



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Zuzana Linhartová, M.Sc.	First name(s), surname, titles of supervisor: Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: Micromanipulation and cryopreservation of germ cells in fish	
REVIEWER:	
Surname: Robles Rodríguez	Institution: Spanish Institute of Oceanography (IEO) El Bocal, Barrio Corbanera s/n 39012 Monte, Santander, Spain
Name: Vanessa	
Titles: Ph.D.	E-mail: robles.vanesa@gmail.com
Please describe your professional relationship to the PhD student: No relationship	Please describe your field of expertise: Reproductive Biology and Cryobiology

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**):

This PhD thesis deals with one of the most promising fields in Aquaculture. Primordial germ cell biology opens new possibilities to develop biotechnological tools and offers a new scenario for actual Aquaculture Industry with techniques such as surrogate production. Therefore, we can consider this thesis in the cutting edge Research in the field of Aquaculture and the results present high potential impact and great relevance in the field.

The thesis has been conducted in a group recognized at international level and leader in the field. Taking this into account, together with the scientific publications in peer-reviewed international journals, some of them in Q1, derived from this thesis, and all the presentations in national and international scientific meetings, the full competitiveness of the PhD thesis in the international context is guaranteed.



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**):

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

In general, the quality of this thesis is very high. It is very well elaborated in scientific terms and also it is well structured and comprehensible for the reader. It is evident from this PhD thesis that the PhD student obtained a high-level scientific training and as a result high quality scientific results were produced. The PhD student is first author in 3 of the publications that conform this thesis, which guarantee her crucial contribution in the work.

The objectives in this study were ambitious but well focused on crucial aspects and the approaches used for their consecution combined originality and scientific rigour.

The thesis is not only focused in one species, but the selection of the different species is justified in each case, as an example, the selection of tench is justified for its importance as a donor species for the future transplantation in a smaller and faster reproducing fish species. In the case of sturgeon, it is an endangered species of large body size and long reproductive cycle that could be use as a donor using sterlet as a host in the surrogate production procedure, which would result in obtaining germinal cells in a shorter time and from smaller individuals (simplifying therefore their maintenance).

The thesis is organized in seven different chapters:

Introduction (chapter 1) covers all the crucial aspects necessary to provide a general overview on the state of the art and most relevant publications on the cited topics are included in the reference list. Introduction is well written and the figures included are very helpful for understanding the text, in particular figure 3.

Chapters 2-5 correspond to 4 articles published in peer-reviewed journals. All of them significantly contribute, from my point of view, to broaden the knowledge in the fish Reproductive Biology field. As expected for a peer-reviewed article, the scientific question in all of them is relevant and the methodology is explained in detail. It is relevant to mention that some of them are particularly laborious work, as an example, micromanipulation techniques required high technical skills from the operator.

Chapter 6 corresponds to a manuscript with the aim of developing a successful sterilization method in sturgeon using a knocking down strategy. Sterilization is required in surrogate



production to avoid the host contribution to the germ line. This study provides promising results and therefore I envision its successful publication.

General discussion (chapter 7) is short but very well written and decisive for providing thesis homogeneity encompassing the different results from the different chapters. The majority of the articles relevant in this area are also reflected in the general discussion reference list.

The PhD student CV (also included in chapter 7) provides important information about the student scientific training. Scientific maturity of the PhD student is also reflected in the fact that she has collaborated in the supervision of other students in the lab and accepted responsibilities on project leadership.

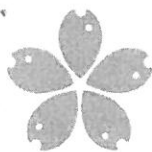
For all the above mentioned reasons, I consider this thesis is a very rigorous and valuable scientific work, of high scientific importance at international level and I fully support the PhD thesis defence.

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

June 5th 2015, Santander, Spain
Date and place

Vanesa Robles
Name and signature



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Review of USB FFPW PhD Thesis

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Title of PhD thesis: Micromanipulation and cryopreservation of germ cells in fish	
REVIEWER:	
Surname: Cabrita	Institution: Faculty of Sciences and Technology (FCT) and Centre of Marine Sciences (CCMAR), University of Algarve Campus de Gambelas 8005-139 Faro Portugal
Name: Elsa	E-mail: ecabrita@ualg.pt
Titles: Assoc. prof.	
Please describe your professional relationship to the PhD student: I have no professional relationship with the PhD student	Please describe your field of expertise: Cryopreservation, spermatogonia, sperm quality, broodstock management

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 work developed by the candidate is original and presents an interesting contribution to the state of art on germ cell biotechnology applications. The basic research developed can have a tremendous impact on applied research, especially with application to endangered species, such as sturgeons, one of the groups studied in this thesis. The results will indeed have an impact on strategies for cryobanking and reproduction, allowing the conservation of these species.

