



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Ievgeniia Gazo, M.Sc.	First name(s), surname, titles of supervisor: Jacky Cosson, Ph.D., Dr.h.c.
Title of PhD thesis: The role of reactive oxygen species and protein phosphorylation in fish spermatozoa	
REVIEWER:	
Surname: Shiba	Institution: Shimoda Marine Research Center University of Tsukuba Shizuoka 415-0025 Japan
Name: Kogiku	E-mail: kogiku@kurofune.shimoda.tsukuba.ac.jp
Titles: Assoc. Prof.	
Please describe your professional relationship to the PhD student: Researcher belong to the host laboratory for her temporal study in Japan, 2013.	Please describe your field of expertise: Cell biology, reproductive biology (sperm motility; cilia and flagella)

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 thesis describes the role of reactive oxygen species (ROS) and protein phosphorylation in fish spermatozoa. Sperm is very specialized cell to be released outside of the individual body and be able to adapt their motility to variable environment. Elucidation of mechanism on the effects of ROS and oxidative stress for sperm is very important not only in basic biological interests but also in applied fields such as fisheries sciences and reproductive technique. The thesis contains three published papers and provides novel and important contribution to the field of sperm biology. The works are scientifically and internationally valuable and the level is high in the relevant scientific 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**):

The organization of this PhD thesis is well considered and well achieved. To clarify the role of reactive oxygen species (ROS) on fish spermatozoa the author showed that ROS production induced DNA fragmentation and suppression of sperm motility in two species, sterlet and common carp. Furthermore, she focused on the protein phosphorylation related to intracellular signaling pathway in the downstream of ROS production and oxidative stress and identified several important proteins by proteomic analysis. The structure of the thesis is well-ordered and the experimental approaches and results are clear.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The thesis is a well written, interesting, and useful contribution, which I think is entirely worthy of defence of PhD thesis.

The thesis is written in accordance with four objectives and following results and discussion;

1. To investigate the effects of xenobiotic-induced oxidative stress on fish spermatozoa after in vitro exposure. Chemicals related to ROS production induced DNA damage and motility suppression in sterlet and common carp. These results suggest that ROS is very important factor to affect sperm parameters and fish spermatozoa could be sensitive models to evaluate the effect of xenobiotics in aquatic field. (Chapter2 and 3)
2. To evaluate the protective properties of antioxidants and seminal plasma against oxidative stress in fish spermatozoa. Some of enzymatic antioxidants such as catalase, superoxide dismutase and glutathione can reduce oxidative stress with seminal plasma in common carp, suggesting seminal plasma possesses the capacity to protect sperm from oxidative attack. (Chapter3)
3. To study the interplay between ROS and protein phosphorylation in fish spermatozoa. The influence of ROS on tyrosine phosphorylation were examined by 2D-PAGE and western blotting and two phosphorylated proteins were identified in common carp spermatozoa. (Chapter4)
4. To describe the role of phospho-proteins in the initiation and regulation of spermatozoa movement. Molecular mechanisms of sperm motility activation and intracellular signaling were analyzed in sterlet and common carp. Motility analysis showed that two protein kinases, PKA and PKC involved in sperm motility and proteins phosphorylated or dephosphorylated after sperm



motility activation were identified in both species. Commonality and differences in molecular mechanism on regulation of sperm motility between two species were discussed. (Chapter 5)

All the objectives have been properly achieved in this thesis. All the data would become an important knowledge for studying the role of ROS and protein phosphorylation in fish spermatozoa.

Although these data are very important, I recommend the candidate to mention a little more about:

1. Positive effect of ROS in sperm motility regulation. For example, in *Chlamydomonas* redox-dependent switching plays an important role in cell swimming during phototaxis (Wakabayashi et al., 2011, Proc Natl Acad Sci U S A. 108(27):11280-4).
2. Whether the differences in the effects and roles of ROS between sterlet and common carp spermatozoa depend on species-specific reproductive behaviour or habitat environments.
3. Direct regulation of axonemal proteins by ROS. Outer arm dynein contain a unique intermediated chain (Sea urchin and ascidian) or light chain (*Chlamydomonas*) with domains of thioredoxin (Ogawa et al., 1996, Mol Biol Cell, 7: 1895-1907, Padma et al., 2001, Gene; 275: 177-183, Patel-King, 1996, J Biol Chem, 271: 6283-6291). Is it possible that ROS directly regulates dyneins not by mediating phosphorylation? Statements should be added concerning how regulation of phosphorylations results in the activation or inactivation of flagellar motility.

Nonetheless, overall the context of this thesis is well organized and well written. The aim is clear and the research outcome in publication is substantial.

