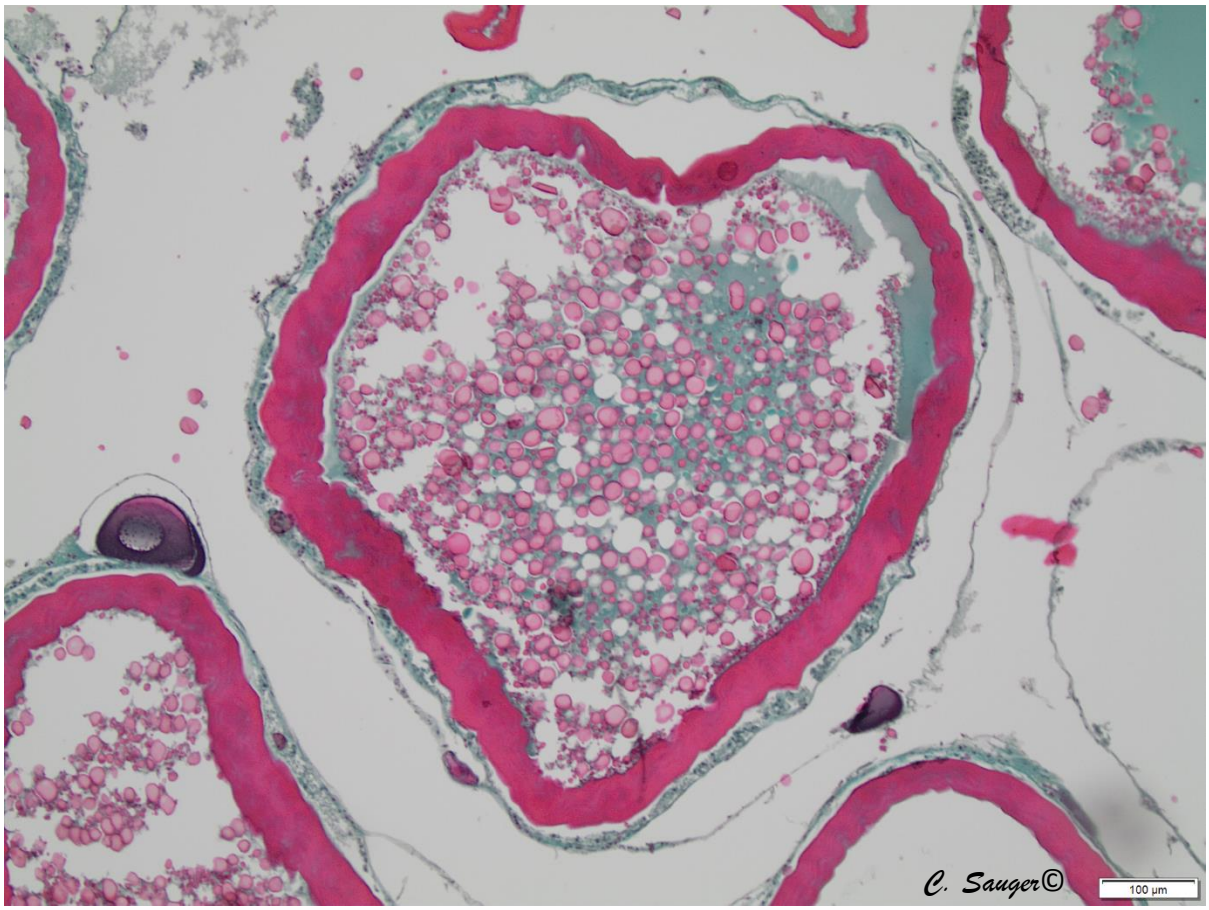
Lexicon of histological structures found in the ovaries and during the oogenesis of the European plaice, *Pleuronectes platessa*, Linnaeus, 1758



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Cover photo: Picture of a hydrating oocyte found in a plaice (*Pleuronectes platessa*) ovary. The follicle's shape was warped during the histological slide preparation. Personal collection. C. Sauger©

Summary

Abbreviations

CA : cortical alveoli
ce : outer cytoplasm
cf : follicular cell
chr : condensed chromosome
chrom : chromatin
ci : internal cytoplasm
cm : macrophage cell
cs : blood vessel
ei : Intercellular space
fc : collagen fiber
gl : lipid droplet
gv : vitellus droplet
i : undetermined structure
L : lysis
N : nucleus
NC : Nucleo-Cytoplasmic
nl : nucleolus
oaA : undischarged atretic oocyte
oaB : discharged atretic oocyte
oca : cortical alveoli oocyte
och : oocyte in hydration
oh : hydrated oocyte
op1 : premature oocyte stage 1
op2 : premature oocyte stage 2
ov : oogonium
pg : gonadal wall
POF : Post-Ovulatory Follicle
SI : sexually immature
SM : sexually mature
som : somatic cell
T : theca
tc : connective tissue
v : unnatural emptiness (from the slide's cut)
vi : vitellus
vit1 : oocyte in stage 1 of vitellogenesis
vit2 : oocyte in stage 2 of vitellogenesis
vit3 : oocyte in stage 3 of vitellogenesis
vit4 : oocyte in stage 4 of vitellogenesis
zr : *zona radiata*

Introduction

During this study, the terminology used was that of Brown-Peterson et al. (2011), since it followed the criteria set by the working groups of the International Council for the Exploration of the Sea (ICES) that defined different maturity scales for European stock species (ICES 2008, 2010, 2013, 2014, 2018).

For the description of the female germinal cells that will become gametes (*Figure 1*), the terms **oogonia** (before meiosis) or **oocyte** (meiosis has begun) will be used. An oocyte is characterized by an **ooplasm** encased in an **oolemma** (plasmic membrane) and a **zona radiata** (Tyler and Sumpter, 1996).

The term follicle will be used to designate an oocyte encased in somatic cells: follicular cells and theca cells (Tyler and Sumpter, 1996).

For the description of gamete development, the term **stage** will be used to designate the different gametogenesis stages (Brown-Peterson et al., 2011) :

- Oogonia
- Primary growth oocytes
- Secondary growth oocytes
- Oocyte maturation
- Ovulation stage

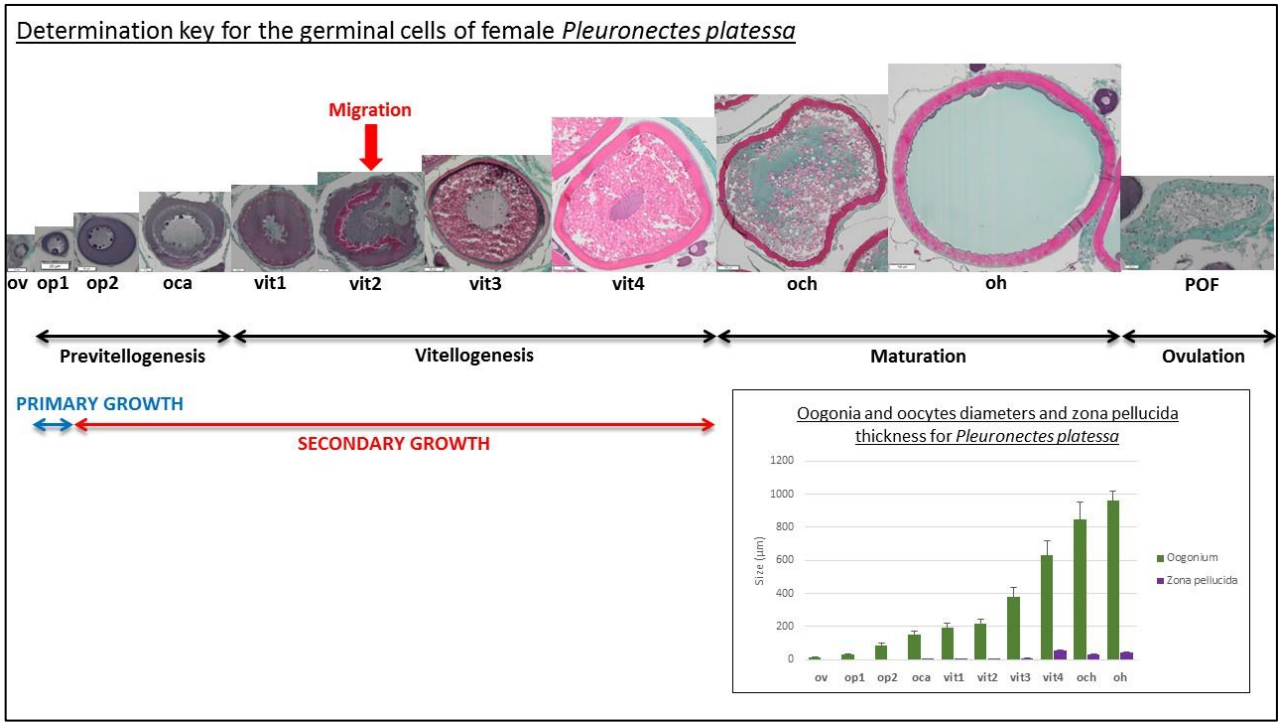


Figure 1 : Determination key of the different female germinal cells that can be found in the ovaries of *Pleuronectes platessa* at different oogenesis stages. With average oogonium and oocyte diameters (µm ± standard deviation), and zona radiata widths (µm ± standard deviation)

The sexual maturity cycle (*Figure 2*) is split into two **states**. The Sexually Immature (**SI**) state and the Sexually Mature (**SM**) state. The terminology **phase** will be used for the gonadal development. The reproduction cycle of fish is divided into different **phases** (Brown-Peterson et al., 2011, ICES, 2018a).

- Immature
- Developing
- Spawning
- Regressing
- Regenerating
- Omitted spawning
- Abnormal

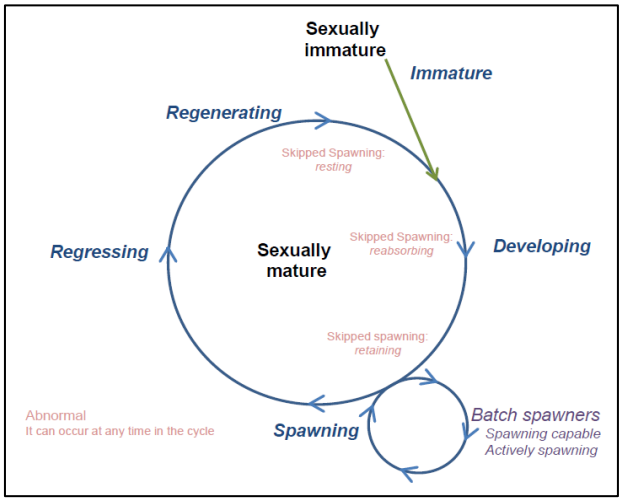


Figure 2 : Different phases found in the teleost maturation cycle, from the ICES (2018a). The phases being: Immature (A), Developing (B), Spawning (C), Regressing/Regenerating (D), Skipped spawning (E), Abnormal (F)

Table 1 : Macroscopic and microscopic descriptions of the phases in the reproductive cycle of female fish, from Brown-Peterson et al. (2011). Timing within each phase is species dependent. Some criteria listed for phases may vary depending on species, reproductive strategy, or water temperature. Subphases that apply to all fishes are listed; additional subphases can be defined by individual researchers. With CA = cortical alveoli, GVBD = germinal vesicle breakdown, GVM = germinal vesicle migration, OM = oocyte maturation, PG = primary growth, POF = post-ovulatory follicle complex, Vtg1 = primary vitellogenic, Vtg2 = secondary vitellogenic, Vtg3 = tertiary vitellogenic

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.

As seen in Table 1, the different **stages** are correlated with the presence or absence of specific cell types, following the terminologies and definitions of Brown-Peterson et al. (2011) and the ICES (2018a). For the North sea plaice, the different cell types that were described for this work will be shown in bold (**ov**, **op1**, **op2**, **oca**, **vit1**, **vit2**, **vit3**, **vit4**, **och**, **oh**) while their matching *Figures* will be indicated in italics.

- Ooginium stage :
    - oogonia (**ov**) (*Figures 7 & 8*)

- Primary oocyte :
    - Premature stage 1 oocyte (**op1**) (*Figures 9* & *10*)
    - Premature stage 2 oocyte (**op2**) (*Figures 11 & 12*)

- Secondary oocyte :
    - cortical alveoli oocytes with lipid droplets (**oca**) (*Figures 13 to 15*)
    - vitellogenic oocytes
        - subphase vtg1 : **vit1** (*Figures 16 & 17*)
        - subphase vtg2 : **vit2** & **vit3** (*Figures 18 to 22*)
        - subphase vtg3 : **vit4** (*Figures 23 to 25*)

- Oocyte maturation :
    - nucleus migration : in the plaice, this occurs during the **vit2** subphase
    - germinal vesicle breakdown : oocyte enters metaphase I of meiosis
    - vitellus coalescence : oocyte in hydration (**och**) (*Figures 26 & 27*)
    - hydration : hydrated oocytes (**oh**) (*Figures 28 & 29*)

- Ovulation stage : discharge of the hydrated oocytes into the ovary's lumen, leaving behind a post-ovulatory follicle (**POF**) (*Figures 30 & 31*)

The histological pictures found in this lexicon were taken with an Olympus AX70 microscope using the Olympus CellSens© software. The measurements of mean cell diameters and mean zona radiata widths were also taken with the Olympus CellSens© software, on a minimum of 20 oocytes found throughout multiple slides.

