How to stage the gonadal cycle in (small) tunas. Histological criteria

UCA Universidad de Cádiz

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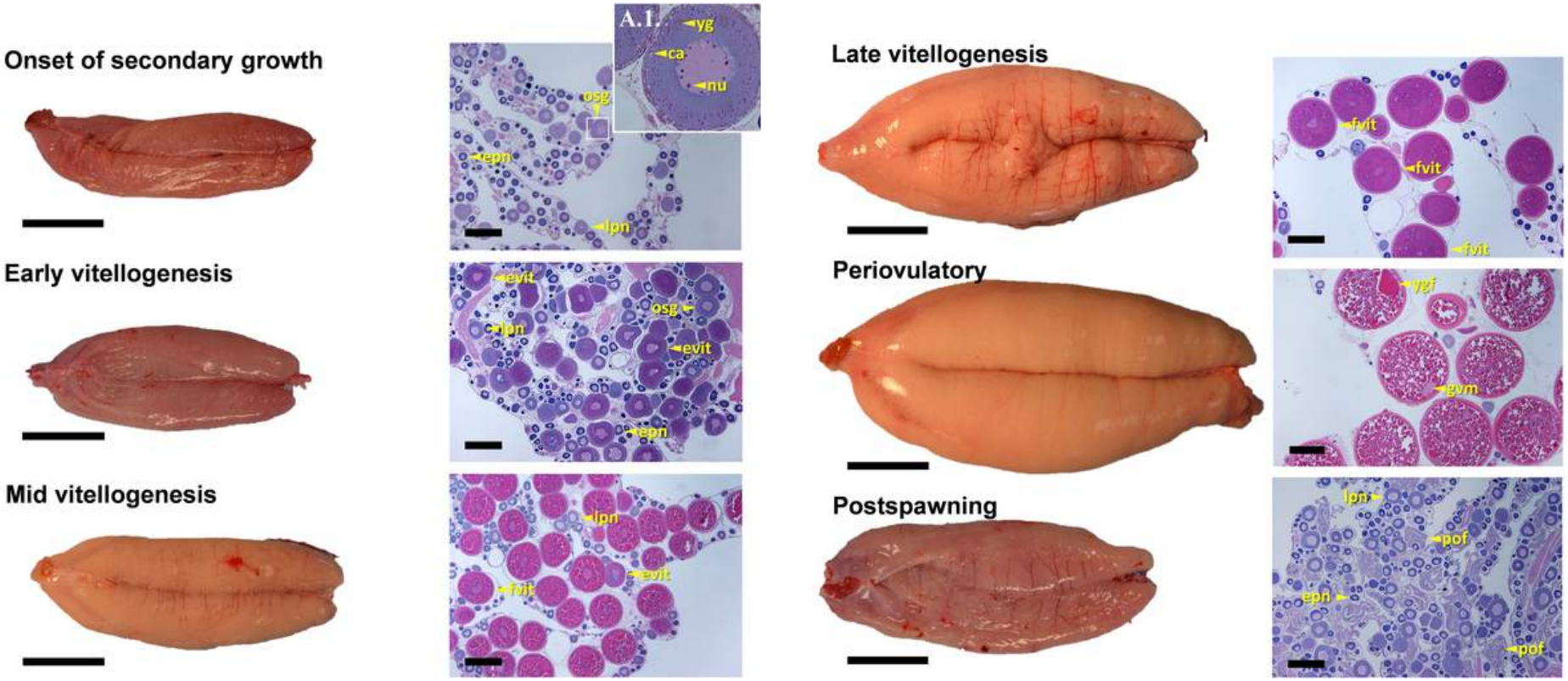
ICCAT Small Tunas Sampling and Biology Workshop
Fuengirola (Malaga, Spain). February 17-21, 2020

Fitting macroscopic appearance to physiological status

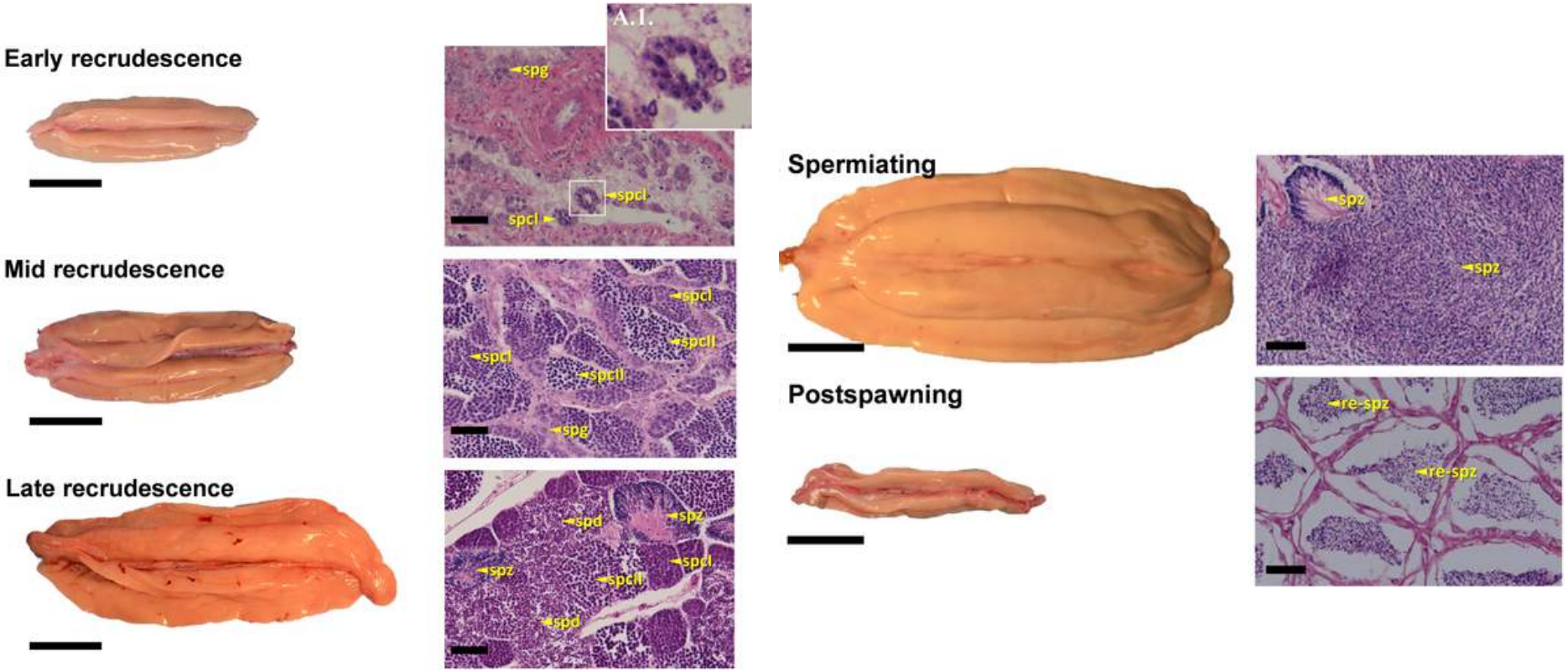
Representative photographs of the external appearance and histological sections of sablefish ovaries at onset of secondary growth, early mid and late vitellogenic stages, periovulatory and postspawning stages.



From Guzmán, JM et al. (2017) Reproductive life history of sablefish (*Anoplopoma fimbria*) from the U.S. Washington coast. PLOS ONE 12(9): e0184413. https://doi.org/10.1371/journal.pone.0184413

Fitting macroscopic appearance to physiological status

Representative photographs of the external appearance and histological sections of sablefish testes at early, mid and late recrudescence stages, spermiating and postspawning stages.



From Guzmán, JM et al. (2017) Reproductive life history of sablefish (*Anoplopoma fimbria*) from the U.S. Washington coast. PLOS ONE 12(9): e0184413. https://doi.org/10.1371/journal.pone.0184413

Sampling

Sampling

Sampling

Sampling

Histological preparation. Bluefin tuna

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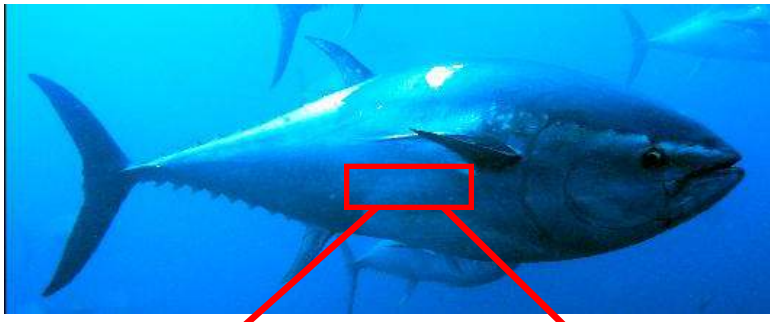

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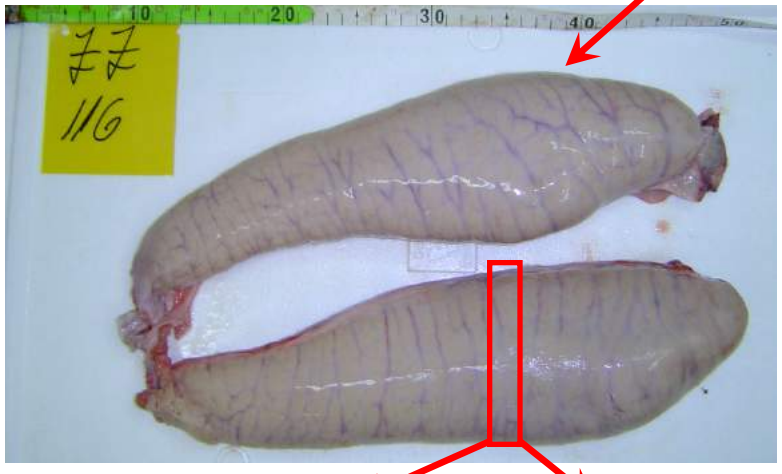
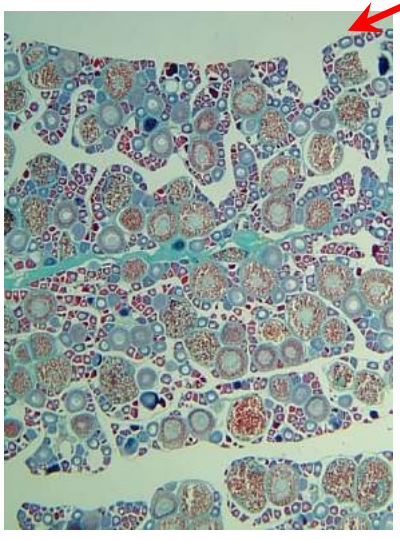
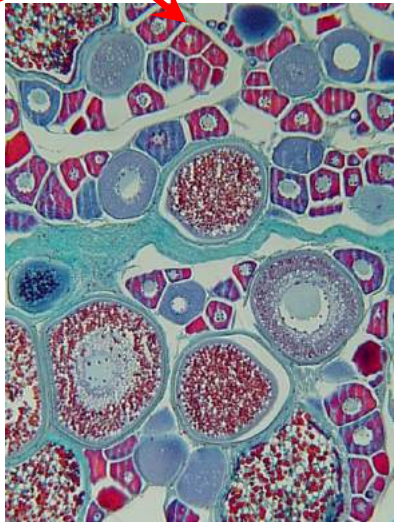
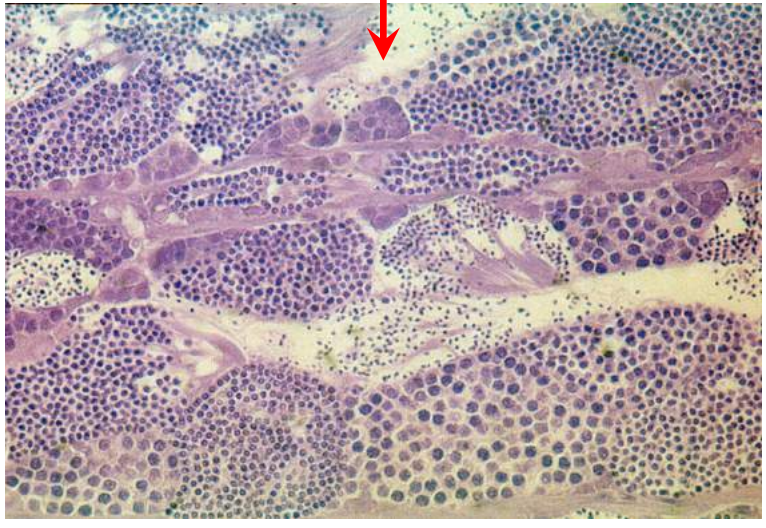
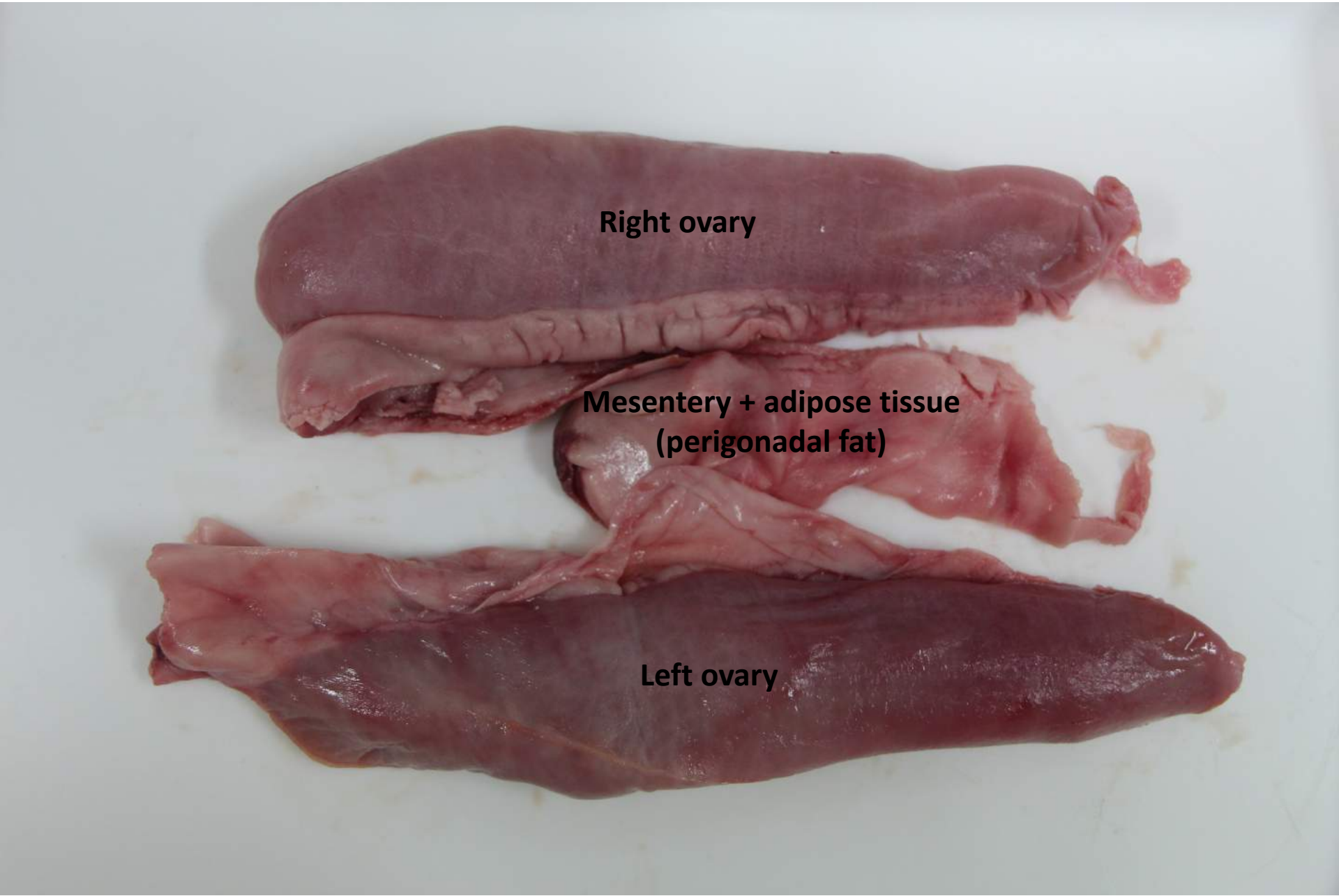




