles.
- Theca is proportionally a higher fraction of the POF.
- Possible confusion with advanced atresia (β and γ).

Stage 6

Resin. PAS-Mallory Trichrome. 40x

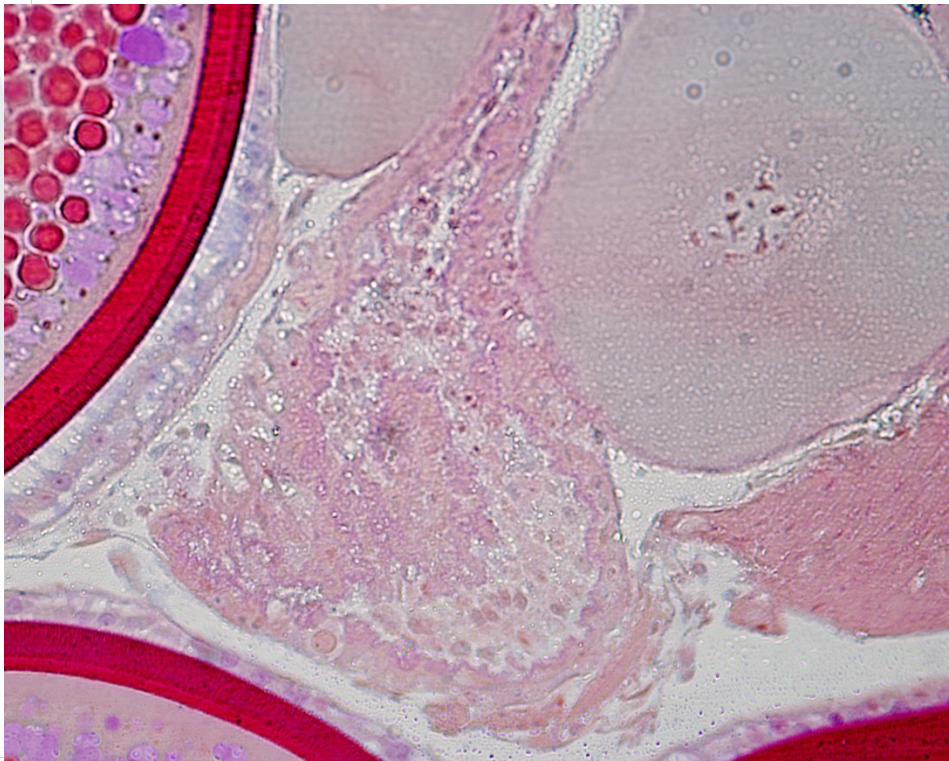
Resin. PAS-Mallory Trichrome. 400x



Stage6

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x



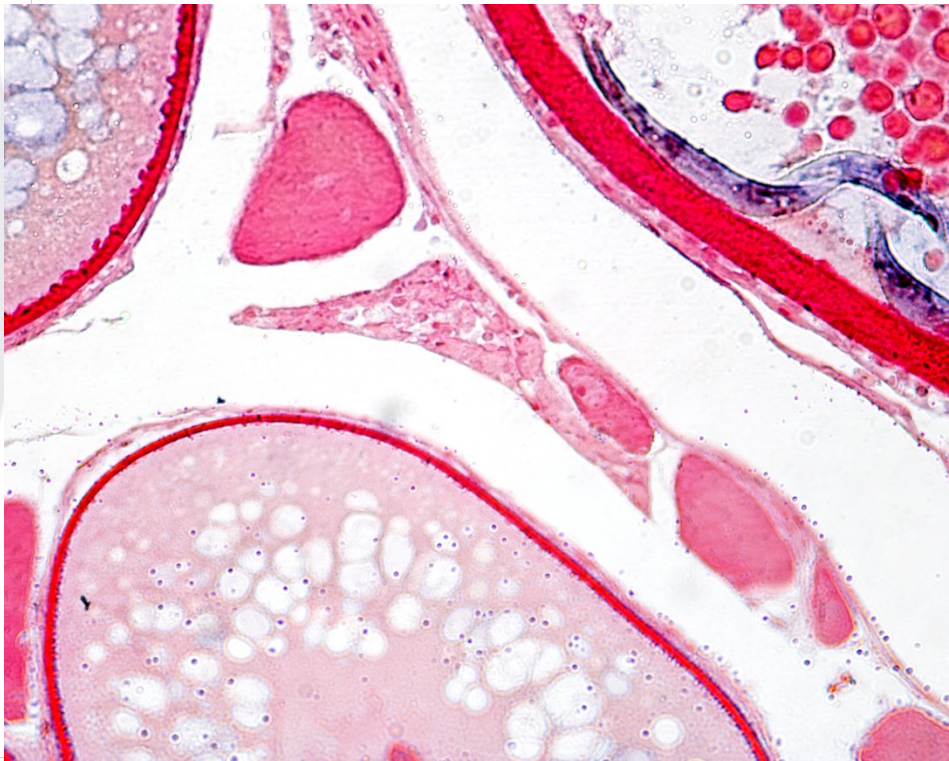
STAGE 7

- Very small POF. Difficult to see with 40x magnification . Their number in the sample is low.
