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Ionic activation of sperm motility in an endangered viviparous fish

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The redtail splitfin *Xenotoca eiseni* is a viviparous species distributed in Mexico and some areas of the United States. It belongs to the family Goodeidae, which contains 40 species within 18 genera. Currently almost all of the species in this family are considered to be endangered in the wild. There are few studies on the reproduction of these fishes, especially on internal fertilization, and this presents great challenges to their conservation. Sperm cryopreservation could be an effective way to preserve the germplasm and restore populations. Motility activation of sperm is the initial step for study of sperm cryopreservation and conservation. In this study, we investigated the effects of osmolality, pH, non-electrolytes, and ions on the sperm motility activation of *Xenotoca eiseni*. Hanks' balanced salt solution (HBSS) with osmolalities ranging from 25-900 mOsmol/kg did not initiate motility (0-1%). Isotonic HBSS at pH values of 6.0, 7.0, 8.0, and 9.0, and three non-electrolytes (mannitol, sucrose and glucose) also did not activate motility. To test specific ions and their interactions, different combinations of $CaCl_2$ (0.1-40 mM) and KCl (2-60 mM) in isotonic TRIS-HCl buffer were tested, and Ca^{2+} activated motility in a concentration-dependent fashion, while the concentration of K^+ showed a potential negative correlation with motility. To further identify the effective range of Ca^{2+} for motility activation, concentrations ranging from 0 to 320 mM were tested. The first pilot observations in March, 2014, showed the highest motility (15-23%) at 1 min for a Ca^{2+} concentration of 200 mM, at 1 hr for 160 mM (48-52% motility), and at 3 hr for 10 mM (35-45% motility). Motility was never activated (<1%) with 0 mM, and at or above 280 mM Ca^{2+} (Figure 1). Bundle-like sperm clusters were observed at 200-× magnification. When activated, sperm within the bundles vibrated strongly. Peripheral sperm swam away from the bundles first, followed by the interior sperm. These same treatments in July, 2014, showed lower maximum motility (13% at 1 min with 160 mM Ca^{2+}) and shorter motility duration (none above 1% at 12 hr) but agreed qualitatively with the initial observations. Compared to the pattern observed in other similar-sized freshwater fishes, such as zebrafish *Danio rerio* and fathead minnow *Pimephales promelas*, and other internally fertilized *Xiphophorus* fishes, sperm motility activation in *Xenotoca eiseni* is distinct and does not rely upon osmolality, but instead upon the ionic composition of the activation media. Further study will focus on ionic mechanisms during motility activation, seasonal variation of sperm quality, artificial fertilization, protocol establishment for sperm cryopreservation, and repository development.

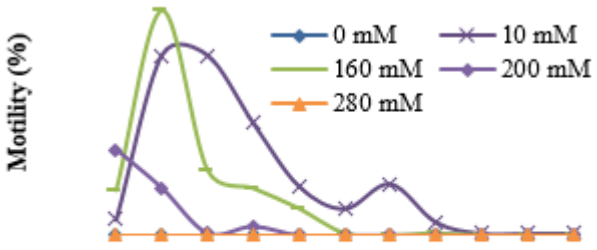

FIGURE 1. Sperm motility activation with Ca^{2+}