Overall ovarian organization

The plaice is a **total spawner** species, meaning that the females will release all of their oocytes as a unique event during the breeding season.

Figure 3 : Dissected plaice, *Pleuronectes platessa,* with the presence of the dorsal ovary

Criteria like the gonad wall (**pg**) thickness, connective tissue (**tc**) quantity, gonad vascularization (**cs**) or the surface area of the **lumen**, will depend on the maturity phase the fish is in. The histological cross sections will visually be quite different, with oocytes at various stages of gametogenesis (*Figure 4*).

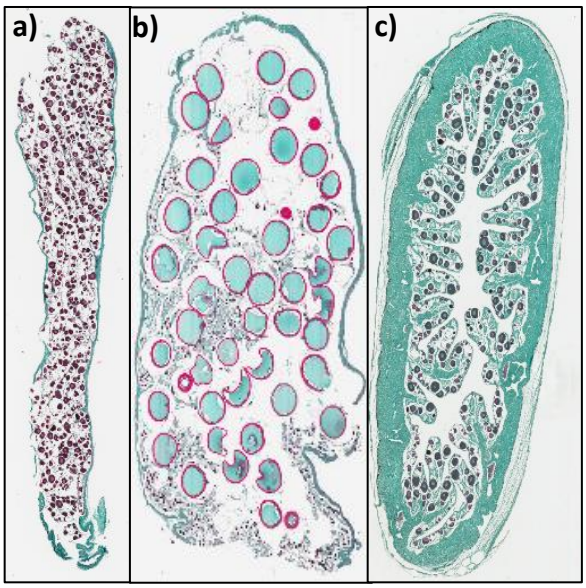


Figure 4 : Cross sections of plaice ovaries at different maturity phases : **a)** immature ;
b) spawning/ready to spawn ;
c) regenerating/finished spawning

Each gonad is organized concentrically (*Figure 5* et *Figure 6*), with the ovarian lamellas delimited by the germinal epithelium. The germinal cells (oogonia and oocytes) are found within these ovarian lamellas, inside follicles, at different stages of gametogenesis. The connective tissue (**tc**) will hold the germinal cells together, and the gonad wall (**pg**) can vary in thickness. Finally, the **lumen** is the naturally empty space found between the germinal epithelium lamellas inside the ovary.

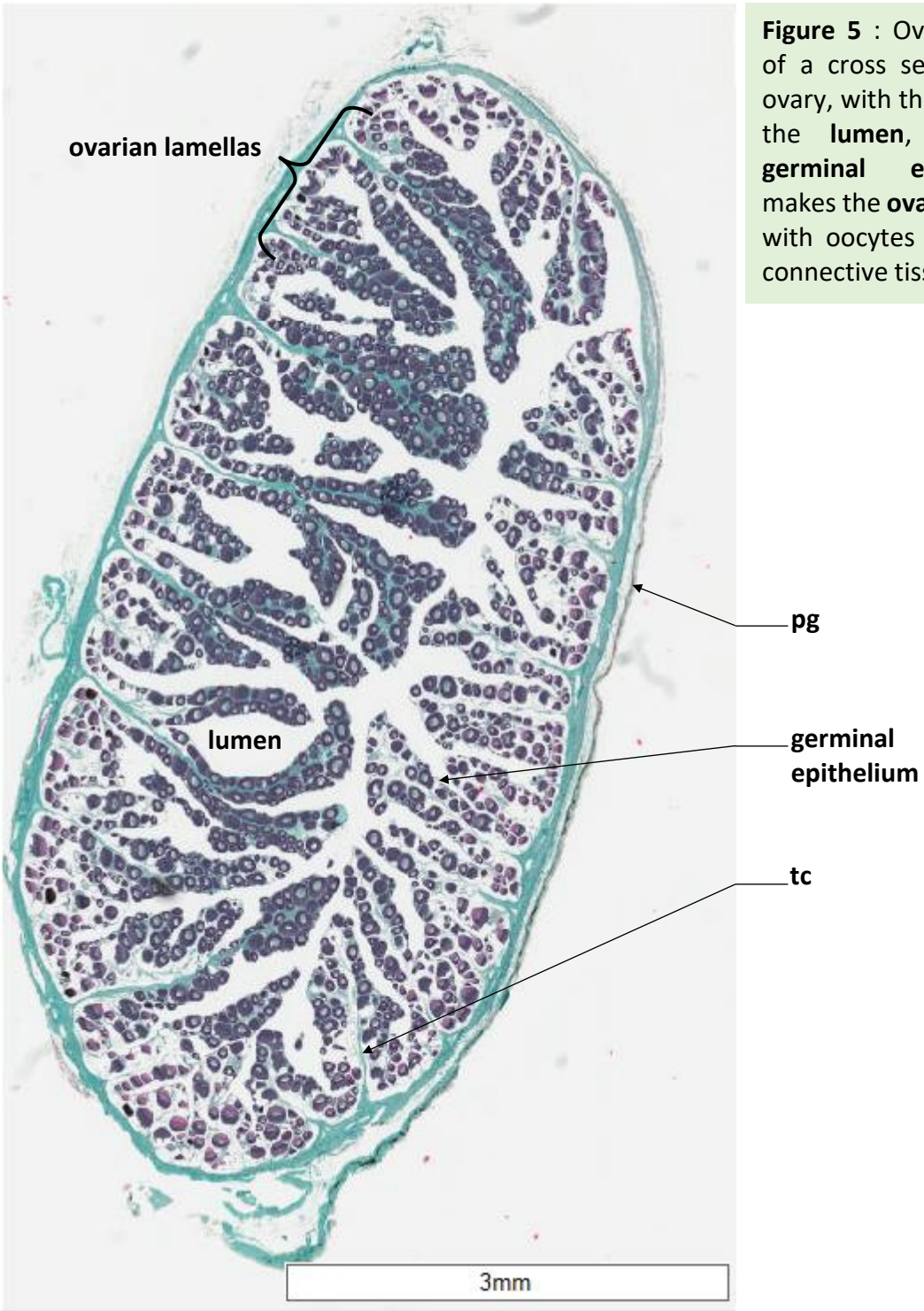


Figure 5 : Overall organization of a cross section of a plaice ovary, with the gonad wall (**pg**), the **lumen**, and a folded **germinal epithelium** that makes the **ovarian lamella** filled with oocytes held together by connective tissue (**tc**)

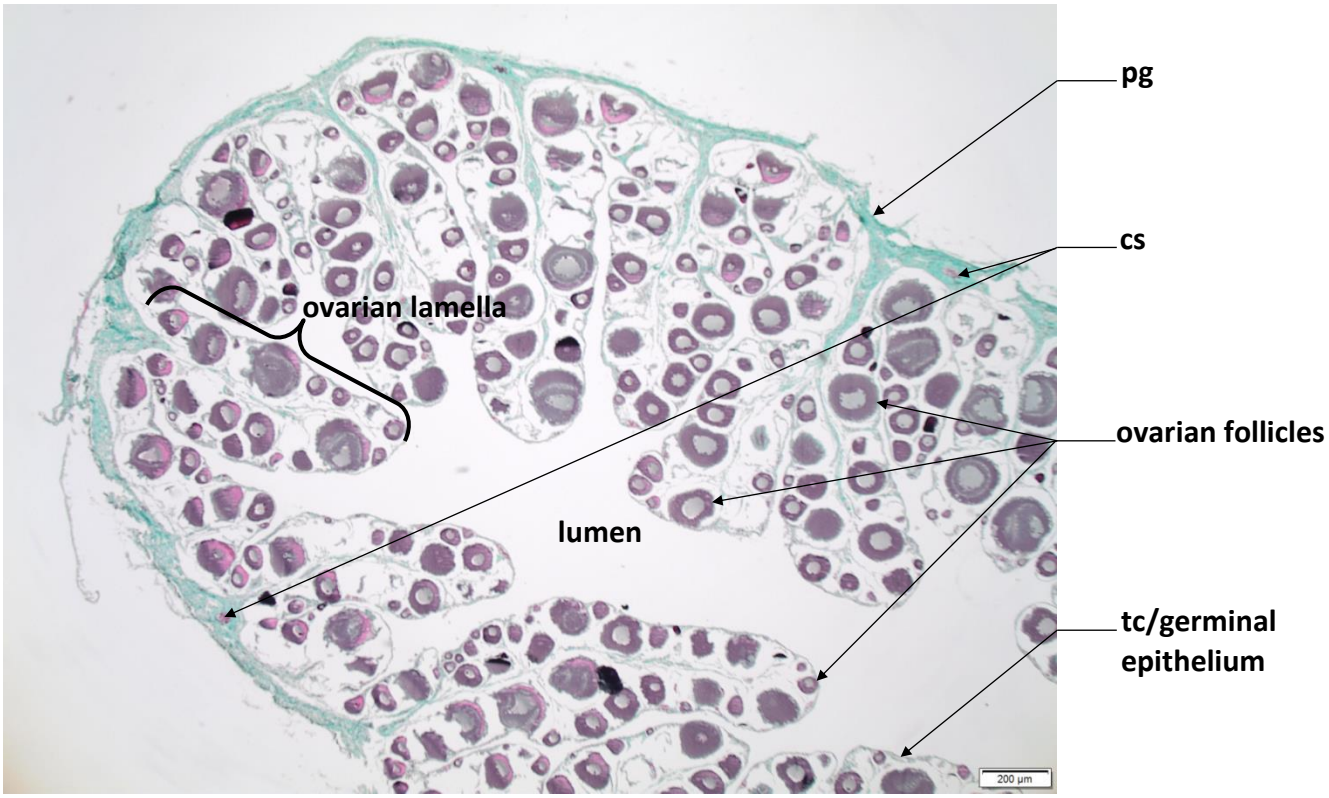
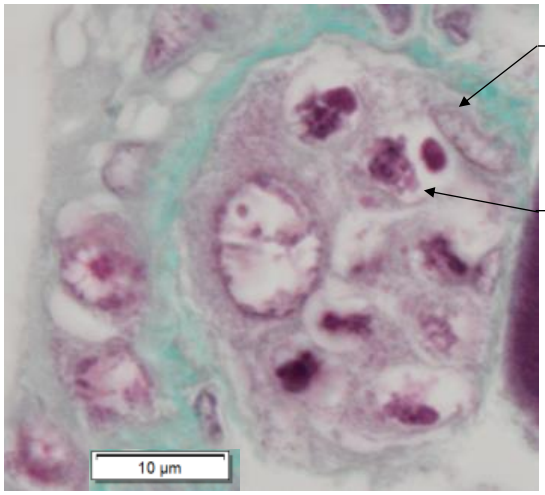


Figure 6 : Partial view of a plaice ovary cross section, with the gonad wall (**pg**), blood vessels (**cs**), the **lumen**, and oocytes in **follicles** held together by connective tissue (**tc**), organized inside **ovarian lamellas**

Oogonium (ov)

An oogonium (**ov**) has a pale and decondensed nucleus (**N**), with a high nucleo-cytoplasmic (NC) ratio (very little cytoplasm). Chromatin clusters can be seen near the edges of the nucleus. Inside this nucleus, a single large nucleolus can generally be found. The cytoplasm (**c**) is light-coloured (*Figure 8*). Oogonia stem from germinal cells through gonial mitosis. They can be found alone or in a germinal niche within the germinal epithelium (*Figure 7*).

Identification : It is uncommon to fall on this very small sized cell. The **nucleus is light-gray and fully apparent** while the **cytoplasm is barely visible and very light-coloured**. The diameter of the nucleus of an oogonium is greater than the surface area of the cytoplasm (>50 %).



Average size

ov : 14.28µm (±3.74µm)

Figure 7 : Stem cell divisions inside a germinal niche with their somatic cells (**som**)

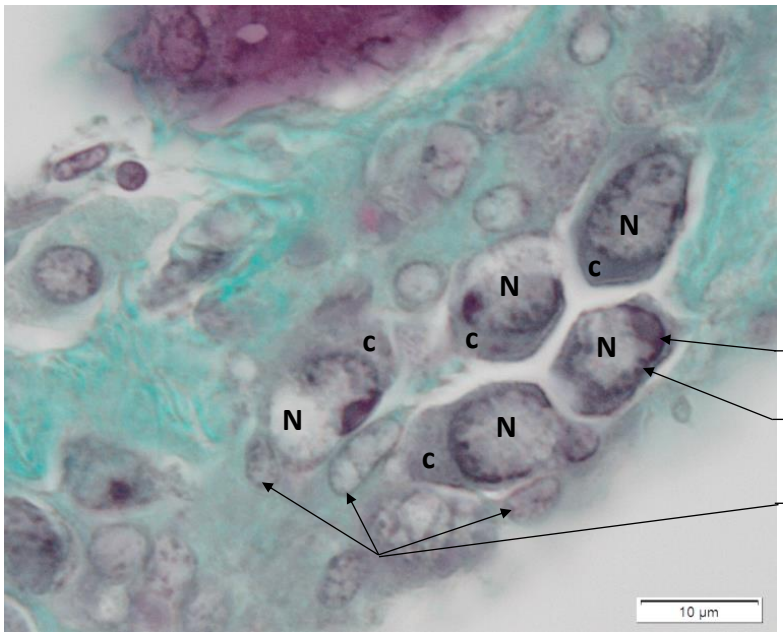
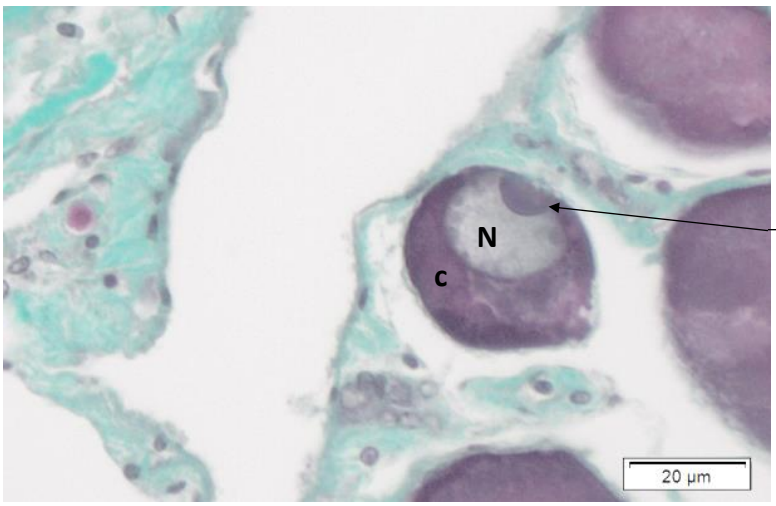


Figure 8 : Oogonia (**ov**) and their apparent, decondensed, nucleus (**N**) with a single large nucleolus (**nl**) and clusters of chromatin (**chrom**). Light-coloured cytoplasm (**c**). Presence of somatic cells (**som**)

Premature stage 1 oocyte (op1)

A premature stage 1 oocyte (**op1**) has a darker cytoplasm (**c**) compared to an oogonium (**ov**), with a smaller **NC** ratio. The diameter of the cytoplasm is inferior or equal to 50 % of the nucleus' diameter. The nucleus (**N**) is spherical, smooth, with 1 (sometimes 2 *cf Figure 10*) nucleolus.