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 15th, 2015, Shimoda, JAPAN

Kogiku Shiba

Kogiku Shiba

.....
Date and place

.....
Name and signature



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Review of USB FFPW PhD Thesis

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Title of PhD thesis: The role of reactive oxygen species and protein phosphorylation in fish spermatozoa	

REVIEWER:

Surname: Ciereszko	Institution: Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Department of Gamete and Embryo Biology Tuwima 10 10-748 Olsztyn, Poland
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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 results of the thesis have been published in peer-reviewed journals ranked very high by the ISI Web of Knowledge (Journal Citation Reports 2013), Chemo-Biological Interactions (IF = 2.98) is ranked 79 (of 256) in the category „Pharmacology and Pharmacy“ and 28 (of 87) in the category „Toxicology“; Fish Physiology and Biochemistry (IF = 1.68) is ranked 17 (of 50) in the category „Fisheries“; Molecular Reproduction and Development (IF = 2.67) is ranked 11 (of 30) in the category „Reproductive Biology“. As such, the results of the thesis were subjected to a very strict peer-review process and thus were validated according to very rigorous standards of the scientific process. Additionally, the manuscript concerning the mechanism of motility activation contains new and original knowledge and certainly will be published in good peer-reviewed journal. In my opinion, this thesis is well focused around the physiological and toxicological events related to reactive oxygen species (ROS) and protein phosphorylation in fish spermatozoa. Results of this thesis significantly extend our knowledge concerning the mechanism of oxidative stress effects on physiology of spermatozoa and identification of key proteins involved in this mechanism. This thesis also adds valuable information important for evaluation pollutants effects on reproduction in aquatic animals and for improvement of sperm handling in fish regarding protection against oxidative attack. Results obtained are highly relevant for reproduction of sturgeon fish (sterlet) and teleost fish as well (carp). Overall, this thesis significantly contributes to the knowledge on male fish reproduction, both regarding basic (oxidative stress, reproductive proteomics) and applied (reproductive biotechnologies, environmental protection) research.



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 main part of the thesis is composed of four chapters (three reprints of published papers and one manuscript). Chapter 2 provides information concerning the mechanism of vinclozolin influence on sterlet spermatozoa, with special emphasis on DNA integrity, oxidative stress indices, antioxidative response and ATP content. Protection of carp spermatozoa against oxidative stress by antioxidants and seminal plasma is presented in chapter 3. A positive effects of antioxidative enzymes and seminal plasma have been demonstrated. Chapter 4 deals with more detailed studies of ROS action on carp spermatozoa especially related to tyrosine phosphorylation status of sperm proteins, such as O-linked N-acetylglucosamine transferase (isoform 4) and septin-8-A. Results of this study allowed to conclude that oxidative stress impairs intracellular signaling patterns. Intracellular signaling in spermatozoa of sturgeon and teleost fish are subject of chapter 5. Participation of protein kinases in sperm activation and numerous protein phosphorylation have been demonstrated. This research has led to identification of numerous sperm proteins involved in metabolic pathways. The scientific part of the thesis is preceded by the introductory chapter 1, in which the author adequately reviews the topics related to fish sperm and importance of studies sperm physiology, description of the mechanism of oxidative stress, intracellular signaling pathway in spermatozoa with the special emphasis on protein phosphorylation. In my opinion, this part would benefit from brief information concerning the mechanism of protein phosphorylation, since this posttranslational modification is an important issue of the thesis. General discussion (chapter 6) follows the scientific part; the main achievements of the thesis are summarized and concluded. The thesis consists of four papers concerning sturgeon and carp which resulted in quite an impressive amount of information. In my opinion, results for both species are well balanced which allows to make general statements regarding sturgeon fish and teleosts. The language of the thesis is clear. My concerns (listed below) are of minor importance and can be easily corrected.

OVERALL COMMENTARY ON THE PhD THESIS



Please write comments in extent of 1-2 pages:

As I mentioned above, this thesis covers a set of original topics related to the mechanism of reactive oxygen species and protein phosphorylation in teleost and sturgeon fish. The thesis is written in a clear manner and the results of the thesis were already published in top peer-reviewed journals. Basic science is main subject of this thesis but applied aspects of fish reproduction and environmental pollution are covered as well.

It needs to be mentioned, that Ms. Ievgeniia Gazo, in addition to the papers included in the thesis, is the coauthor of 9 papers. This number is notable and confirms the excellence of her research and her competence in scientific work.

In conclusion, my overall grade of the thesis is excellent and I strongly recommend the thesis for the defense of PhD thesis.

Minor corrections that can be used to improve the text are listed below.

P11 Fig. 1 should be centered.

P12, first paragraph, I do not think that xenobiotics are stored in seminal vesicle in fish.

P12, 1.3. protective role of seminal plasma should be mentioned in this chapter.

P13, 1.3.1 I would show Haber-Weiss reaction

P16, other calcium binding proteins should be mentioned as well, for example parvalbumin

P18 change "digestive enzymes" to "proteolytic enzymes"

P22- References should be presented in uniform way, because for some references volumes and issues are shown whereas for other references only volumes are shown.

P29 relevant

P107 last sentence of 1st paragraph in not clear

P107 last sentence of 2nd paragraphseveral freshwater fish species...just sterlat and carp?

P111 the name of this city is OLSZTYN!:-)

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

Olsztyn, 8 June 2015.....
Date and place

Name and signature