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

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

Confidential

Supervisor´s Review of USB RIFCH PhD Thesis

Surname of the PhD student: Iegorova	Name of supervisor: Taiju Saito
Title of PhD thesis: "Polyspermy produces viable haploid/diploid mosaics in sturgeon"	

OVERALL COMMENTARY ON THE PhD THESIS

Ms. Viktoriia Iegorova conducted a detailed study of the mechanism that produces abnormally divided (AD) embryos, which have for example three cells at the two cell stage, in sturgeon. The present research began when she realized that some sturgeon embryos showed abnormal division during early cleavage stages. In the literature, abnormal divisions in early sturgeon embryos had already been described by some researchers in the 1950s, but the mechanism underlying this phenomenon had not been explained with any evidence. In addition to this, it had been believed that AD embryos could not develop beyond the hatching stage due to their abnormality. However, Viktoriia doubted the commonly accepted view, and she found that AD embryos were able to develop more than several months.

Since that discovery, to uncover the mechanism of this unique phenomenon in sturgeon, she compiled a great deal of data using well-designed experiments, including animal rearing experiments, cytological and histological observations, flowcytometry analysis, molecular parentage analysis using microsatellite markers, and statistical analysis. As a result, she found that AD embryos could be induced through insemination with a large amount of sperm, and she discovered that the additional blastomeres have unusual haploid nuclei, meaning that AD embryos are a mosaic of haploid and diploid cells. The haploid cells were unevenly distributed in the organs of AD fish, and sometimes an organ, including the gonad, was solely composed of haploid cells.

Based on these results, she concluded that the additionally formed blastomeres in AD embryos originated from supernumeral spermatozoa by polyspermy. This conclusion is supported by a vast quantity of evidence obtained from many well-designed experiments. Moreover, surprisingly, she demonstrated that it is possible to produce a single embryo with the genome of three parents, just by inseminating with mixed sperm from two sturgeon species. She has summarized the above results into two papers published in peer-reviewed journals with high impact factors.

The findings of her PhD study are completely novel, not only in sturgeon but also in vertebrates. Thus, her study has provided vital information for sturgeon propagation programs in hatcheries. Additionally, it has opened a new avenue related to sturgeon biotechnologies, because by applying this knowledge, it will be possible to produce "clone" sturgeon with cryopreserved sperm in a short period, an idea proposed in her thesis. Given the strength and novelty of her research, I recommend that she be awarded a PhD degree.

FINAL RECOMMENDATION

- [x] **can be recommended for defence of PhD Thesis**
- [ ] **can be recommended with reservations for defence of PhD Thesis**
- [ ] **can not be recommended for defence of PhD Thesis**

10/8/2018, Japan
Date and place

Taiju Saito
surname and signature