



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Swapnil Gorakh Waghmare, Ph.D.	First name(s), surname, titles of supervisor: M.Sc. Azin Mohagheghi Samarin, Ph.D.
Title of PhD thesis: Fish oocyte ageing and histone modifications	

REVIEWER:

Surname: de Oliveira Fernandes	Institution: Nord University Bodø Norway
Name: Jorge Manuel	
Titles: Prof.	E-mail: jorge.m.fernandes@nord.no
Please describe your professional relationship to the PhD student: None	Please describe your field of expertise: Epigenetics, Genomics, Aquaculture, Immunology and Muscle Development

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

The doctoral dissertation submitted by Mr. Waghmare represents an independent and original scientific work of high academic standard at an international level.

The topic of this thesis is timely and relevant from both basic and applied research perspectives. Post-ovulatory oocyte ageing results in a progressive loss of quality, which impairs fertilisation rates and increases the frequency of developmental abnormalities. Determinants of fish egg quality have been extensively studied in laboratory models and various species of commercial importance but our current knowledge about the epigenetic mechanisms involved in post-ovulatory ageing in fish is very limited.

In spite of being purely descriptive, this is one of the first studies in fish to reveal that histone acetylation may be an important epigenetic regulator during fish oocyte ageing. Hence, it clearly contributes to advance the state of the art. In particular, this thesis sheds light on the molecular mechanisms involved in oocyte ageing, and may have an impact in aquaculture and even assisted reproduction technology in other vertebrates.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):

This PhD thesis consists of an introductory section, a general discussion and three experimental chapters. In spite of being well structured and logically organised, there are numerous typos and grammatical mistakes scattered throughout the thesis. Also, the quality of the figures is not acceptable.

The approach and methodology are classical (e.g., Western blotting and enzymatic activity assays) but appropriate to achieve the overarching goal of the thesis, which is to investigate the possible alteration in histone modifications during fish post-ovulatory oocyte ageing. The results are generally sound but would be more convincing if more biological replicates had been used.

Two papers have been published in reputable journals with peer review and the candidate is first author in both articles. He is third author in the multi-author manuscript that is unpublished. The papers are original and have a significant impact in the field.

The fifth objective "Examining the expression level of genes involved in DNA methylation (*dnmt1*, *dnmt3*, *tet3*), histone modification (*hat1*, *myst1*, *hdac1*, *hdac9b*, *sirt1*) as well as the transcripts associated with fertilization and embryo development (*igf2*, *oct4*, *npm2b*, *dicer*, *trim35* and *vasa*) in zebrafish" is not fulfilled. This is a flaw that must be corrected, either by including the corresponding experimental chapter or excluding this objective from the thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

Introduction

The introduction is generally well written, providing a good and up to date background of the most important subjects dealt with in the subsequent papers. Nevertheless, it could be more comprehensive at times. In particular, the thesis would benefit from an introduction to oocyte development in fish, which is central to all experimental chapters.

Most references cited are relevant and up-to-date. However, it is not always clear if the statements in the introduction refer to fish or mammalian literature. The background review should be revised to correct this and appropriate references in fish should be included, when available. For example, Zhu et al (2019, PMID: 30922236) could be cited in section 2.2 on histone methylation.

The bibliography needs attention, since the references do not have a uniform style (e.g., journal names). The figures used are appropriate but the citation in the legend of figure 3 is incorrect.



The aims are clearly stated after the general introduction and four out of the five objectives are adequately linked to the three experimental chapters.

*Paper I - Oocyte ageing in zebrafish *Danio rerio* (Hamilton, 1822) and its consequence on the viability and ploidy anomalies in the progeny*

This paper was peer reviewed and published in *Animals* (MDPI) in 2021. It is a sound descriptive study, in which the authors examined the duration and effect of oocyte ageing in zebrafish (*Danio rerio*). One of the main findings was that post-ovulatory oocyte ageing in zebrafish has a considerable influence on the developmental competence of the embryos.

*Paper II - Histone acetylation dynamics during in vivo and in vitro oocyte aging in common carp *Cyprinus carpio**

This study was peer reviewed and published in the *International Journal of Molecular Sciences* (MDPI) in 2021. Investigation of oocyte aging on specific histone acetylation in common carp *Cyprinus carpio* revealed that global H4K12ac levels increased significantly at 28 h post-stripping. It is noteworthy that this is the first report of histone modifications as possible epigenetic regulators during oocyte ageing in fish.

*Paper III - In vitro post-ovulatory oocyte ageing affects H4K12 acetylation pattern and histone acetyltransferase activity in grass carp *Ctenopharyngodon idella**

This manuscript is unpublished. The aim of this study was to investigate the dynamics of histone acetylation in grass carp oocytes, in order to better understand the underlying molecular mechanisms of post-ovulatory oocyte ageing.

There are several methodological details missing, namely i) the origin of the fish, including what is known about their genetic background, ii) husbandry conditions, iii) the rate of water temperature increase to 25 °C, iv) the positive and negative controls used in quantification of histone acetylation levels from Western blots, and v) the number of embryos examined to determine survival rates.

The discussion is at times repetitive (e.g., the roles of *sirt2* and *rpd3* in oocyte ageing). Also, the differences H4K12ac during oocyte ageing between mammals and fish, and between common and grass carp are not sufficiently discussed.

General discussion and conclusions

The discussion reads well but it does not go much further than what is already stated in the introduction and in the experimental chapters. It would have been relevant to discuss the results of paper I in the context of circadian rhythmicity of the oocytes during ageing and the limited number of replicates in paper II, given the individual variation in histone acetylation levels. In particular, the discussion would benefit from a critical evaluation of the methods used in the thesis and their comparison with the state-of-the-art (e.g., ChIP-seq and ATAC-seq).

The conclusions provide a good summary of the main findings. The apparent contradiction between the decrease in H4K12ac levels with oocyte ageing in grass carp and the concomitant increase in histone acetyltransferase activity needs to be elaborated. Stating



that “the results of our study suggest the possible development of an epigenetic marker for fish oocyte ageing” is too vague. It should be further explained how such marker can be developed and used, since there are few consistent changes in acetylation levels with oocyte ageing and they vary between fish species.

Required corrections

1. Address the issues above regarding the fifth objective of the thesis, introduction and general discussion.
2. Replace the current figures with high-resolution images.
3. Format the bibliography.
4. Proofreading by a native English speaker.

FINAL RECOMMENDATION

- ☐ PhD thesis can be recommended for defence
- ☒ PhD thesis can be recommended for defence with reservations
- ☐ PhD thesis cannot be recommended for defence

Jorge M. O. Fernandes

Bodø, 29 December 2021

.....
Date and place


.....
Name and signature