- POF reduced to almost only the theca. Granulosa, if present, is residual.
- Using low magnification, is possible confuse it with old atresias, blood capillaries or smooth muscle.

Stage 7

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x



Stage 7

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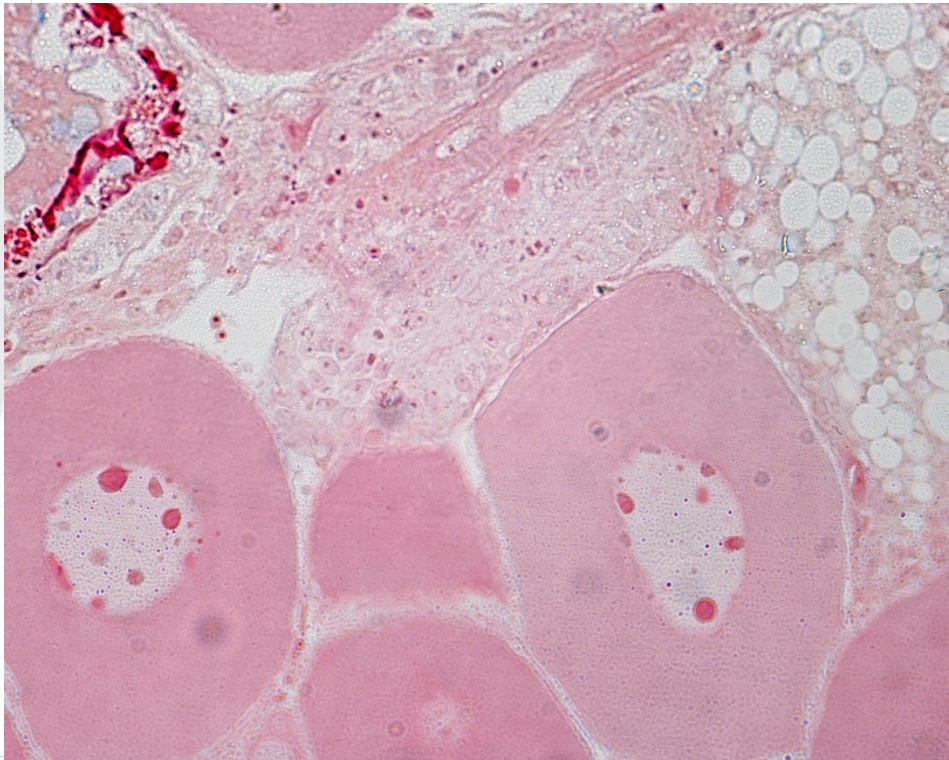
Resin. PAS-Mallory Trichrome. 400x

