

Abstract

Reproduction Techniques Applied to Chondrichthyans Conservation †

Pablo García-Salinas , Victor Gallego and Juan F. Asturiano ^{*}, † 

Grupo de Acuicultura y Biodiversidad, Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, 46022 Valencia, Spain; pablo.g.salinas@outlook.com (P.G.-S.); vgalbiach@ualg.pt (V.G.)

^{*} Correspondence: jfastu@dca.upv.es; Tel.: +34-96-387-9385

† Presented at the IX Iberian Congress of Ichthyology, Porto, Portugal, 20–23 June 2022.

‡ Presenting author (Poster presentation).

Abstract: Chondrichthyan fishes, which comprise sharks, rays, and chimaeras, are one of the most threatened groups of vertebrates. Given this situation, one possible strategy for the protection of these species could be the use of ex situ conservation projects. However, to develop sustainable ex situ conservation programs, captive breeding techniques, such as sperm extraction and its preservation, should be used. Two main obstacles must be overcome to develop these techniques: first, the lack of knowledge and the scarce previous work focused on the conservation of gametes from these animals; secondly, the peculiarities of the reproductive anatomy of each particular species. Through a detailed description of their reproductive anatomy, we have been able to develop the best techniques to obtain viable sperm from 17 species. Extraction has been performed in both live and dead animals, using cannulation, abdominal massage, and dissection. Exceptionally, we have even been able to recover viable sperm from the reproductive tract of females. Moreover, we have formulated artificial seminal plasma that can be used as an extender to maintain sperm motility for 36 days at 4 °C. By supplementing this extender with different combinations of cryoprotectants, i.e., methanol, dimethyl sulfoxide (DMSO), and fresh egg yolk, we were able to successfully cryopreserve (for the first time in most of these species) the sperm of 14 chondrichthyan species. Sperm samples were frozen inside a styrofoam box using the vapour of liquid nitrogen and were preserved in liquid nitrogen. The sperm quality was assessed by studying the motility and membrane integrity post thawing, demonstrating its effectiveness in the 14 species tested. In rays, the use of 10% DMSO or 10% methanol rendered post-thawing motility values higher than 40%. In sharks and the chimaera species, the best post-thawing motility values were obtained with a combination of 5% DMSO, 5% methanol and 10% egg yolk, which induced mean values close to 35%. All this information broadens our knowledge on the reproductive techniques that can be applied to chondrichthyans, laying the foundations for the first cryobanks for their sperm.

Keywords: anatomy; sperm extraction; cryopreservation; sharks; rays; chimaeras; assisted reproduction techniques



Citation: García-Salinas, P.; Gallego, V.; Asturiano, J.F. Reproduction Techniques Applied to Chondrichthyans Conservation. *Biol. Life Sci. Forum* **2022**, *13*, 19. <https://doi.org/10.3390/blsf2022013019>

Academic Editor: Alberto Teodorico Correia

Published: 6 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Author Contributions: Conceptualization, P.G.-S. and J.F.A.; sampling, P.G.-S. and V.G.; methodology, P.G.-S. and V.G.; writing—review and editing, P.G.-S. and J.F.A. All authors have read and agreed to the published version of the manuscript.

Funding: Study partially funded by the Fundación Biodiversidad (PRCV00683). P.G.-S. had a contract from the European Union through the Operational Program of the European Social Fund (ESF) of the Comunitat Valenciana 2014–2020 ACIF 2018 (ACIF/2018/147). V.G. had a postdoc contract from the MICIU, Programa Juan de la Cierva-Incorporación (IJCI-2017-34200).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Animal Care and Welfare Committee of Fundació Oceanogràfic (Project reference: OCE-16-19 on 1 August 2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: Experimental data can be demanded to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.