



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Yu Cheng, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Otomar Linhart, DSc.
Title of PhD thesis: Sperm quality affects the fertilization in freshwater fishes	

REVIEWER:

Surname: Dietrich	Institution: Polish Academy of Sciences in Olsztyn, Poland
Name: Mariola	
Titles: Asst. Prof.	E-mail: m.dietrich@pan.olsztyn.pl
Please describe your professional relationship to the PhD student:	Please describe your field of expertise: Fish semen 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 the thesis have been published in peer-reviewed journals ranked high by the ISI Web of Knowledge (Journal Citation Reports 2021), Animal Reproductive Science (IF = 2.22; Q1), Aquaculture Reports (two papers, IF = 3.385; Q2), Animals (IF = 3.231; Q2), Aquaculture (IF = 5.135; Q1), Theriogenology (IF = 2.923; Q1) and International Journal of Molecular Sciences (IF = 6.208, Q1). 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. It should be underlined that out of seven publications, Yu Cheng is the first author of four publications. In my opinion, this thesis is well focused around the fish gamete quality including management practices such as optimization and standardization of fertilization procedures (egg incubation, activation and fertilization) and sperm storage as well as epigenetic alteration behind sperm aging. Results of this thesis contributed to a better practical application in aquaculture and laboratory conditions for scientific study, and significantly extend our knowledge concerning mechanism of sperm aging including DNA methylation associated with sperm phenotype. Although author did not find any functional enrichment for identified differentially methylated regions, this study provides new data useful for understanding the mechanism of sperm aging and have a potential to be used as epigenetic markers in the future. This thesis also adds valuable information concerning characterization of seminal plasma of good and poor quality semen and for improvement of short-term sperm storage and sperm handling in fish regarding protection against spontaneous motility and possibility to revitalize low quality semen in sterlet. The important finding is also reduction of sperm concentration during *in vitro* storage. Results obtained are highly



relevant for reproduction of sturgeon fish (sterlet) and teleost fish as well (carp, zebrafish, catfish). Overall, this thesis significantly contributes to the knowledge on male and female fish reproduction, both regarding basic (epigenetic modification, sperm phenotype, sperm aging, sperm revitalization) and applied (reproductive biotechnologies) 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 seven chapters (chapters 2, 3, 5 and 6 contain one reprint of published paper and chapter 4 contains three reprints of published papers). Chapter 2 aims to develop the simple but efficient method of incubation of starlet eggs using tabletop static Petri dish system which can be used in laboratory conditions for scientific study. The standardization method of egg activation and fertilization in starlet is presented in Chapter 3 indicating importance of sperm concentration in the activation medium during fertilization. Chapter 4 provides information concerning sperm management and maintenance (semen collection and short-term storage), and optimization of fertilization procedures in zebrafish, European catfish and sterlet. This study also report for the first time the capability to revitalize low quality sterlet semen and characterize seminal plasma composition of good and bad quality semen. The investigation of different activation solutions (fertilization medium) on aging sperm of common carp during short-term storage (for 7 days) is the subject of Chapter 5. The influence of activation media and its osmolality on motility, kinetic parameters and fertilization capacity of aging sperm as well as reduction of sperm concentration during storage has been demonstrated. Chapter 6 deals with more detailed studies of mechanism of sperm aging in carp, with special emphasis on sperm phenotype (motility, velocity, concentration, viability, fertilization, hatching and malformation rates) and epigenetic changes including global and gene-specific DNA methylation. This research showed fluctuation in global DNA methylation level over storage time and has led to identification of differentially methylated regions. The scientific part of the thesis is preceded by the introductory Chapter 1, in which the author adequately reviews the topics related to importance of optimization and standardization of the egg incubation, activation and fertilization, and sperm management, description of the mechanism of sperm molecular changes during short-term storage with the special emphasis on sperm phenotype and epigenetic modification such as DNA methylation. General discussion (chapter 6) follows the scientific part; the main achievements of the thesis are summarized and concluded. The thesis consists of seven papers concerning sperm quality sturgeon and teleost species which resulted in quite an impressive amount of information. In my opinion, the results are more concentrated on application aspect than basic science. 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 in the box specific comments concerning the PhD thesis in extent of 1-2 pages:



As I mentioned above, this thesis covers a set of original topics related to the mechanism of sperm aging and revitalization as well as improvement of fish management 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. Both basic science and applied aspects of fish reproduction are presented.

