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IONIC ACTIVATION OF SPERM MOTILITY IN THE LIVEBEARING FISH *Xenotoca eiseni*

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ABSTRACT

Motility activation of sperm from redbelly splitfin *Xenotoca eiseni* (Family Goodeidae) 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, with three non-electrolytes (mannitol, sucrose and glucose) also did not activate motility. Combinations of CaCl_2 (0.1–40 mM) and KCl (2–60 mM) in isotonic TRIS-HCl buffer activated motility in a concentration-dependent fashion, while the concentration of K^+ showed a potential negative correlation with motility. Ca^{2+} concentrations ranging from 0 to 320 mM were tested next. The first pilot observations 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, nor at or above 280 mM Ca^{2+} (Figure 1). Bundle-like sperm clusters were observed at 200 \times magnification. Compared to the pattern observed in other similar-sized freshwater fishes, such as zebrafish *Danio rerio* and fathead minnow *Pimephales promelas*, and other internal fertilizing *Xiphophorus* fishes, it appears that sperm motility activation in *Xenotoca eiseni* is distinct and does not rely upon osmolality, but instead upon the ionic composition of the activation media.