



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Olga Bondarenko, M.Sc.	First name(s), surname, titles of supervisor: M.Sc. Boris Dzyuba, Ph.D.
Title of PhD thesis: The role of osmotic and ionic agents in fish sperm physiology	
REVIEWER:	
Surname: Yoshida	Institution: Toin University of Yokohama Biomedical Engineering Center Yokohama, Kanagawa-ken, Japan
Name: Kaoru	
Titles: Assoc.prof., Ph.D.	E-mail: yoshidak@toin.ac.jp
Please describe your professional relationship to the PhD student: sharing the same academic field of study about sperm physiology	Please describe your field of expertise: Reproductive biology, biochemistry, physiology, and molecular 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**):

Sperm physiology is important for dealing with sperm from a field of study of reproductive health, animal husbandry, and to aquatic products; nevertheless there are not enough researches in the field. In that sense, the PhD thesis showed originality and scientific importance. The PhD thesis showed a notable perspective for basic research, which common molecules participating in sperm activation play a different part adaptively to environment. Moreover the results also suggest the application for in vitro fish sperm maturation and cryopreservation. The technology used in the PhD is not most advanced one, however the level of research compares favorably with the current state of the art in sperm physiology field.



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**):

Formally, the PhD thesis has 107 pages and consists of eight chapters. Text is divided into parts: General introduction, five recent publications, General discussion and Conclusion. General introduction provides a very useful up-to-date overview about the topic of study including information about water transport, osmotic regulation and ion transport of sperm membrane in relation to sperm motility. All experimental methods used in this study are described in Materials and Methods in each chapter from two to seven. Experiments are well organized to solve the objectives and data are very nicely presented. This is a well written thesis which perspicuously understandable and valuable results of high quality. I suppose that Chapter 2-7 remained as publication style depending on the institutional rules. However, I recommend the author use of "I" instead of "We" in Chapter 1 and 8.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The PhD thesis with the role of osmotic and ionic agents in fish sperm physiology. The use of high quality gametes from captive fish brood-stock is of great importance for ensuring the production of valuable offspring for aquaculture. Therefore, Improved knowledge of the sperm biochemistry, physiology, and that of the mechanisms of reproduction will be beneficial for the improvement of artificial fertilization procedures in fish breeding. The main aim of this work was to identify and characterise the mechanisms underlying fish spermatozoa maturation as well as motility activation via changes in environmental factors vary among species and have not yet been fully investigated. For this purpose, the author focused on clarification of the roles of environmental osmolality and ion composition in the initiation of spermatozoa motility in different freshwater fish species. For the studies of osmotic regulation of spermatozoa volume alterations in different fish species (Chapter 2), nephelometry, light microscopy, electron microscopy, and spermatocrit methods were used for quantitative assessment of cell volume changes in media of different osmolalities. The author discovered that spermatozoa swelling are species-specific. For the studies about application the capacity for swelling in carp spermatozoa to improve the cryopreservation procedure relative to post-thaw sperm quality (Chapter 3), the author demonstrate that spermatozoa ability for hypotonic changes could be used for improvement of cryopreservation procedure. For the studies of the role of environmental osmolality and ion composition in fish sperm motility (Chapter 4-7), sturgeons and salmonids sperm was activated in media of differing ionic and osmotic composition. The author discovered that; 1) the presence of environmental Ca²⁺ or Na⁺ is required for motility initiation of salmonids and sturgeons spermatozoa, 2) even though environmental Ca²⁺ concentration is not crucial in conditions of Na⁺ presence, a minimal intracellular free Ca²⁺



concentration should be present in sturgeon spermatozoa for initiation of motility, 3) trout sperm sensitivity to environmental ionic composition is not affected by osmotic pressure, 4) Alteration of sperm sensitivity to Ca^{2+} was detected during spawning, according to seasonality. For the studies of the role of osmotic and ionic agents in sterlet spermatozoa maturation (Chapter 6-7), the author demonstrate that; 1) sturgeon testicular spermatozoa lack capability for motility activation and fertilization, 2) testicular sperm incubation with urine controls *in vivo* maturation, but can be performed *in vitro* as well, 3) sperm maturation does not occur under conditions of Ca^{2+} deficiency, 4) a gradual decrease of spermatozoa sensitivity to environmental Ca^{2+} . Therefore, the author hypothesize that maturation process might be controlled by Ca^{2+} influx into the cell with subsequent loading of some Ca^{2+} stores, and provide that evidence for the presence of Ca^{2+} stores in sturgeon spermatozoa, suggesting that these stores regulate not only the sperm maturation process, but are also involved in the mechanisms of spermatozoa motility.

I have these questions related to the PhD work:

1. The author claims that spermatozoa swelling are species-specific. I suppose that the word "species-specific" used as general meaning. Even though, is there any possible explanation by phylogenetic classification?

2. The manuscript in Chapter 7 contains several typographic errors. i.e.; some parenthesis are not properly used in line 11, page 74, and in line 2-3, page 75; What is stand for "Sucr" ? Sucrose? ; Capital letters are not properly used in the indication of Figure, etc. Please reconfirm proofreading.