Identification : This cell type is slightly bigger than an oogonium, but is still relatively small in size compared to the other cellular structures found inside the ovary. Falling on this follicle in stereology is still uncommon. The **nucleus must be fully visible, smooth,** and the **cytoplasm is darkly-coloured**. The nucleus diameter of an **op1** is inferior or equal to 50 % of the cytoplasm's diameter. If there's an hesitation between an **op1** and an **op2**, the cell size can be taken into consideration.



Average size

op1 : 29.64µm (±5.45µm)

Figure 9 : Premature stage 1 oocyte (**op1**) with a spherical and smooth nucleus (**N**) with a single nucleolus (**nl**). The cytoplasm (**c**) is darker compared to that of an oogonium

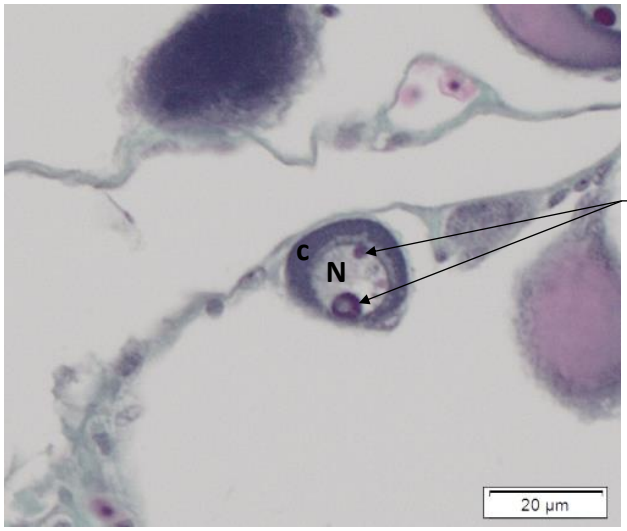
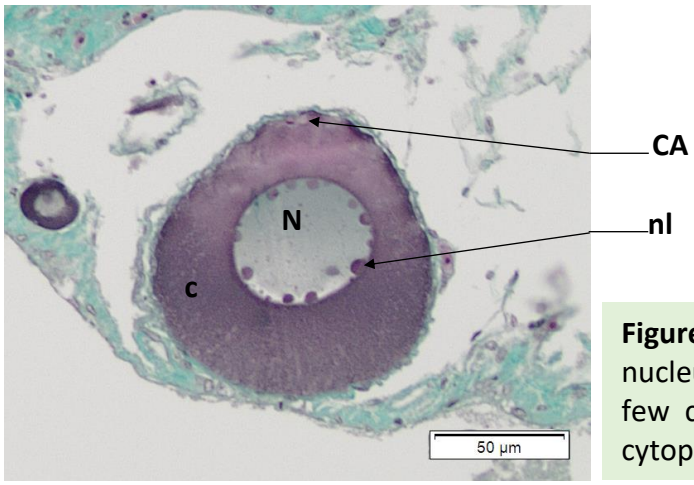


Figure 10 : Premature stage 1 oocyte (**op1**) with a spherical and smooth nucleus (**N**) with two nucleoli (**nl**). The cytoplasm (**c**) is darker compared to that of an oogonium (**ov**)

Premature stage 2 oocyte (op2)

Inside the cytoplasm (**c**) of a premature stage 2 oocyte (**op2**), rare cortical alveoli (**CA**) and lipid droplets (**gl**) may be seen. The cytoplasm (**c**) can sometimes be divided into two somewhat distinct areas. Moreover, it is possible to see lampbrush chromosomes inside the nucleus, typically found in immature diplotene oocytes, from time to time.

Identification : This ovarian follicle varies in size, between the **op1** and **oca** stage, and is largely present inside gonads of immature individuals. This cell can have either a **single cytoplasm** or a **cytoplasm divided into two parts** that will become more distinct the closer the follicle is to the **oca** stage. The **nucleus is spherical, smooth**, and usually shows multiple nucleoli. A few, but rare, lipid droplets and cortical alveoli can be seen inside the cytoplasm.



Average size

op2 : 82.70µm (±15.89µm)

Figure 11 : A **op2**, with a spherical and smooth nucleus (**N**) filled with multiple nucleoli (**nl**). A rare few cortical alveoli (**CA**) can be seen inside the cytoplasm (**c**)

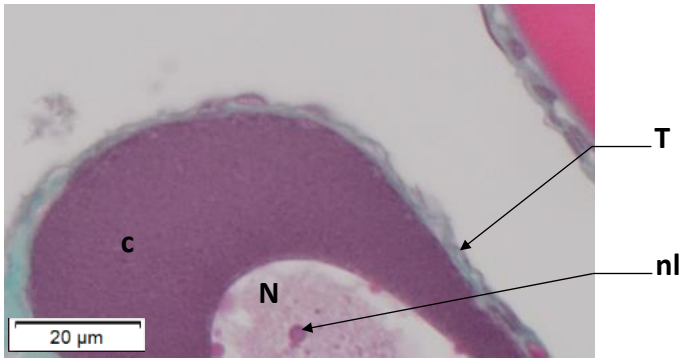


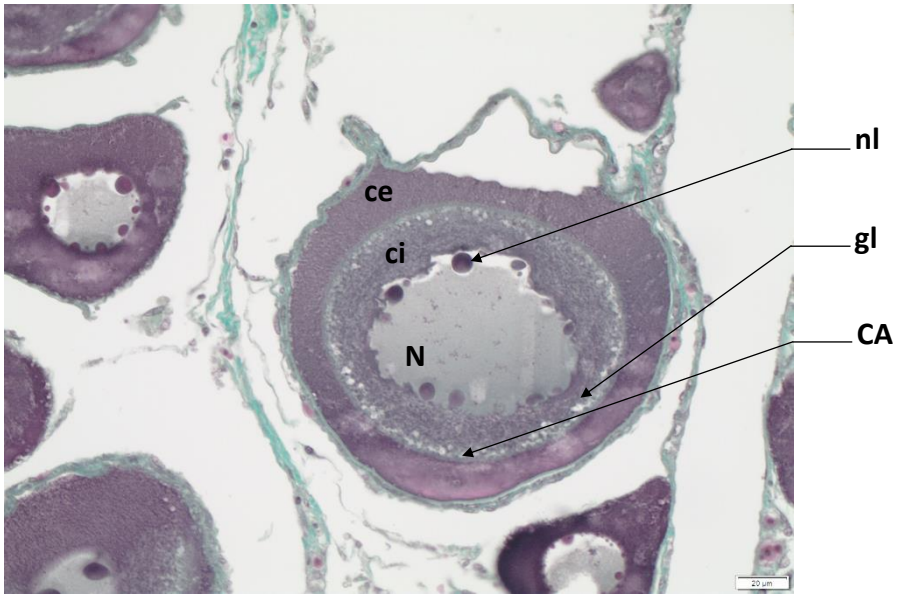
Figure 12 : Cell wall of a **op2**, with a spherical and smooth nucleus (**N**) filled with multiple nucleoli (**nl**). The theca (**T**) encases the cytoplasm (**c**)

Cortical alveoli oocyte (oca)

An oocyte with cortical alveoli (**oca**) is defined by : (1) a cytoplasm (**c**) divided in two areas : internal cytoplasm (**ci**) and external cytoplasm (**ce**) ; (2) a first appearance of lipid droplets (**gl**) inside the cytoplasm (**c**) and around the nucleus (**N**), as well as cortical alveoli (**CA**) near the cell wall ; (3) a nucleus (**N**) in the center of the cytoplasmic mass, with a scalloped nuclear envelope and multiple nucleoli (**nl**) against the envelope. Sometimes, it is possible to see lampbrush chromosomes inside the nucleus, typically found in immature diplotene oocytes. Finally, the *zona radiata* (**zr**) will start developing between the cytoplasm and the follicular cells (**cf**) + theca (**T**).

The **CA** will take a lot of space inside the cytoplasm and can be mistaken with lipid droplets (Anderson, 1968 ; Tyler et Sumpter, 1996). Moreover, before the beginning of the vitellogenic stage (**vit1**) the internal cytoplasm (**ci**) will expand, making it difficult to see the external cytoplasm (**ce**). At this stage, the **CA** can be found along the *zona radiata* (**zr**).

Identification : An oocyte with cortical alveoli is primarily defined by its **nucleus in the center of the follicle, with a scalloped envelope** and multiple nucleoli along said nuclear envelope. A **two-zoned cytoplasm is commonly observed**, with lipid droplets at the limit of these two zones. It is possible to see condensed chromosomes (small irregular dark-gray lines inside the nucleus), as well as cortical alveoli. Finally, around the oocyte, **the zona ratiata will start to form**.



Average size

oca : 153.07µm (±17.83µm)

zr : 0.91µm (±0.23µm)

Figure 13 : Oocyte with CA (**oca**), the nucleus (**N**) is scalloped and holds multiple nucleoli (**nl**). Lipid droplet (**gl**) build-up between the internal (**ci**) and external cytoplasm (**ce**). Presence of **CA** in the **ce**

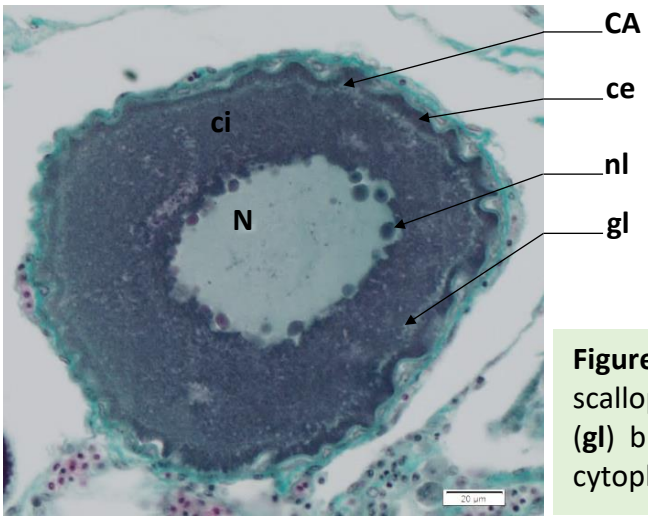


Figure 14 : Oocyte with CA (**oca**), the nucleus (**N**) is scalloped and holds multiple nucleoli (**nl**). Lipid droplet (**gl**) build-up between the internal (**ci**) and external cytoplasm (**ce**). Here the **ci** is pushing back the **ce**

