IMPROVED STERLET STURGEON SPERM CRYOPRESERVATION PROCEDURE





SUMMARY

This output demonstrates a novel method for increasing sterlet sturgeon (Acipenser ruthenus) sperm concentration via centrifugation without a decrease in sperm quality. This reduces the volume of sperm sample required for fertilisation of large numbers of eggs in aquaculture, and reduces the space required for cryostorage. This output will be of interest to both sturgeon breeders and marine biologists concerned with the restoration of wild sturgeon stocks.

KNOWLEDGE NEED

Wild populations of sturgeons have historically been overfished for their eggs, which are processed and sold as black caviar. The boneless meat acquired from sturgeons is also highly prized. Overexploitation and illegal fishing have led to the closure of nearly all legal wild fisheries, and sturgeon is now listed as vulnerable on the IUCN red list of threatened species. The demand for sterlet sturgeon aquaculture has increased worldwide in recent times, and is becoming economically important. Sturgeon aquaculture can contribute to the protection of wild populations by providing a safe market supply. Cryopreservation allows the long-term storage of fish sperm and is a very useful tool for breeding programmes and hatcheries. However, there is an expense associated with the storage of large specimens. In sturgeon, including the sterlet species, sperm is naturally mixed with urine, which leads to high-volume samples with lower sperm concentrations. This can result



in differences in cryopreservation outcomes between samples of different sperm concentrations. It also results in the need for a greater amount of storage space and labour for the cryopreservation of samples.



- This output could contribute to the successful restoration of wild sturgeon stocks, particularly in the future, when inbreeding has become an issue.
- Reduced space requirement for cryostorage of sperm is expected to improve financial viability of sturgeon breeders.

EATIP - Strategic Research and Innovation Agenda (SRIA) Thematic Area 3 – Managing the biological lifecycle; Goal 3. To see the full list and descriptions of the thematic areas and goals, please visit: **eatip.eu/?page_id=46**



UNDERLYING SCIENCE

This research examined whether artificial modification of sterlet spermatozoa concentration before freezing is beneficial for sturgeon sperm cryopreservation. The sperm was first concentrated by centrifugation of normal sperm samples and removal of the excess seminal fluid. The concentrated sperm samples were then diluted with the same seminal fluid to obtain different sperm concentrations in suspension, and these samples were subjected to freeze-thawing. Samples with concentrations of 0.2, 1 and 3×10^9 spz ml⁻¹ were analysed for post-thaw sperm fertilisation ability. Normally, sperm concentration does not exceed 2×10^9 spz ml⁻¹ and frequently it is in range of 0.4-1.5 $\times 10^9$ spz ml⁻¹.

RESULTS

- This novel method artificially increased sperm concentration by approximately three to seven times more than standard methods.
- Sperm concentrations of 3 x 10⁹ spz ml⁻¹ (which is higher than the normal sperm concentration found naturally in sturgeon) were found to be appropriate for use in cryopreservation procedures. At this concentration, the sperm fertilising ability remained at a high level even though a significant decline in sperm motility percentage was observed.
- The results of this study indicate that sturgeon sperm of smaller volume but higher concentration can be prepared for cryopreservation. Artificially increasing the sperm concentration using the method above does not result in sperm quality deterioration.
- The post-thaw spermatozoa motility percentage depended on the sperm concentration in the samples. Better motility was found in sperm samples with a concentration between 0.2 and 1×10^9 spz ml⁻¹.
- This technological approach allows effective reduction of the storage volume required for sperm cryobanking.

END-USERS & POTENTIAL APPLICATIONS

END-USER 1: Sturgeon breeders

APPLICATION: New procedure for cryopreservation that allows for a reduction in the amount of sperm to be collected, thereby reducing the volume of storage space and amount of labour required.

○ END-USER 2: Marine biologists

APPLICATION: Cryopreservation procedures which are more efficient, that could be used to re-establish populations of wild sturgeon.

STATUS

Technology Readiness Level (TRL) 2 - technology concept formulated

- The results were conclusive and the host institution has already used the new procedure for cryobanking.
- Further research will be carried out to establish if there are any negative impacts on the resulting progeny.
- Research is being carried out on cryo-technics for the sperm of sturgeon species of different ploidy levels (possessing spermatozoa of different size). Additional research is also focused on DNA damage in sturgeon spermatozoa after cryopreservation.
- Further research on economic aspects, including a cost / benefit analysis, are required.



TITLE: Improved sterlet sturgeon sperm cryopreservation procedure

KNOWLEDGE TYPE: Scientific publication WHERE TO FIND IT: Contact details below

STATUS: Manuscript submitted

TNA FACILITY USED: University of South Bohemia in České Budějovice

CONTACT DETAILS: Assoc. Prof. Borys Dzyuba, Ph.D.; University of South Bohemia in České

Budějovice; bdzyuba@frov.jcu.cz

PATENTS OR OTHER IPR EXPLOITATIONS: No