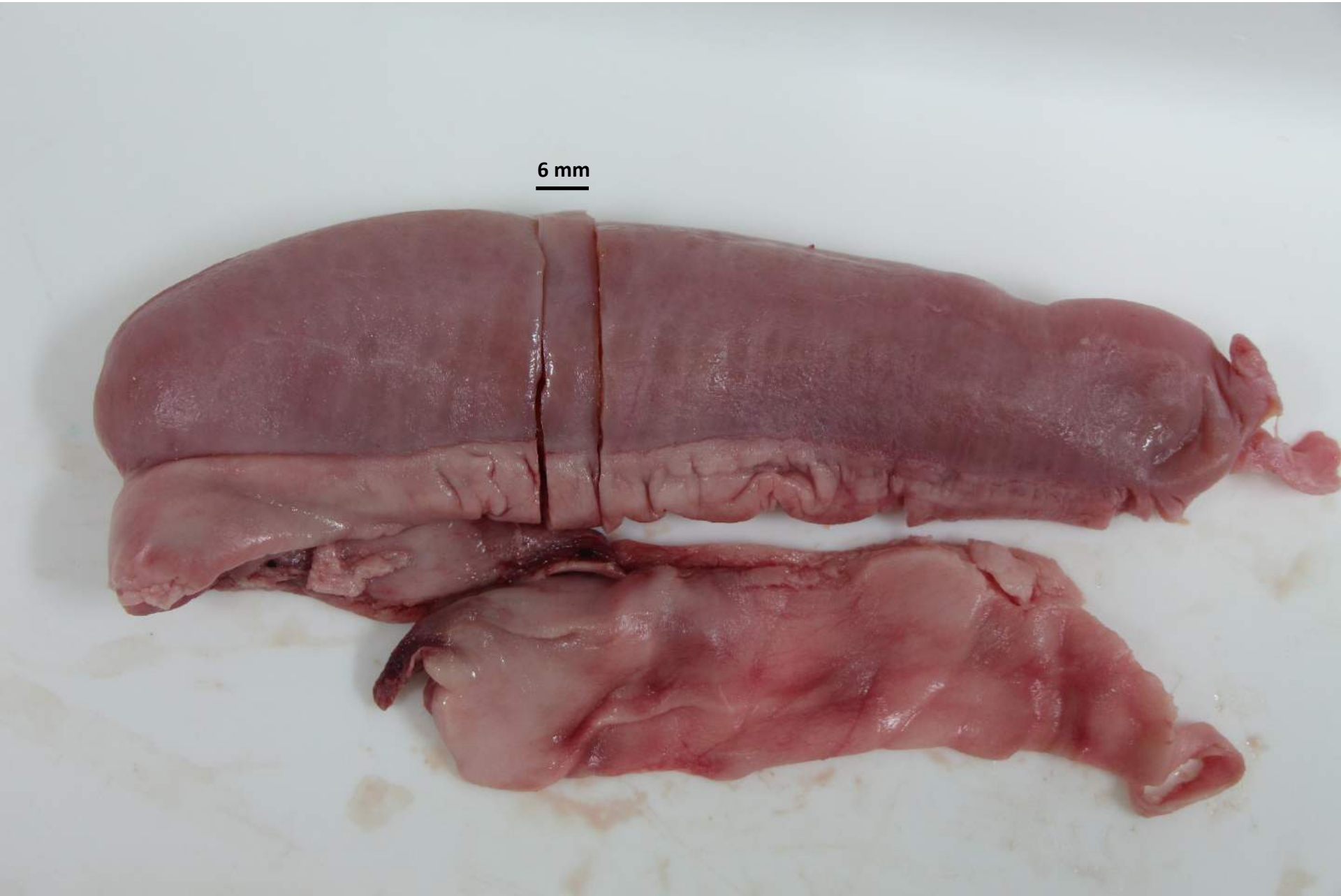




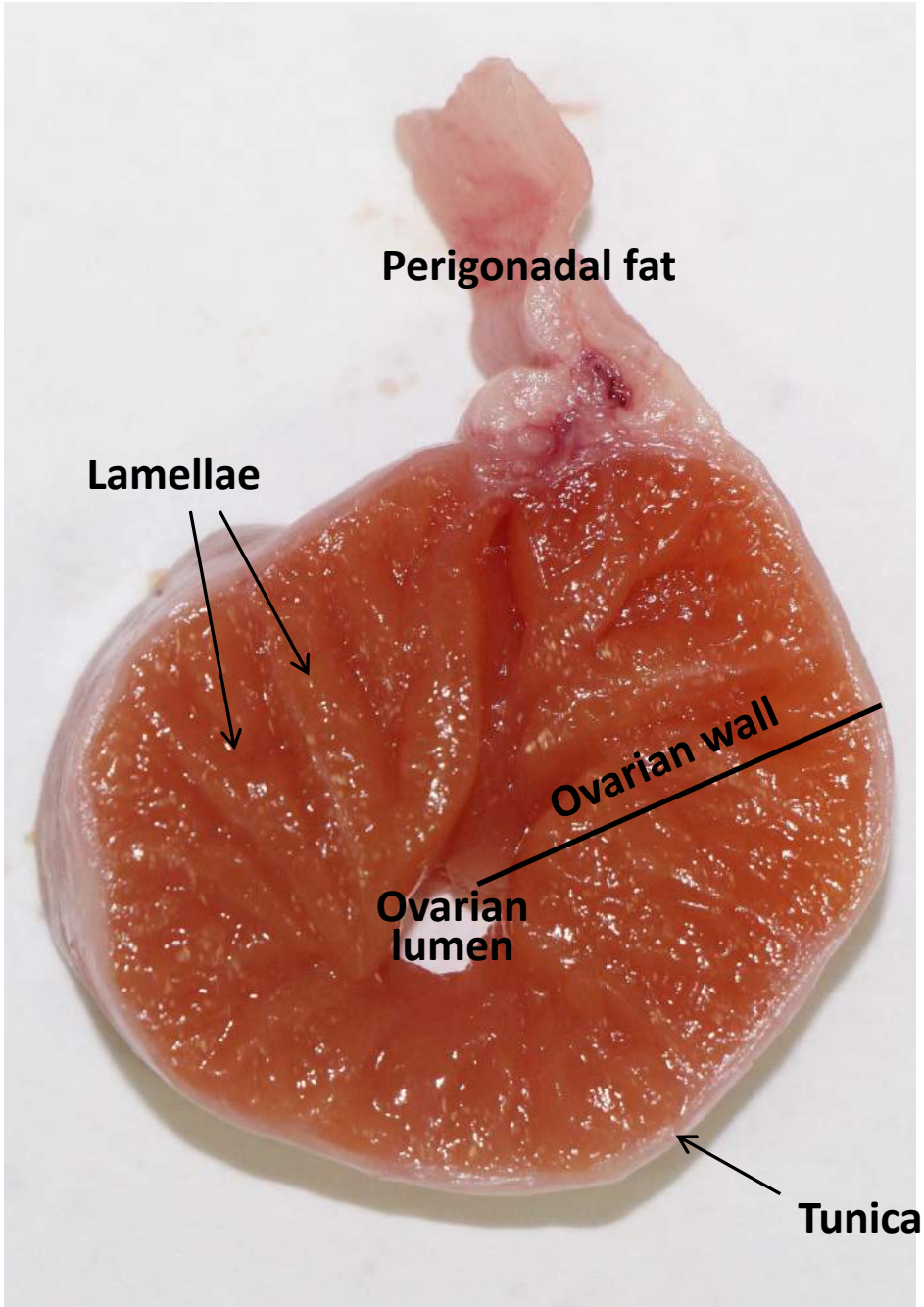
Ovaries collected on January 16, 2020

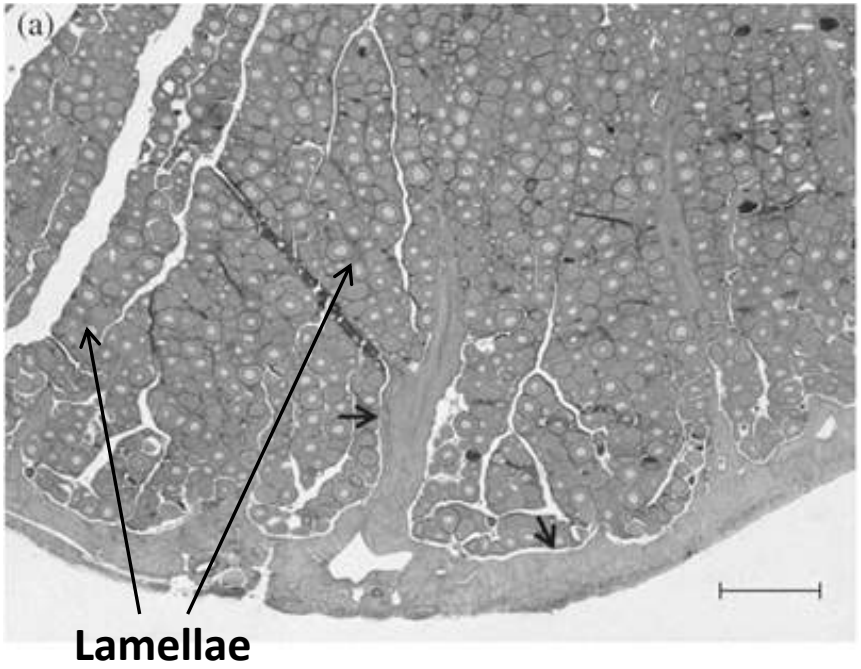


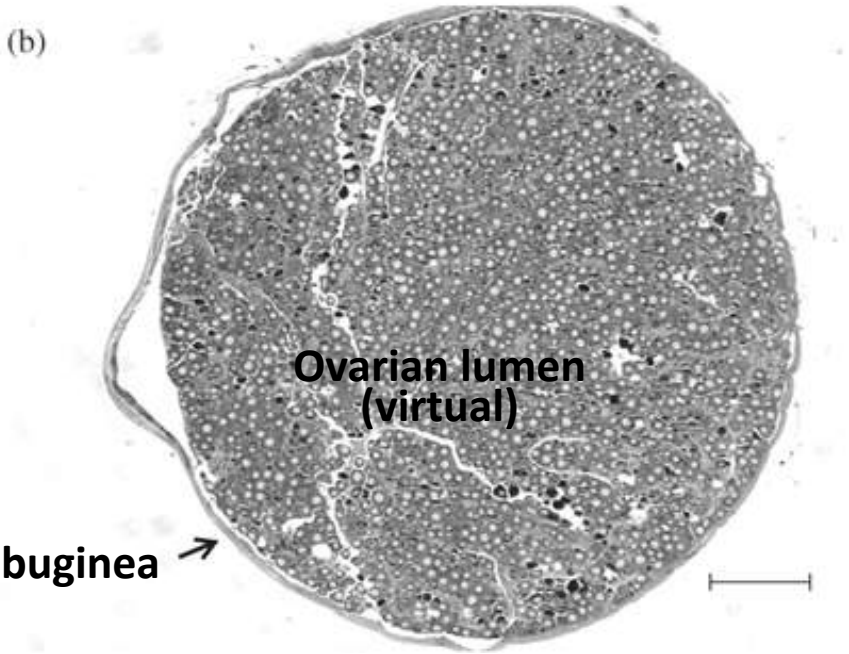
Transverse cuts



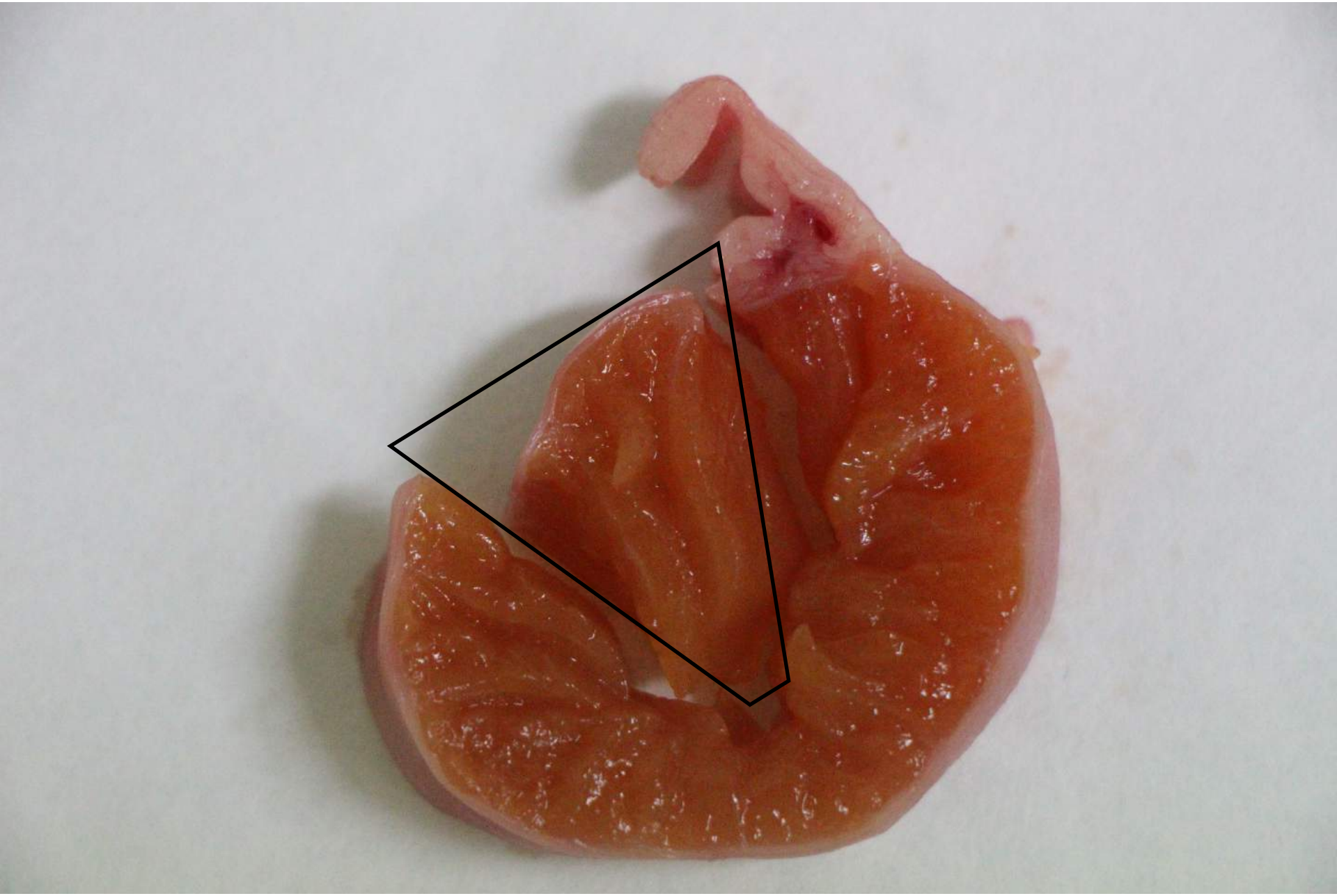
Whole cross section of the ovary



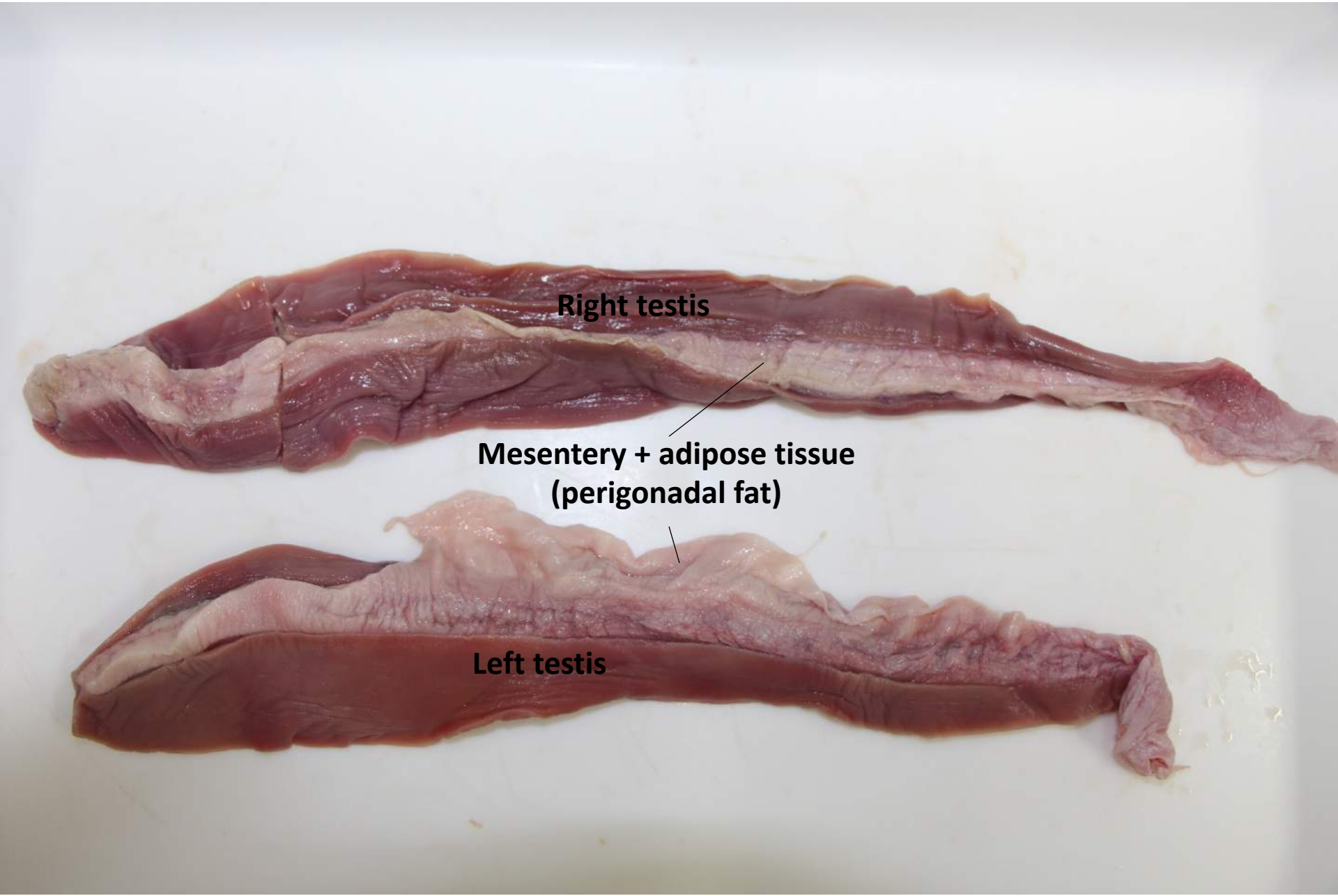




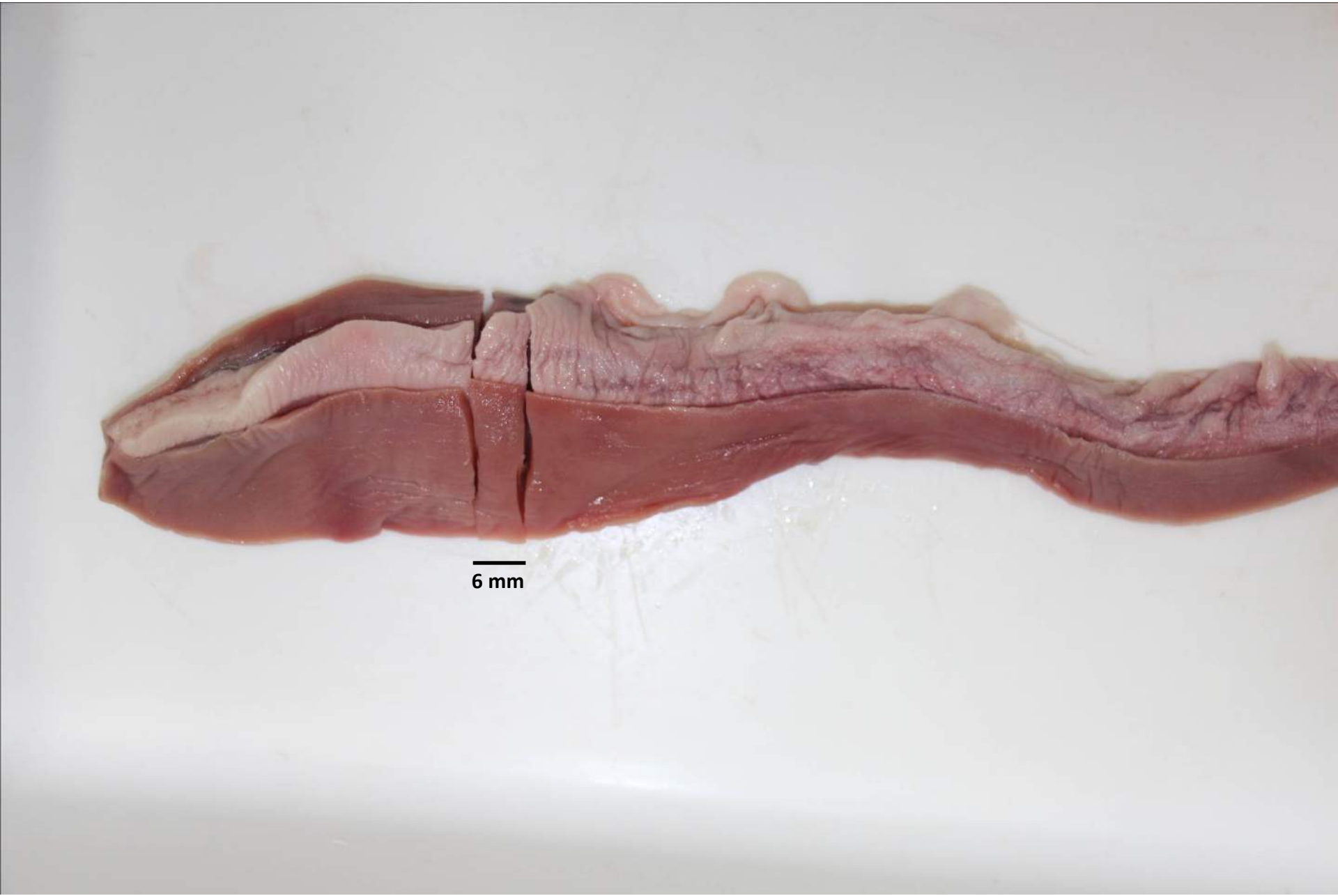
Selection of tissue sample



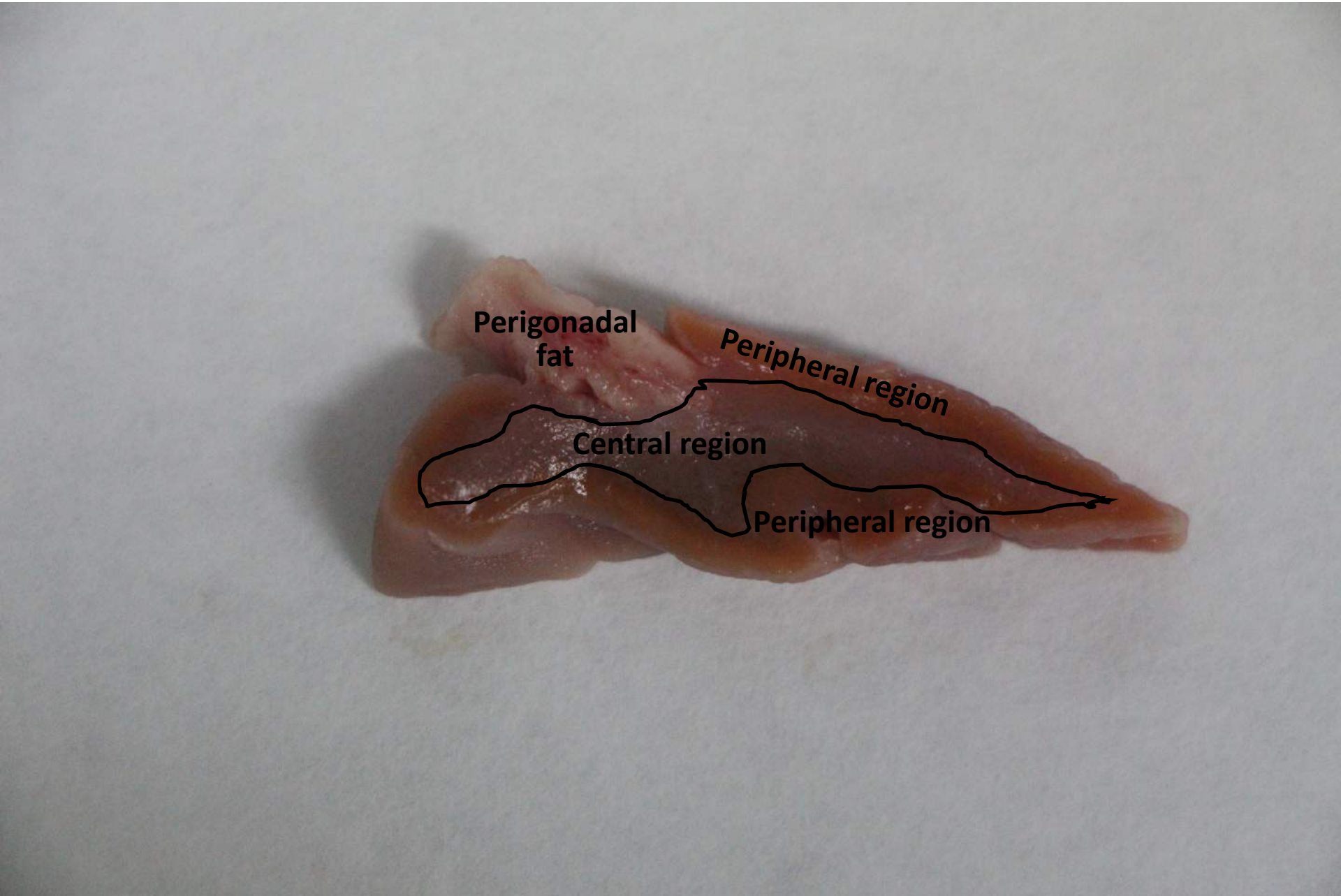
Testes collected January 16, 2020



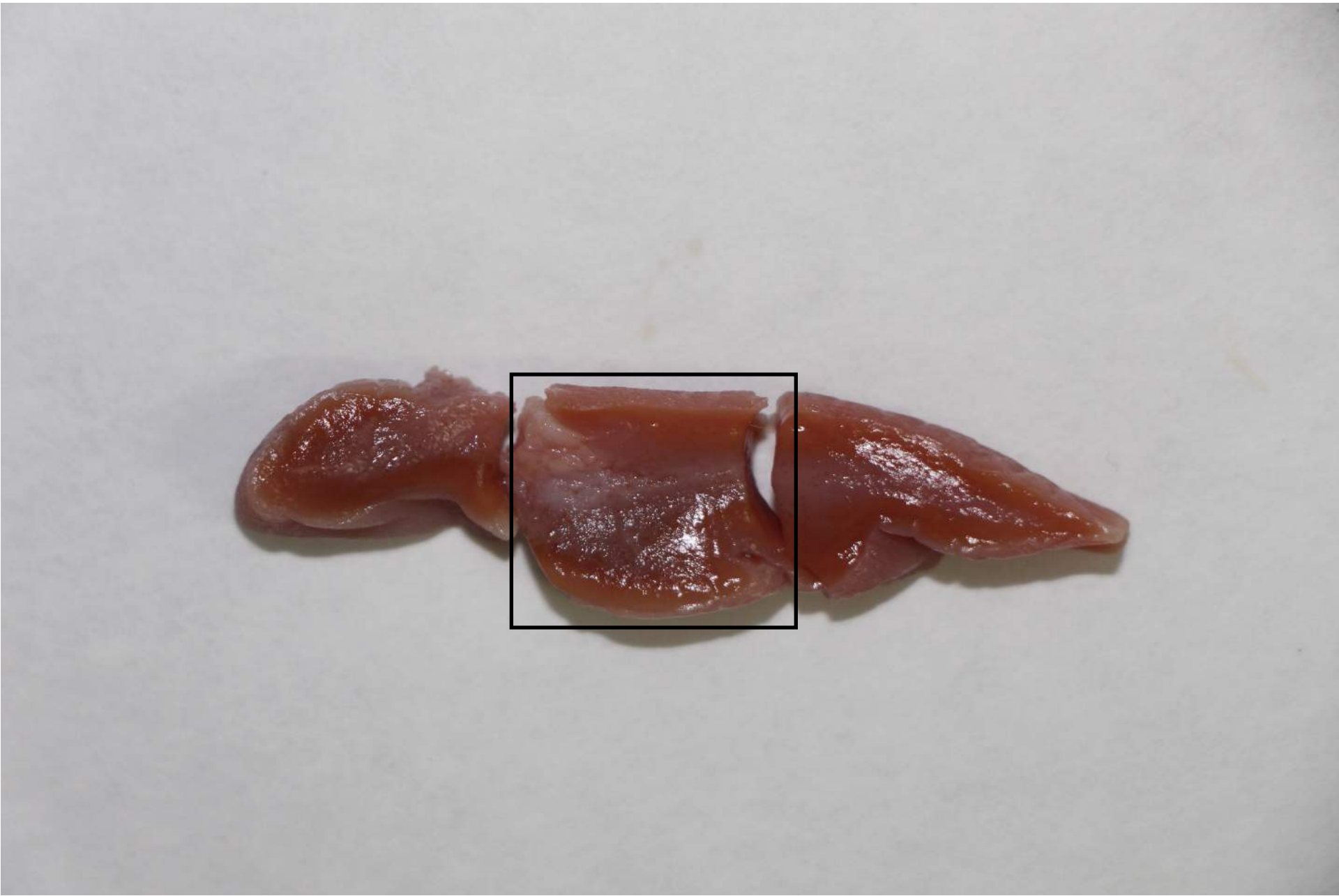
Transverse cuts



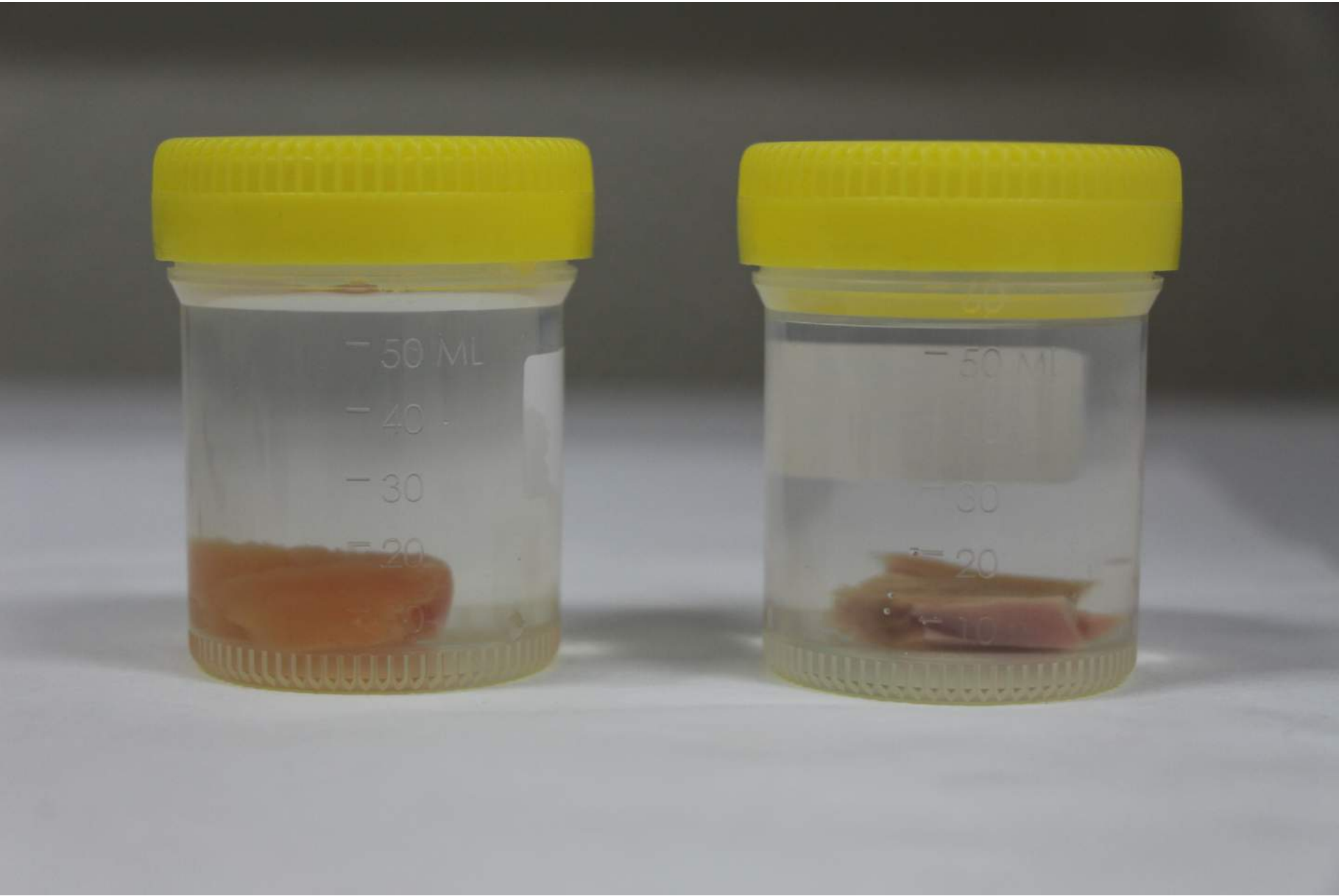
Whole cross section of the testis



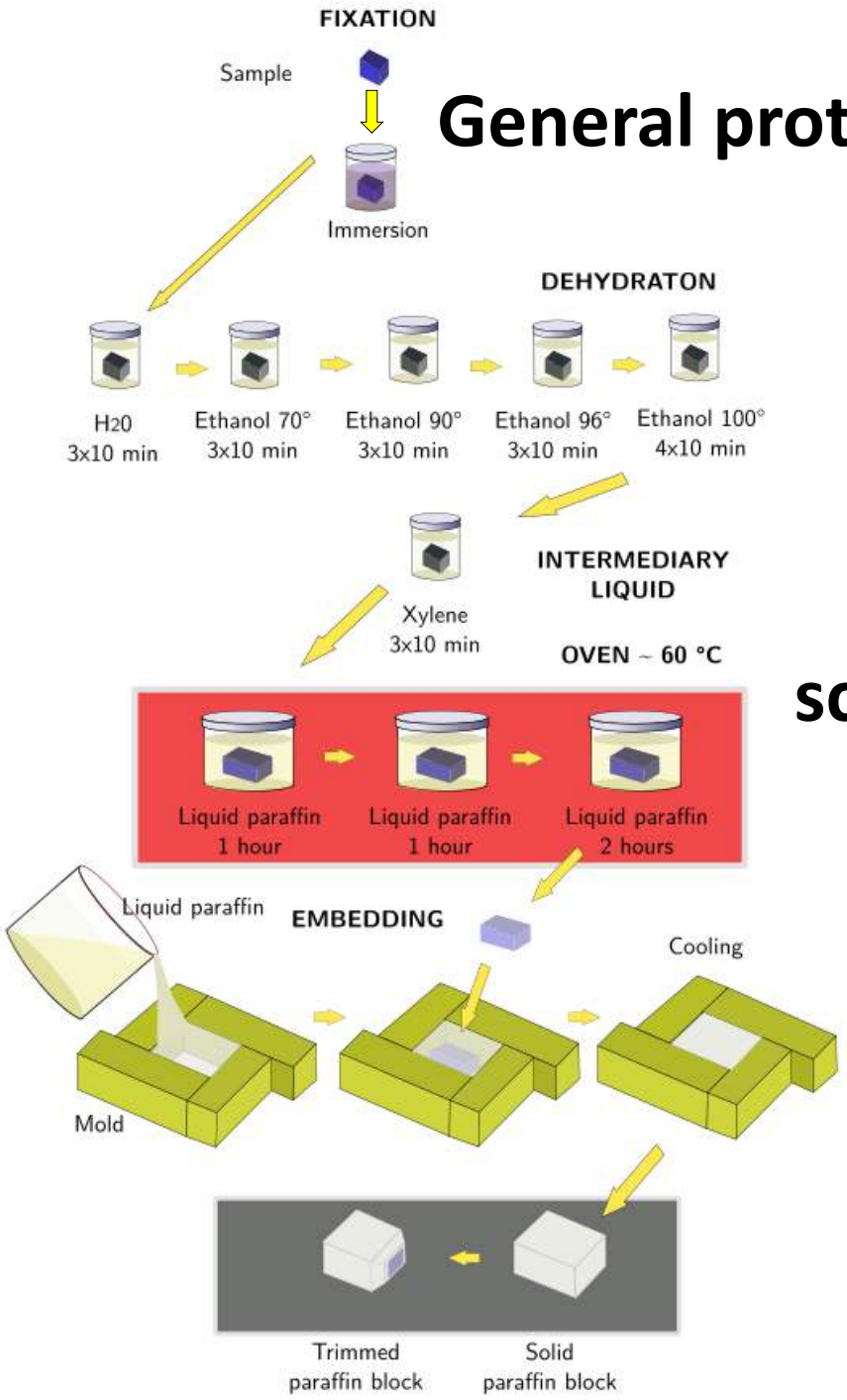
Proper selection of tissue sample



