



Review of Habilitation Thesis entitled „Osmotic combined to ionic control of sperm motility in taxonomically distant fish models: relations with cryopreservation“ authored by Borys Dzyuba, M.Sc., Ph.D.

Submitted Habilitation Thesis is based on a set of 11 selected papers authored and/or co-authored by the applicant. Thesis includes brief Introduction into topics of Thesis and other relevant comments. The selected papers constituting Thesis basically deals with i) spermatozoa motility is a prerequisite for natural fertilization in fishes, ii) specific environment signals such as osmolality and ionic composition regulate that regulate sperm motility, iii) species-specific signals determining either “osmotic” or “ionic” mode of sperm motility activation; all these aspects in very different and phylogenetically distant fish species. As given above, the main body of Thesis forms already published, i.e. peer-reviewed papers in quite excellent journals at the field of fish reproduction. This aspect, i.e. already anonymously reviewed texts, naturally restricts the possibilities of reviewer to write kind of thorough review of Thesis. As such, my review deals just with some formal aspects of submitted Thesis. Introduction and other introductory comments are given in 16 pages, written in decent English (in Acknowledgment one can found that Bill Shelton helped with editing and hence the language used has some aspects of American English). In spite of that, I have some small comments. The term habilitation (used at least 2x) in fact means “a process”, while correct term is to be “habilitation thesis”, i.e. noun. The term “sea-water” is of course intelligible but the term “marine” is much more frequented. The usage of plural vs singular of the word fish (several places of the text): fish versus fishes - by convention, “fish” refers to one or more individuals of a single species, “fishes” is used when discussing more than one species, regardless of the number of individuals involved (The diversity of fishes/Gene Helfman, [et al., eds]. – 2nd ed., 1997, p. 7). The term “taxonomically” was correctly used in most places of the text, but one must remind that the terms taxonomy, systematics and phylogeny refers to another subjects of study; in some places the term phylogenetically would be more appropriate. Another formulation problem is cumulating nouns in adjective position (e.g. p. 7: ...fish spermatozoa motility activation.), which is considered not grammatical (Heatwole, H., 2008: A plea for scholarly writing. Integrative and Comparative Biology, 48: 159–163). On the other hand author is evidently well oriented in last development on phylogeny of higher-rank fish taxa as well as in difficult and various problems of fish sperm and related cryobiology. Thus all selected of articles in this Thesis illustrates well the species-specific interrelations between modes of activation of spermatozoa motility,



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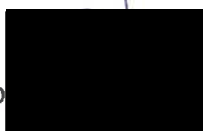
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characteristics of reproductive organ anatomy in different fish species under study, spawning environment and aspects of spermatozoa cryopreservation. Though in many aspects are clearly related to basic knowledge of biology of fish spermatozoa, other aspects are highly important to develop cryopreservation of fish sperm, and important tool in fish breeding and/or conservation. Applicant showed thus full ability and to progress this research direction. I fully recommend this Habilitation Thesis for next proceeding as competent basis for Assistant Professor Degree.

Liběchov 16.12. 2016

Prof. Petr Ráb





Review of the habilitation thesis by Juan F. Asturiano

Introduction

As one of the opponents of the habilitation thesis of MSc. Borys Dzyuba, Ph.D., I have read both the habilitation thesis and the supplementary documents submitted by the applicant, and evaluated both the content of the main document as well as his merits (CV).

Evaluation

Merits (CV)

Once revised in the supplementary documents the information regarding the different criteria suggested by the USB FFPW, these were the found scores:

- Publications on Web of Science (according to WOS):43/20
- Outputs of applied research:
 - Certificated methods and technologies: 5
 - Patent: 2 (in stage of consideration)5+2/3
- Number of times cited without self-citations:223/50
- Courses given:
 - Bachelor and Master students (Ukraine): Pond aquaculture, Aquaculture of natural waterbodies, Industrial aquaculture; Master students (FFPW, USB): Fish gamete cryopreservation; PhD students (FFPW, USB): Fish reproduction, Basics of scientific communication.....Total of at least 1539 h (in Ukraine) and Czech Republic (68?)
- Advisory of defended bachelor, master or doctoral theses:
 - Bachelor thesis (8 defended), Master thesis (2 defended), PhD study (1 defended, 3 in progress)11/5
- Presentations of scientific work results at international conferences:11/2
- Presentations of scientific work results at international conferences abroad: .10/2
- Continuous foreign internship (months)....1 year stay in London (12/6)
plus several short stays (1-2 m)

In short, the applicant exceeds the minimum values of each one of the recommended criteria suggested by the USB FFPW.

