Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Xie Xuan, M.Sc.	**First name(s), surname, titles of supervisor:** Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: In vitro culture of sturgeon germ stem cell	

REVIEWER:

Surname: Herráez	**Institution**: University of Leon, Spain
Name: María Paz	
Titles: Prof. Dr.	**E-mail**: paz.herraez@unileon.es
Please describe your professional relationship to the PhD student: We do not have any professional relationship	**Please describe your field of expertise:** I'm researcher in the field of fish reproduction, particularly on spermatology and germ cell biology

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 development of reproductive biotechnologies is a very dynamic field which is boosting the advances in human and animal reproduction. The management of fish populations, either from the wild or from aquaculture, also could benefit from these technologies. Among them, the surrogate production is considered a very promising tool to be applied in conservation or farming programs. Considering that fish are the vertebrate goup including a higher number of species, showing very diverse reproductive strategies, and that our knowledge about basic aspects of their reproductive control is still poor, the development of specific reproductive biotechnologies to be applied in a particular group of species, requires still an important effort on basic research.

This thesis contributes to the field providing a detailed work on the identification, isolation and culture of germ stem cells from sturgeon, one of the best target species for the application of surrogate production. The obtained results represent an important step in the development of this technology, because one of the bottleneckes is the unsuficient supply of germ cells. The possibility to isolate the earlier germ cells (spermatogonia A) from sturgeon and to expand them in culture, would provide the scientists with the required matherial not only for the development of surrogate production, but also for in vitro studies on the control of gametogenesis. The applied aspects of this thesis are thus clear and relevant.

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, logicality 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)**:

The objectives of the thesis were clear and match perfectly with the chapters. The first one, which is necessary in any thesis was "Summarize current knowledge regarding…" I consider that this kind of review is not a specific objective of the thesis, and better corresponds to a good introduction which shows the rational for the choice of the real objective: "to establish efficient methods with respect to identification, enrichment and long-term culture condition of germ cells in sturgeon". These 3 aspects (identification, enrichment and long-term culture) were covered by the 3 actual objectives. The 3 aspects were formulated in the more rational order but were approached in the reverse: culture, enrichment, identification. The organization of the experimental procedures is always dependent on such a number of conditioning factors, that is perfectly understandable that the "optimal" has been substituted by the "possible". Once the procedures for the identification, isolation and culture have been stablished, the culture can be performed with the properly isolated cells to optimize the whole protocol.
The applied methods have been similar to those previously developed by other researchers in other species. There are not "original" approaches, but the obtained results are unique for sturgeon and provide interesting and translational information.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The thesis is made of a general introduction, 4 chapters -two of them already published in indexed journals- a general discussion and the conclusions.
The introduction is the weakest part of the thesis, because is not well written, do not define the concepts with clarity, the english should be reviewed and not even is respectful with the guideliness related to citations (citations are not homogeneous, some times the name and surname of the authors are quoted, some references with 3 authors include the 3 names, etc). As some examples: the subjeading 1.1.1. shoud define the main characteristics of germ stem cells, but is a mixture of different concepts not well related among them, omminting that they are the germ cells which are commited to develop in the gametes; Figure 2 is not original and has no reference in the legend; some sentences lack verb, such as „Structural changes during the transformation of PGC into oogonia"; Surrogate production, a central topic of the thesis is not defined, etc. The weakness of the introduction is surprising, because the next chapter (Chapter 2) is a very good review of the same topics, and it is updated, well written and covering all the required aspects.

Moreover, it has been published in a reputed journal (Biomolecules, IF 4,082). Considering that the guidelines stablish that "If the part or a ll literature review on the topic of the Ph.D. thesis was published in a journal with IF, it is possible to shorten this chapter and replace it by the published review in a journal with IF", I strongly recommend to review and shorten the introduction (Chapter 1).

The 3rd chapter has been already published in a JCR journal and represent a very detailed work on germ cell culture. A huge number of conditions have been assayed in order to determine the optimal culture conditions for these cells. The objective of obtaining the highest proliferation rate without differentiation has been achieved after a really hard work. Moreover, the transplantation approach evidenced that the cultured cells remained perfectly functional after 40 days. As it is well discused, the finding that feeder cells do not improve SCGs proliferation is very interesting for the implications about cell signalling during spermatogenesis in sturgeons. As suggestions for further studies I could mention i) trying the optimal culture contidions with isolated ASGs (following your own method of isolation), ii) include some approach for the analysis of cell death and apoptosis, iii) checking the expresion of germ cells genes after 40 days in culture and iv) provide some value about the number of obtained cells (how much were they expanded?)

In the next chapter, the analysis of testicles from sterlet in different maturity stage, containing different subsets of cells, seems a good approach to identify whether the spermatogona Aund have particular characteristics of size and complexity which can be used to define a cell population easy to be identified by FACS. The signal provided by flow citometer effectively allowed the identification of this subpopulation with higher SSC and FSC signals than the rest of the germ cells in the testicle. The method is easy and simple (for a trained person) and represent an very interesting tool. The percentage of vasa positive cells in P2 of the immature testicles could reveal the presence of some Aund spermatogonia in this region, imposible to be isolated from the rest of germ cells in P2 when mature testicles are under study. Nevertheless, the possibility to obtain a so pure fraction of ASG compensates from the potential lose of some of them.

In the last chapter a high number of monoclonal antibodies developed against Pacific blue tunna and trout germ cells were assayed in sterlet. The work developed has been hard and imply the training in several immunodetection methods. The results show that one of the tested antibodies identify Aund spermatogonia, but epitopes seems to be located in the cytoplasmic instead of in the membrane. This antibody would thus be particularly usefull for the identification of ASG post transplantation, nor for their use in sorting using MACS. The images suggest that also endothelial cells are positive to the same antibody. Considering that these two chapters are closely related, I suggest to consider to join them for publication.

The discussion is focussed on the relevant aspects of the thesis and the conclusions summarize all the relevant findings.

I consider that the thesis represent a significative progress and an important contribution to the field.

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FINAL RECOMMENDATION

X PhD Thesis can be recommended for defence

☐ **PhD Thesis can be recommended with reservations for defence**

☐ **PhD Thesis can not be recommended for defence**

Maria Paz Herráez

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……León, 5 July 2020……
Date and place

…María Paz Herráez…
Name and signature