



**Confidential**

**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Amin Golpour Dehsari, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
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<b>Title of PhD thesis:</b> Functional regulation of subcellular calcium during fish gametogenesis
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**REVIEWER:**

<b>Surname:</b> Hatef	<b>Institution:</b> Department of Veterinary Biomedical Sciences, Western college of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7H0J7, CANADA
<b>Name:</b> Azadeh	
<b>Titles:</b> Dr.	<b>E-mail:</b> azadeh.hatef@usask.ca
<b>Please describe your professional relationship to the PhD student:</b> I have no professional relationship with Amin Golpour	<b>Please describe your field of expertise:</b> Reproductive Endocrinology

**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 (2 papers) and/or accepted (one paper) in peer-reviewed journals. According to the ISI Web of Knowledge (Journal Citation Reports 2016) have ranked as following:

**Microscopy and Microanalysis** (IF = 1.89) is ranked 131 out of 275 in the category of Materials Science and Multidisciplinary, 4 out of 10 in the category of Microscopy.

**Journal of Morphology** (IF= 1.65) is ranked 7 out of 21 in the category of Anatomy and Morphology.

**Micron** (IF=1.92) is ranked 3 out of 10 in the category of Microscopy.

All papers are placed in second quartile (Q2), which denote middle-high position of IF positions and have passed a peer-review process.

There is a published paper in the first chapter of thesis that it should be deleted from thesis, as it is not related to the defined aims of thesis. However, Mr. Golpour have 4 more papers as first author and also he is co-author of 8 papers, which confirms his competence in scientific work.



Overall, this thesis provides basic information about distribution of Calcium in fish gametes during gametogenesis. Similar results had been reported in other vertebrate and this basic information has the potential to help further research for understanding of Calcium signalling in gametogenesis. It would have been good if Mr. Golpour were able to measure some Calcium signalling pathway during his Ph.D study to show a better approach of mechanism of calcium function during gametogenesis.

***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 thesis is composed of 3 chapters (two reprints of published papers and one manuscript). Both papers and the manuscript deal with the subcellular distribution of calcium during gametogenesis. As all three papers follow similar patterns and results, It is not logical to separate them in different chapters. At least chapter 4 and 5 can be organised at one chapter titled: Subcellular distribution of calcium during zebrafish gametogenesis.

General discussion is not comprehensive; the main achievements of thesis are not summarized and concluded. The thesis consists of three studies that localized calcium distribution in different stages of gametogenesis and it is quite similar in both fishes examined and previous studies. As there is no signalling pathway study to show mode of calcium action on gametogenesis, in all 3 papers it was speculated that changes in calcium localization from deposit to unbound during gametogenesis reflect changes in its physiological function during gametogenesis. Therefore, the results do not cover the title of thesis, which is "functional regulation of subcellular calcium during fish gametogenesis".

Besides, objectives of thesis must be carefully modified. At the current format of your thesis, results of your thesis are not covering your entire objectives. For instance, you did not find links between regulations of calcium dynamics in subcellular levels with the possible role of calcium in cell functions during different developmental stages of gametogenesis in fish. So please concise your objective part according to what results are presenting in the thesis.

**OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

- 1- Your PhD defence is going to be in 2017, please correct graduation year from 2016 to 2017. For instance pages 2, 3 and 71
- 2- Page 3: your thesis reviewers' affiliation is not written in a correct way. Please replace it with "Dr. Azadeh Hatef, Ph.D. Western College of Veterinary Medicine, University of Saskatchewan.
- 3- Page 4 (thesis content): I highly recommend deleting chapter 2 from your thesis. It is not directly related to your thesis title and aim. In addition, you did not provide any information regarding correlation of 11KT or other reproductive hormones with calcium signalling in gametogenesis in general introduction, which is an overall picture of your thesis.
- 4- Page 6: Why introduction has number 1.1? I recommend keeping number 1, 2 and so on.
- 5- Page 6 part 1.2. Line 8: delete " the" after motile
- 6- Page 7 part 1.3. Line 17: accumulation OF higher
- 7- as mentioned in page 30, Chapter 4 of thesis is an accepted manuscript. I wonder if it is corrected according to referee's comments? I have noticed many statements in the discussion part are concluded from mammalian studies but it is not mentioned in the text. It is recommended to specify the species in your statements for better understanding.
- 8- Page 36 line 6: please provide a reference for your statement.
- 9- Page 59: General discussion must be rewrite again. First paragraph of general discussion should be start with the overall view of the results of the present study. First 2 paragraphs of discussion are related to chapter one of thesis as it is recommended to omit. (Histology of starlet gamete is not the aim of this thesis).
- 10- There is no need to mention the method used for the study in discussion section (For instance, line 5, paragraph 3 page 59). Also, line 5-8, paragraph 3 page 59 gives some introductory information about calcium role in reproduction, which should be mention in introduction section not in discussion.  
In general, in discussion you should compare your results with previous studies that investigated calcium role in gametogenesis and reproduction. Also, you can compare the distribution of calcium deposit in 2 species used in your study (zebrafish and Starlet), which is discussed in paragraph 1 and 2 page 60 briefly.
- 11- Please avoid using unbound and free together in every time you mention it in



discussion. You can define it for one time and use one of them in the rest of your thesis. Use (Deposit and free) or (bound and unbound). For instance, Line 6, 8,15 and 19.