Habilitation thesis

- General comments

The habilitation thesis, entitled "Osmotic combined to ionic control of sperm motility in taxonomically distant fish models: relations with cryopreservation", is a set of eleven scientific papers published by the applicant, with a general introduction and discussion.

- Specific comments

Introduction

The introduction starts with a definition of some basic concepts and then brings the reader to the main area of research: the establishment of relationships between urogenital system anatomy, sperm maturation and sperm motility activation mode, as well as sperm cryoresistance and cryopreservation.

The second part of that section is dedicated to review the results of the presented papers that are then discussed in relation with bibliography. This is a brief subsection, but gives a good overview of the state of the art in the different subjects mentioned through the work.

The choice of the eleven papers has resulted in a solid group of works evidencing the most important lines of research of the candidate. The papers have been published in a total of 9 different journals, most of them in the first quartiles of their categories, demonstrating that this is a wide area of research.

Attachments 1-4

These works show how fish species have different sperm motility activation mechanisms that can involve activation media being hypotonic, isotonic, or hypertonic in comparison with the seminal plasma.

Suggested questions:

-Why do you think that tilapia results (paper 1) are different to those obtained by Morita?; what is the relationship of these results with the sperm activation?, do you think that is similar to the salmonids one (exit of K^+) or cyprinids-like?

-As the sterlet sperm is activated after dilution with hypotonic, isotonic, or hypertonic media (paper 3). Can the activation be due to the dilution effect?. In general, studies on the quiescent stage (roles of ions) should be done.

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Attachments 5-7

These works describe the specific mechanism of sturgeon sperm maturation, based on the mixing of sperm and urine, and how this mechanism implies energy from spermatozoa respiration, being dependent of external Ca^{2+} and K^+ ions.

Moreover, the cryoresistance and post-thaw fertilizing ability of sturgeon testicular spermatozoa subjected to a maturation phase before and after cryopreservation were tested, without finding differences. Thus, the sperm cryopreservation and its artificial maturation can be combined to use the testicular sperm in cases such as accidental death of valuable broodstock.

Suggested questions:

- Explain how the process of sperm maturation through the kidney depends on mitochondrial oxidative phosphorylation, and how is the relationship between osmolality conditions and energy generation.
- Under a practical point of view, is better maturing the sturgeon sperm before or after the cryopreservation?

Attachments 8-11

Basic aspects of fish sperm cryopreservation and species-specific cryoresistance are reviewed in the articles 8 and 9. The ability to preserve cellular volume due to changes of osmotic pressure during the freezing-thawing process seems to be determinant for cell survival (paper 8).

Freeze-thawing can cause different levels of spontaneous sperm activation in a species-specific manner (paper 9).

Moreover, the effectiveness of sequential stripping of sturgeon males after a single hormonal treatment was estimated, using different sampling timings and finding different sperm characteristics (paper 10).

Finally, for carp sperm, being sensitive to osmotic changes, it was demonstrated that spermatozoa cryoresistance can be improved by short-term treatment with moderately hypotonic media prior to freezing (paper 11).

Suggested questions:

- Describe which could be the physiological mechanism inducing the


spontaneous sperm activation (cryoactivation)?

-Considering aquaculture purposes, is the sequential stripping the most efficient system to obtain good sperm quality from sturgeons? Could hormone (progestins) administration help increasing the volume and quality of obtained sperm in the secondary samplings?

