

# Characterization of stallion PLC $\zeta$ as a model for evaluation of male fertility

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## Objectives

1. To clone and sequence equine phospholipase C zeta (PLC $\zeta$ ).
2. To characterize the ontogeny of expression and immunolocalization of PLC $\zeta$  in equine testis and sperm.

## Background and Justification

- PLC $\zeta$  is the postulated sperm-delivered factor responsible for the initiation of intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) oscillations at fertilization leading to oocyte activation in all mammalian species studied thus far.
- Microinjection of mouse PLC $\zeta$  cRNA induces [Ca<sup>2+</sup>]<sub>i</sub> oscillations and embryonic cleavage in horse oocytes; however, rates of embryonic development to the blastocyst stage are low.
- Characterization of equine PLC $\zeta$  may allow the development of better methods for parthenogenetic activation of mare oocytes and the investigation of possible causes of stallion subfertility.

## Results and Conclusions

Fig. 1: Immunoblotting of equine sperm protein yields a band at ~73 kD corresponding to PLC $\zeta$  and is detectable at concentrations as low as 0.1 x 10<sup>6</sup> sperm.

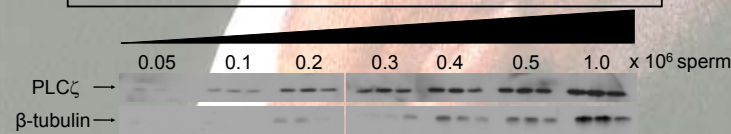


Fig. 2: Equine sperm contain more PLC $\zeta$  than murine sperm.

Fig. 3: Our clone yields <sup>35</sup>S Methionine labeled equine PLC $\zeta$  protein.

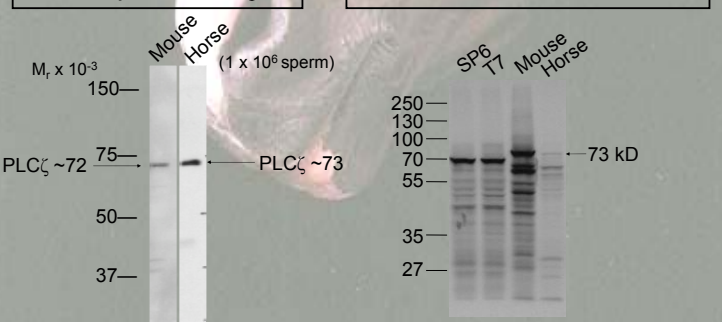


Fig. 4: Predicted equine PLC $\zeta$  protein sequence shows 71.3 - 82.5 % homology with other species.

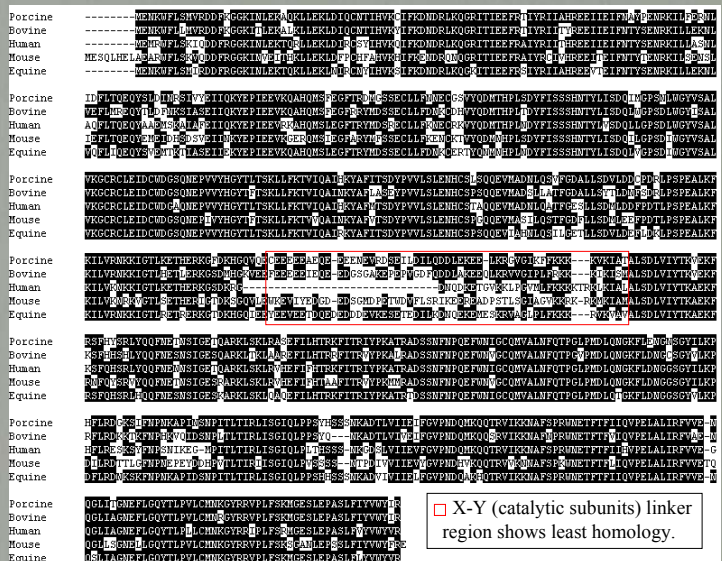


Fig. 5: Based on immunohistochemistry in testicular tissue from mature fertile stallions, expression of PLC $\zeta$  begins at the round spermatid stage.