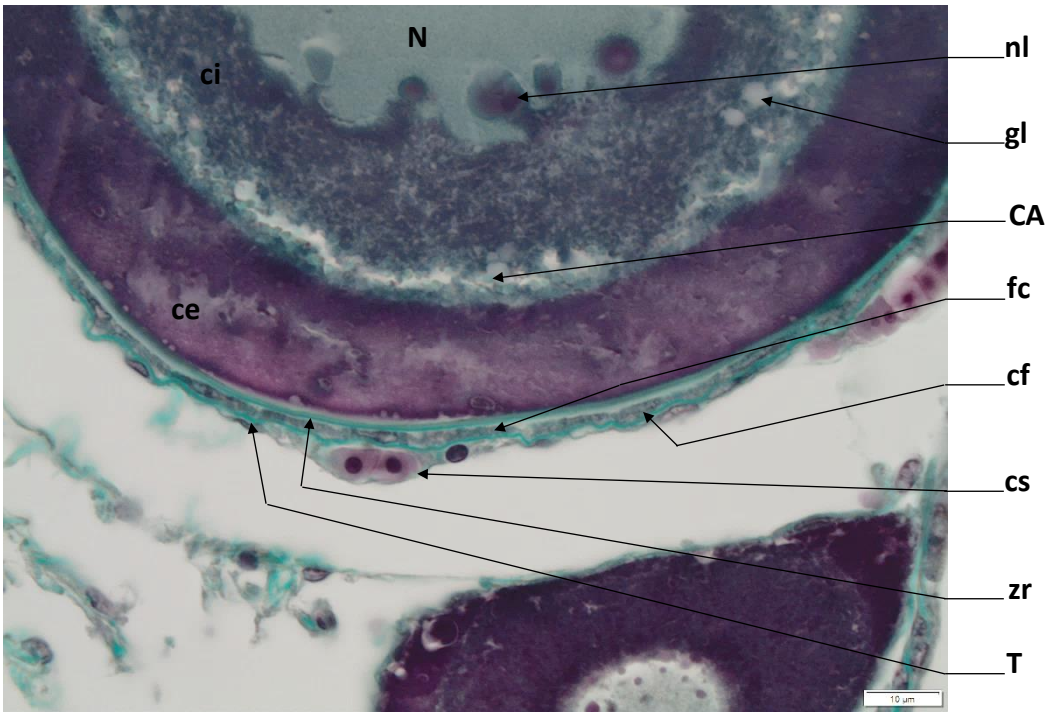


Figure 15 : Cell wall of an oocyte with cortical alveoli (**oca**), the nucleus (**N**) is scalloped and contains multiple nucleoli (**nl**). Lipid droplet (**gl**) build-up between the internal (**ci**) and external cytoplasm (**ce**). Beginning of the *zona radiata* (**zr**) growth, presence of cortical alveoli (**CA**), follicular cells (**cf**), collagen fiber (**fc**) and the theca (**T**) with blood vessels (**cs**)

Oocyte in early vitellogenesis (vit1)

An oocyte at the beginning of the vitellogenic stage (**vit1**) is defined by the presence of a ring of vitellus droplets (**gv**), pink eosinophilic droplets, on the edge of the cytoplasm, along the *zona radiata* (**zr**). A few lipid droplets (**gl**) can still be seen in the cytoplasm (**c**). The nucleus (**N**) is still centered in the follicle, with multiple nucleoli (**nl**) along the scalloped nuclear envelope. The *zona radiata* (**zr**) is thin and green (not acidophilus).

Identification : This oocyte in early vitellogenesis is easily recognizable with the appearance of a **peripheral ring of pink vitellus droplets in the cytoplasm**. At the **first appearance of a vitellus droplet**, the oocyte is considered to be in the **vit1** stage. The **nucleus is still scalloped and centered inside the follicle** and the **ring of vitellus will run along the edge of the cell wall** (along the zona radiata). Even if the nucleus is not visible, if the ring of vitellus is visible and adjoined to the cell wall, this follicle should be placed in the **vit1** category.

Average size

vit1 : 191.52µm (±29.08µm)

zr : 1.43µm (±0.32µm)

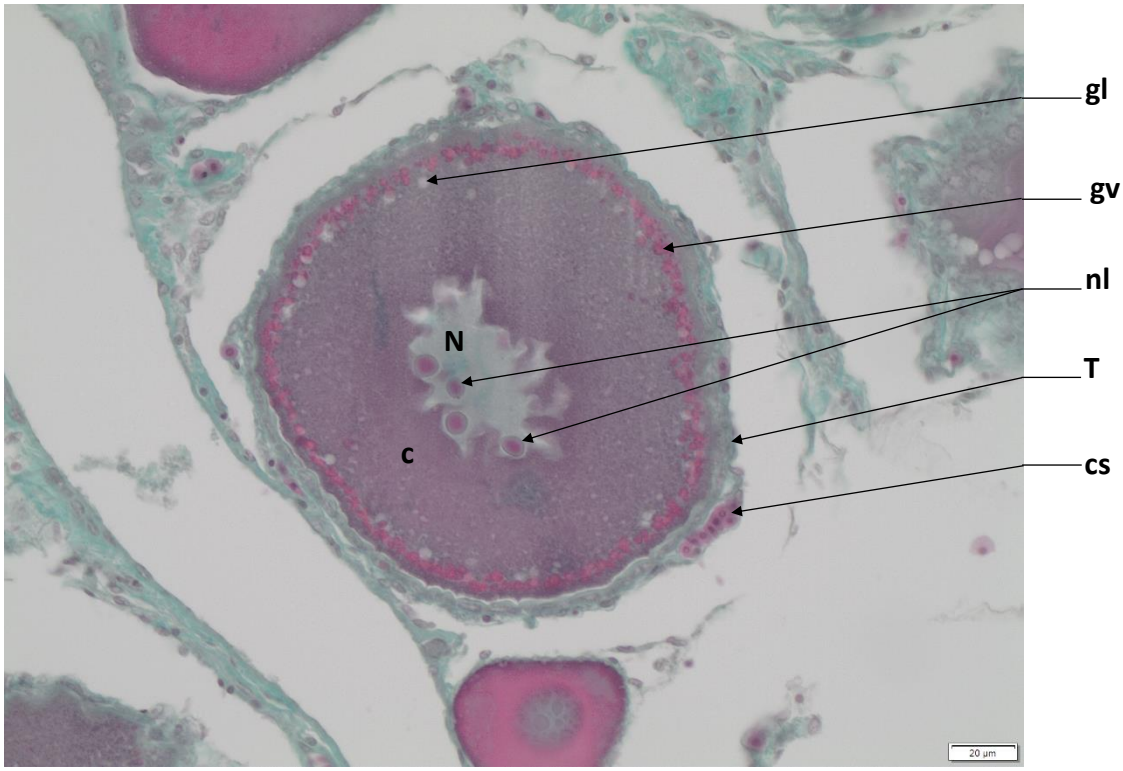


Figure 16 : A stage **vit1** oocyte, with a scalloped nucleus (**N**) envelope containing multiple nucleoli (**nl**). A ring of vitellus droplets (**gv**) will form on the edge of the cytoplasm (**c**). Lipid droplets (**gl**) can still be seen at this stage. Blood vessels (**cs**) are present in the theca (**T**)

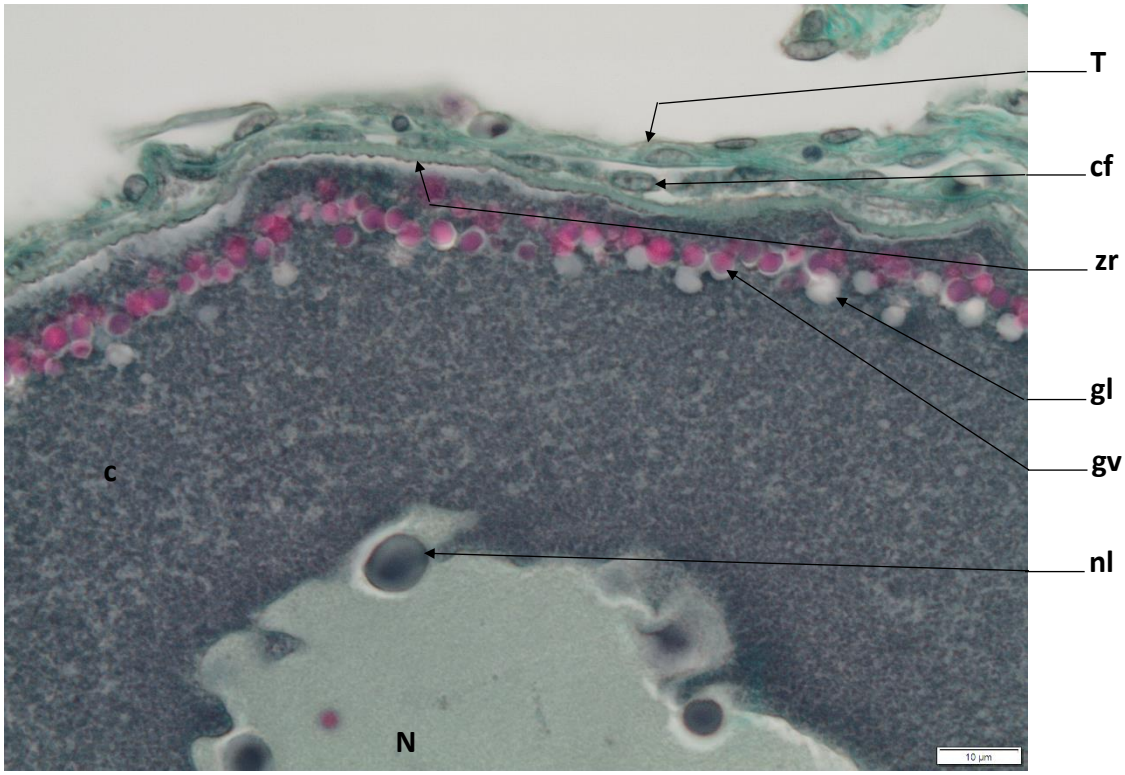


Figure 17 : Cell wall of a **vit1** oocyte with a scalloped nucleus (**N**) envelope containing multiple nucleoli (**nl**). A ring of vitellus droplets (**gv**) will form on the edge of the cytoplasm (**c**). Lipid droplets (**gl**) can still be seen at this stage. Follicular cells (**cf**) are present in the theca (**T**), with the *zona radiata* (**zr**) under these cell layers

Oocyte in vitellogenesis with nucleus migration (vit2)

During the **vit2** stage, the ring of vitellus droplets (**gv**) migrates from the external area of the cytoplasm to the medial area of the follicle ; the nucleus (**N**) progressively approaches the oocyte's periphery, comes up against the ring of vitellus droplets that will open up to let it through. The *zona radiata* (**zr**) has the same aspect as in the **vit1** stage.

A migrating nucleus will have a warped shape and be off-centered in the follicle. Depending on the angle of the cross section, the nucleus can be unapparent. Moreover, during the nucleus migration, the ring of vitellus can be in disarray after being broken through.

A nucleus that has finished its migration will be pushed up against the zona radiata on one side of the follicle while the vitellus droplets will stack up on the other side. This structure is uncommonly seen, especially since it is easy to miss the nucleus if the cut of cross section is not at the right angle.

Identification : This cell type is similar to the **vit1** stage, but is differentiated by either a **ring of vitellus around the nucleus, with a zone of cytoplasm between the zr and the vitellus**, or by a **nucleus pushed up against the zr on one side of the follicle and the gv stacked up to the other side**. It is preferable, but not mandatory for the nucleus to be visible. The nucleus can be centered, migrating or finished migrating.

Average size

vit2 : 220.19µm (±25.28µm)

zr : 1.45µm (±0.20µm)

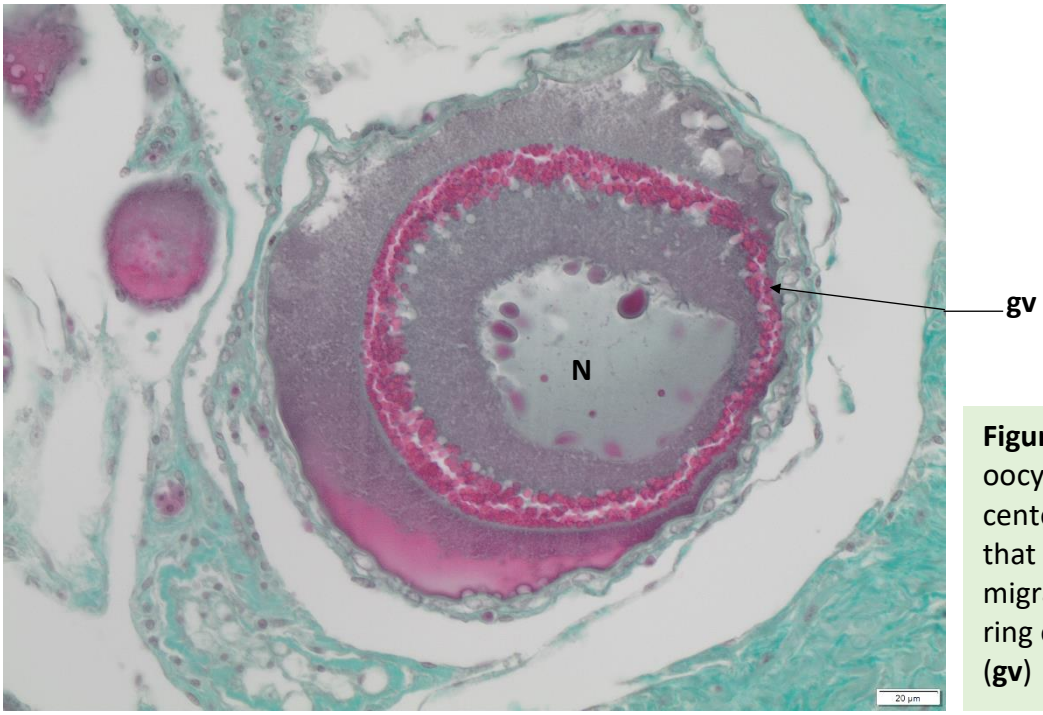


Figure 18 : A stage **vit2** oocyte with an off-centered nucleus (**N**) that has started migrating towards the ring of vitellus droplets (**gv**)