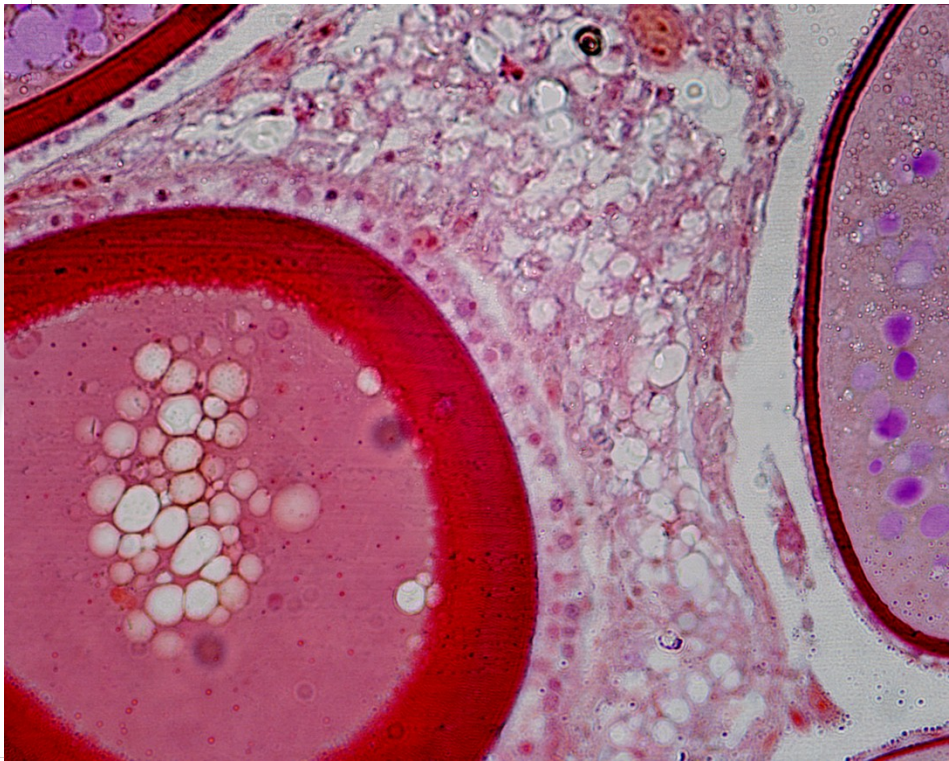


STRUCTURES THAT CAN CAUSE CONFUSION

OLD ATRESIAS

Resin. PAS-Mallory Trichrome. 40x

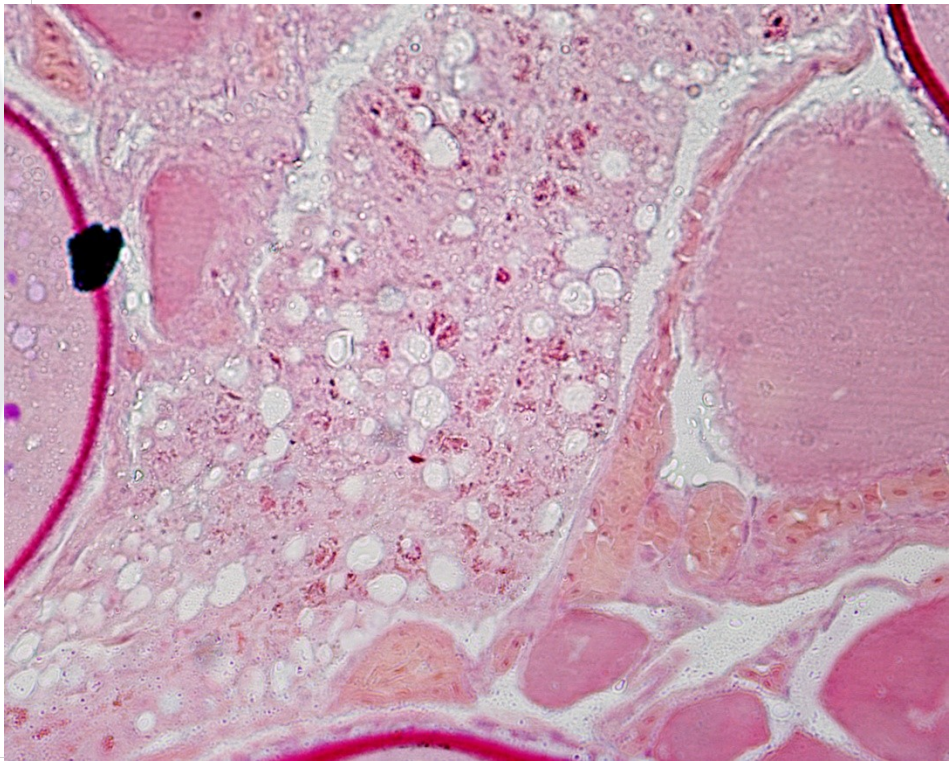
Resin. PAS-Mallory Trichrome. 400x



OLD ATRESIAS

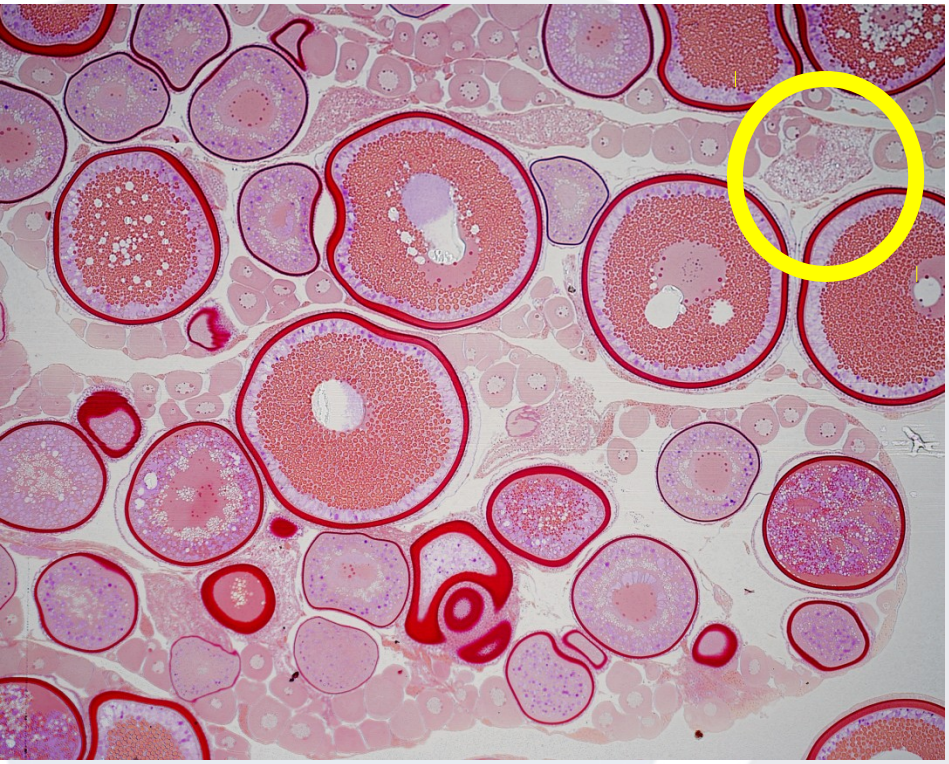
Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x

