Article about cryopreservation of zebrafish's semen is the cover of an internat[illegible]

A study conducted by the research group AQUAM from UFRGS[1], specialized in biodiversity and aquatic species conservation, analyzed cryopreservation protocols of zebrafish gametes. The species is a biological model used in research in several areas

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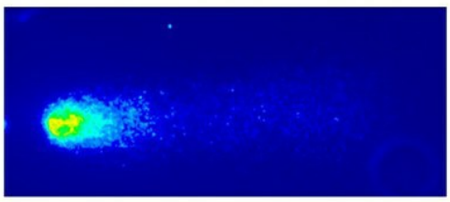
The Zebrafish journal issue of April 2021 highlights the study carried out at the AQUAM Research Group - Conservation of aquatic species biodiversity at UFRGS about cryopreservation of zebrafish's semen. The article "Oxidative Stress and DNA Damage of Zebrafish Sperm at Different Stages of the Cryopreservation Process'', highlighted on the cover of the newest issue, is the result of Rômulo Batista Rodrigues' doctoral research at the Postgraduate Program in Animal Science. The thesis "Cryopreservation of zebrafish's semen", defended in 2020 and nominated to represent the Animal Science Postgraduate institute at the 2021 edition of the CAPES[2] Thesis Award, addresses an important topic not only for research using zebrafish as a biological model, but also for the reduction of damage in cryopreservation processes of cells of other species, like mammals – humans included.



DNA fragmentation in zebrafish spermatozoa after cryopreservation. Alkaline comet assay and enzymatic comet assay (Formamidopyrimidine Glycosylase and endonuclease III) applied - Photo: Rômulo Batista Rodrigues/AQUAM

Rômulo's thesis consists of three scientific articles: the first is a systematic review of the historical and contemporary scenarios of protocols at the zebrafish cryopreservation, pointing paths and limitations; the second presents an evaluation of different cryoprotectants for the zebrafish's semen, aiming at a protocol's improvement; and the third article deals with the evaluation of oxidative and DNA damage at different moments of cryopreservation. Rômulo, who is a zootechnician and is currently pursuing a postdoctoral degree at AQUAM, was already dedicated to the study of other species for the fish industry, and he decided to research the protocol of cryopreservation of the semen of zebrafish because, according to him, there were no studies, not even for mammals or humans, about what happens to the offspring coming from cryopreserved semen. As zebrafish has high genetic homology with humans (about 70% of its genome is equal to ours) and, therefore, it is widely used as a biological model in studies of several areas, the research on the cryopreservation of this species allows us to make inferences about what may or may not happen with mammalian pups, for example. The researcher adds that the results are also useful for working with other fish species at AQUAM, both related to aquaculture and the preservation of endangered species. "Having zebrafish as a model, the protocol we use in it can be tested on other species to see if it works. It is a broadly acceptable model; everyone is using it. There are thousands of lineages created in laboratories that need to be preserved for future studies, so cryopreservation is an important method, but so far, the protocols used need improvement." emphasizes Rômulo.

The group of researchers gathered the main cryopreservation protocols already published, the two main extenders used and the main cryoprotectants and then combined all the characteristics of these protocols to see which ones would present the best result. Specifically in the article published in the Zebrafish Journal, the research presented sought to understand when the main damages resulting from the cryopreservation process occur - for example, if it is the toxicity of the cryoprotectant, that is, it occurs when the semen sample is put in contact with it or if it is in the freezing stage itself – and then observe the oxidative and DNA damage in these samples." The main point of the study is this, to see when most of the damage occurs, because this type of test doesn't occur frequently," says Rômulo. The group identified that the freezing/unfreezing moment of the samples is when the deepest damage occurs to spermatozoa and that using skimmed powdered milk in the cryoprotective solution reduces the damage, increasing the number of viable spermatozoa after cryopreservation. It was possible to observe that the use of powdered milk decreased the generation of reactive oxygen species and DNA fragmentation, in addition to promoting greater antioxidant activity.

The powdered milk is a cryoprotectant which has been used in the cryopreservation process for many years now but, according to Rômulo, there are researchers who contraindicate its use, because they understand that the product may end up fouling the sample, then making it difficult to analyze. "Many researchers have reported that powdered milk would not have a very positive influence on the protection of sperm and discourage its employment, claiming that it blurs the sample, but we have identified that it plays an important part, especially in protecting against DNA damage. When you are thinking about doing fertilization in vitro with this sperm and generating new individuals of zebrafish, we must avoid the use of samples that are with damaged DNA, as it can badly affect the development of the offspring."

Rômulo explains that powdered milk is a type of external cryoprotectant, which functions as a protective layer for the frozen cell. "Many studies do not use an external cryoprotectant, but we have seen that powdered milk is fundamental, it proved to be the most effective protection, so our suggestion is this: use powdered milk or test new external and impermeable protectors for cryopreservation zebrafish's semen," he says. In the AQUAM study, two internal cryoprotectants, methanol and DMSO, were also used, with no relevant discrepancy between them." In the end, the factor that influenced the result the most was whether to use or not powdered milk," he says.

Although the results obtained so far contribute significantly to the development of a more accurate cryopreservation protocol, Rômulo believes that there is still more progress to come. One of the important points, in his view, is the assessment of which genetic damage can occur in the offspring coming from the fertilization of cryopreserved semen. According to him, much attention has been so far given to assess if the sperm is still viable at the end of the process, but the study is still to advance on determining the genetic damages that can occur in this cell – damages that are not seen at the first moment, but that may appear in the long run. Rômulo explains that such observation should include not only the embryonic development but also the offspring after birth. His postdoctoral project at AQUAM aims, precisely, at the evaluation the offspring derived from cryopreservation and their development. He intends to complete his study by December 2021, performing at AQUAM the cryobiology part and using partner laboratories to evaluate the behavior and development parts of the offspring.

Translator's note:

[1] UFRGS stands for Universidade Federal do Rio Grande do Sul in Portuguese or Federal University of Rio Grande do Sul in English. It is a Brazilian public university subsidized by the federal government.

[2] CAPES is the abbreviation for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior or Coordination of Superior Level Staff Improvement in English. It's a Brazilian federal agency responsible for evaluating the postgraduate programs, scientific dissemination investing and promoting international scientific cooperation

Translated into English by Manuela Pereira Kliemann, under the supervision and translation revision of Elizamari R. Becker (PhD) – IL/UFRGS