Fakulta rybářství   Jihočeská univerzita
a ochrany vod   v Českých Budějovicích
Faculty of Fisheries   University of South Bohemia
and Protection   in České Budějovice
of Waters   Czech Republic

Confidential

Supervisor´s Review of USB RIFCH PhD Thesis

Surname of the PhD student: Fatira	Name of supervisor: **Taiju Saito**
Title of PhD thesis: Nuclear transplantation in sturgeon eggs	

OVERALL COMMENTARY ON THE PhD THESIS

Ms. Effrosyni Fatira conducted a detailed study on animal cloning, which is realized by the nuclear transfer of a donor species into an oocyte of a recipient, in sturgeon species for the first time. Acipenseriformes, including sturgeons, is frequently labeled "living fossils". All 27 sturgeon species are on the Red List of Threatened Species, with 17 categorized as critically endangered and 4 considered extinct. Cloning, which can revive individuals from a single somatic cell, could help restore the genetic diversity and boosting propagation of endangered sturgeons. However, even in model species, such as medaka and zebrafish, there are still many biological and technical constraints, and cloning success rates have been low. Thus, I expected that the application of cloning to sturgeons would be a challenging topic for her. However, she overcame difficulty with her carefully designed research.

She started her research from the basic optimization of experimental conditions in each cloning step, such as harvesting method of somatic cells from a fin, storage method for keeping sturgeon oocytes in saline solution, make-up of workspace for efficient cell transplantations, and testing a position of the oocyte for nucleus transplantation. With a method she established by intensive trials, she transplanted a cell from a Russian sturgeon fin into an oocyte of a starlet sturgeon; 6.7% of the embryos developed until the blastula stage, and 0.5% reached the feeding larval stage. Therefore, she could improve the cloning technique in sturgeons at least to the level of that in model species. However, she was not satisfied with this result and attempted to improve the technique to obtain more sturgeon clones. Her hypothesis for the low cloning success rate was that the oocyte area that can accept and reprogram a transplanted nucleus is small, which caused difficulties in positioning the donor nucleus to the area. To surmount this, she transplanted a large number of nuclei into a single oocyte with the expectation that any of them can localize in the narrow area. As a result, she could obtain a 66.4% developing rate of transplants to the blastula stage in the Russian-to-sterlet clones. Moreover, DNA microsatellite parentage analysis revealed that some embryos were genuine clones, that is, they solely had donor-derived genomes.

She 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 many other animals. Her study will provide vital information for sturgeon propagation programs in the future. 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**

28, October, 2019
....
Date and place

....
surname and signature