Placement into fixative solution

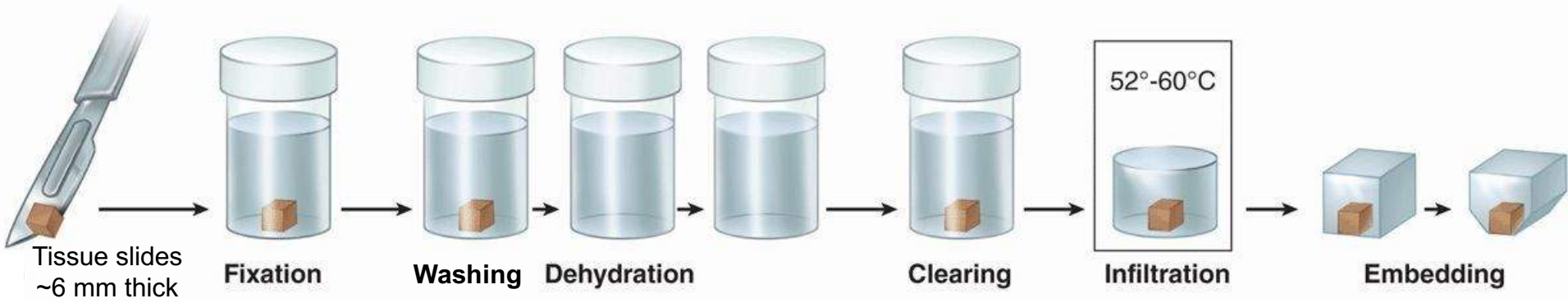


General protocol for histological processing



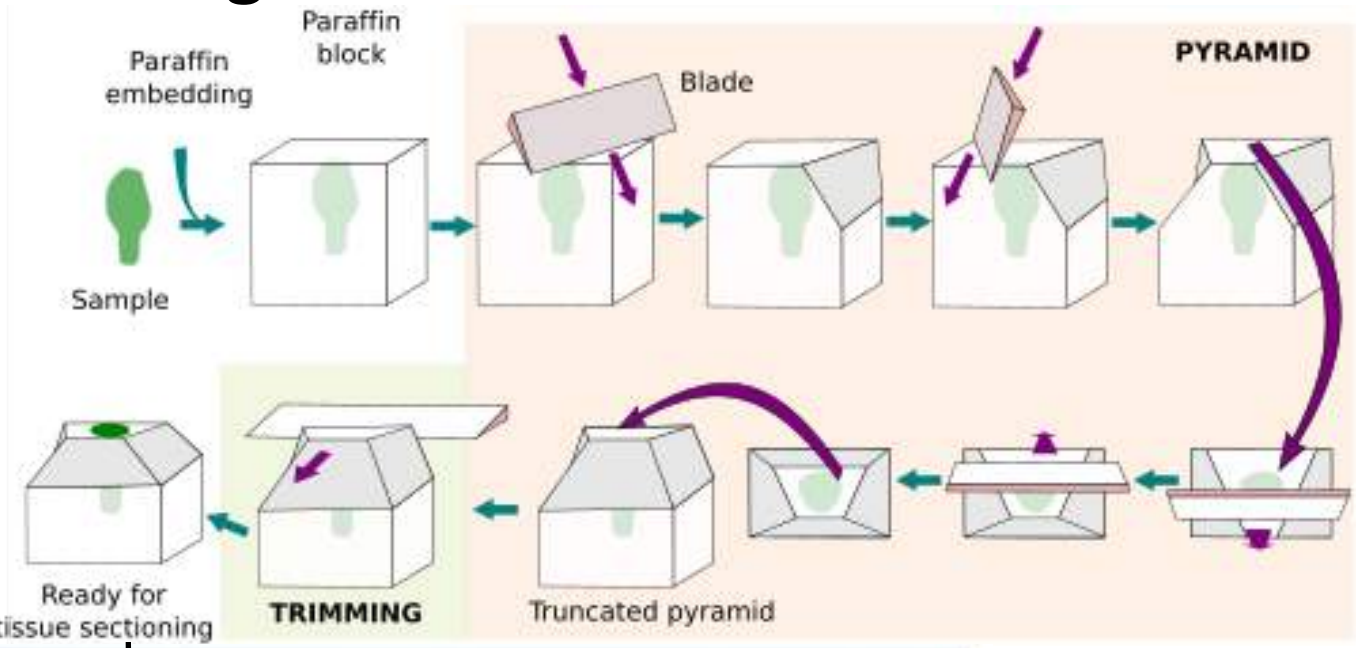
You may/should adapt the schedule to your particular case

Protocol for tissue processing. An example

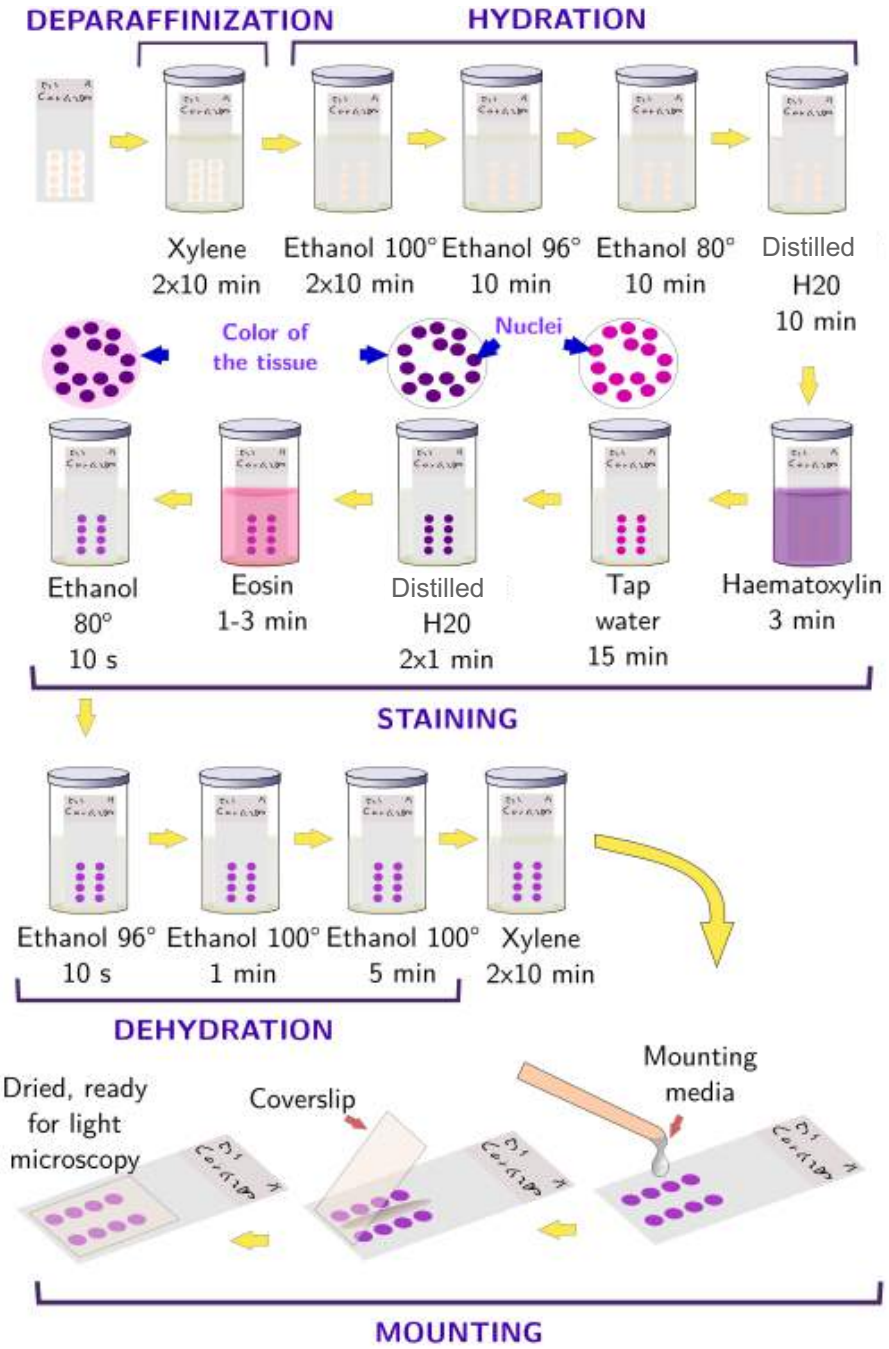


24-48 h (may be extended)	3 × 10 min	50% 1 × 1 h 70% 1 × 1 h 90% 1 × 1 h 95% 1 × 1 h 100% 2 × 1 h	2 × 20-30 min	1 × 20-30 min Overnight	Blocks with paraffin-embedded tissue

Trimming of blocks and microtome sectioning



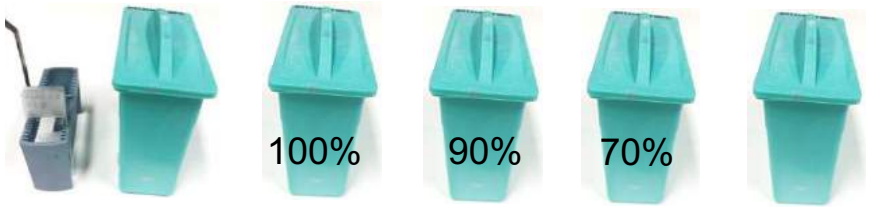
Staining with haematoxylin-eosin



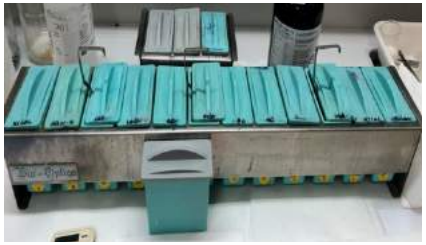
The schedule may change depending on tissue types and dye manufacturers, etc.

