COLLEGE DOCTORAL BRETAGNE SCIENCES DE LA VIE ET DE LA SANTE

Year 2022-2023

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☐ **Requested** ☒ **Acquired**	**Funding origin: INRAE/ANR**
Thesis title: **Role of miRNAs in the control of oocyte reserve in fish (RESOV)**		3 keywords: Oogenesis MicroRNA 3D bio-image analysis
Research Unit / team: **LPGP, INRAE**		
Director's name: **Violette THERMES (directrice) et Frédérique CLÉMENT (co-directrice)**		Phone number: 02.23.48.57.20 Email address: violette.thermes@inrae.fr Year of HDR : 2022

Socio-economic and scientific context (approximately 10 lines):

In fish, female fecundity is mainly based on the primary oocyte stock within the ovary, from which the mature oocytes laid during each reproductive cycle (*i.e.,* eggs) are formed. The establishment of this reserve and its regulation determines the number of eggs laid during the life cycle (size and frequency of laying) and thus determines the fecundity of females. Our research aims to understand the fundamental processes that govern this oocyte reserve in order to identify potential levers for improving the management of both wild and farmed fish.

In iteroparous fish, and in contrast to mammals, it is well accepted that the oocyte reserve is in constant renewal, but the mechanisms underlying the establishment of this reserve and its regulation in the adult are still not elucidated. One likely hypothesis is that, as in mammals, there is an inter-follicular dialogue through paracrine factors such as anti-müllerian hormone (AMH), which would allow the regulation of the reserve status. There is also another class of molecules, small non-coding RNAs (or miRNAs) whose involvement in the ovary has long remained uncertain, but which are now emerging as important novel players in the control of oogenesis. In the laboratory, we have recently identified that two miRNAs, miR-202 and miR-187, are key regulators of female fecundity in Medaka and that they are potentially involved in the control of the oocyte reserve.

Working hypothesis and aims (approximately 8 lines):

The research issue is the establishment of the oocyte reserve in the larva and its regulation in the adult. The aim is to determine the role of the two miRNAs identified in the laboratory in the formation of the reserve in the larva and its renewal in the adult, and to understand the underlying mechanisms. We thus hypothesize here that these two miRNAs (miR-202 and miR-187) are involved in the regulation of oocyte reserve and the objective of the project will be to:

1) Describe the contribution of germline stem cells in reserve formation in the larva and its renewal in the adult.

2) Understand how miR-202, miR-187 and AMH influence the state of the reserve (formation, renewal).

3) Determine by which molecular mechanisms these two miRNAs regulate these processes.

Main milestones of the thesis (approximately 12 lines):

The project will be conducted in four steps:

- **Task 1:** First, we will focus on the description of the development and maintenance of the oocyte reserve during the female life span (larvae, juvenile and adult stages), and in particular we will describe the evolution of germline stem cells (number, distribution and cellular activity) by 3D imaging approaches (confocal microscopy) on ovaries after specific fluorescent labeling (transgenic line *sox9::gfp*, immunohistochemistry, RNAscope *in situ* hybridization).

- **Task 2:** In parallel and with the same approaches as in task 1, we will study the reserve status and its evolution in *miR-202 -/-*, *miR-187 -/-* and *AMHR2 -/-* (Hotei) mutant lines, at larvae, juvenile and adult stages.

- **Task 3:** We will also investigate the molecular pathways targeted by miR-187 and miR-202. We will search for direct targets using a transcriptomic approach (RNAseq), which will consist in identifying candidate genes showing low inter-individual variability in WT, over-expression in the mutant and that possess the miRNA binding site in their 5'- or 3'-UTR region (*in silico* analyses).

- **Task 4:** Finally, we will validate the most promising targets (2 or 3 per miRNA) by analyzing their cellular expression in the ovary, in order to validate their co-expression with miRNAs, and by analyzing the reproductive phenotype of females after depletion of the miRNA binding site (by CRISPR/Cas9 knock-out).

Scientific and technical skills required by the candidate (2 lines):

A strong background in developmental, cellular and molecular biology, basic knowledge in microscopy, and knowledge in image bioinformatics will be welcome.

3 publications from the team related to the topic (last 5 years):

(1) M. Lesage, J. Bugeon, T. Pécot, M. Thomas, T.-K. Ly, N. Hinfray, R. Beaudouin, M. Neumann, R. Lovell-Badge, **V. Thermes**, An end-to-end pipeline based on open source deep learning tools for reliable analysis of complex 3D images of ovaries. https://doi.org/10.1101/2022.08.03.502611. (*In revision in Development, Technics and Ressources)*

(2) S. Gay, J. Bugeon, A. Bouchareb, L. Henry, C. Delahaye, F. Legeai, J. Montfort, A. Le Cam, A. Siegel, J. Bobe, **V. Thermes** (2018-09-10).

MiR-202 controls female fecundity by regulating medaka oogenesis. *PLoS Genetics,* 14 (9), 1-26, https://dx.doi.org/10.1371/journal.pgen.1007593, https://hal.inrae.fr/hal-01871468

(3) **F. Clément**, D. Monniaux. Mathematical modeling of ovarian follicle development: A population dynamics viewpoint. *Current Opinion in Endocrinology and Metabolism Research*, 2021, 18:54-61. https://doi.org/10.1016/j.coemr.2021.02.003, https://hal.inria.fr/hal-03146955

National and international collaborations:

Minoru Tanaka, Laboratory of Reproductive Biology, Nagoya University, Japan