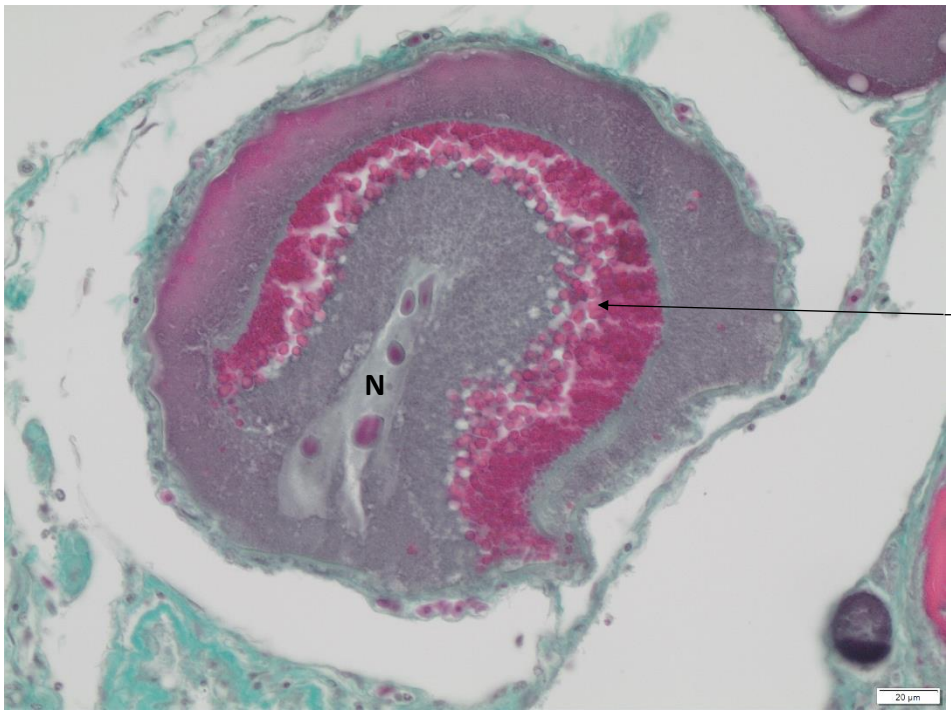


Figure 19 : A stage **vit2** oocyte with a warped nucleus (**N**) that has started migrating through the ring of vitellus droplets (**gv**)

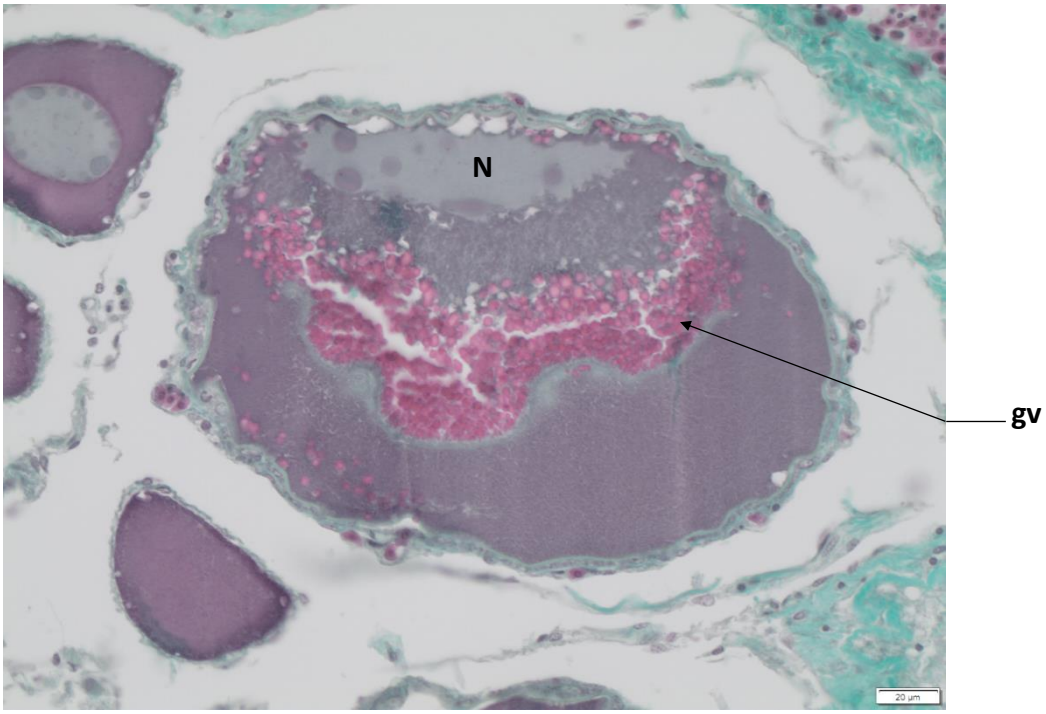
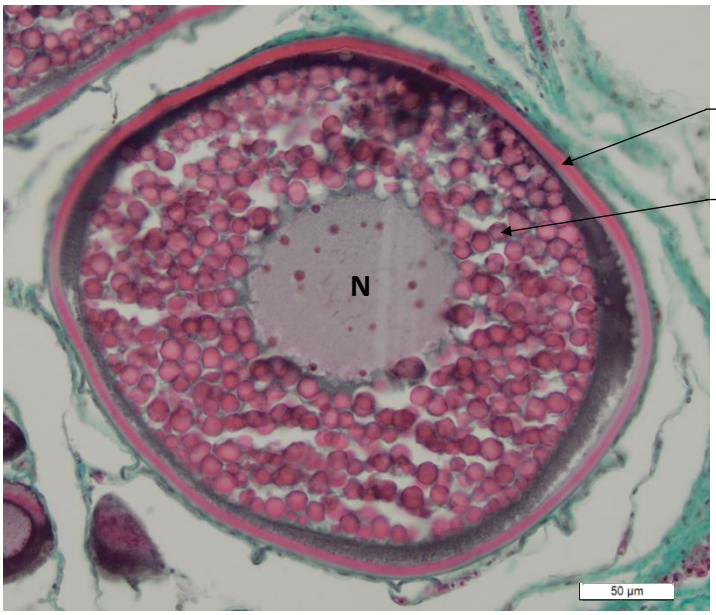


Figure 20 : An oocyte near the end of stage **vit2**, with a nucleus (**N**) that has finished migrating : **N** is outside of the **gv** mass, and is polarized

Oocyte in vitellogenesis with zona radiata growth (vit3)

An ovarian follicle enters stage 3 of vitellogenesis (**vit3**) when the *zona radiata* (**zr**) becomes acidophilus. The **zr** is slightly striated and takes on a pink tinge as it grows in size. The vitellus droplets (**gv**) will take up the entire space inside the follicle as they start to fuse together and grow in size. This stage seem to occur in a short period of time.

Identification : A oocyte in vitellogenesis with a zona radiata in expansion is defined by a **pink, thin, and slightly striated zr**, as well as **small to medium sized gv that take up the entire space inside the follicle**. Seeing the nucleus is not mandatory for the identification of this stage.



Average size

vit3 : 380.02µm (±57.16µm)

zr : 9.21µm (±1.63µm)

Figure 21 : A stage **vit3** oocyte with a growing *zona radiata* (**zr**) that takes on a pink tinge and is slightly striated, a nucleus (**N**), and lots of vitellus droplets (**gv**) of various size

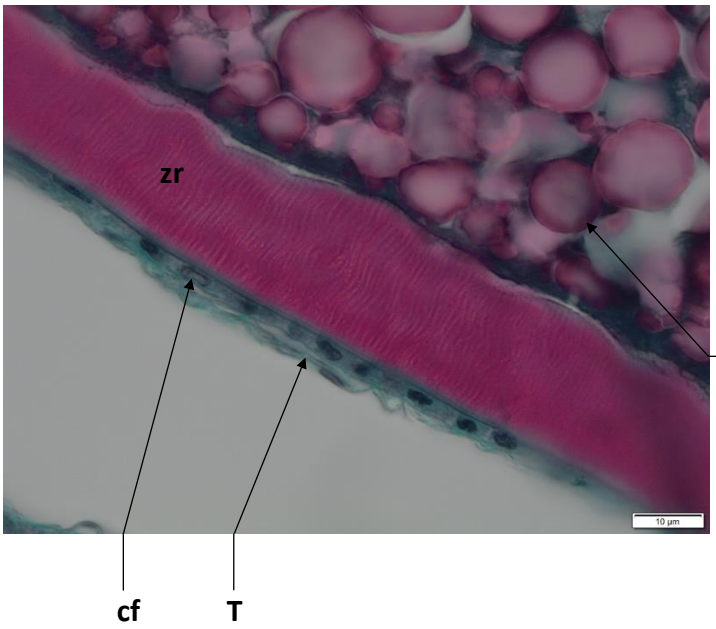


Figure 22 : Cell wall of a **vit3** oocyte with a growing *zona radiata* (**zr**) that takes on a pink tinge and is slightly striated, a nucleus (**N**), a lot of vitellus droplets (**gv**) of various size, and a theca (**T**) with follicular cells (**cf**)

Oocyte at the end of vitellogenesis (vit4)

Follicles near the end of the vitellogenis stage (**vit4**) are polygonyal shaped and are nested together. The *zona radiata* (**zr**) is thick, very eosinophilic, and takes on a deeply streaked appearance (radial striae). The oocyte is filled with eosinophilic vitellus droplets (**gv**). The nucleus (**N**) is rarely visible. Around the **zr** follicular cells (**cf**) can be found, while the theca (**T**) can be associated with blood vessels (**cs**).

The follicles in vitellogenesis are very brittle, and will easily lose their vitellus and other cellular material during the histological cutting process. These oocyte fragments that have been ripped away can be found out of the theca. In such cases, if the absence of a theca still allows the identification of the cell type, then they can be classified as such.

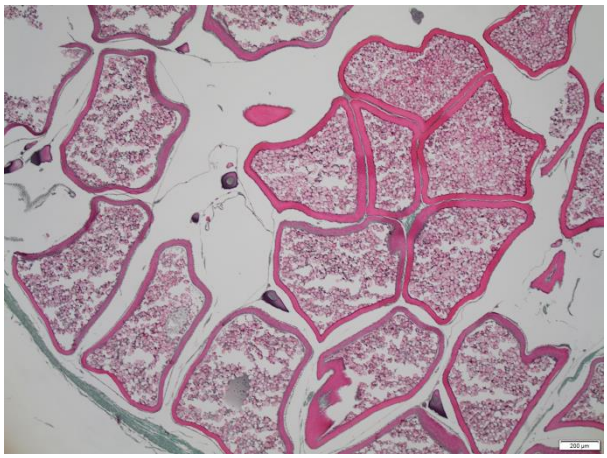


Figure 23 : Nested oocytes near the end of their vitellogenesis (**vit4**)

Average size

vit4 : 629.47µm (±88.45µm)

zr : 53.81µm (±5.10µm)

Identification : These large cells have a **thick and distinct pink zr**. Being such large cells, more than one sampling point can fall inside a same cell. Each of these sampling points will be categorized under the **vit4** cell type. **The oocyte is filled with pink gv**. The nucleus can sometimes be seen, but its visibility is not mandatory for the identification of this stage.

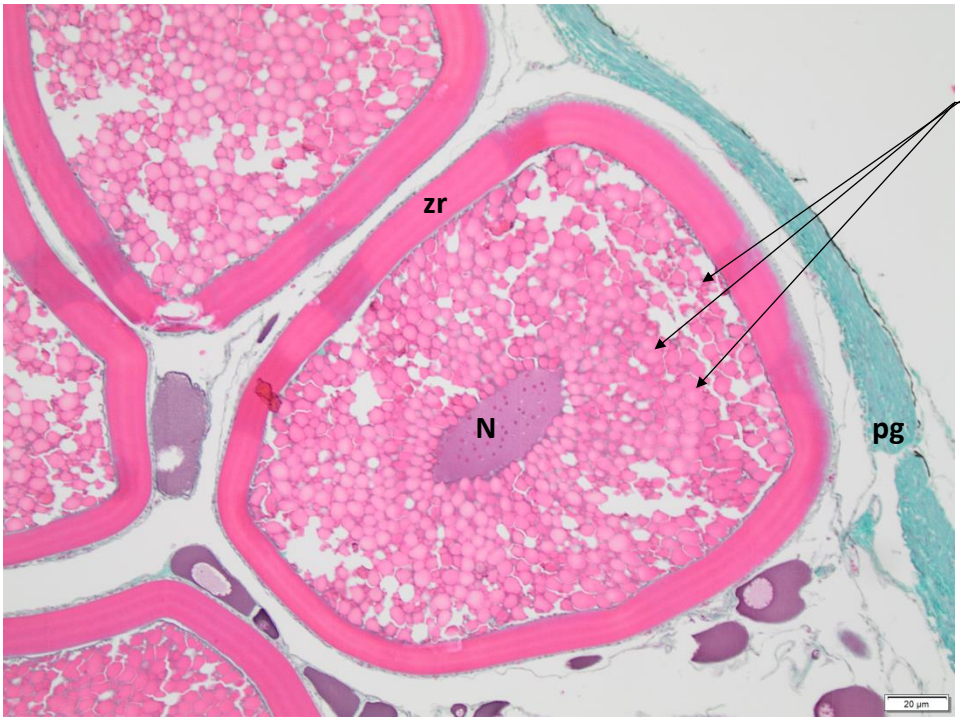


Figure 24 : End of vitellogenesis oocytes (**vit4**) with a thick *zona radiata* (**zr**), a nucleus (**N**) and large sized vitellus droplets (**gv**). Presence of the gonad wall (**pg**)