Haematoxylin-eosin staining (protocol used at UCA lab)

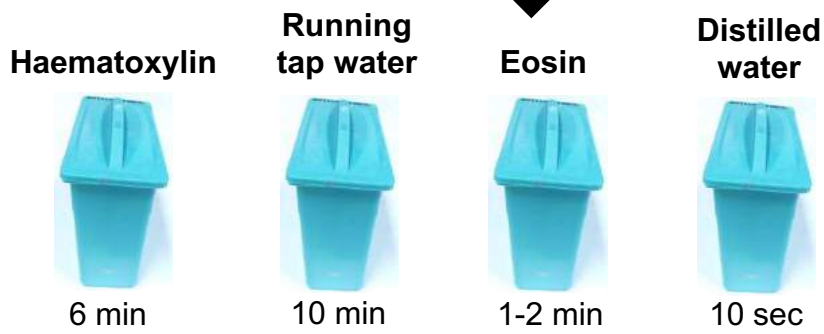
Slides with paraffin sections



HYDRATION

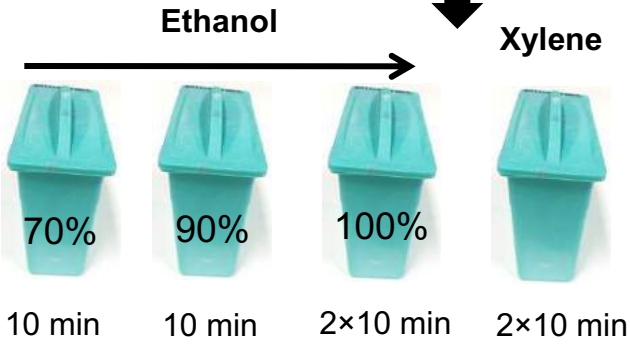


Coplin staining jars



STAINING

Coverslips + mounting medium



DEHYDRATION → MOUNTING

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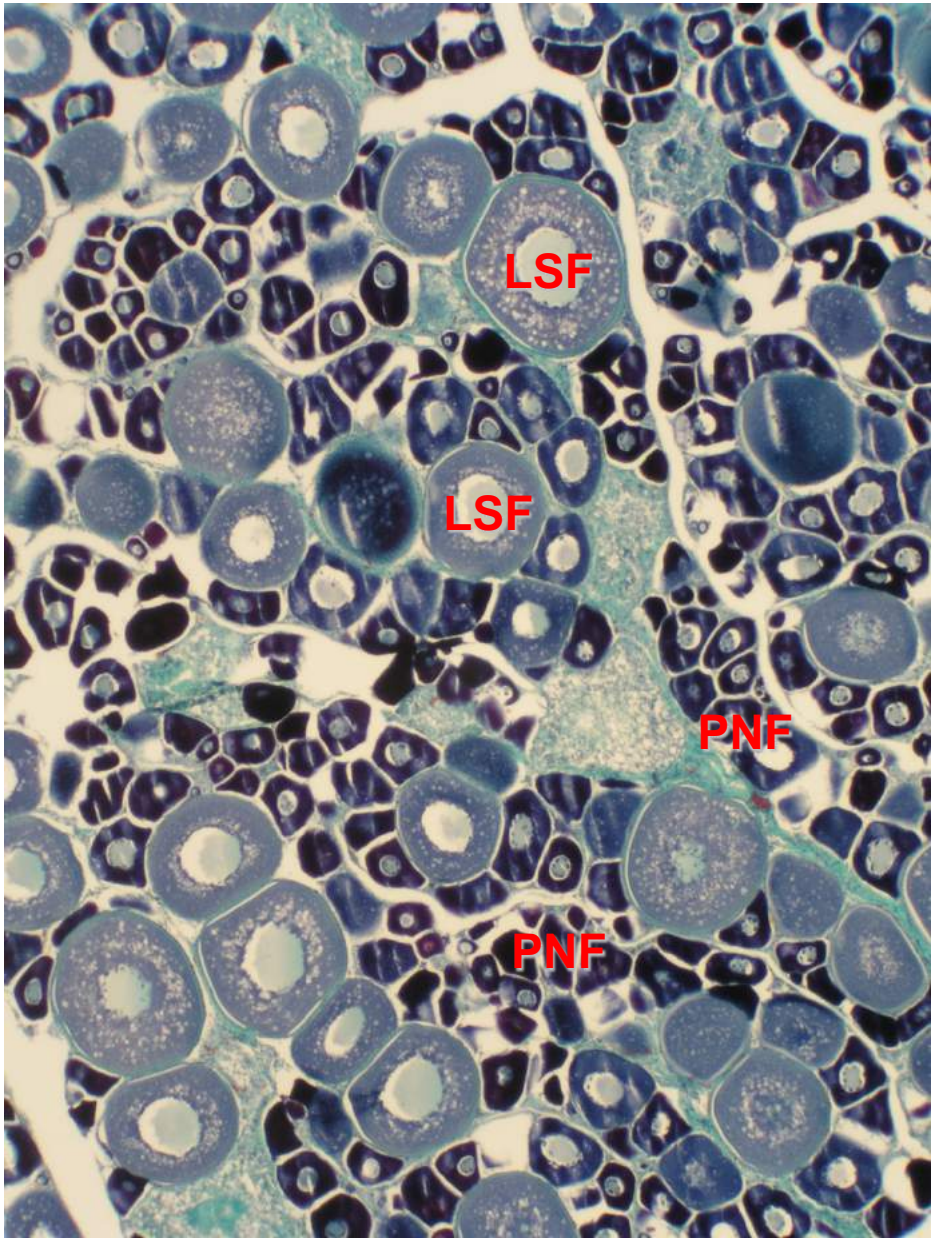
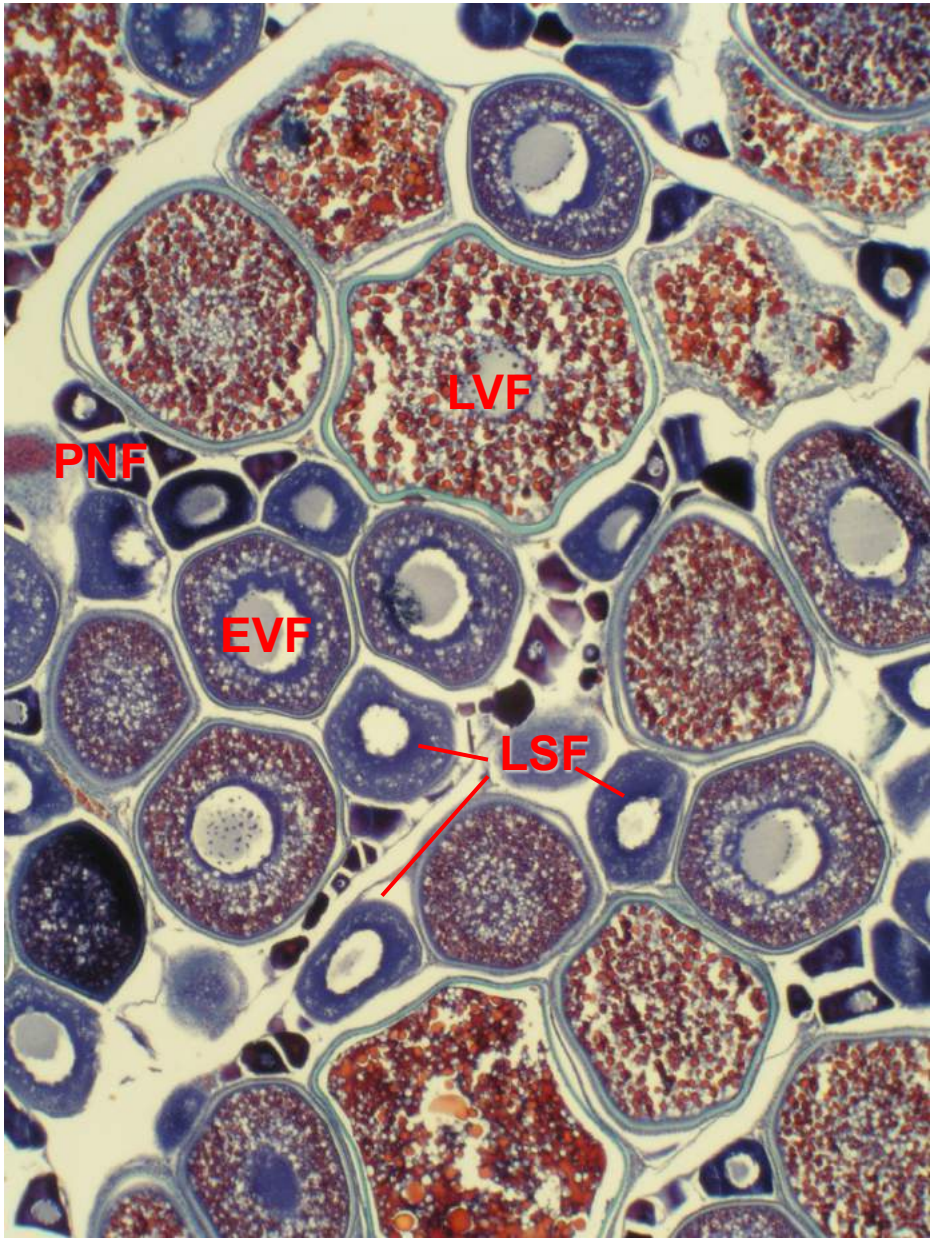
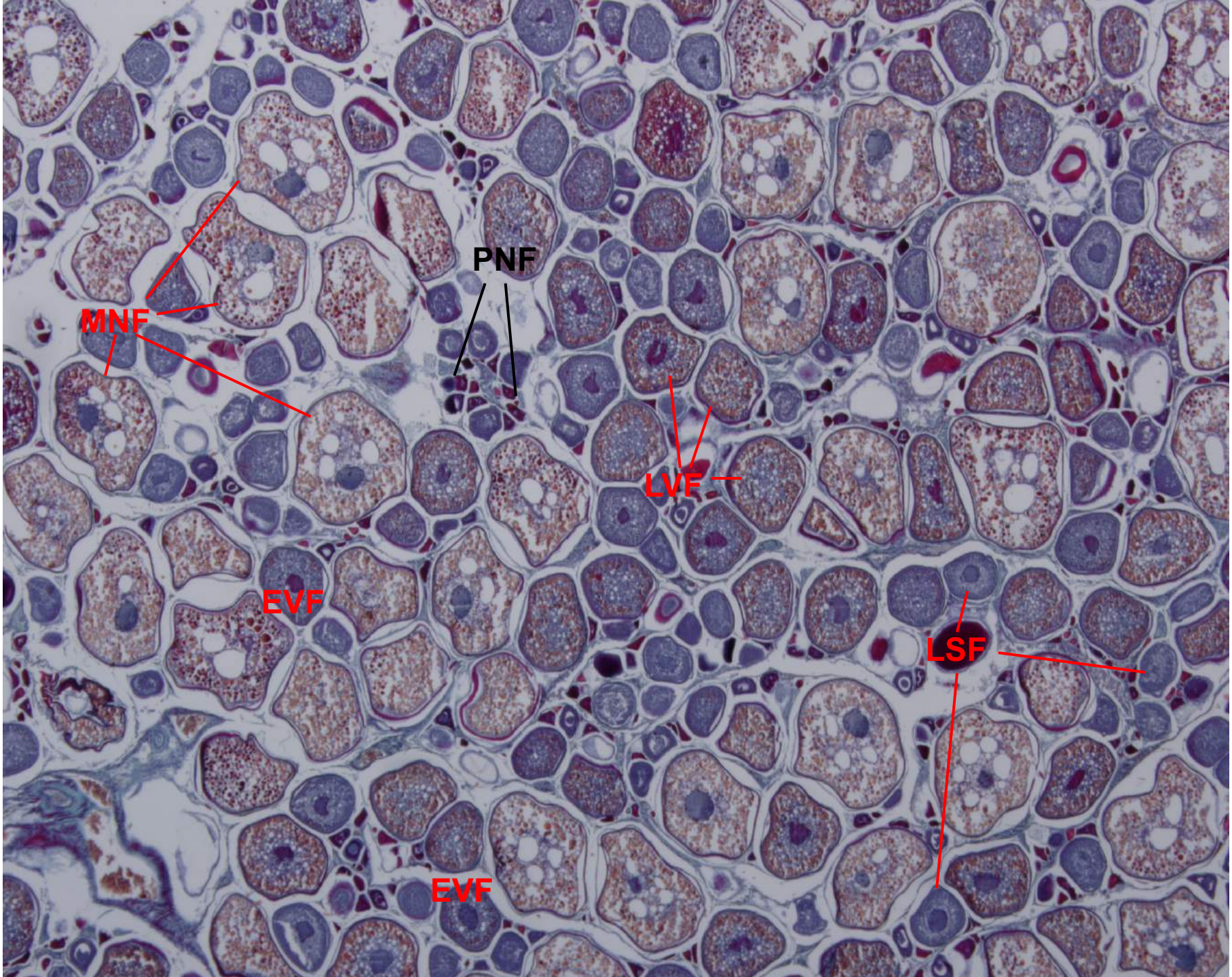
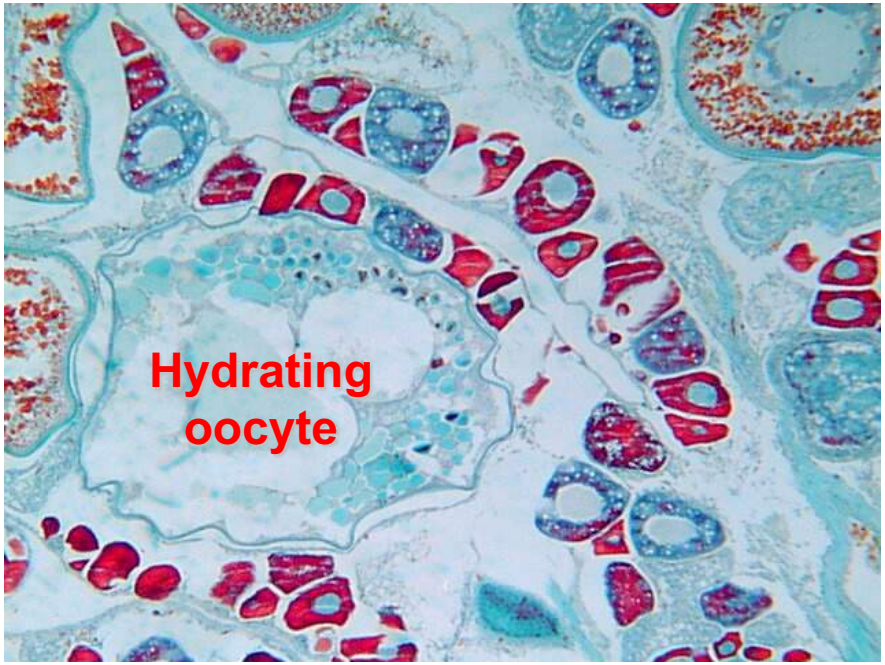
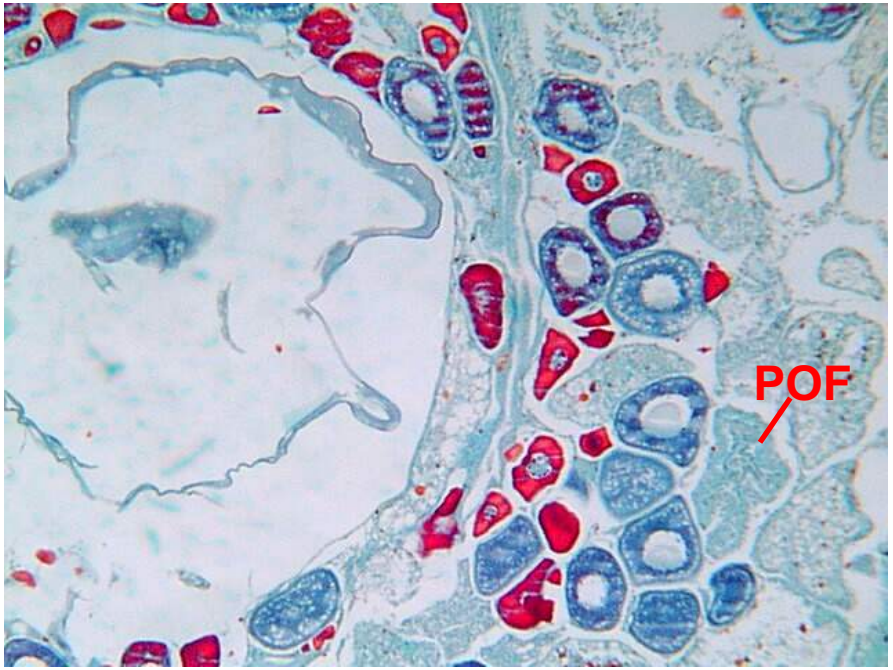
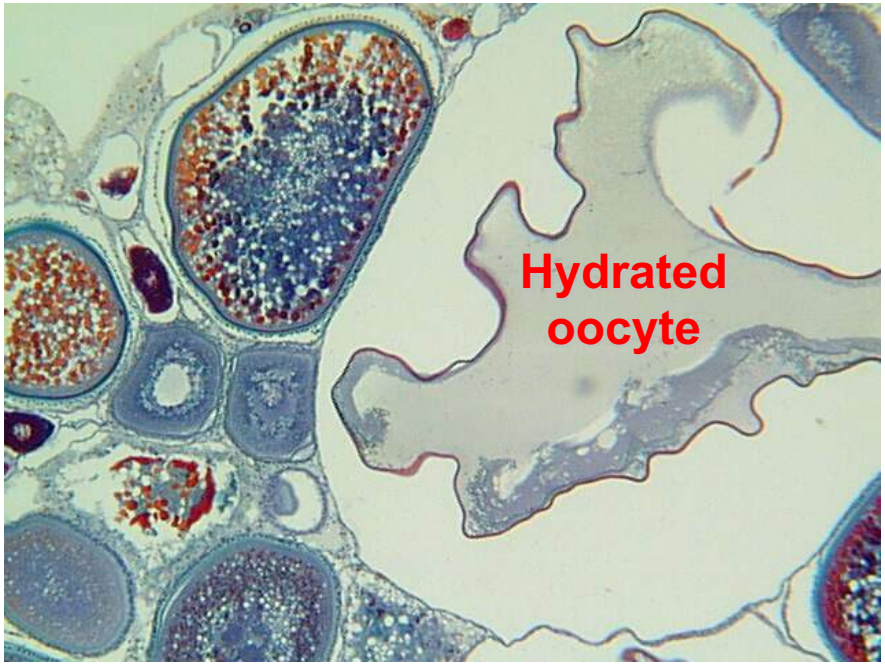

