

## Abstract

# Another Tool for Chondrichthyan Ex Situ Conservation: First-Time *Chimaera monstrosa* Sperm Cryopreservation <sup>†</sup>

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**Abstract:** Chondrichthyans, which comprises elasmobranchs (sharks and rays) and holocephalans (chimaeras), are one of the most endangered group of vertebrates on the planet. Ex situ conservation programs, such as captive breeding, are tools that can be used to improve the status of some of the most sensitive species belonging to these groups. However, the use of reproductive techniques is necessary when planning sustainable breeding programs in controlled environments. In recent years, our group has described the protocols necessary to achieve viable sperm extraction and its cryopreservation in 13 species. However, the use of these techniques in the holocephalan group has not yet been explored. Here, the process of obtaining viable sperm in a holocephalan species, *Chimaera monstrosa*, is presented for the first time. The sperm was obtained from animals recovered from bottom trawling bycatch. It was possible to recover sperm from both males (n = 3), using cannulation and abdominal massage, and females (n = 2), by directly accessing their oviductal glands. Sufficient sperm was obtained from the males to apply cryopreservation protocols developed for elasmobranchs. For this purpose, the sperm was diluted in an extender for elasmobranchs (1 sperm:9 extender) previously developed by our group. The cryopreservation of sperm was achieved through the addition of different cryoprotectants to the extender: methanol, dimethyl sulfoxide (DMSO) and fresh egg yolk. Samples were frozen inside a Styrofoam box using vapor of liquid nitrogen and preserved in liquid nitrogen. Sperm quality was assessed by studying motility and membrane integrity post-thawing. The initial motility and membrane integrity values were close to 54%. The best post-thawing motility values were obtained with a combination of 5% DMSO, 5% methanol and 10% egg yolk, which induced motility values close to 25% and membrane integrity values close to 24%. This is the first time that sperm from this group of animals has been cryopreserved, expanding our knowledge on their reproductive biology and the tools available for their conservation.



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