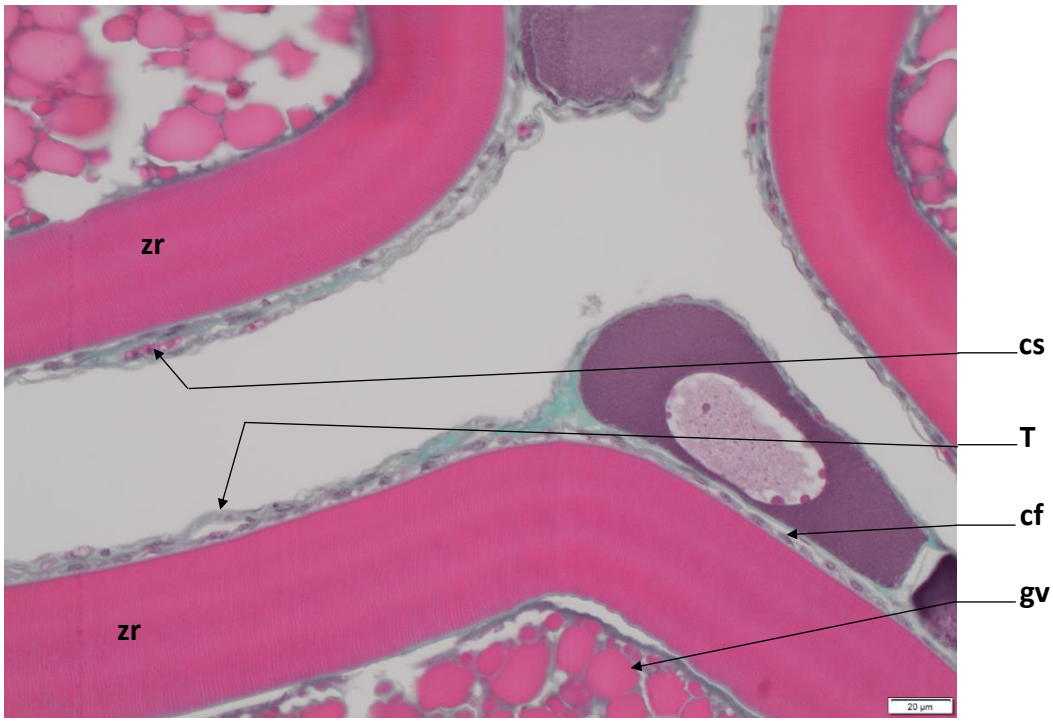
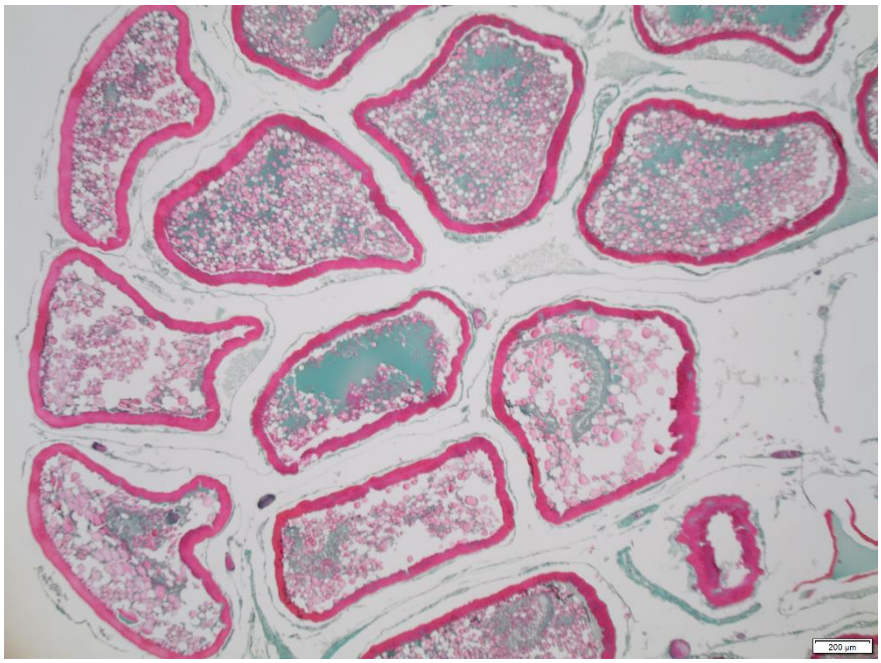


Figure 25 : Cell wall of a **vit4** oocyte around large vitellus droplets (**gv**). The membrane is made up by a *zona radiata* (**zr**), follicular cells (**cf**) and a theca (**T**) encasing blood vessels (**cs**)

Oocyte in hydration (och)

The vitellus droplets will fuse together, becoming a zone of homogeneous basophilic vitellus (**vi**) (greenish-blue areas). A oocyte in hydration (**och**) is encased in a thick *zona radiata* (**zr**). These cells are still polygonyal and nested together.

Identification : This cell looks like a **vit4**. The **gv** start to break down to form a **zone of homogeneous vitellus (greenish-blue areas)**. The nucleus can sometimes be seen, but its visibility is not mandatory for the identification of this stage.



Average size

och : 847.21µm (±104.01µm)

zr : 33.11µm (±3.50µm)

Figure 26 : Cross section of an ovary with oocytes in hydration (**och**)

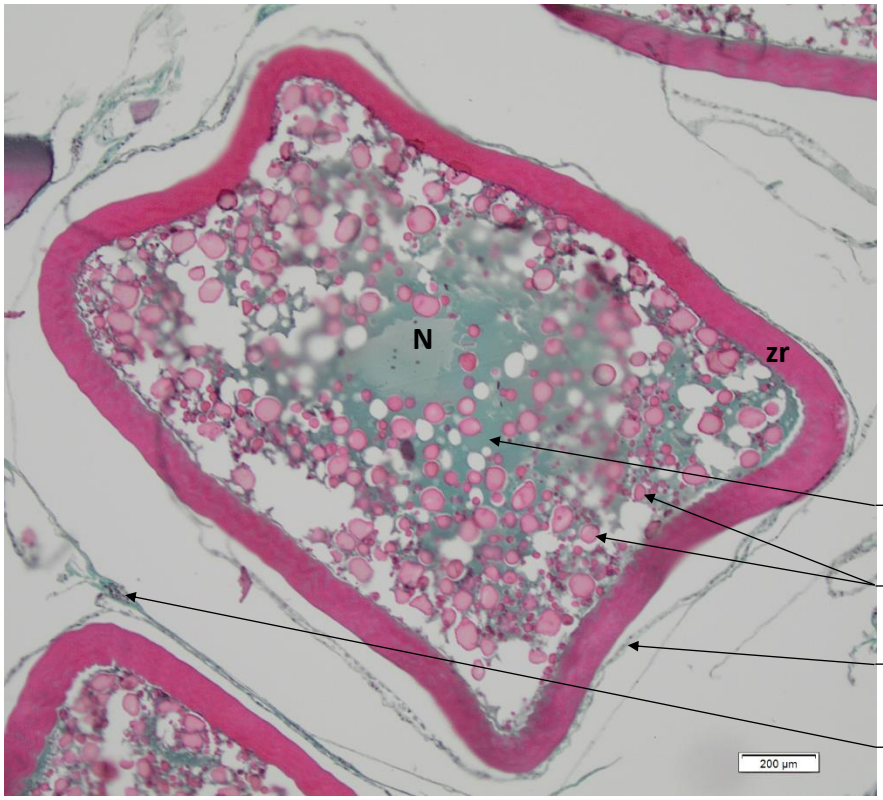
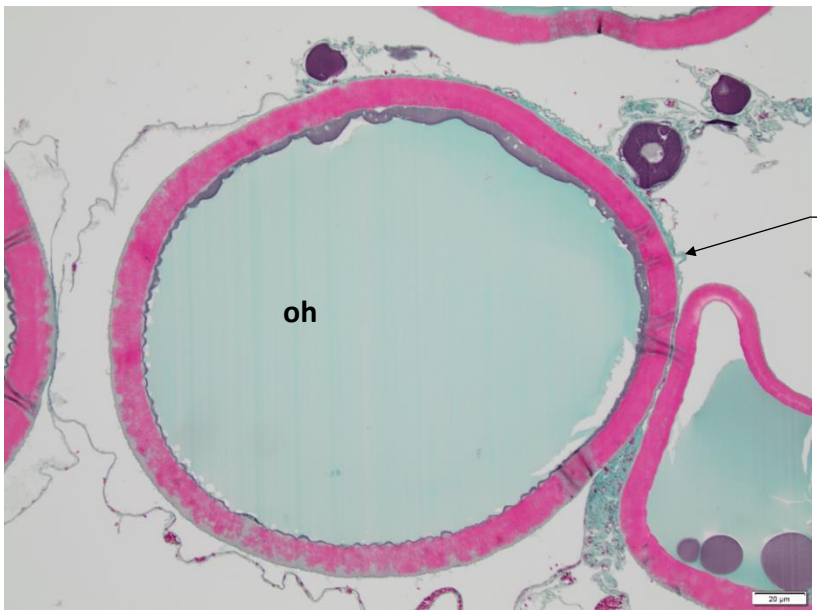


Figure 27 : Oocyte in hydration (**och**) with a thick *zona radiata* (**zr**), a nucleus (**N**), vitellus droplets (**gv**), a blue zone of homogeneous vitellus (**vi**) and a theca (**T**) with blood vessels (**cs**)

Hydrated oocyte (oh)

A hydrated oocyte (**oh**) is defined by a light blue homogeneous vitellus (**vi**) that takes up the entire volume of the oocyte. A few lipid droplets (**gl**) can sometimes be seen near the think and deeply grooved *zona radiata* (**zr**).

Identification : Biggest cell type found in the ovary, this oocyte will most often than not have a warped shape after the dehydration process used before the coloration of the histological slide. Whether this oocyte has been discharged from its follicle in the lamella or not, this cell type will be categorized as **oh**. The **zr is pink, thick, with deep radial grooves**, and the **oocyte is filled entirely with homogeneous vitellus (greenish-blue color)**.



Average size

oh : 958.66µm (±60.66µm)

zr : 40.41 µm (±5.00µm)

Figure 28 : Hydrated oocyte (**oh**) that has not been discharged yet : encased in a theca (**T**)

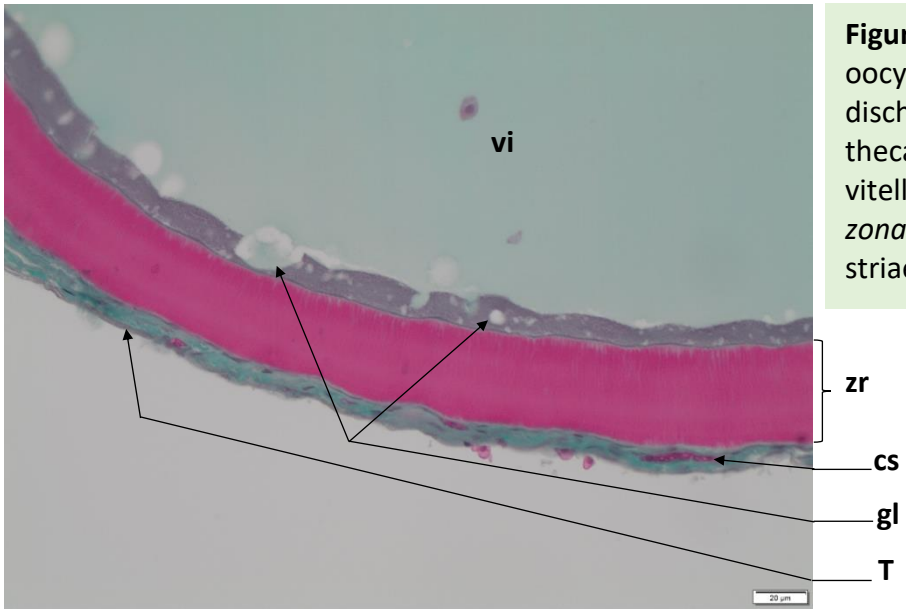


Figure 29 : Cell wall of a hydrated oocyte (**oh**) that has not been discharged (still encased in a theca (**T**)), with homogeneous vitellus (**vi**), lipid droplets (**gl**), a *zona radiata* (**zr**) with radial striae, and blood vessels (**cs**)

Post-Ovulatory Follicle (POF)

A Post-Ovulatory Follicle (**POF**) can show signs of lysis (presence of macrophage cells (**cm**) inside them). A **POF** indicates that a hydrated oocyte (**oh**) has been discharged into the lumen.

A **POF** is composed of all of the somatic tissues of the follicle (theca (**T**) and follicular cells (**cf**)) that have left behind after the mature oocyte (**oh**) has been discharged into the lumen. Their green dye is identical to that of connective tissue (**tc**). Be careful not to put a **POF** into the **tc** category. **POF** vary in size and shape, and are essentially empty sockets that have receded onto themselves.

Identification : A **POF** is **a sack of somatic tissue** that can eventually be identified by the **presence of macrophage cells inside its cavity**. Macrophage cells (**cm**) are very small, gray tinged, and more often than not are clustered together.