LSF
LSF
PNF
PNF

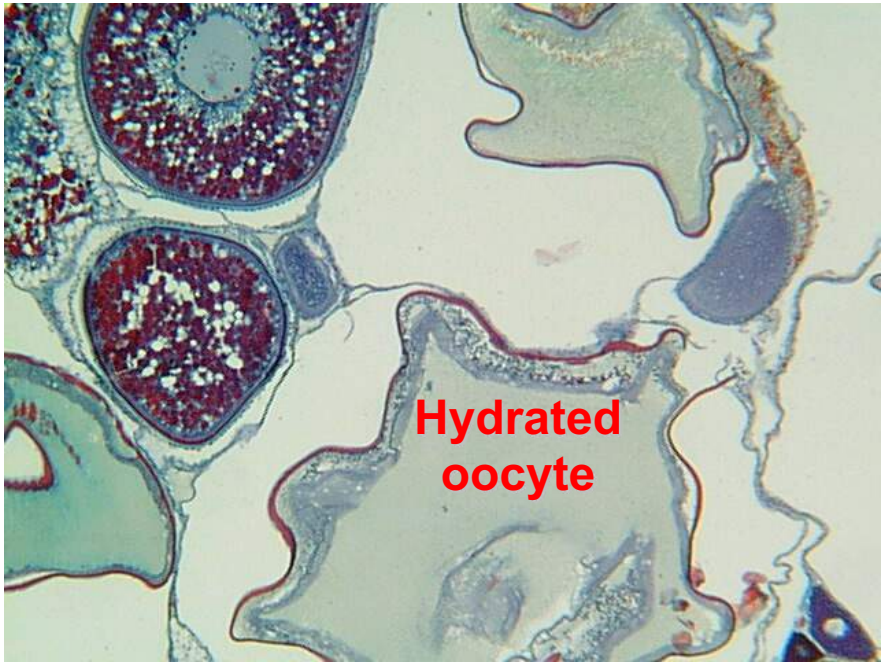

LVF
PNF
EVF
LSF

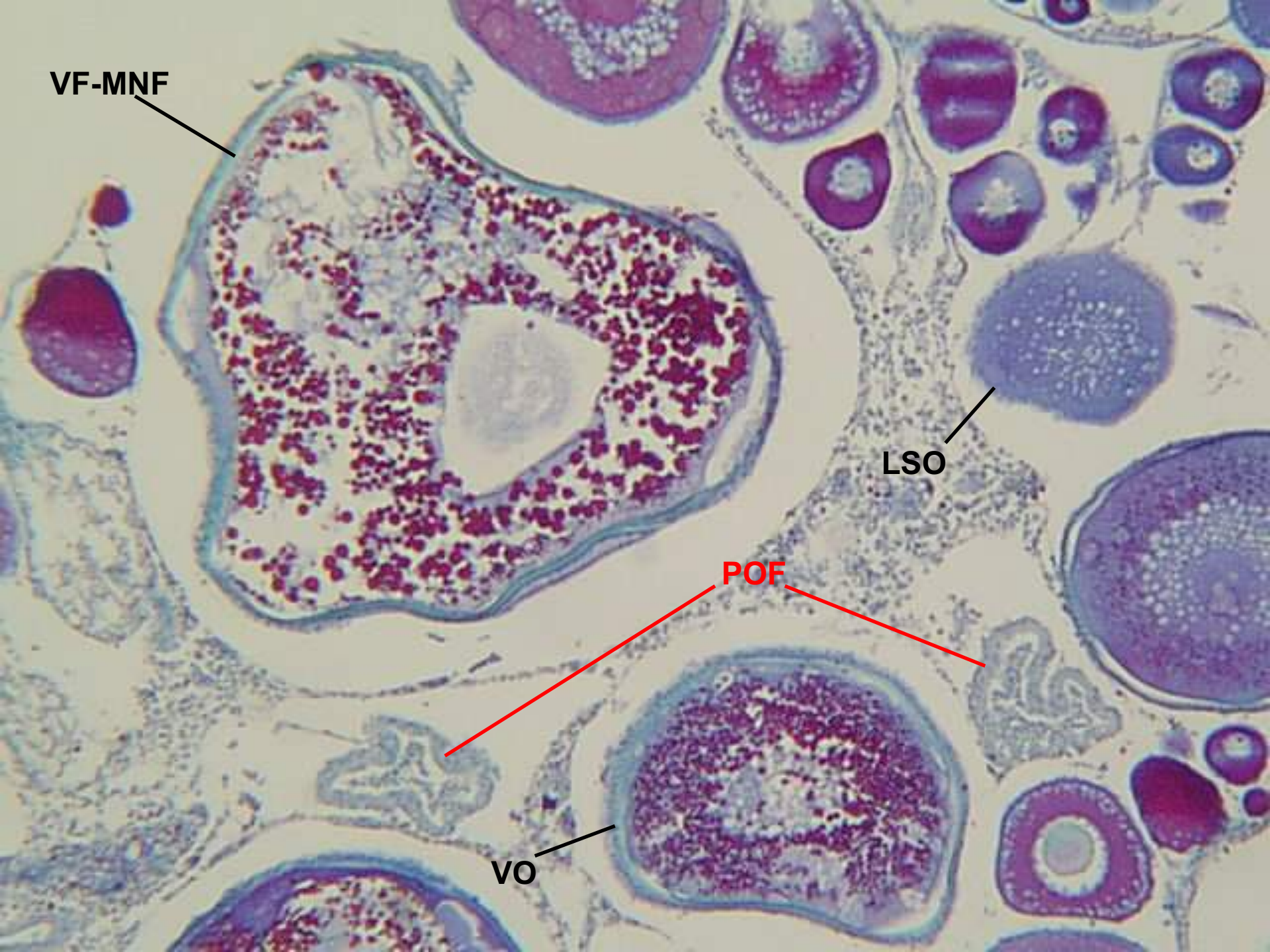

PNF
MNF
LVF
EVF
LSF
EVF

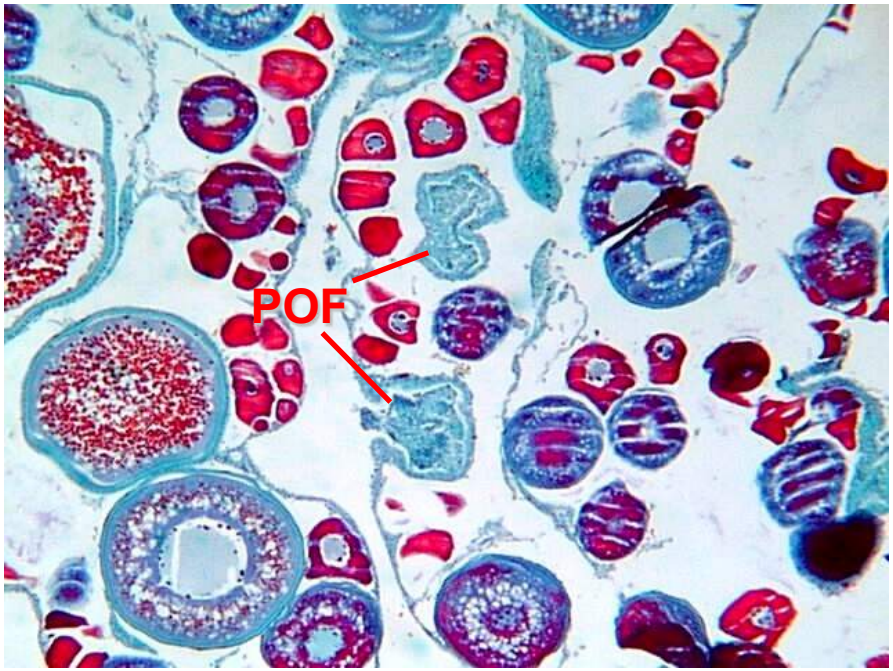

Hydrating
oocyte

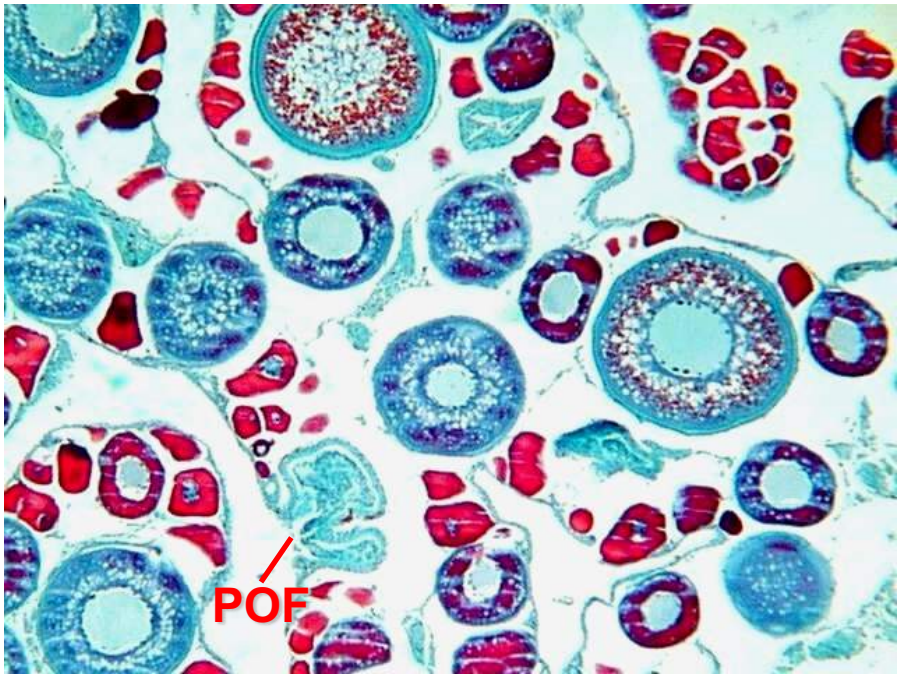

POF


Hydrated
oocyte


Hydrated
oocyte


VF-MNF
LSO
POF
VO


POF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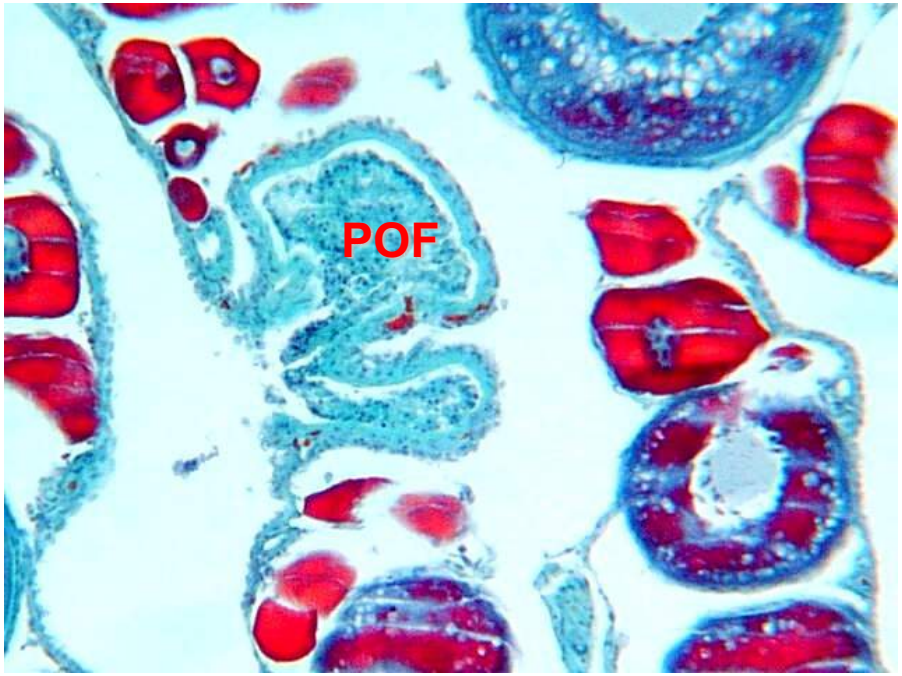
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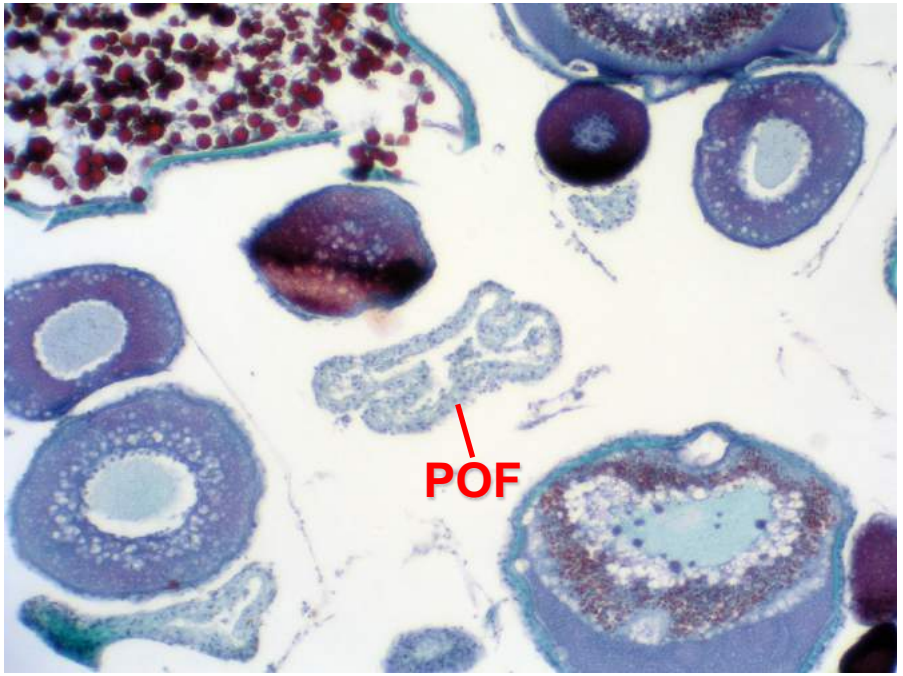
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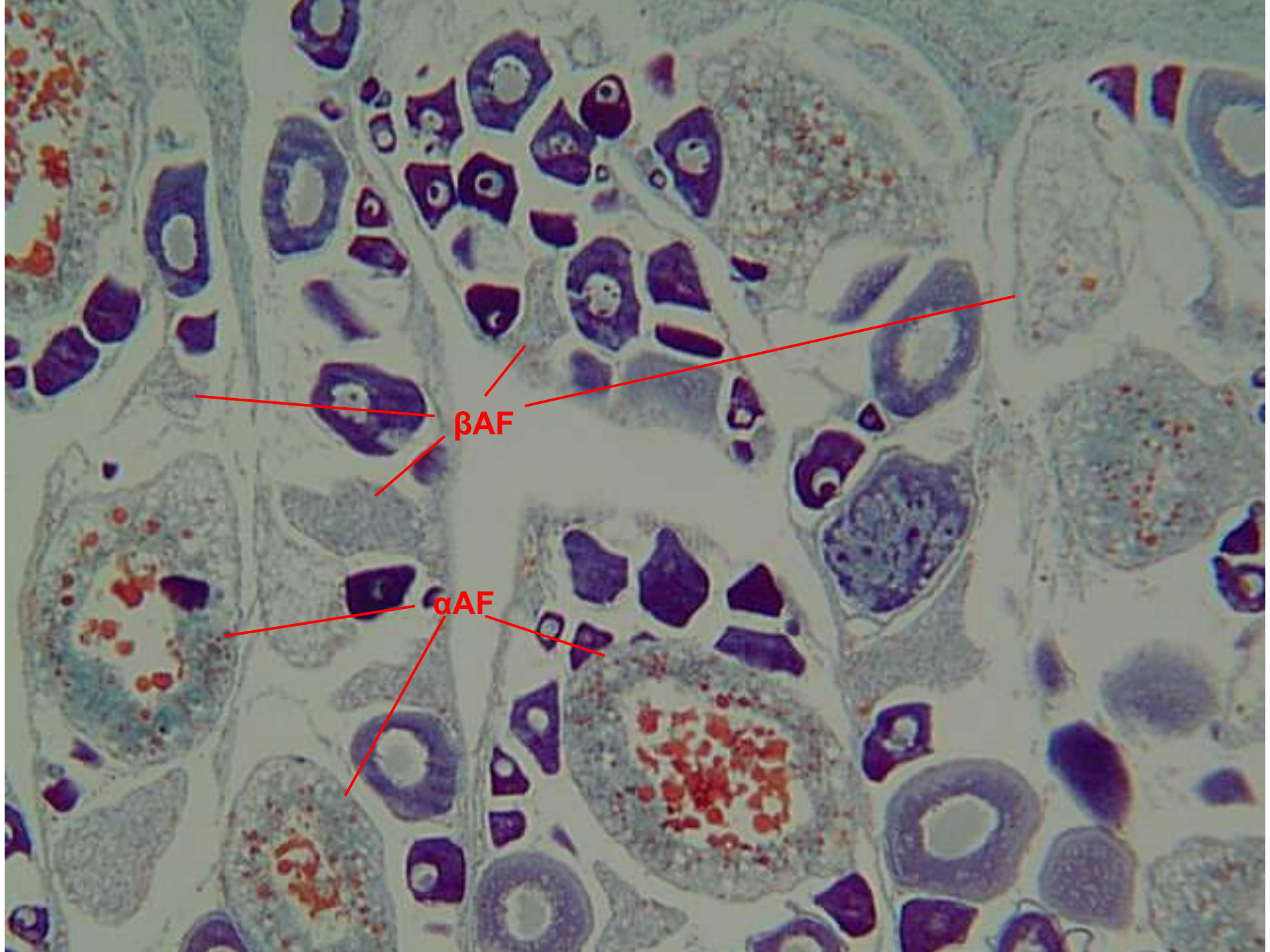
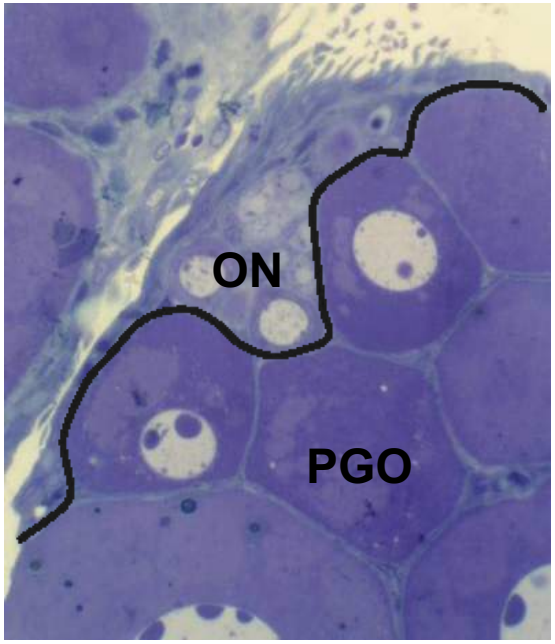
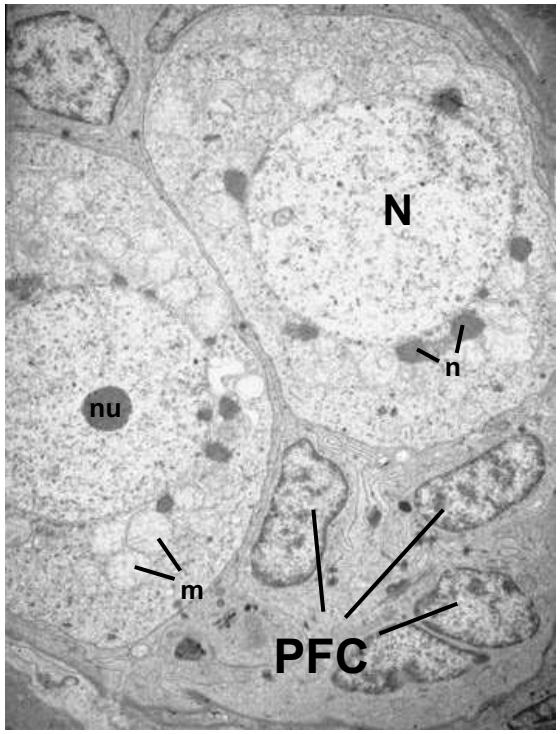
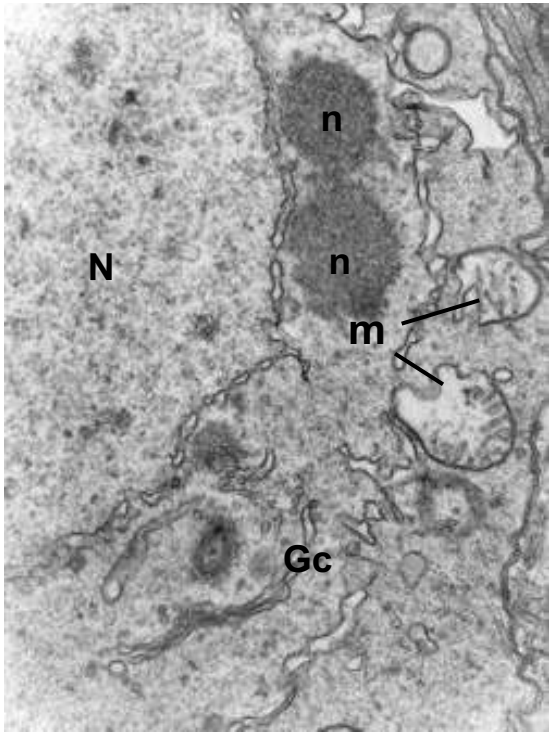