Conclusions

In conclusion, in my opinion, the candidate: MSc. Borys Dzyuba, Ph.D., has probed his professional maturity and demonstrated his scientific and academic background. Considering that, I **RECOMMEND** the **Habilitation Committee to proceed with his habilitation defense.**

Valencia (Spain), December 4th, 2016



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Assessment of the Habilitation Thesis by Borys Dzyuba PhD entitled: Osmotic combined to ionic control of sperm motility in taxonomically distant fish models: relations with cryopreservation

Firstly I must state that I have received no guidance as to how to perform this assessment, therefore, I must apologise if it does not comply with the regulations (if there are any). I will follow common sense and evaluate the thesis according to my own logic.

Regarding the formal requirements, there can be no question about the capability of the candidate to perform teaching duties as a professor. He has the necessary qualifications, an outstanding publication and citation record, more than sufficient teaching experience and a successful career devoted to science.

The thesis itself is composed of 11 scientific articles authored by the candidate and his colleagues as well as a common introduction of the problems and a summary of the results presented in the papers, merged into a single organic structure. The experiments presented here combine into a very well-organised chain of scientific studies that in the end build up a very interesting story about the forms of sperm activation and practical aspects related to it. I have found it very interesting how the authors modify and fine-tune our previous concepts regarding the role of osmolality and K^+ in the activation of teleost spermatozoa. Based on the results of the authors, it seems that the presence of Ca^{2+} is stronger prerequisite of fish sperm activation than low K^+ or hypo-osmotic conditions. Interestingly, I have found no reference to the work by Krasznai et al. (PNAS 2000 97(5):2052-7.) who have found that the sperm of common carp also remains inactive in Ca^{2+} -free solutions, regardless whether they were hypo-osmotic or iso-osmotic. All this in a species that was considered a classical case of osmotic activation pattern. The results obtained in the blackchin tilapia seem to confirm this, although the findings of Morita et al. (J. Exp Biol, 2004 207, 337-345) on the Mozambique tilapia seem to complicate this pattern. Obviously this is an interesting topic that still needs a lot of research but the candidate has contributed significantly to the clarification of this problem. Perhaps our knowledge on the role of Ca^{2+} in the activation of salmonid sperm needs revision, too.

The role of sperm maturation in the fertilising capacity of sturgeon sperm has been discussed by the candidate in several of his works in sufficient detail. As previously, I find it highly interesting because in Hungary, it has even been recommended to hatchery managers to sacrifice sterlet males if sperm is not available in sufficient volume and use sperm extracted directly from the testes – which in this case should not work. The candidate even makes a reference to this work by Horváth et al. (2002). Anecdotal information from hatchery managers has confirmed that testicular sperm was indeed used successfully in the sterlet to produce larvae. Whatever the reason, the topic is worthy of investigation.

Activation of sperm cells in various fish species due to dilution with cryoprotective media or cryopreservation itself is another interesting topic to which the candidate contributed significantly. These observations have very important implications in the adaptation of cryopreservation into hatchery practice and combined with differences in cryoresistance among species and among individuals within species can further complicate this story.



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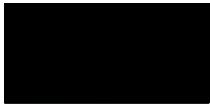
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Interestingly, the candidate states that the sperm of species with osmotic activation pattern (perch, common carp) can be cryo-activated while that of species with ionic activation pattern (such as salmonids) cannot. The candidate explains this with the presence of K^+ in the extender. How would then the candidate explain the observations of Ciereszko et al. (Aquaculture 420-421 (2014) 275-281) who used a simple 0.18 M glucose extender with methanol as cryoprotectant (no K^+ supplementation in the extender) for rainbow trout sperm and had very high post-thaw and post-activation motility and fertilisation rates with this sperm? Trout sperm would normally require the presence of potassium in the extender to suppress motility, yet, here it was not present.

The applied aspects of sperm cryopreservation and presented in a concise way and through a series of very interesting experiments. Indeed, they could be used in the practice of handling fish sperm in hatcheries, thus, they provide very important information for both sturgeon and carp farmers.

In light of what is written above, I have found Dr. Borys Dzyuba's Habilitation Thesis an outstanding evidence of a rigorous and highly organised scientific career and I support his application without any doubts.

Sincerely yours,



Ákos Horváth PhD