The work also opens new perspectives of utilization of the techniques developed to other species, which would solve the reproductive problems in other genera. This could be important not only from a productive point a view but also from a conservation point of



view.

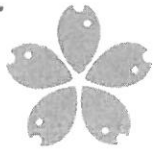
Elaboration of the PhD thesis, objectives of the work and deliverables

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**):

In the present thesis the objectives presented by the candidate were all achieved successfully as it can be seen by the publications presented in chapter 3, 4, 5 and 6. The work is well structured by a synthetic introduction to topics, that in some cases could have been somehow extended specially in topics on “**Micromanipulation of germ cells**” and in “**Isolation and cryopreservation of germ cells**”. Then the thesis presents 5 chapters, corresponding to the scientific publications resulted from the work, where two are from sturgeon species and another two from cyprinids, all of them in the topic of stem cells (primordial or spermatogonial). Chapter 2 (**Morphology and ultrastructure of beluga (*Huso huso*) spermatozoa and a comparison with related sturgeons**) is slightly moved from the objectives of the thesis since as mentioned they are:

- 1) To document the embryogenesis and larval development of selected fish species, along with the origin and migration routes of germ cell lineage.
- 2) To develop a practical technique for isolation and cryopreservation of early stages of germ cells of selected fish.
- 3) To identify germ cell responsible genes and develop an efficient method of sturgeon sterilization.
- 4) To investigate germ-line chimerism within fish.

I agree with the statement of the thesis that says that this thesis is composed by “*several studies with differing focus of research but with one target goal – to induce germ-line chimerism in fish. All these results are prerequisite of future application and development of surrogate production in these species*”. According to this statement on final page of discussion, chapter 2 was not necessary to achieve the goals proposed on the presented objectives.



The approaches followed to achieve the main objectives were very innovative as is demonstrated in chapter 6. After the scientific chapters, the thesis is followed by a general discussion (Chapter 7), that integrates all the chapter information and main conclusions/ achievements demonstrated.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This thesis gathers all the scientific information to achieve the main goal, which was highlighting the procedures for germ cell isolation, manipulation and transplantation in two species with different background and interests. The *Tinca tinca* is a species with important commercial interest in aquaculture, and the presented work will contribute to the improvement and development of new reproductive strategies for this species production increase. This is highlighted in chapters 3 and 4, where the work developed have contributed to the basic research on the ontogeny of germ cell development and migration. In parallel the work assured the preservation of this material using techniques such as cryopreservation to maintain the germline. The work developed in sturgeon species from the genus *Acipenser* (chapter 5 and 6) will contribute to the knowledge needed for the development of strategies for the conservation of these species. Several sturgeon species are categorized in the Red List (IUCN 2014) as critically endangered and new approaches need to be developed to reproduce successfully these species in captivity. This will certainly contribute also to the production and restocking of these individuals. The research developed under this thesis opens a window of knowledge to the implementation of such techniques at a higher scale.

The thesis is therefore innovative and has a substantial impact in the fields of aquaculture and conservation, especially for the production, protection and conservation of the target species. The subject is worth of being investigated and can contribute to several areas: the improve knowledge by fundamental research, create conservation measurements and restock endangered populations. This is a



comprehensive, well written (especially the chapters corresponding to the scientific publications) and organized study. The state of art presented is cohesive, although as referred previously, some parts could have been extended. The outcomes will be important for the research in the area and will be fundamental for future research. The scientific chapters were published in relevant high impact journals (*Animal Reproduction Science, Journal of applied Ichthyology, Czech Journal of Animal Science, Theriogenology*), which will contribute to the dissemination of the research developed. The candidate is first author in four of the manuscripts (one still in revision) and co-author in another one. This thesis research involved multidisciplinary subjects and most of the work is supported by three main areas, cryopreservation, cell biology and molecular biology. For all these reasons I strongly recommend the defense of this thesis.

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

FAB, 15th JUNE 2015

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Date and place

ELSA CABRITA

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Name and signature