βAF
αAF

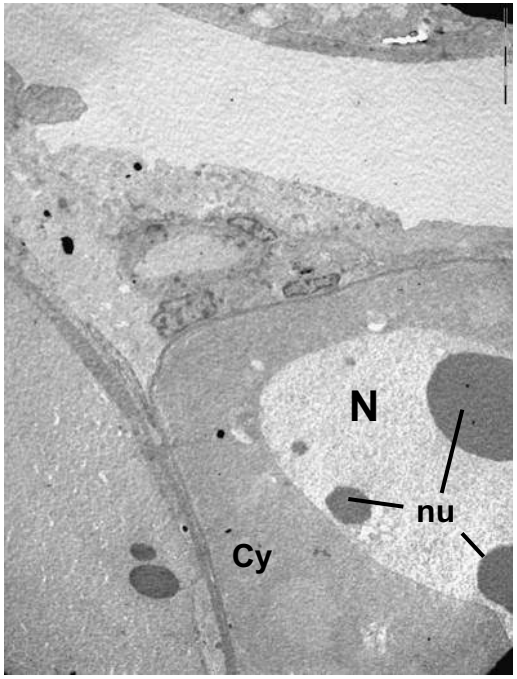
Ultrastructure

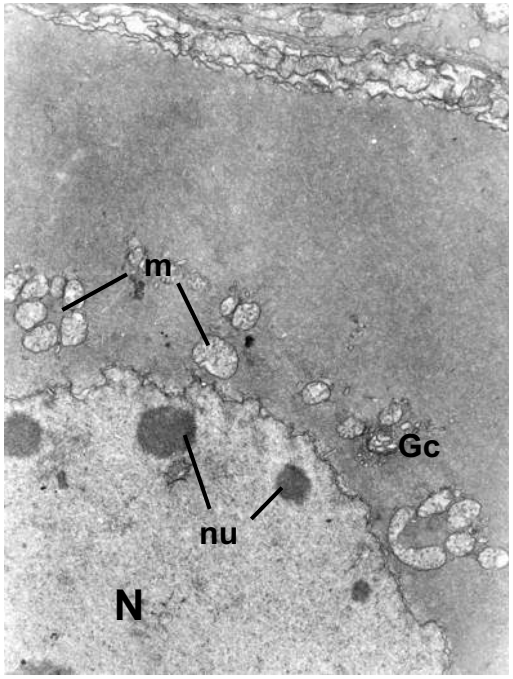
Ultrastructure. Previtellogenesis

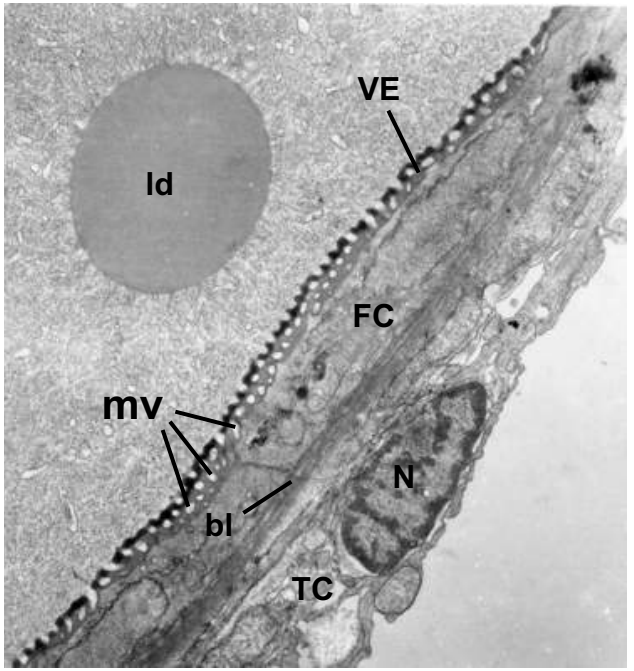




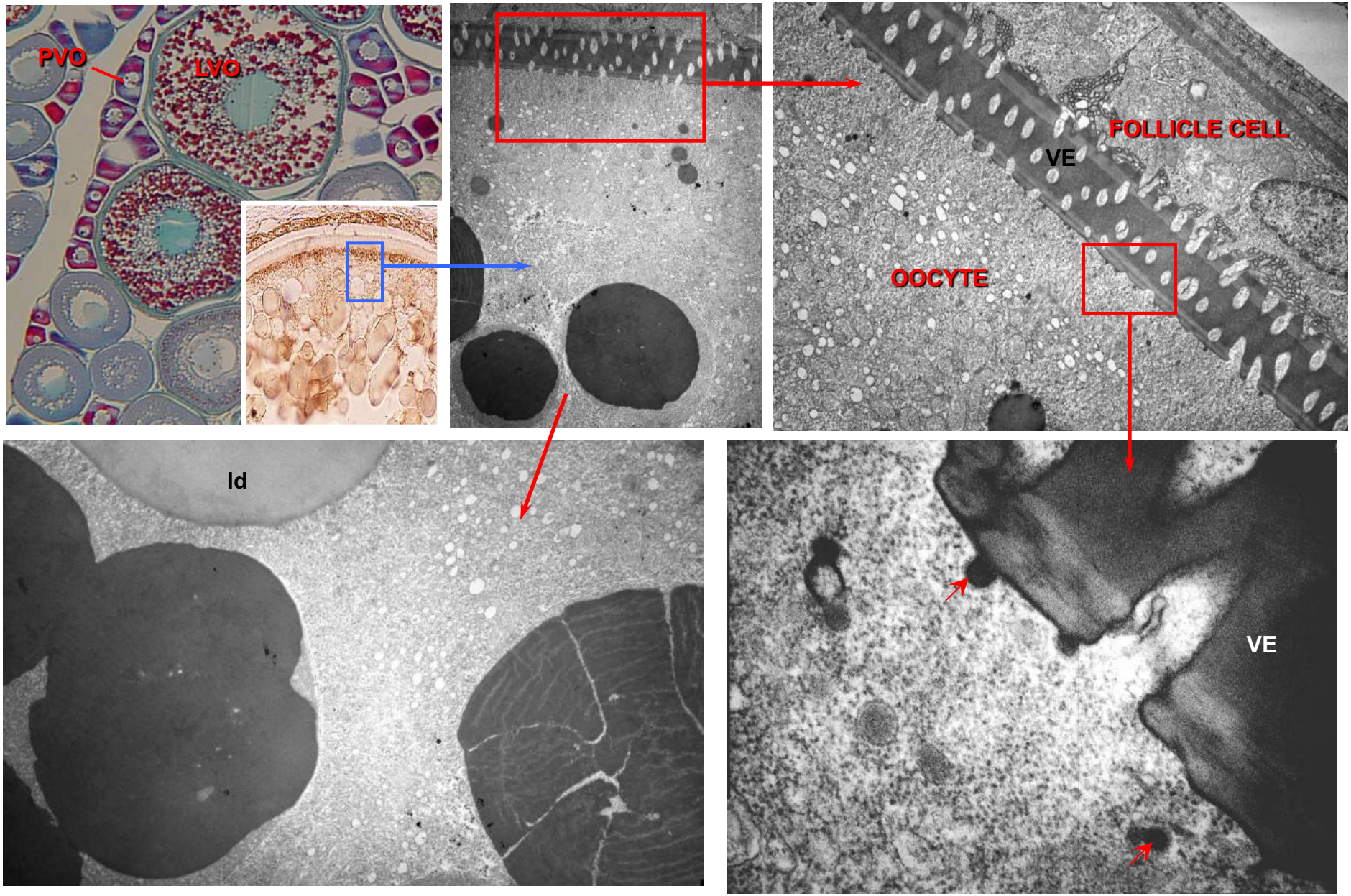




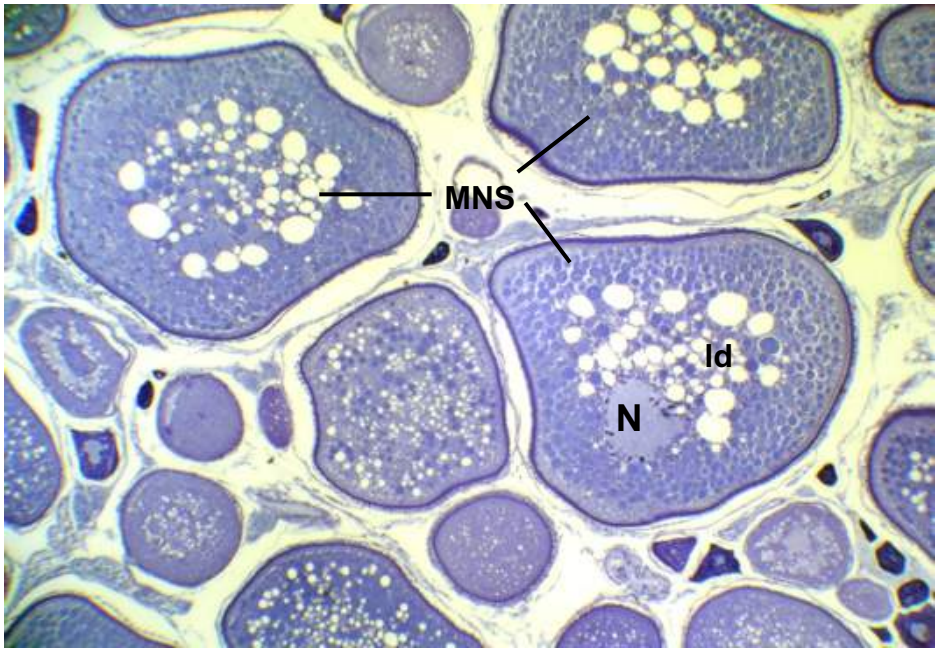


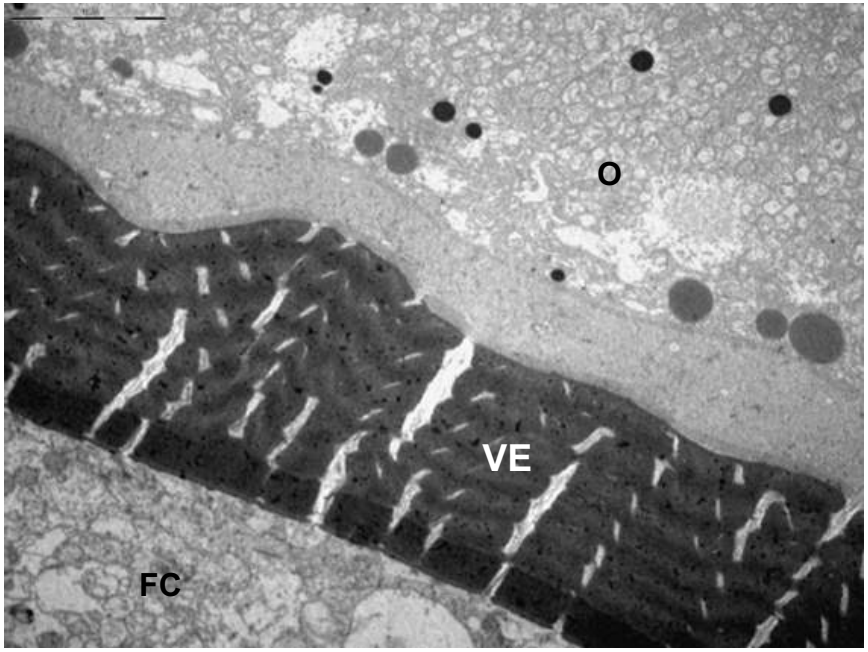


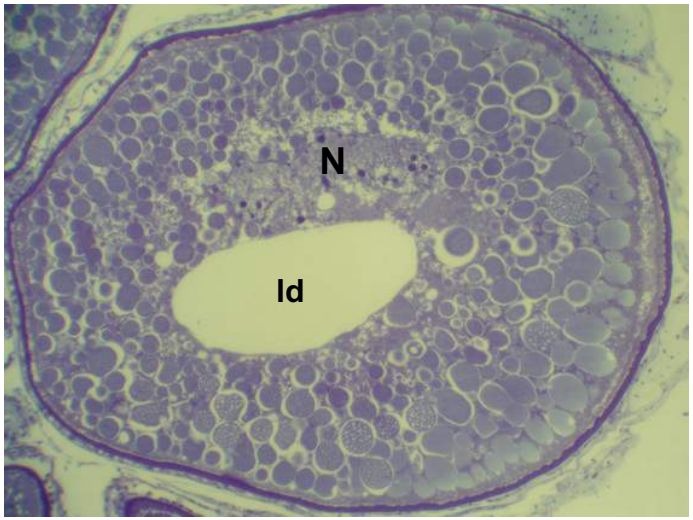
Ultrastructure. Vitellogenesis

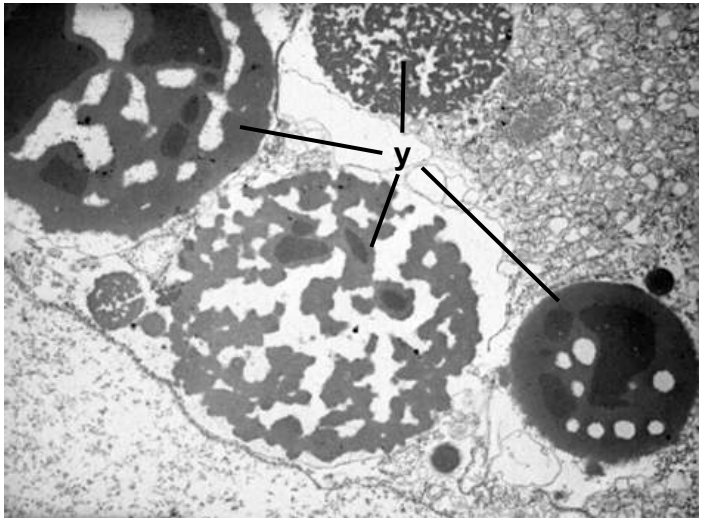


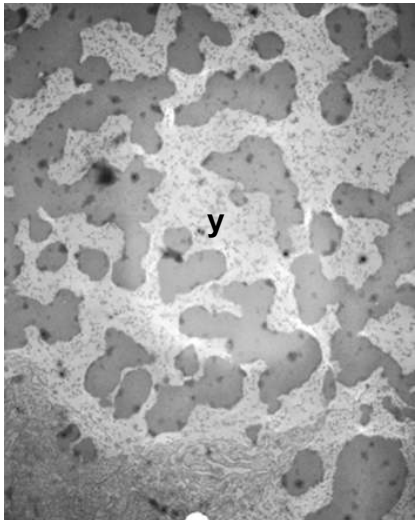
Ultrastructure. Oocyte maturation and hydration











Peripheral region

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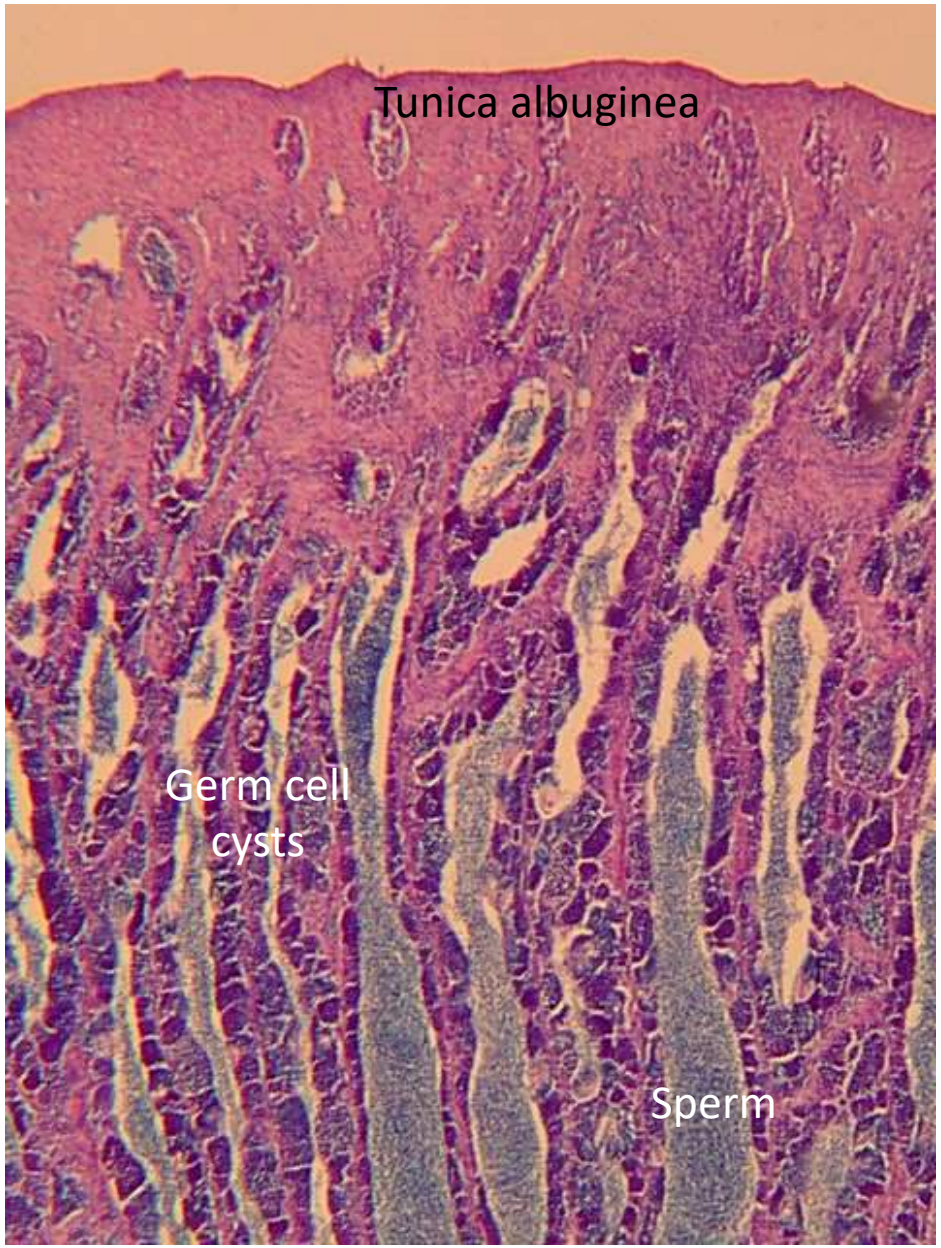
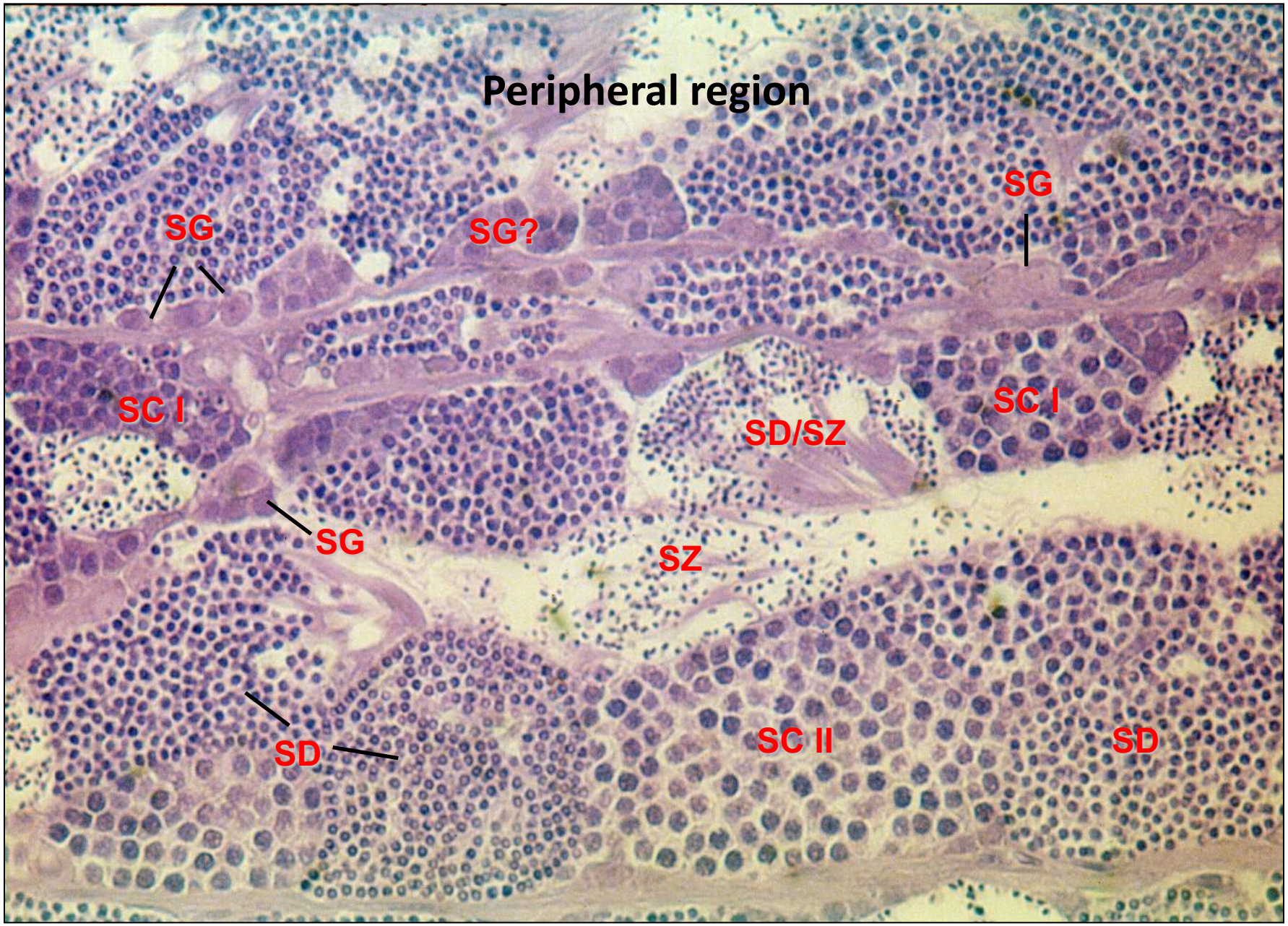
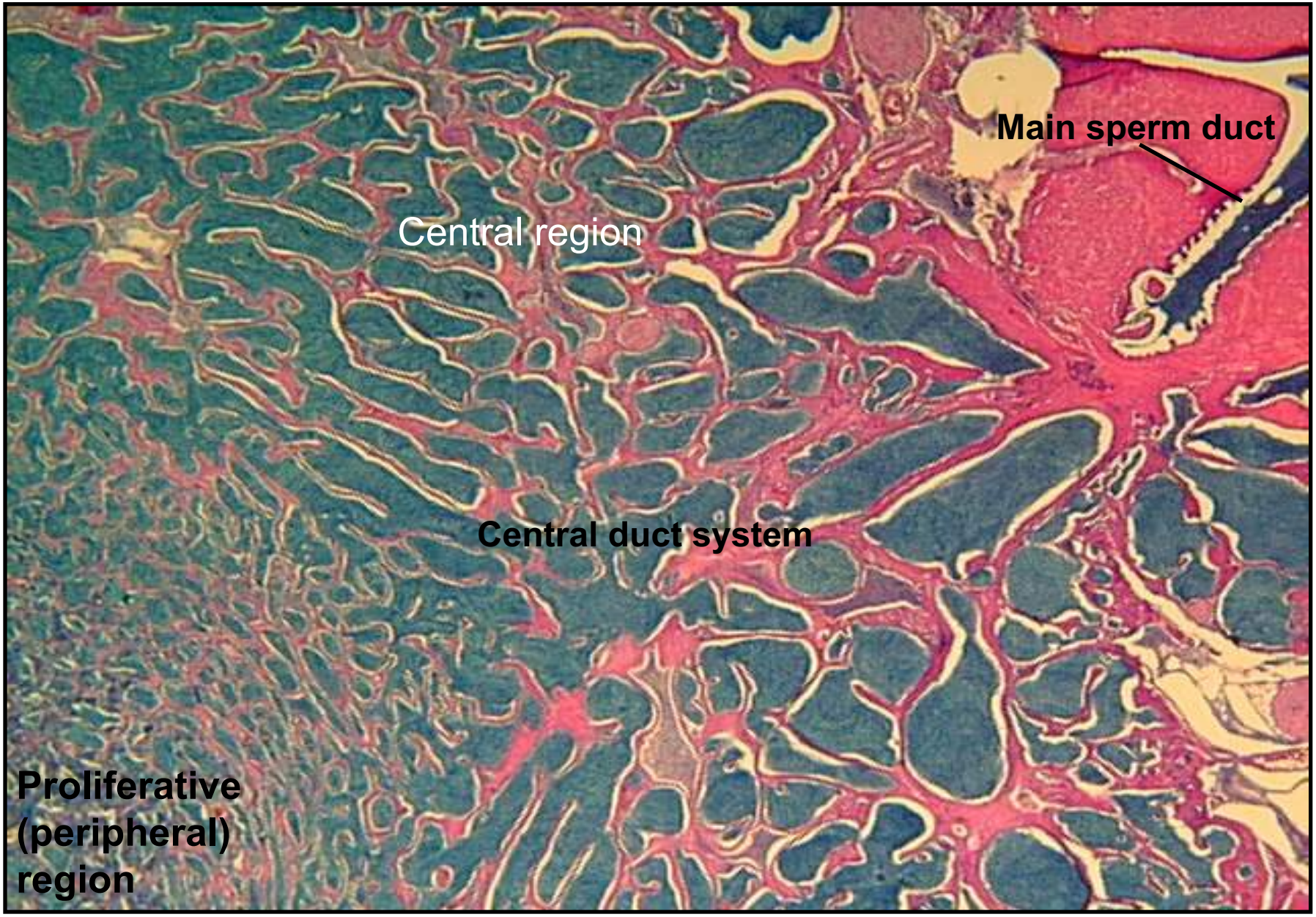
Central region