12- Page 60 line 12: impairment of cell function

13- Page 60, paragraph 2 line 1: stage OF spermatogenesis IN Sterlet

14- Page 72: last line: gonadal development OF

15- The CV attached to last page of thesis has some mistake. Please go through it and correct the mistakes. For example:

- Your education period in B.Sc. (2006- 2010) and M.Sc. (2009- 2011) are overlapping. Please correct it.
- Training SCHOOL
- Introduction and discussion need English correction

### ***FINAL RECOMMENDATION***

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

June 15, 2017. Saskatoon

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Date and place

Azadeh Hatef

.....

Name and signature



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**Review of USB FFPW PhD Thesis**

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<b>Title of PhD thesis:</b> Functional regulation of subcellular calcium during fish gametogenesis	
<b>REVIEWER:</b>	
<b>Surname:</b> Pěkníková	<b>Institution:</b> Institute of Biotechnology AS CR, v. v. i. Prumyslova 595 252 50 Vestec
<b>Name:</b> Jana	<b>E-mail:</b> Jana.Peknicova@ibt.cas.cz
<b>Titles:</b> doc. RNDr., CSc.	
<b>Please describe your professional relationship to the PhD student:</b> I don't have a relationship with the student	<b>Please describe your field of expertise:</b> Reproductive 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 results of this thesis provide new insight into information about the regulation and functional roles of calcium during fish gametogenesis in the international context. During early stages of gametogenesis most parts of intracellular calcium is sequestered in isolated deposits to keep the level of free (unbound) calcium. On the other hand, the distribution of calcium as unbound pool (free) in the advanced stages of spermatogenesis (spermatid and spermatozoon) and late stages of oogenesis is probably due to facilitate release of calcium from related organelles (nucleus and cortical alveoli) as the main internal stores of calcium for rapid transport of calcium during egg and sperm fusion and egg activation.

**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 work is well and clearly structured with a clear goal, a description of the results, and a dedicated discussion. The objectives presented have been met and published in the submitted publications.

The presented work has a high professional level, not only proves the hard work of the candidate, but also its ability to analyze and combine the acquired knowledge.

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**OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

Calcium as a biogenic element is one of the basic building stone of cells, it plays a variety of vital regulatory functions in physiological and biochemical events in the cell.

To find out the role of calcium in fish spermatogenesis, oogenesis and fertilization is the aim of the presented work.

The Ph.D. thesis is divided into six chapters. The first presents a clear general introduction to the issue and summarizes the current knowledge in the given field. The second to fifth chapters are based on published articles. The first two presented papers concern the localization of intracellular calcium during the reproduction cycle and during spermatogenesis on the starlet sturgeon model (*Acipenser ruthenus*). The other two papers presented are on the zebrafish model (*Danio rerio*) and deal with spermatogenesis and oogenesis. The last chapter contains a general discussion and author's publications.

The goals presented were:

1. Histological study of different developmental stages of spermatogenesis in starlet (*Acipenser ruthenus*).
2. Ultrastructural localization and quantification of subcellular calcium during different developmental stages of spermatogenesis in starlet (*Acipenser ruthenus*).
3. Ultrastructural localization and quantification of subcellular calcium in zebrafish (*Danio rerio*) spermatogenesis
4. Ultrastructural localization and quantification of subcellular calcium in zebrafish (*Danio rerio*) oogenesis.

The objectives presented have been met and published in the submitted publications.

General discussion and Summary are also clearly broken down and highlight the important conclusions of the work. The intracellular distribution of calcium during different developmental stages of spermatogenesis has been clearly described and reflects its physiological function and homeostasis during gamet production in sturgeon and zebrafish. In addition, it is described subcellular distribution of calcium deposits in the course of the ogenesis of zebrafish.

The results of this thesis provide new insight into information about the regulation and functional roles of calcium during fish gametogenesis. During early stages of gametogenesis most parts of intracellular calcium is sequestered in isolated deposits to keep the level of free (unbound) calcium. On the other hand, the distribution of calcium as unbound pool (free) in the advanced stages of spermatogenesis (spermatid and spermatozoon) and late stages of oogenesis is probably due to facilitate release of calcium from related organelles (nucleus and cortical alveoli) as the main internal stores of calcium for rapid transport of calcium during egg and sperm fusion and egg activation.

Amin Golpor Dehsari, M.Sc. presents a total of 16 publications, as the first author is seven times. An overview of the literature (a total of 51 and others in the submitted publications) proves the candidate's wide theoretical knowledge.

The role of the opponent is facilitated because all manuscripts have undergone a demanding revision in international journals.

I read the work myself with great interest and thank you for completing my knowledge of fish reproduction and the ability to compare it with reproduction in mammals.

I put two questions in the discussion:

1. The fertilization process in fish and mammals is different, mainly related to the specific binding of sperm surface and acrosomal proteins on zona pellucida of oocyte.. In sturgeon fish it is also acrosom. Is its role known in fish fertilization? Does the acrosomal proteins of sturgeon fish participated on the sperm/oocyte binding?

2. Can you explain why the calcium deposit is in the nucleus? What is the molecular mechanism of its release?

The presented work has a high professional level, not only proves the hard work of the candidate, but also its ability to analyze and combine the acquired knowledge.

**I recommend this work to award Ph.D.**

Prague, June 24, 2017

Assoc. Prof. Jana Pěkníková, Ph.D.

**FINAL RECOMMENDATION**

**PhD Thesis can be recommended for defence**

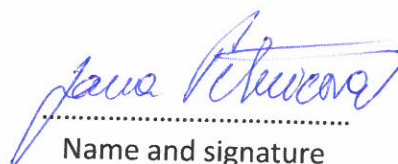
*PhD Thesis can be recommended with reservations for defence*

*PhD Thesis can not be recommended for defence*

Prague, June 24, 2017

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Date and place



.....  
Name and signature