3D imaging-based analysis of the germline in teleost

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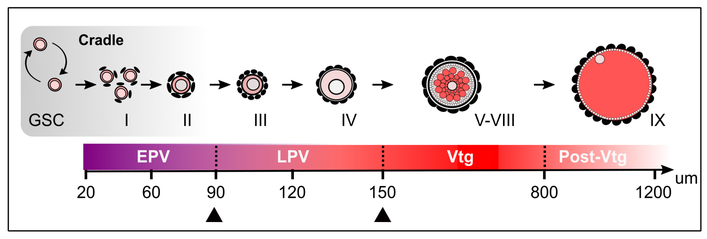
Context and objectives

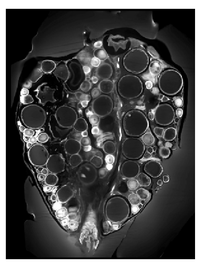
- In mammals, the stock of oocytes is predetermined at birth, whereas in teleosts, the oocyte reserve is thought to have the potential to be renewed throughout life
- The reserve is used in adults to produce mature oocytes and replenished from germline stem cells
- Germline stem cells are organized in germline cradles surrounded by somatic cells expressing sox9 [1]

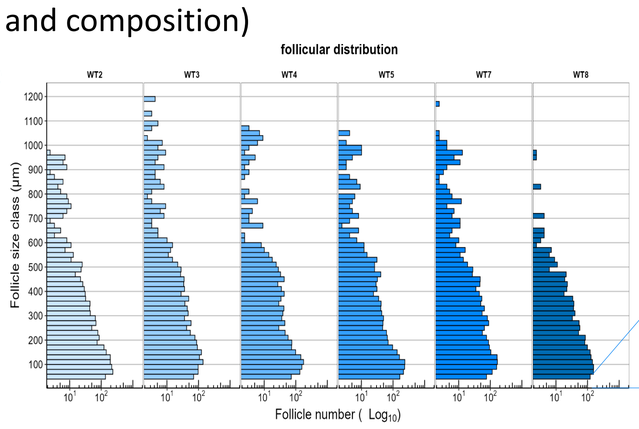


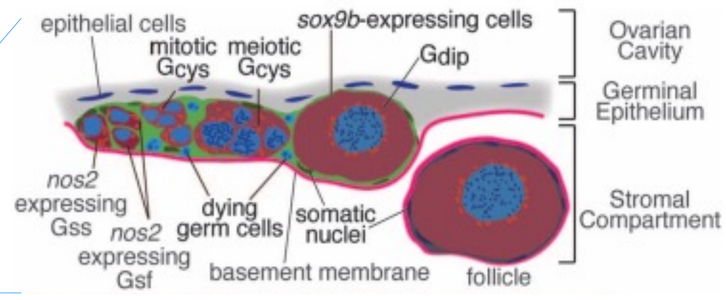
Is the renewal of the oocyte reserve by germline stem cells continuous throughout the female lifespan, and how is it controlled?

Exploration of the oogenesis renewal process and its control using the medaka as a model organism and the development of a 3D imaging strategy for the whole ovary, to provide **quantitative data to study the cell dynamics of germinal cradles**.

- Study oocyte reserve formation and renewal (size, distribution and composition)
- Identify the molecular regulators impacting the oocyte reserve





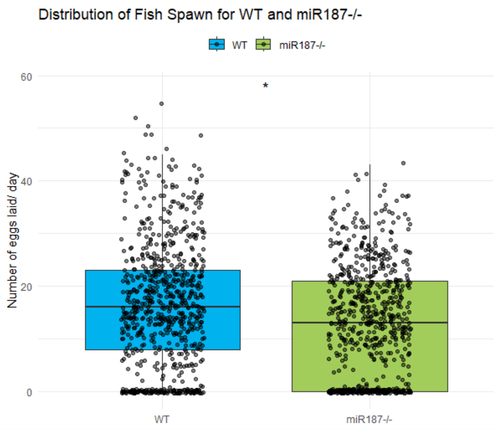


Changes in follicle distribution under the influence of hormonal regulators (FSH, LH, AMH): what regulatory mechanisms affect germline cradles (feedback loops? miRNAs?) and hence maintenance of oocyte reserve?

A potential regulator of this reserve

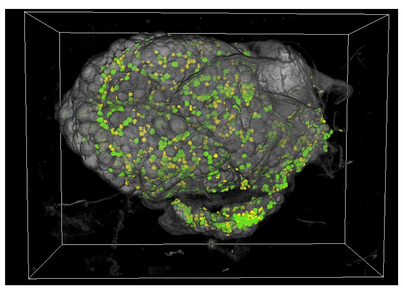
KO of miR-187 :

- Fewer eggs laid, lower female fecundity
- Increase in the number of stage I follicles



Yellow = Stage I follicles (20µm to 60µm)
Green = Stage II follicles (61µm to 90µm)