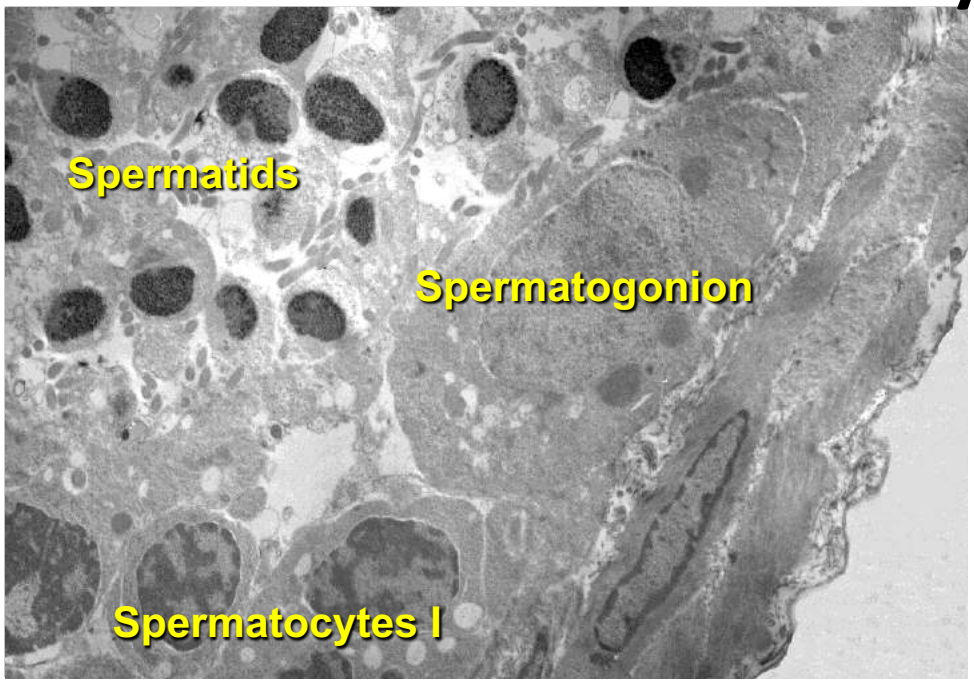


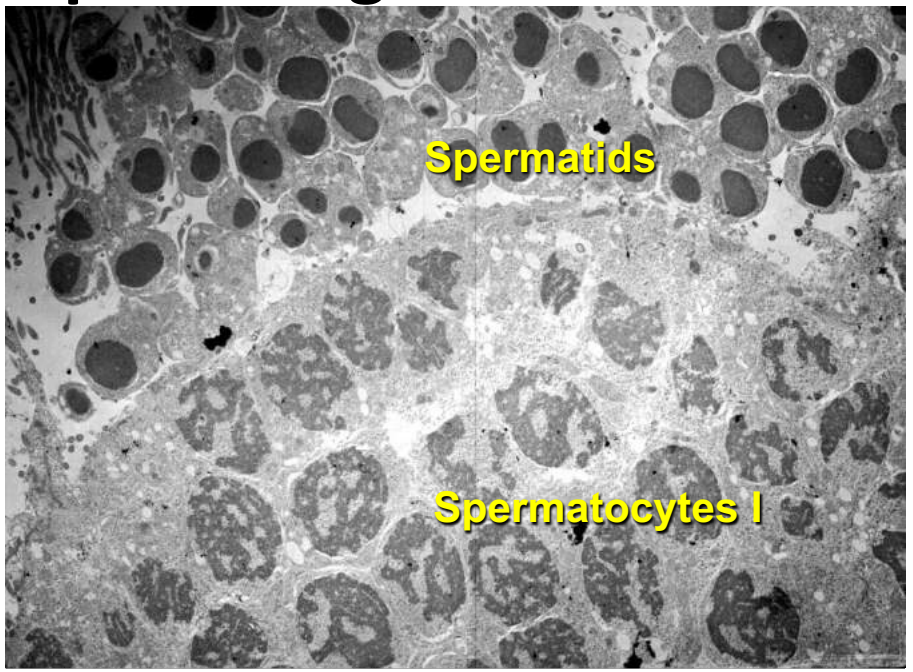
Testis, peripheral region. Migrant tuna from the Strait of Gibraltar. SC I: primary spermatocytes; SC II: secondary spermatocytes; SD: Spermatids (flagellate and aflagellate); SG: spermatogonia; SZ: spermatozoa. Toluidine blue-stained plastic section.

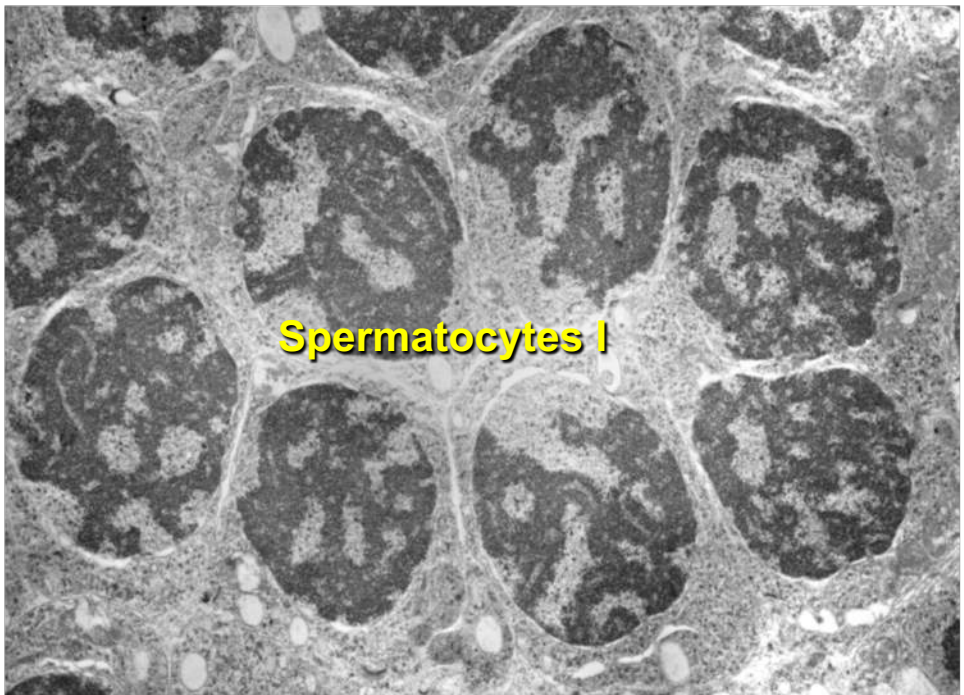


Testis, central region. Migrant tuna from the Strait of Gibraltar. Paraffin section; haematoxylin-eosin staining.

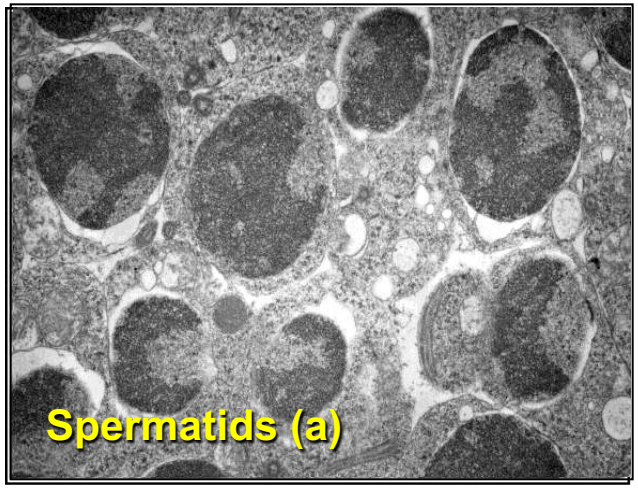
Ultrastructure. Early spermatogenesis

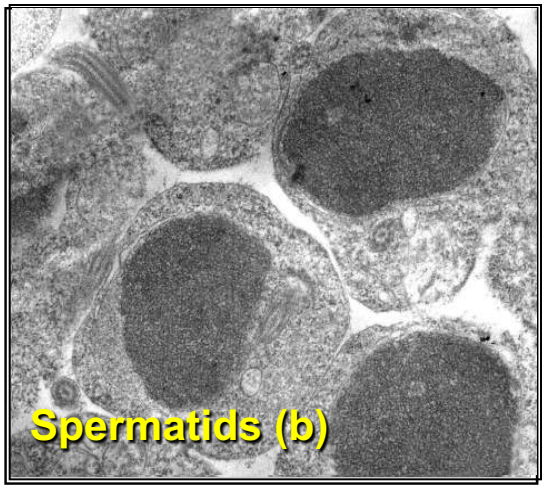


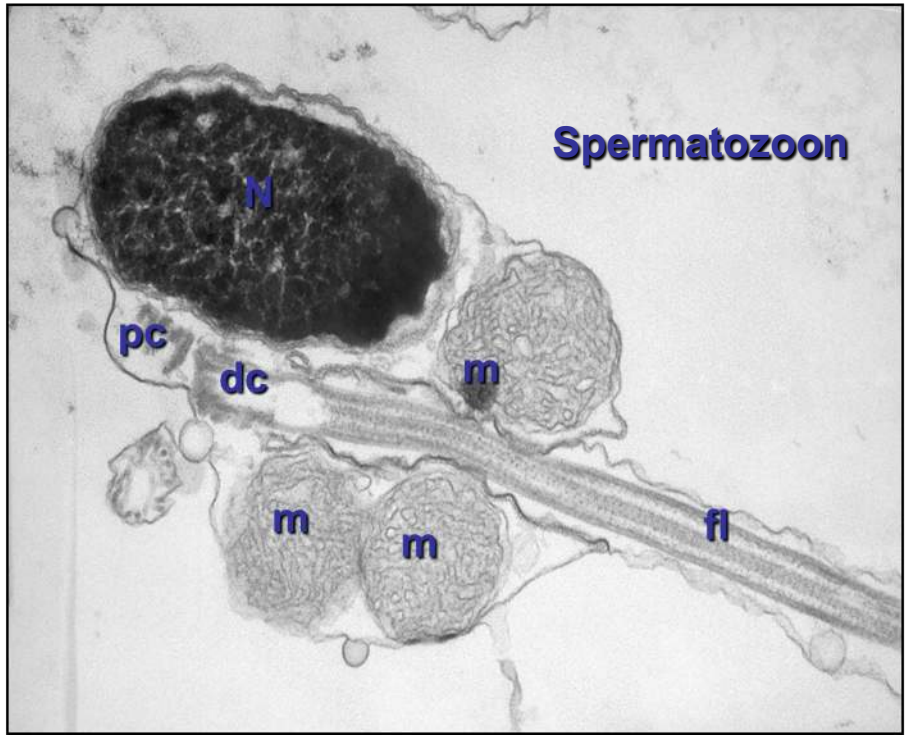


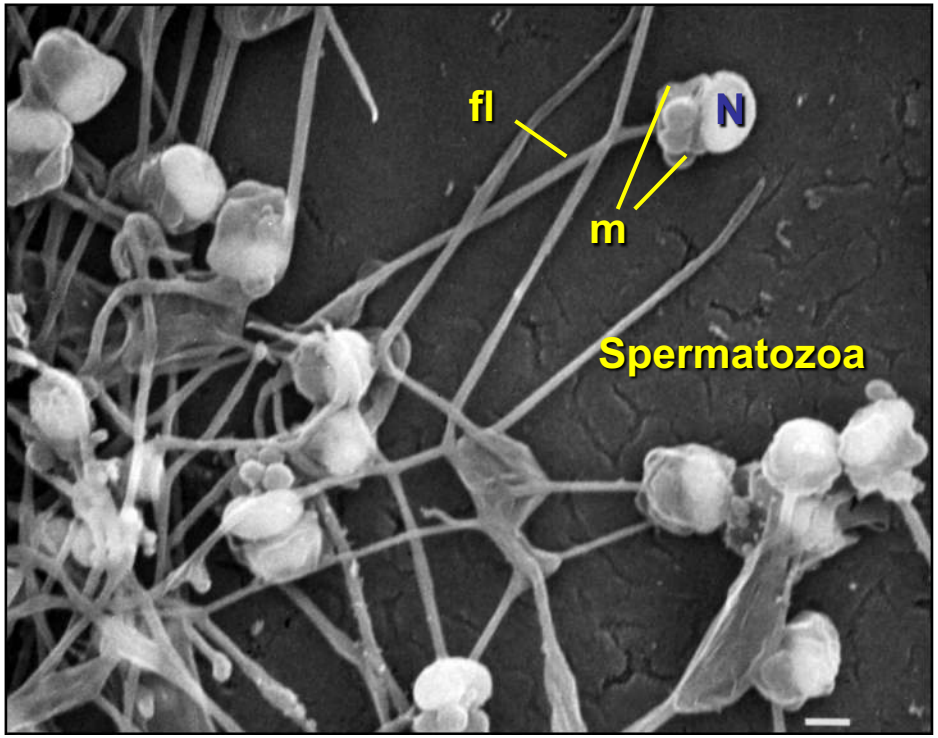


Ultrastructure. Spermiogenesis

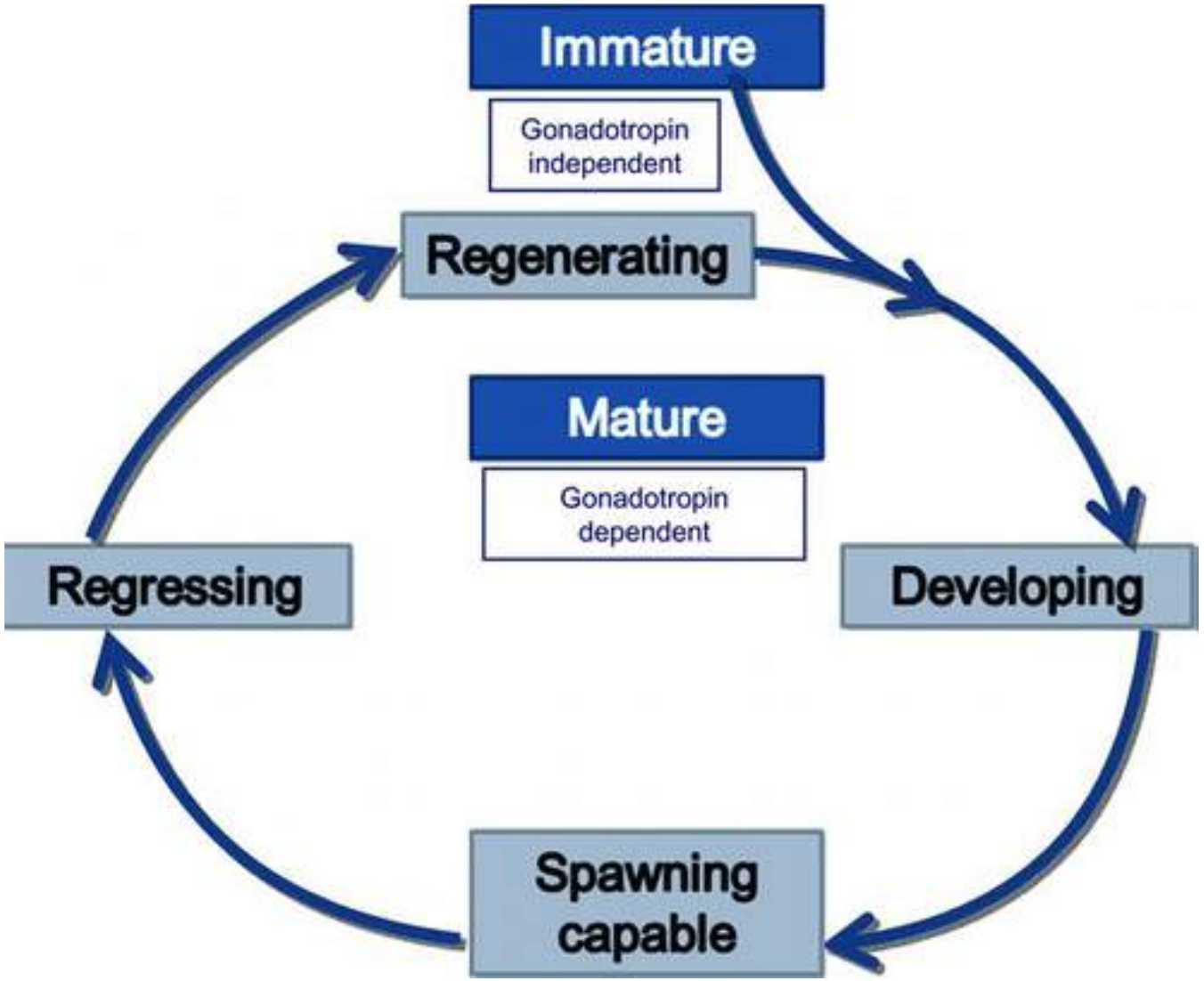








Conceptual model of fish reproductive phases



From Brown-Peterson, N. J. et al. (2011). A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science, 3:52–70, 2011

Ovary classification after Brown-Peterson et al. (2011)

Phase	Previous terminology	Macroscopic and histological features
Immature (never spawned)	Immature, virgin	Small ovaries, often clear, blood vessels indistinct. Only oogonia and PG oocytes present. No atresia or muscle bundles. Thin ovarian wall and little space between oocytes.
Developing (ovaries beginning to develop, but not ready to spawn)	Maturing, early developing, early maturation, mid-maturation, ripening, previtellogenic	Enlarging ovaries, blood vessels becoming more distinct. PG, CA, Vtg1, and Vtg2 oocytes present. No evidence of POFs or Vtg3 oocytes. Some atresia can be present. *Early developing subphase*: PG and CA oocytes only.
Spawning capable (fish are developmentally and physiologically able to spawn in this cycle)	Mature, late developing, late maturation, late ripening, total maturation, gravid, vitellogenic, ripe, partially spent, fully developed, prespawning, running ripe, final OM, spawning, gravid, ovulated	Large ovaries, blood vessels prominent. Individual oocytes visible macroscopically. Vtg3 oocytes present or POFs present in batch spawners. Atresia of vitellogenic and/or hydrated oocytes may be present. Early stages of OM can be present. *Actively spawning subphase*: oocytes undergoing late GVM, GVBD, hydration, or ovulation.
Regressing (cessation of spawning)	Spent, regression, postspawning, recovering	Flaccid ovaries, blood vessels prominent. Atresia (any stage) and POFs present. Some CA and/or vitellogenic (Vtg1, Vtg2) oocytes present.
Regenerating (sexually mature, reproductively inactive)	Resting, regressed, recovering, inactive	Small ovaries, blood vessels reduced but present. Only oogonia and PG oocytes present. Muscle bundles, enlarged blood vessels, thick ovarian wall and/or gamma/delta atresia or old, degenerating POFs may be present.

From Brown-Peterson, N. J. et al. (2011). A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science, 3:52–70, 2011

Ovary histo classification for tunas after Schaefer (1989)

Reproductive stage	Histological features
Active nonspawning	Advanced yolked oocytes and no atresia or minor (< 50%) α atresia
Active spawning	Avanced yolked oocytes and no or minor α atresia plus postovulatory follicles and/or migratory-nucleus oocytes
Inactive mature	Previtellogenic or early yolked oocytes plus α and/or β atresia, or advanced yolked oocytes plus major (> 50%) α atresia
Resting/regenerating*	Unyolked (previtellogenic) or early yolked oocytes and no atresia

* Referred to as inactive immature in Schaefer's original classifications.

Based on histological features according to Schaefer, K. (1998). Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. Inter-Amer. Trop. Tuna Comm. Bull., 21: 205-249.