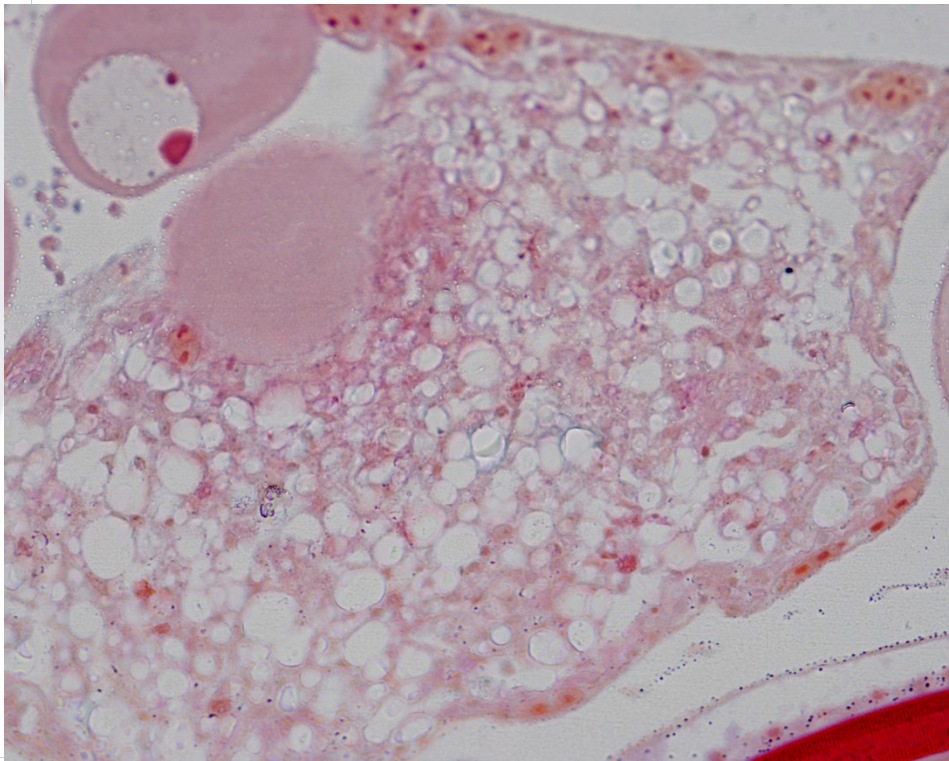


OLD ATRESIAS

Resin. PAS-Mallory Trichrome. 40x



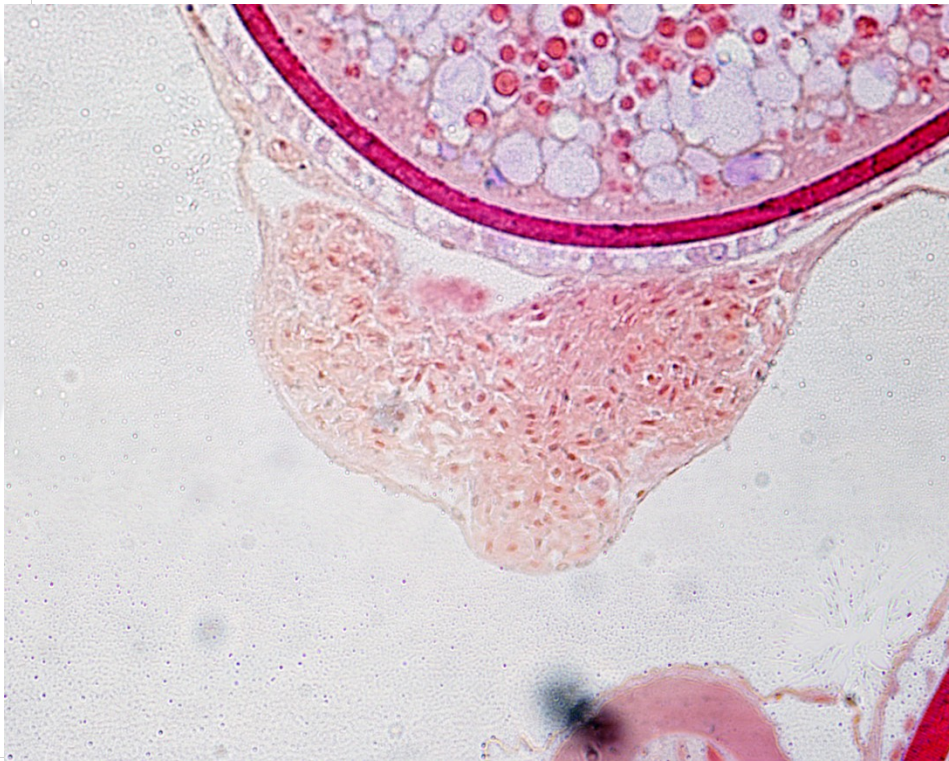
Resin. PAS-Mallory Trichrome. 400x



BLOOD CAPILLARIES

Resin. PAS-Mallory Trichrome. 40x

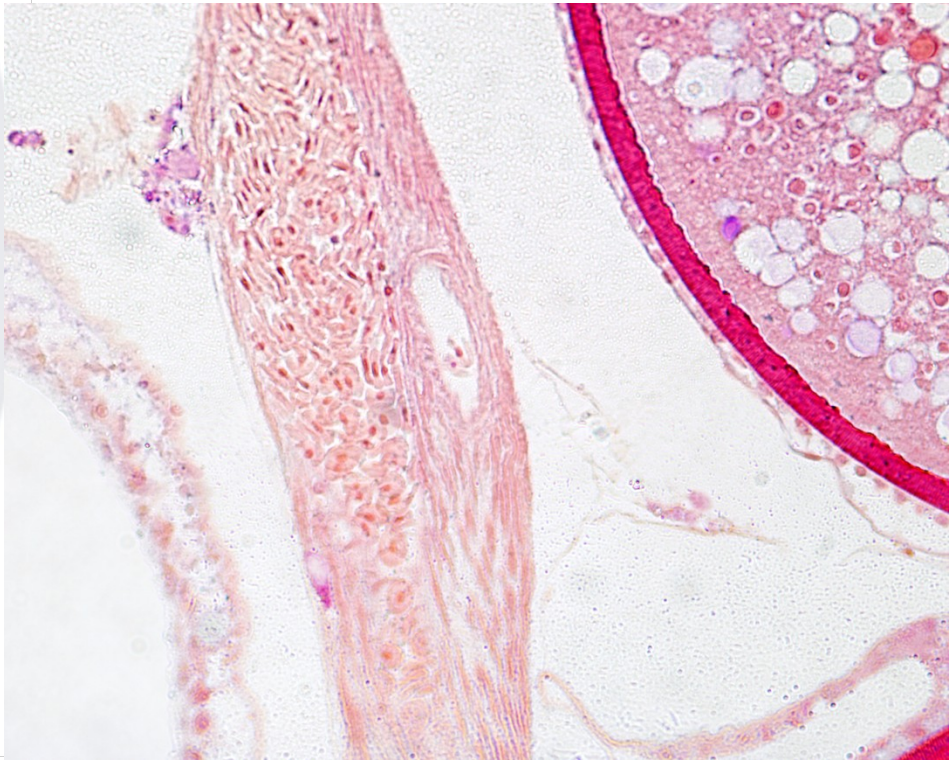
Resin. PAS-Mallory Trichrome. 400x



BLOOD CAPILLARIES

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x



ARTEFACTS

This is an empty follicle, but it’s a sample manipulation artefact. It is not a POF.

Resin. PAS-Mallory Trichrome. 40x

Resin. PAS-Mallory Trichrome. 400x