90 days juvenile



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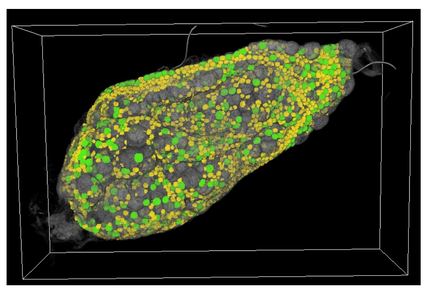

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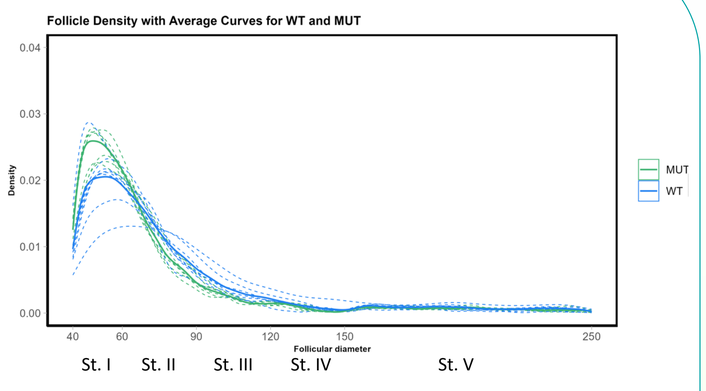


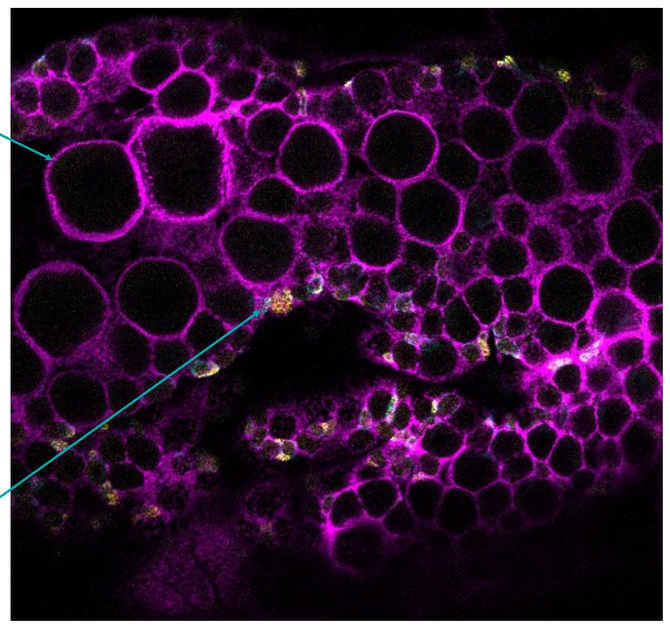
Hypothesis : involvement of miR187 in the transition from stage I to stage II, that is, in the exit from the germinal cradle and consequently in the formation of the oocyte reserve

- Study of germ cell proliferation (PH3 labeling)

Imaging and image analysis

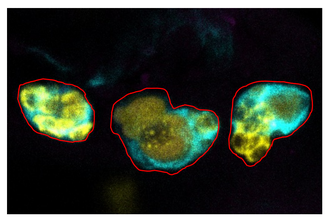
Development of a whole ovary immunolabeling protocol combined with the C-ECi clearing protocol to image the germinal cradles and follicles. Completion of the protocol followed by the transfer of samples to agarose for **3D imaging using Light Sheet Blaze imaging** (APEX platform, ONIRIS, Nantes).

Denoising and segmentation of images using the **N2V** module and Cellpose for the study of follicle distribution, followed by error correction and diameter measurement with **AMIRA software**. Adaptation of these analysis steps for the quantitative study of germinal cradles.



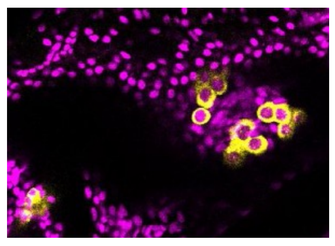
Nuclei in magenta / somatic cells expressing sox9 in cyan / germ cells expressing vasa in yellow

1- Study the distribution and number of cradles in medaka ovaries



- Denoising with N2V
- Segmentation of vasa-positive germline stem cell clusters surrounded by somatic cells expressing sox9 with Cellpose_Napari

2- Study the composition of germinal cradles



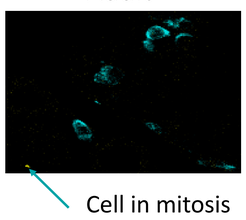
- Difficulty : vasa-labeling is cytoplasmic so that these cells are difficult to segment individually
- Use of MG core labeling to individually separate vasa-positive cells with Cellpose_Napari

3- Study the proliferation of germ cells

Juvenile

Cell in mitosis within a cradle

Adult



Cell in mitosis (out of the germinal cradles)

- Combination of PH3-labeling with vasa-labeling to verify that the mitotic cells in germinal cradles are indeed germ cells
- Segmentation of PH3-positive germ cells to count the number of dividing germ cells per germinal cradle

Prospects

- Develop workflow for germline cradles image analysis (numbering, distribution and composition)
- Develop workflow for study germ cells proliferation by using PH3 labeling

References

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