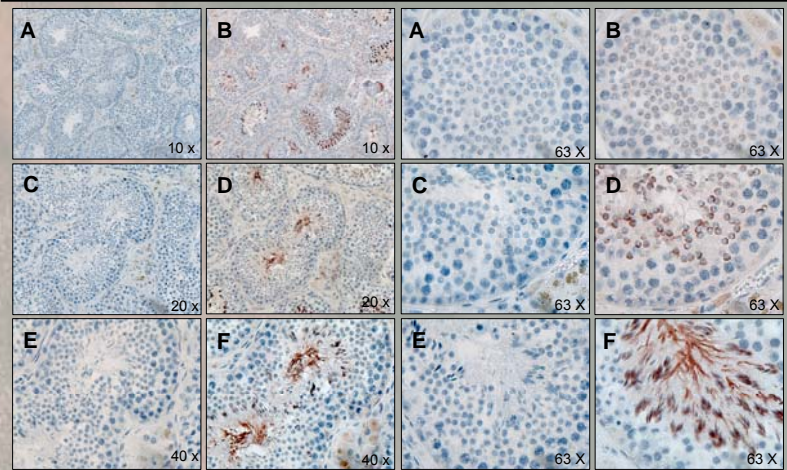


Fig. 6: Based on immunofluorescence and immunoblotting, PLC $\zeta$  is expressed in both the sperm head and flagellum (400 X).

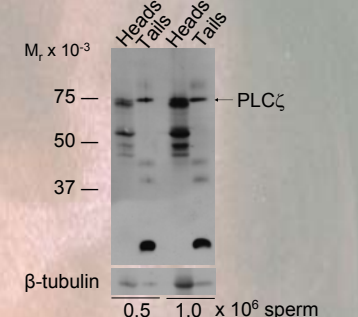
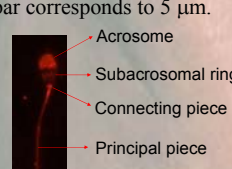
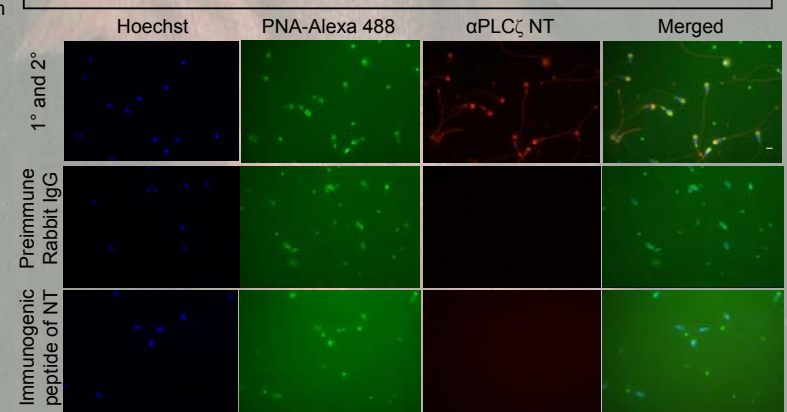


Fig. 7: PLC $\zeta$  localized to the flagellum of stallion sperm is biologically active as injection of sperm tails into fura-2 loaded mouse oocytes triggers [Ca<sup>2+</sup>]<sub>i</sub> oscillations.

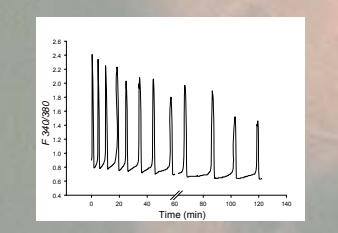
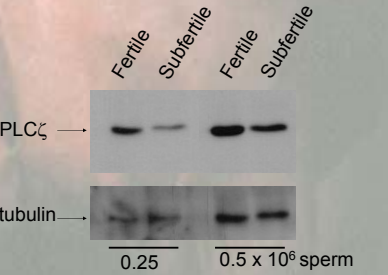


Fig. 8: Sperm from a subfertile stallion had lower relative amounts of PLC $\zeta$  than sperm from a fertile stallion.



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## Future Directions

- To semiquantitate and investigate the regional expression and bioactivity of PLC $\zeta$  in stallions with high (>60%) and low (<30%) relative fertility.