3. I suppose "if" is typographic error in line10, page 91. Please indicate the reference in line 16, page 92 and "efflux" should be "influx" because NCX works as a "reverse-mode" in this case.

Olga Bondarenko learned and successfully used several physiological and morphological assessment methods. This thesis convincingly documented that she acquired necessary theoretical knowledge and practical skills and that she can build up working hypotheses and test them with appropriate set of methods. Importantly, she includes necessary controls in experimental design and is careful with data interpretation. Thus, author of this thesis showed her capability for independent scientific work.

Olga Bondarenko co-author on three publications in international journals, and which are directly related to her PhD topic. Most importantly, she is the first author on the key publication about sperm cell volume changes in Theriogenology, comprehensive journal dealing with domestic animals. She is also the first author on another two publications about the role of Ca^{2+} on sperm motility in brook trout, and is also the first author on another manuscript about the role of Ca^{2+} on sperm motility in sturgeon, which is submitted recently. She presented eight oral presentations and posters on international conferences, attend three international training courses, and conducted a research in foreign stay twice.

Overall, this PhD thesis has very good level. Olga Bondarenko showed all necessary practical skills, theoretical knowledge and high motivation for research work. Quality of this thesis is fully in agreement with requirements for PhD thesis in the field of fish sperm physiology. Therefore, after successful defence of the PhD thesis, I recommend to award PhD degree to Olga Bondarenko.



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

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

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 15, 2015
Yokohama, Japan

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Date and place

Kaoru Yoshida, Ph.D

Kaoru Yoshida

Name and signature



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Title of PhD thesis: The role of osmotic and ionic agents in fish sperm physiology	
REVIEWER:	
Surname: Krasznai	Institution: University of Debrecen, Medical and Health Science Center, Faculty of Medicine Department of Biophysics and Cell Biology 4032 Debrecen, Nagyerdei krt. 98, Hungary
Name: Zoltan	
Titles: Assoc. prof., Ph.D. C.Sc.	E-mail: krasznai@dote.hu
Please describe your professional relationship to the PhD student: no any close professional relationship	Please describe your field of expertise: reproduction biology, cell biology, biophysics electrophysiology

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**):

Artificial reproduction is commonly used in commercial fisheries. Sperm quality is a key issue in this process. O. Bondarenko's work provides better knowledge in fish sperm motility initiation, original ideas about ionic and osmotic control of motility. The thesis provides a detailed summary of the knowledge in the field and contributes with remarkable results to the better understanding of the process to control sperm motility. Some of the results can be directly used in fisheries practice.



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**):

The structure of the thesis is good. It is based on 6 per reviewed original publication in the best journals of the field.

The aims of the thesis is clear and in the thesis O. Bondarenko gives adequat answers for the objectives. The attached publications are directly related to the objectives.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The thesis seems to generalize the previously obtained observation of the osmotic and ionic mode of the initiation of sperm motility. Gives new information about sperm volume regulation and in vitro maturation of sterlet sperm. Supplemented with a manuscript about the role of Ca²⁺ ions in sterlet spermatozoa in vitro maturation.

Comments to the Ph.D. thesis:

The title of the thesis is to general does not give information about the content. I understand that the content is very wide but a more informative title would be helpful.

Introduction

Page 10, 3rd paragraph from bottom: (sucrose) (Perchec et al., 1995) the first description was given by Morisawa using mannitol in 1980.

Page 13 Last paragraf at the bottom: In mammals should be omitted. This thesis deals with fish sperm motility.

Page 17 last paragraph from bottom: The largest should be omitted fish sperm does not express ion channels participating in action potential characteristic for excitable cells. The chapter should be rewritten and instead of Hille's otherwise excellent book relevant publications should be cited if any!

Page 19 first paragraph 4th row from its bottom: „Action potential required for sperm motility activation“ I have never heard about action potential in fish sperm activation!

Page 20 first sentence: What do you want to say?

Cited published papers are perfect.

Manuscript

Page 72 :“Addition of Ca²⁺ channel inhibitor or EGTA to the incubation madium during in vitro maturation induced or completely suppressed motility.“

My comments tell what happened did it induced motility or it did completeli suppressed motility?



On the other hand the channel inhibitor should be named. Verapamil is an inhibitor of a wide group of Ca²⁺ channels but it also affects other ion channels.

Page 78: you use calcium and EGTA together. There are programs available to calculate the free calcium concentration which also depends upon other components like ionic strength, pH, temperature etc. Can you give free Ca²⁺ concentration.

Page 80: Discussion first sentence: In carp spermatozoa motility initiation Ca induced Ca release from stores occurs and the increased calcium concentration is maintained in a relatively narrow window by the Na/Ca exchanger.

Page 85: The same paper of Krasznai et al. is cited twice but unfortunately none of the citations are correct. In one the publication date, in the other the authors not cited correctly.

General conclusion:

The thesis written by Olga Bondarenko is a nice piece of work I like it very much. Regardless of my critical comments, I have the very strong opinion that it is suitable for the defence and I strongly support to honour this work with the Ph.D. title.

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



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Jihočeská univerzita
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Czech Republic

16 June, 2015 Debrecen, Hungary
Date and place


Zoltan Krasznai
Name and signature