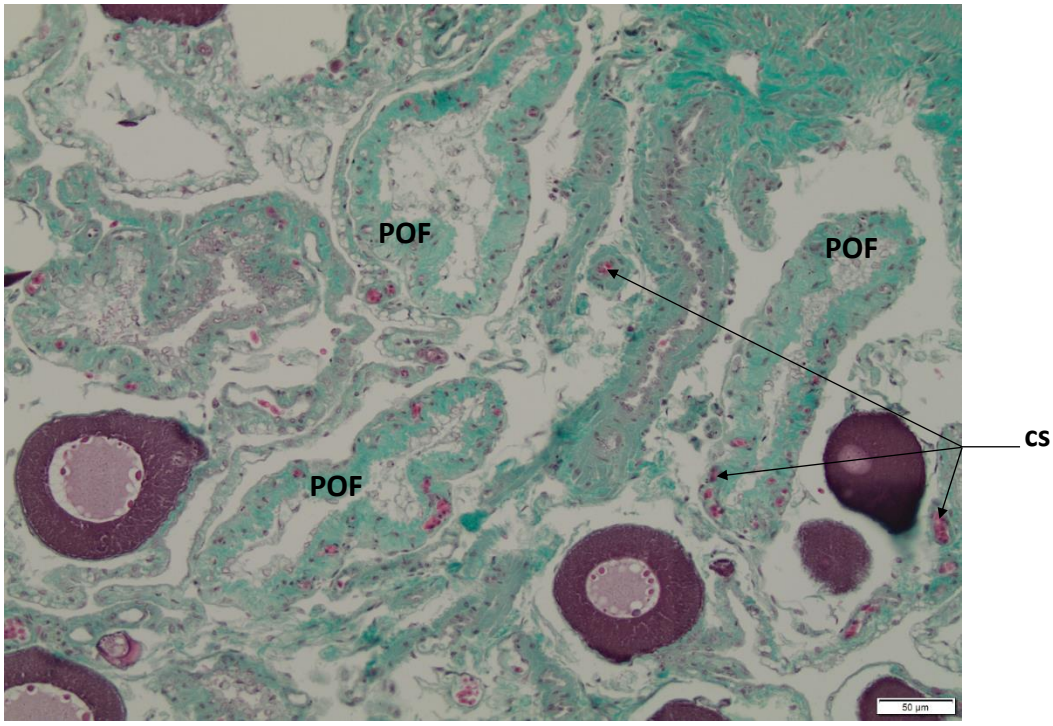


Figure 30 : Post-Ovulatory Follicles (**POF**) with blood vessels (**cs**)

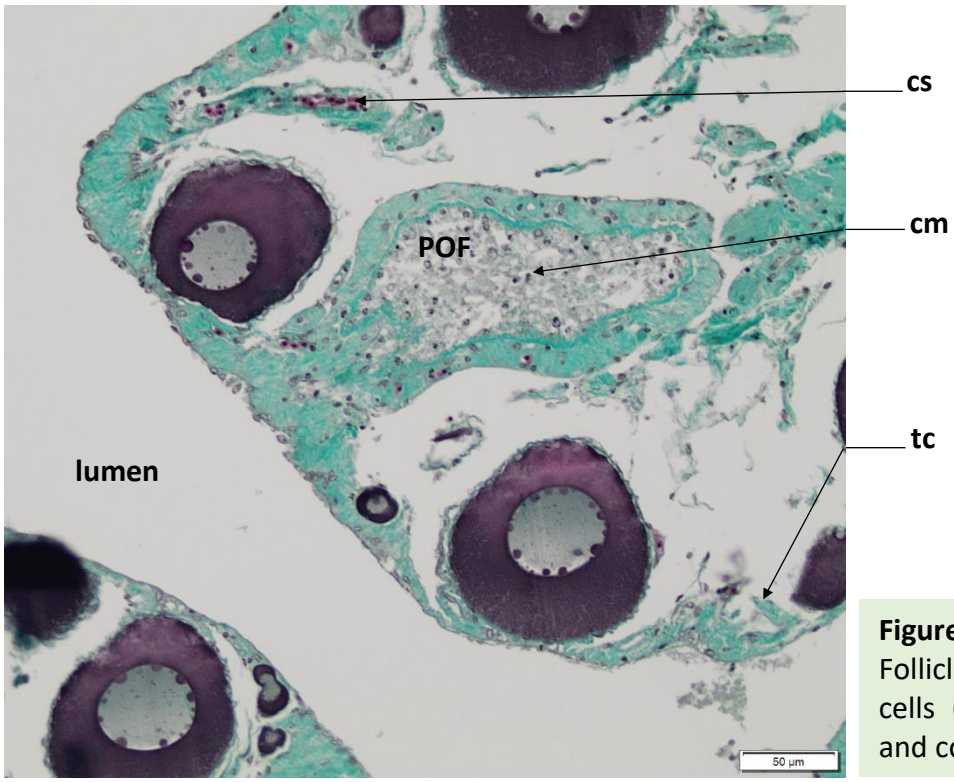


Figure 31 : Post-Ovulatory Follicle (**POF**) with macrophage cells (**cm**), blood vessels (**cs**) and connective tissue (**tc**)

Atresia (oaA & oaB) and Lysis (L)

Torres-Martìnez et al. (2017) define atresia as the degeneration, reabsorption, or even ablation, of ovarian follicles. In general, the first sign of atresia is the degeneration of the germinal vesicle of the oocyte, followed by the fragmentation of the *zona radiata* (**zr**) that will take an irregular appearance. This will lead to an hypertrophy of the follicular cells. There can be cell differentiations of the follicular cells (**cf**) into phagocytes and/or macrophages.

In this study, we will define two types of atresia. Oocytes in early atresia (**oaA**) and oocytes in late atresia (**oaB**). **oaA** are all germinal cells in lysis, from the oogonim (**ov**) stage until the **undischarged** hydrated oocyte (**oh**) stage. They will always be encased in somatic cells (follicular cells (**cf**) and/or theca (**T**)). **oaB** are all hydrated oocytes in lysis that have been **discharged**, and are thus not encased in somatic cells anymore. Since a **oaB** is a cellular structure in lysis in the middle of the **lumen**, their shapes will be more often than not warped, scattered and surrounded by lysis (**L**).

Identification : An **oaA** is a **follicle in lysis**. This means that the **oocyte is still encased inside a theca**, **within the ovarian lamella**.

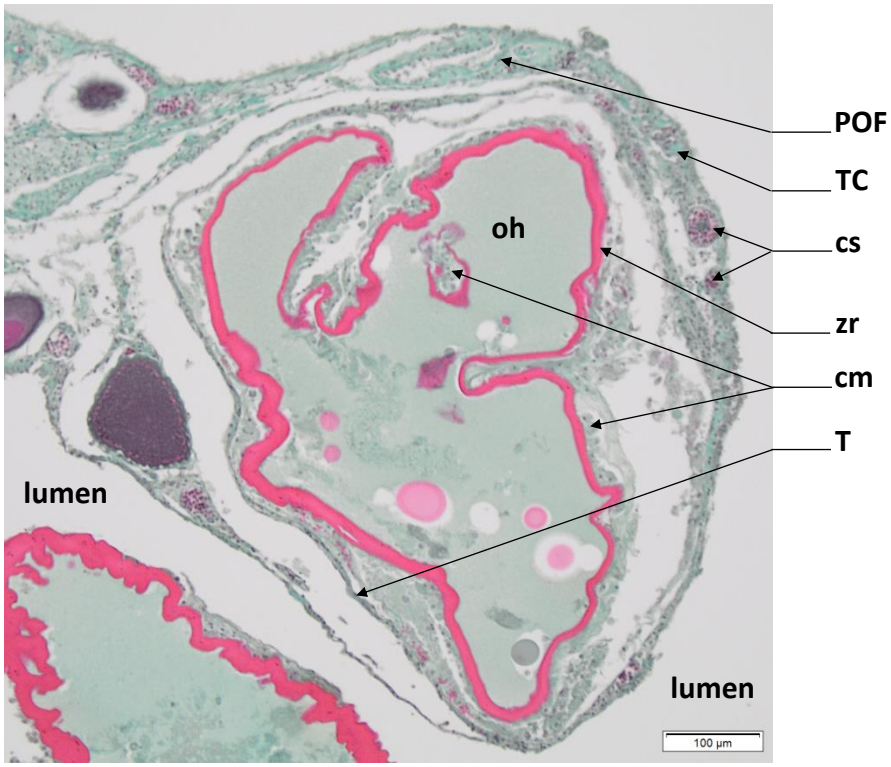


Figure 32 : Hydrated oocyte in early atresia (**oaA**). The hydrated oocyte (**oh**) has not been discharged into the **lumen**, and the follicle is still within the connective tissue (**tc**) of the ovarian lamella, encased by its theca (**T**) and surrounded by macrophages (**cm**) that are between the theca and the **zr**. Presence of **POF** and blood vessels (**cs**)

<u>Identification</u> : An **oaB** is an **oocyte in lysis**. This means that the **oocyte is not encased in a theca** anymore, and will be found **in the lumen of the gonad**. An **oaB will be encased in only its zr**. If a sampling point lands in a « white zone », even if it is surrounded by **oaB** structures, that sampling point will be categorized under **ei**. Moreover**, all lysis near the atretic mass of the oaB with a zr will be part of the oaB**.

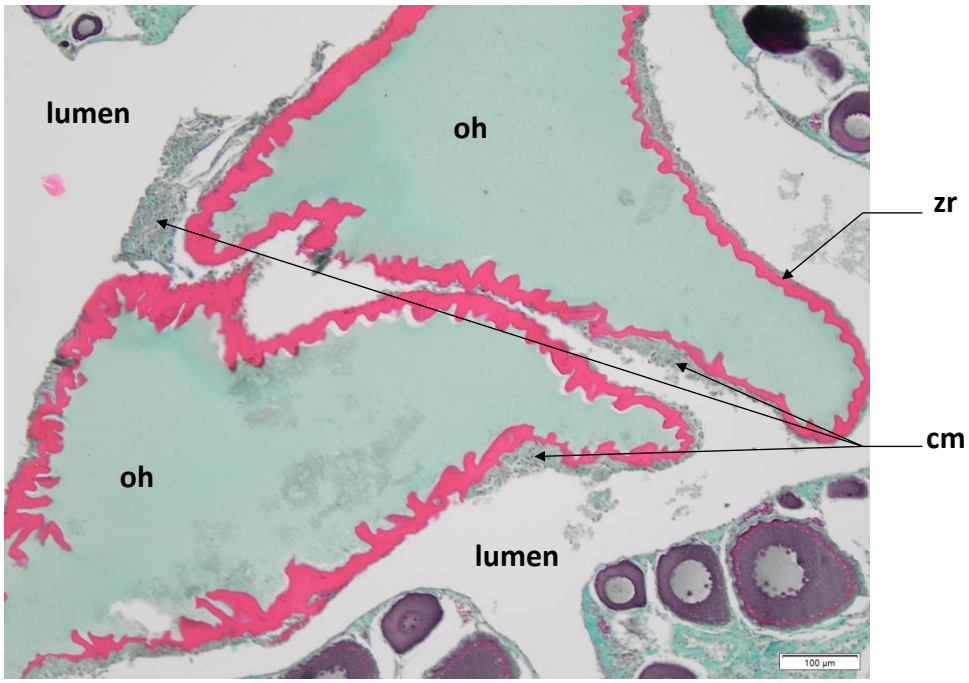


Figure 33 : Hydrated oocyte in late atresia (**oaB**). The hydrated oocyte (**oh**) has been discharged and is now in the **lumen** of the gonad, surrounded by macrophages (**cm**)

Identification : Lysis can be difficult to identify, especially with the presence of atresia. They can be found **anywhere inside the gonad**, **at any maturity phase**. This **cluster of cells in lysis** can contain macrophages, are of **different shapes and sizes**, and do **not possess a theca and/or zona radiata**

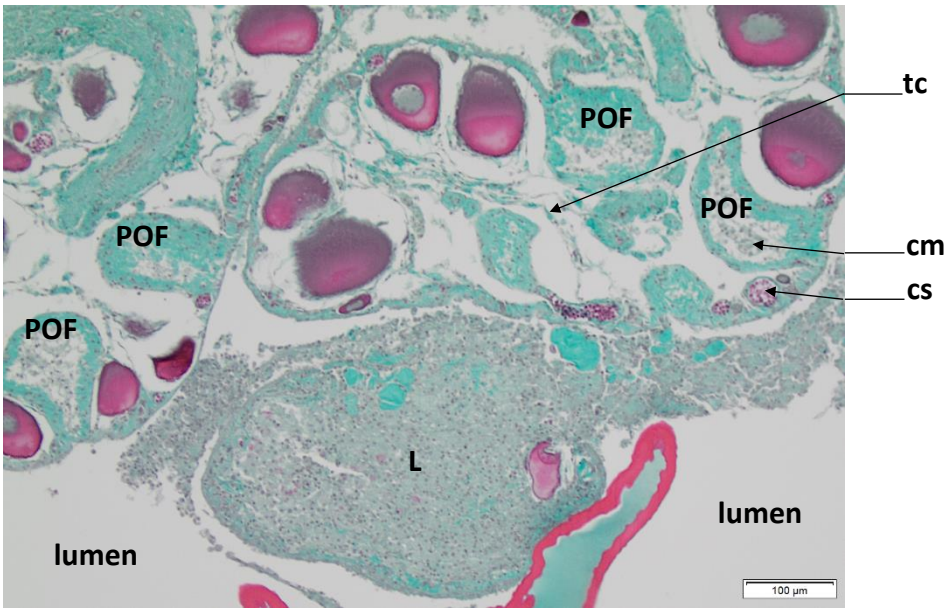


Figure 34 : Lysis (**L**) in the **lumen**, and post-ovulatory follicles (**POF**) with macrophages (**cm**) in their central cavity. Connective tissue (**tc**) and blood vessels (**cs**) are also present