It needs to be mentioned, that Ms. Yu Cheng, in addition to the papers included in the thesis, is the coauthor of 5 papers and first author of one paper as well as co-author of additional three papers with status under review. 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.

P9 L25 please delete „in“ after study

P11 L38 please change Contreras et al., 2019 to 2020

Fig. 1 I suggest to add proteome changes among phenotype changes

Chapter 1.6.1. I suggest rewriting the subtitles in this chapter; the term „damage“ has been unfortunately used in the context of sentences like „damage to kinetic parameters or damage to viability“. I propose the following subtitles:

„Changes of sperm kinetic parameters“; Sperm plasma membrane damage“

P13 L12 please change „reproduction season“ to „reproductive season or spawning season“

P13 L23-24 please modify as „alteration in sperm morphology and mitochondrial damage“

Chapter 1.6.2. Please change „sperm molecular level“ to „sperm at molecular level“

Again, as I suggested above rewrite the subtitle in this chapter. I propose the following subtitles „Nuclear DNA fragmentation or Nuclear DNA damage“ and „Epigenetic changes“

In addition try to make clear which subtitles belong to sub-chapter Epigenetic changes. If I correctly understand the next sections belong to this sub-chapter. I suggest to use italic or other clear way such as numbering.

P13 L 19 DMRs is not defined

P13 3rd paragraph the second sentence „In zebrafish.....“ is unclear

P13 last paragraph Please explain abbreviation DNMT, the first time it appears in the text, at present the definition DNMT is provided later in last sentence.

P16 L2 Please explain 5mC

P16 second paragraph 6 line 5-hydroxymethylcytosine

P17 4th paragraph L13 please change „fertility ability“ to „fertilizing ability“

P19 1st paragraph L8 Please put PTMs after post-translational modification

P19 1st paragraph the last sentence is not clear

P19 3rd paragraph line 5, Please describe LncRNAs

Reference

Please change Contreras et al 2019 to Contreras et al 2020

P115 1st paragraph 3rd sentence from the bottom Please use italic for *Flavobacterium*



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P116 1st paragraph L4 Citation Linhart et al., 2004 is not provided in Reference list
P116 1st paragraph L6 Please specify if 80% is related to fertilization rate or hatching rate
P116 3rd paragraph L2 Citation Asturiano et al., 2004 is not provided in Reference list
P116 4th paragraph L1 from the bottom Citation Hagedorn and Carter, 2011 is not provided in Reference list
P117 L12 please delete comma after hormonal,
P117 L13, and 20 Citation Fauvel et al., 2010 is not provided in Reference list
P117 L22-23 Citations Morisawa and Suzuki, 1980; Alavi et al., 2007 and Wilson-Leedy, 2009 are not provided in Reference list
P117 L27 Citation Zarski et al., 2015 is not provided in Reference list
P117 last line Contreras et al., 2020 is not provided in Reference list
P118 L3 Please define HPS
P118 L11-13 Citations Cejko et al., 2022; Ubill et al., 2015 and Aramli et al., 2017 are not provided in Reference list
P118 2nd paragraph L4 Citation Labbe et al., 2017 is not provided in Reference list
P118 2nd paragraph L9 Citation Woods et al., 2018 is not provided in Reference list
P118 2nd paragraph L 11 the description of DMRs should be provided earlier in Line 8
P118 3rd paragraph all cited literature in this paragraph (Hammoud et al., 2010; Jenkins et al., 2013, Reik and Walter, 2001, Marques et al., 2004, Haberg et al., 2022, Depince et al., 2020, Herranz-Jusdado et al., 2019) is not listed in Reference
P119 L1 description of ART assisted reproductive technology was provided earlier on P118 3rd paragraph
P119 concluding paragraph what does it mean „sperm mechanism“ in last line of this paragraph
P120 Conclusion of Chapter 4 first sentence is not clear
P120 Conclusion of Chapter 5 L6 please change „confirmed and that“ to „confirmed that“

FINAL RECOMMENDATION

- ☒ PhD thesis can be recommended for defence
☐ PhD thesis can be recommended for defence with reservations
☐ PhD thesis cannot be recommended for defence

18.07.2022, Olsztyn

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Date and place

Monika Dietrich

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Name and signature