Ovary histo classification for skipjack (after Ashida, 2020)

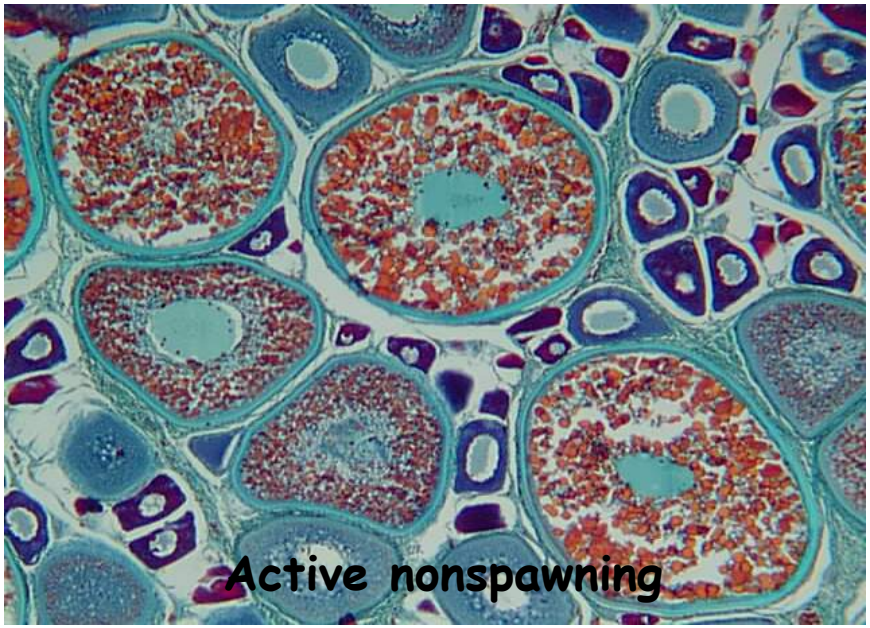
Histological characteristics of the ovarian maturity phase, and gonad index of female skipjack tuna (*Katsuwonus pelamis*) caught in the subtropical and temperate western Pacific Ocean.

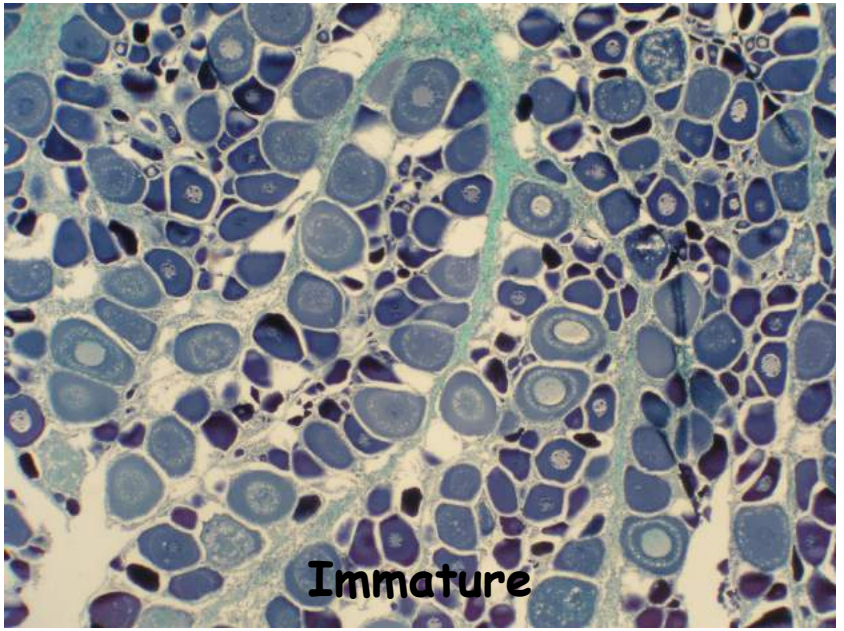
Maturity phase	*n*	MAGO	POF	IA (%)	Mean GI ± SD (Range)
Immature-Regenerating	2783	Pn or Ca	Absent	Absent	0.94 ± 0.53^{c} (0.03–5.84)
Developing	413	Py or Sy or Ty	Absent	IA < 50	3.31 ± 1.47^{b} (1.08–10.90)
Spawning capable	203	Gvm or Hy	May be present	IA < 50	5.13 ± 2.38^{a} (1.07–15.50)
Spawning capable	283	Py or Sy or Ty	Present	IA < 50	
Regressing	37	Py or Sy	Absent	IA ≥ 50	2.69 ± 0.99^{b} (1.41–6.17)
Regressing	19	Pn or Ca	Absent	IA = 100	

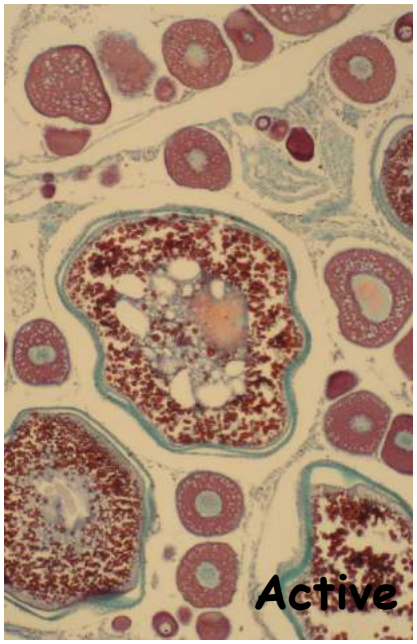
Ca, cortical alveoli; Gvm, germinal vesicle migration; Hy, hydrated oocytes; IA, relative intensity of atresia; MAGO, most advanced group of oocytes; POF, postovulatory follicles; Pn, perinucleolus oocytes; Py, primary yolked oocytes; Sy, secondary yolked oocytes; Ty, tertiary yolked oocytes.

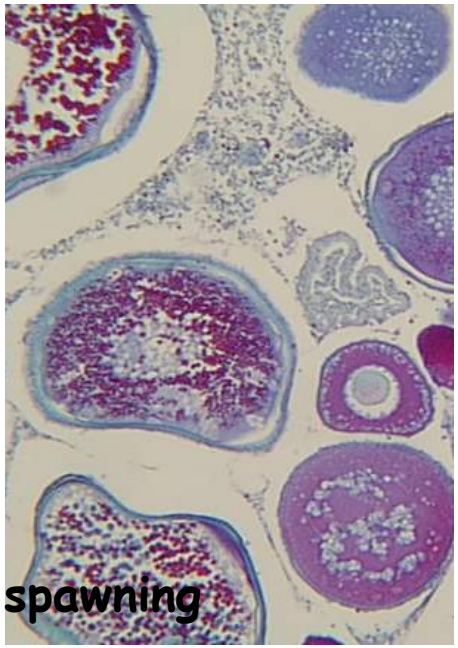
From Ashida, H. (2020). Spatial and temporal differences in the reproductive traits of skipjack tuna *Katsuwonus pelamis* between the subtropical and temperate western Pacific Ocean. Fisheries Research 221, 105352.

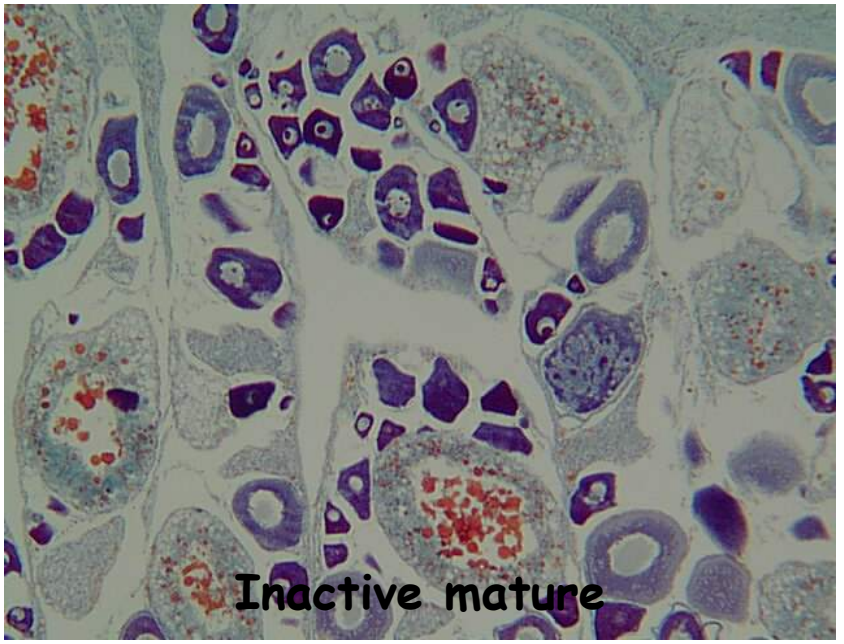
Different stages of ovarian development (histology)

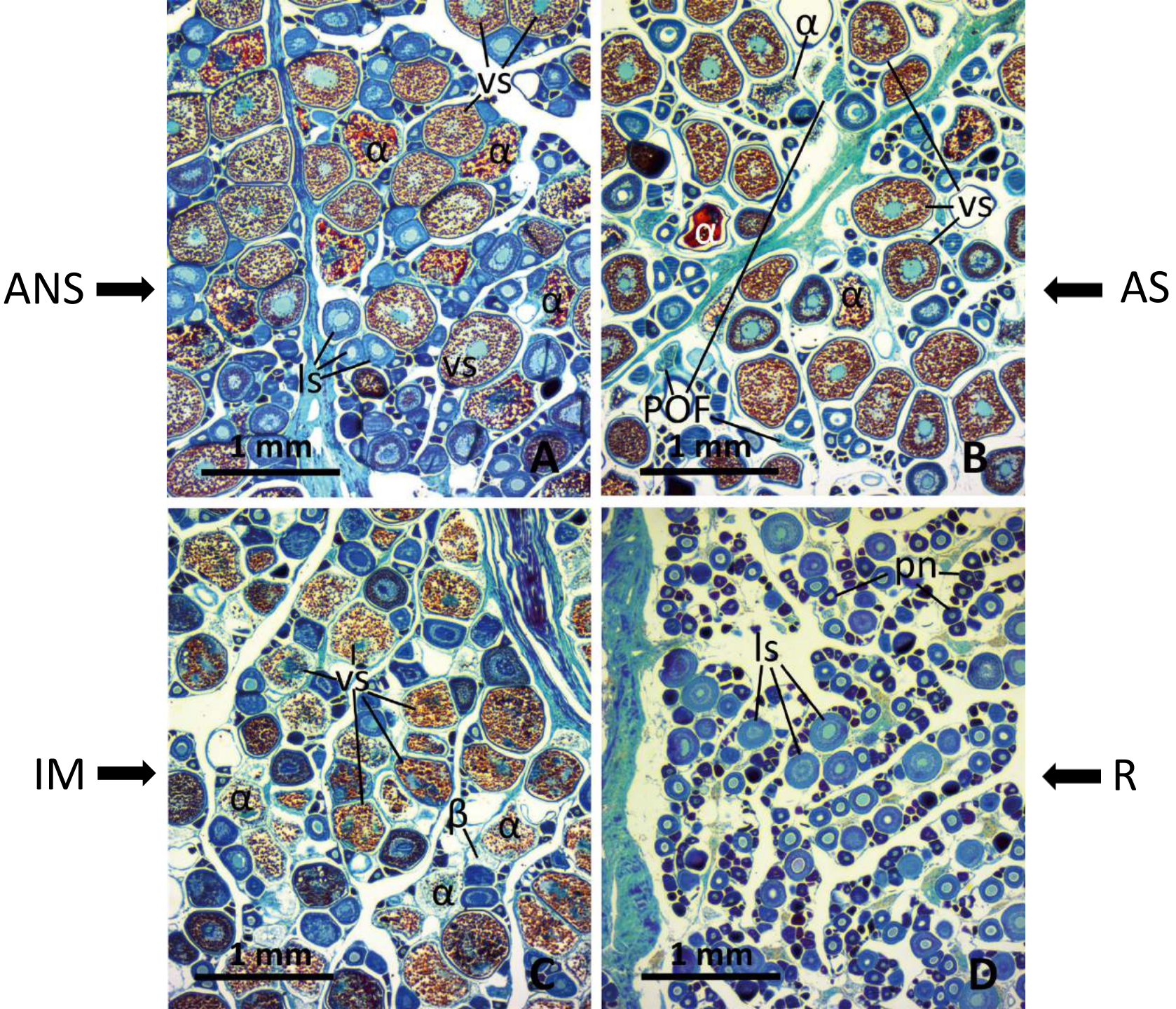








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Testis classification after Brown-Peterson et al. (2011)

Phase	Previous terminology	Macroscopic and histological features
Immature (never spawned)	Immature, virgin	Small testes, often clear and threadlike. Only Sg1 present; no lumen in lobules.
Developing (testes beginning to grow and develop)	Maturing, early developing, early maturation, ripening	Small testes but easily identified. Spermatocysts evident along lobules. Sg2, Sc1, Sc2, St, and Sz can be present in spermatocysts. Sz not present in lumen of lobules or in sperm ducts. GE continuous throughout. *Early developing subphase*: Sg1, Sg2, and Sc1 only.
Spawning Capable (fish are developmentally and physiologically able to spawn in this cycle)	Late developing, mid-maturation, late maturation, late ripening, ripe, partially spent, running ripe, spawning	Large and firm testes. Sz in lumen of lobules and/or sperm ducts. All stages of spermatogenesis (Sg2, Sc, St, Sz) can be present. Spermatocysts throughout testis, active spermatogenesis. GE can be continuous or discontinuous. *Actively spawning subphase* (macroscopic): milt released with gentle pressure on abdomen. Histological subphases based on structure of GE. *Early GE*: continuous GE in all lobules throughout testes. *Mid-GE*: continuous GE in spermatocysts at testis periphery, discontinuous GE in lobules near ducts. *Late-GE*: discontinuous GE in all lobules throughout testes.
Regressing (cessation of spawning)	Spent, regression, postspawning, recovering	Small and flaccid testes, no milt release with pressure. Residual Sz present in lumen of lobules and in sperm ducts. Widely scattered spermatocysts near periphery containing Sc2, St, Sz. Little to no active spermatogenesis. Spermatogonial proliferation and regeneration of GE common in periphery of testes.
Regenerating (sexually mature, reproductively inactive)	Resting, regressed, recovering, inactive	Small testes, often threadlike. No spermatocysts. Lumen of lobule often nonexistent. Proliferation of spermatogonia throughout testes. GE continuous throughout. Small amount of residual Sz occasionally present in lumen of lobules and in sperm duct.

From Brown-Peterson, N. J. et al. (2011). A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science, 3:52–70, 2011

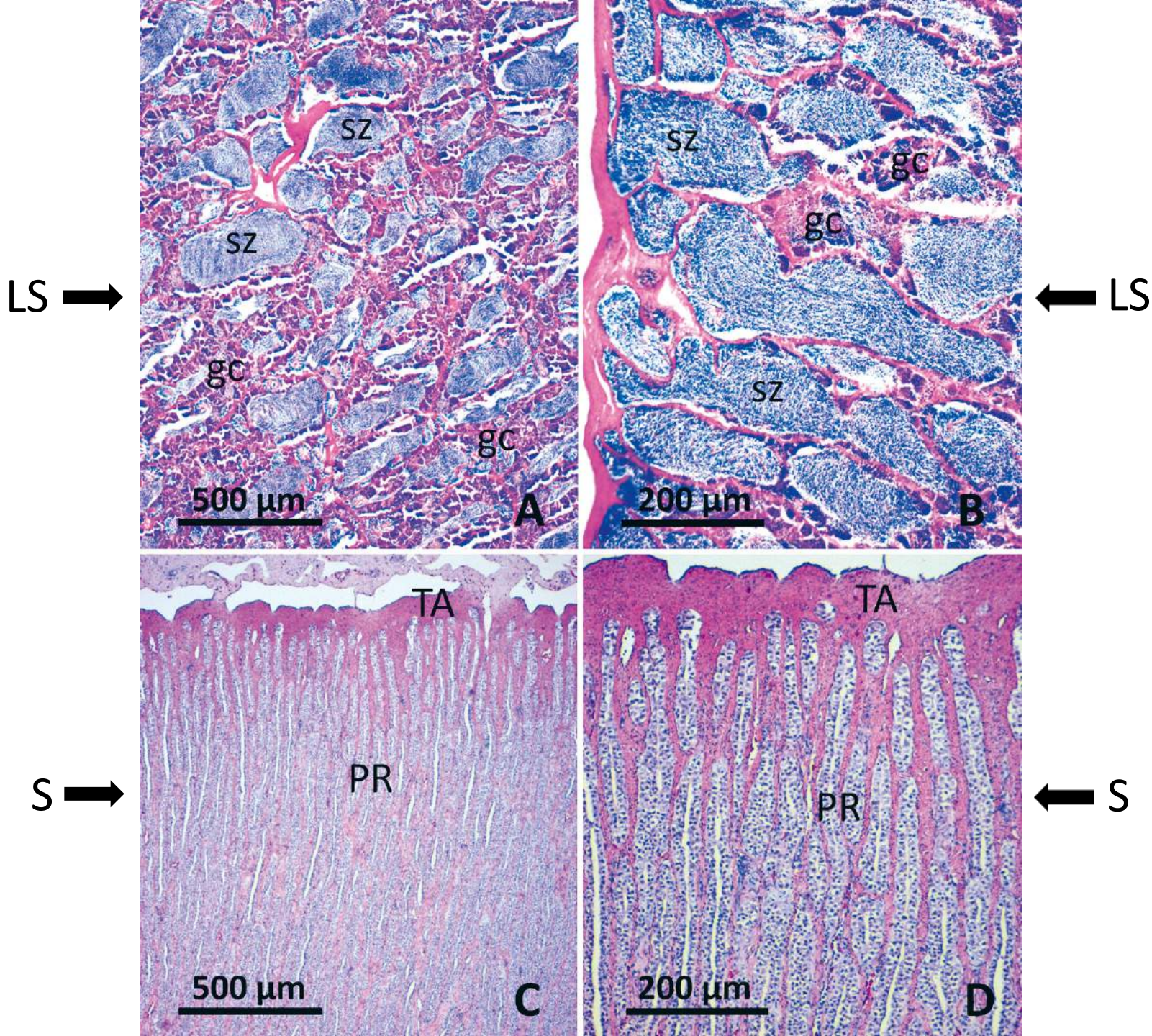
Testis histo classification for skipjack (after Ashida, 2020)

Histological characteristics of the testicular maturity phase, and gonad index of male skipjack tuna (*Katsuwonus pelamis*) caught in the subtropical and temperate western Pacific Ocean.

Maturity phase	*n*	Germ cell	GE state	Mean GI ± SD (Range)
Immature	341	SG	CGE	0.15 ± 0.19^{d} (0.02–2.23)
Developing	933	SG, SC, ST. SP may be present.	CGE	0.90 ± 1.95^{b} (0.02–15.70)
Spawning capable	885	SG, SC, ST, SP	CGE and DGE	2.22 ± 1.95^{a} (0.04–11.80)
Spawning capable	530	SG, SC, ST, SP	DGE	
Regressing-regenerating	219	SG, RSP	CGE or DGE	0.29 ± 0.37^{c} (0.02–3.16)

CGE, continuous germinal epithelium; DGE, discontinuous germinal epithelium; GE, germinal epithelium; GI, gonad index (mean ± SD); RSP, residual sperm; SC, spermatocyte; SG, spermatogonia; ST, spermatid; SP, sperm.

From Ashida, H. (2020). Spatial and temporal differences in the reproductive traits of skipjack tuna *Katsuwonus pelamis* between the subtropical and temperate western Pacific Ocean. Fisheries Research 221, 105352.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Maturity stage classification for visual examination of gonads (both sexes)

Stage	*Criteria*	
	Males	*Females*
I	Gonads small ribbon-like, not possible todetermine sex by gross examination	Gonads small ribbon-like, not possible todetermine sex by gross examination
1	Immature; testes extremely thin, flattened and ribbon-like, but sex determinable by gross examination	Immature; gonads elongated, slender, but sex determinable by gross examination
2	Enlarged testes, triangular in cross section, no milt in central canal	Early maturing; gonads enlarged but individual ova not visible to the naked eye
3	Maturing; milt flows freely if testes pinched or pressed	Late maturing; gonads enlarged, individual ova visible to the naked eye
4	Ripe; testes large, milt flows freely fromtestes	Ripe; ovary greatly enlarged, ova translucent, easily dislodged from follicles or loose in lumen of ovary
5	Spent; testes flabby, bloodshot, surface dull red, little or no milt in central canal	Spawned; includes recently spawned and postspawningfish, mature ova remnants in various stages of resorption, and mature ova remnantsabout 1.0mm in diameter

From Diaha, N.C. et al. (2015). Present and future of reproductive biology studies of yellowfin tuna (*Thunnus albacares*) in the eastern Atlantic Ocean. Collect. Vol. Sci. Pap. ICCAT, 71(1): 489-509.

A useful guide by David G. Itano including nice pictures:

http://www.soest.hawaii.edu/PFRP/biology/itano/oocytes.html

From Itano (2000). The reproductive biology of yellowfin tuna (*Thunnus albacares*) in Hawaiian waters and the Western Tropical Pacific Ocean: Project Summary. SOEST 00-01, JIMAR Contribution 00-328.