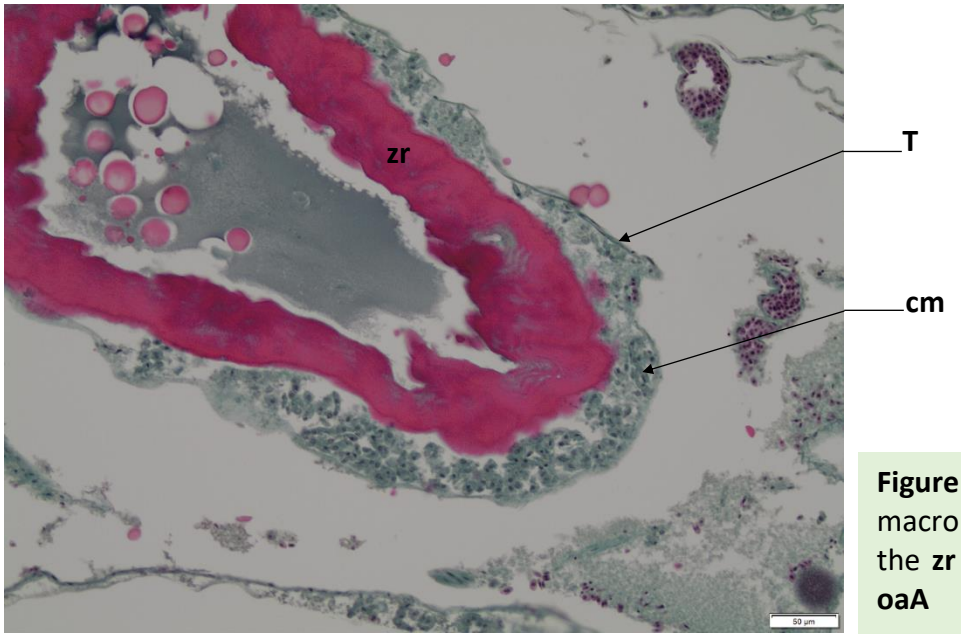


Figure 35 : Zoom on macrophages (**cm**) between the **zr** and the theca (**T**) of an **oaA**

Connective tissue (tc) & Gonadal wall (pg)

The connective tissue (**tc**) links all of the ovarian structures together to form the ovarian lamellas. Follicles can be found inside the ovarian lamellas, encased in **tc**.

Connective tissue (**tc**) is made of green-coloured cells, just like the cells that make up the gonad wall (**pg**). Gonad wall cells are muscular tissue while **tc** is not. Be wary not to mix the both up! Even if a sampling point falls on a very small piece of **tc** in the middle of a wide expanse of intercellular space (**ei**), this sampling point will be classified under **tc**.

The gonad wall will have the same green tint as the **tc**. Muscle bundles and blood vessels (**cs**) can be found in the **pg**. If a sampling point falls on muscular tissue within the **pg**, then the sampling point will be put into the **pg** category. On the other hand, if a sampling point within the **pg** falls on a blood vessel (**cs**), then this structure will be classified under **cs**.

The boundary of the **pg** starts from the outer most cell layer of the gonad and ends at the inner most cell layer of the gonad wall (**pg**), just before entering into the ovarian lamellas. If the **pg** has been stretched, ripped or spread, but that the sampling point lands between the inner and outer most cell layers before the ovarian lamellas, then the sampling point will be classified under the **pg** category. Do not hesitate to unzoom in order to obtain a more general view of where the sampling point is in the gonad. This will allow the reader a better view of where the **pg** ends and the **tc** starts.

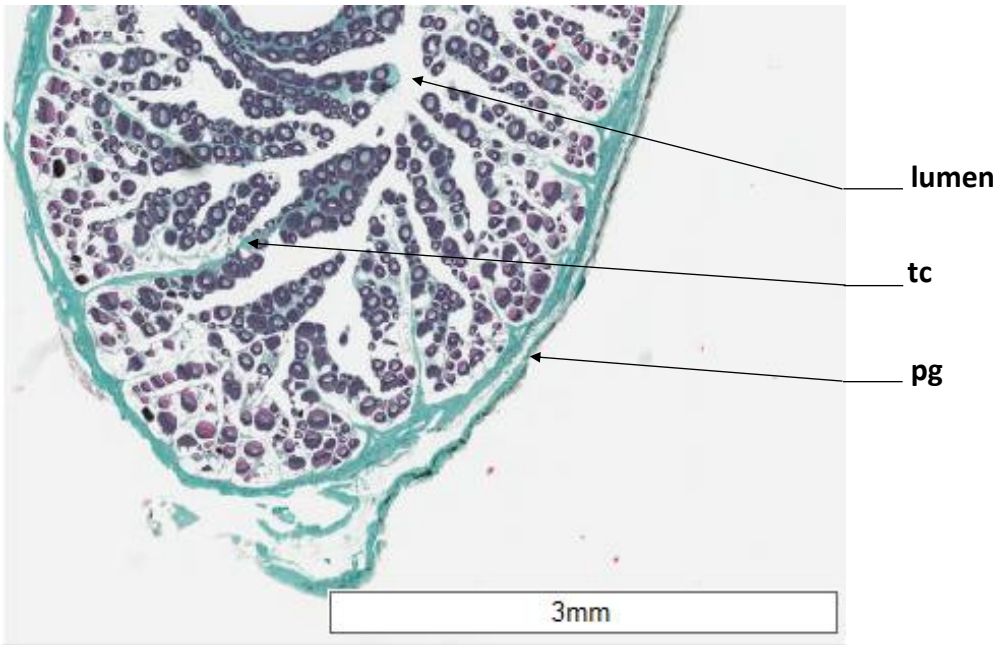


Figure 36 : Cross section of a plaice ovary encased in its gonad wall (**pg**). The ovarian lamellas are held together by connective tissue (**tc**), delimiting the **lumen**

Blood vessel (cs)

Blood vessels (**cs**) are more or less small, with a size that can vary from the size of a single cell to over 100µm.

Found within the theca (**T**), inside the connective tissue (**tc**) or the gonad wall (**pg**), blood vessels (**cs**) are identifiable by the presence of blood cells (small red cells with a darker nucleus). If a sampling point falls in the « white zone » inside a cavity containing blood cells, this sampling point will be classified under the **cs** category.

Intercellular space (ei) and Unnatural emptiness (v)

Inside this category (**ei**) are all of the sampling points that, to a pixel precise scale, will fall on a « white zone » within the gonad wall (**pg**) and/or connective tissue (**tc**).

In the case where cells are found outside of the gonad wall **pg** boundary, all « white zones » between the cells/follicles, or inside the **tc**, will be considered as **ei**.

Are set into the **v** category all sampling points that, to a pixel precise scale, will fall in a « white zone » that is outside of the gonad. This « white zone » is the space found between the **pg** the outline of the sampling grid around the gonad.

In the case that cells are found outside of the **pg** boundary, if the sampling point lands between the outline of the sampling grid and **tc** / **pg**, then this sampling point will be classified under the **v** category.

If a sampling point falls in a « white zone » surrounded by a theca and/or zona radiata, (the inner material of the oocyte having been ripped away), then put this sampling point under the **v** category.

Undetermined (i)

This category allows us to regroup all of the structures that can not be identified into one of the 20 structures cited previously. **When in doubt, it is preferable to use the undetermined (i) category, rather than placing a structure into a category it does not belong in.**

If two structures overlap one another under a single sampling points, the structure to identify will be the one that is the most underneath. If it is impossible to identify this structure (view obstructed by the structure on top), then place this sampling point in **i**.

If the sampling point falls on two different structures, or that the resolution of the picture does not allow to see what structure is pointed out, or that the reader takes more than 2 minutes to identify the structure, put those sampling points in the **i** category.

Categorize in **i** :

- blurry structures
- cells that do not have a clear and fully visible nuclear membrane
- structures that look like an **ov** but have no nucleus
- structures that look like an **op1** but have no nucleus
- structures that look like an **op2** but have no nucleus
- structures that look like an **oca** but have no nucleus
- all structures that do not appear in this lexicon or reading protocol (Sauger *et al.*, 2019)
- pieces of cells that have no theca, and that cannot be identified

Bibliography

Alonso-Fernández, **A.**, **Villegas-Ríos**, **D.**, **Valdés-López**, **M.**, **Olveira-Domínguez**, **B.**, **Saborido-Rey**, **F.**, 2013. Reproductive biology of pollack (*Pollachius pollachius*) from the Galician shelf (north-west Spain). J. Mar. Biol. Assoc. U. K. 93, 1951-1963.

Anderson, **E.**, 1968. Cortical alveoli formation and vitellogenesis during oocyte differentiation in the Pipefish, *Syngnathus fuscus*, and the Killifish, *Fundulus heteroclitus*. J. Morphol. 125, 23-60.

Aragón, **L.**, **Aranda**, **G.**, **Santos**, **A.**, **Medina**, **A.**, 2010. Quantification of ovarian follicles in bluefin tuna *Thunnus thynnus* by two stereological methods. J. Fish Biol. 77, 719-730.

Barr, **W.A.**, 1963. The endocrine control of the sexual cycle in the Plaice, *Pleuronectes platessa* (L). I. Cyclical changes in the normal ovary. Gen. Comp. Endocrinol. 3, 197-204.

Bazzoli, **N.**, **Godinho**, **H.P.**, 1994. Cortical alveoli in oocytes of freshwater neotropical teleost fish. Boll. Zool. 61, 301-308.

Bromley, **P.J.**, **Ravier**, **C.**, **Witthames**, **P.R.**, 2000. The influence of feeding regime on sexual maturation, fecundity and atresia in first-time spawning turbot. J. Fish Biol. 56, 264-278.

Brown-Peterson, **N.J.**, **Wyanski**, **D.M.**, **Saborido-Rey**, **F.**, **Macewicz**, **B.J.**, **Lowerre-Barbieri**, **S.K.**, 2011. A Standardized Terminology for Describing Reproductive Development in Fishes. Mar. Coast. Fish. 3, 52-70.

Brule, **T.**, 1987. The reproductive biology and the pathological changes of the plaice *Pleuronectes platessa* (L.) after the 'Amoco Cadiz' oil spill along the north-west coast of Brittany. J. Mar. Biol. Assoc. U. K. 67, 237-247.

Domínguez-Castanedo, **O.**, **Uribe**, **M.C.**, **Rosales-Torres**, **A.M.**, 2016. Morphological development of the structures related to annualism in the ovarian follicle of the killifish *Millerichthys robustus* (Costa,1995) (Teleostei: Cyprinodontiformes). J. Morphol. 277, 1219-1230.

Gabe, **M.**, 1968. Techniques Histologiques, 2ème ed. Masson et C., Paris.

ICES, 2008. Report of the Workshop on Maturity Ogive Estimation for Stock Assessment (WKMOG), 3-6 June 2008, Lisbon, Portugal. ICES CM2008/ACOM:33. 72 pp.

ICES, 2010. Report of the ICES/HELCOM Workshop on Flatfish in the Baltic Sea (WKFLABA), 8-11 November 2010, Öregrund, Sweden. ICES CM 2010/ACOM:68. 85pp.

ICES, 2013. Report of the Workshop on sexual maturity staging of cod, whiting, had-dock, saithe and hake (WKMSGAD), 4-8 November 2013, San Sebastian, Spain. ICES CM 2013:57. 51pp.

ICES, 2014. Report of the Workshop for maturity staging chairs (WKMATCH), 11-15 June 2012, Split, Croatia. ICES CM 2012/ACOM:58. 57 pp.

ICES, 2018. Working Group on Biological Parameters (WGBIOP), 1-5 October 2018. Ghent, Belgium. ICES CM 2018/EOSG:07. 186pp.

Lincoln, **R.F.**, 1981. Sexual maturation in female triploid plaice, *Pleuronectes platessa*, and plaice x flounder, Platichthys flesus, hybrids. J. Fish Biol., The Fisheries Society of the British Isles 19, 499-508.

Lowerre-Barbieri, **S.K.**, **Brown-Peterson**, **N.J.**, **Murua**, **H.**, **Tomkiewicz**, **J.**, **Wyanski**, **D.M.**, **Saborido-Rey**, **F.**, 2011. Emerging Issues and Methodological Advances in Fisheries Reproductive Biology. Mar. Coast. Fish. 3, 32-51

Miossec, **L.**, 1984. Altération de l'ovogénèse des Plies *Pleuronectes platessa* L. capturées dans les abers Wrac'h et Benoit, depuis la pollution de l'Amoco-Cadiz. Rev. trav. Inst. pêches marit. 46, 195-207.

Sauger, **C.**, **Quinquis**, **J.**, **Dubroca**, **L.**, **Kellner**, **K.**, **Lepoittevin**, **M.**, **Heude-Berthelin**, **C.**, **Elie**, **N.**, 2019. Protocol for the determination of histological structures found in the ovaries and during the oogenesis of the European plaice, *Pleuronectes platessa* (Linné, 1758). https://doi.org/10.13155/61235

Torres-Martínez, **A.**, **Hernández-Franyutti**, **A.**, **Uribe**, **M.C.**, **Contreras-Sánchez**, **W.M.**, 2017. Ovarian structure and oogenesis of the extremophile viviparous teleost *Poecilia mexicana* (Poeciliidae) from an active sulfur spring cave in Southern Mexico. J. Morphol., Wiley Periodicals 278, 1667-1681.

Tyler, **C.R.**, **Sumpter**, **J.P.**, 1996. Oocyte growth and development in teleosts. Rev. Fish Biol. Fish., Chapman & Hall 6, 287-318.