ABSTRACTS

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PLCζ: A marker of fertility for dogs?

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OBJECTIVES AND METHODS: In mammalian oocytes e.g., mice, boars, bulls, and stallions, the initiation of intracellular calcium (Ca²⁺) oscillations at fertilization is essential for oocyte activation and embryo development. A sperm-specific phospholipase C, PLCζ (PLCζ), trigger the Ca²⁺ oscillations. Because many causes of infertility are due to a “male factor” (~30% and contributions of > 50% in humans), and given that many infertility cases are unexplained, decreased levels/absence of PLCζ may explain some cases of undiagnosed infertility, even if the presence of morphological normal sperm. Reduced expression/absence of PLCζ in the sperm could result in suboptimal oocyte activation, which may lead to development failures and lack of conception. It has recently been shown in the mouse that decreased levels of PLCζ in sperm results in litters of reduced number of offspring. In this study we investigated the presence of PLCζ in canine sperm. Our hypothesis was that the expression of PLCζ would be conserved among mammalian sperm. To evaluate species specificity, we i) injected canine sperm intracytoplasmic sperm injection (ICSI) into mouse MII oocytes (IACUC compliant), and ii) establish its presence using Western blots. These blots were loaded with equal numbers of sperm from fertile dogs, mice, boars, bulls, and stallions and used anti-PLCζ antibodies produced against different domains of PLCζ of different species.

RESULTS: Our data show that PLCζ is conserved in the species considered in this study, and, more importantly, it was detected for the first time in dog sperm (Fig. 1). The ICSI results provide functional supporting the biochemical evidence shown above (Fig. 2). The pattern of oscillations initiated in mouse eggs by dog sperm seems different than that initiated by mouse, bovine or human sperm in those oocytes, and more like the pattern generated by injection of rat sperm, which is characterized by an uneven pattern of high frequency oscillations (Fig. 2).

CONCLUSION: The present results constitute an initial demonstration of PLCζ expression in canine sperm, which raises the possibility that expression of this protein could be used as an indicator of infertility in this species. The methods used in this study could easily be translated to standardized tests in the clinical laboratory. Furthermore, we could assay the levels of PLCζ in fresh and post thawed sperm to optimize sperm preservation protocols.

Figure 1: PLCζ detection in mouse and dog sperm: Western blot analysis of dog sperm shows up to a two fold decrease in the levels of PLCζ detected between dog and mouse. Tubulin was used as a loading control protein. Immunoblotting (IB).
Figure 2: Intracellular calcium (Ca\textsuperscript{2+}) pattern of oscillations triggered by intracytoplasmic sperm injection (ICSI). Left panel: Mouse sperm injected into (8) mouse MII oocytes. Right panel: Dog sperm injected into (11) mouse MII oocytes.

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