New Insights in Porcine Zonadhesin Protein Under Different Physiological Conditions

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Mammalian fertilization involves complex molecular and cellular interactions, and deviations from this orchestration can be detrimental to reproductive success. During spermatozoa-zona pellucida interaction, Zonadhesin (ZAN), a glycoprotein expressed in the acrosomal region of spermatozoa, plays a crucial role. A detailed study of zonadhesin may allow the improvement of artificial insemination protocols, the optimization of sperm selection methods, and an increase in the efficiency of swine breeding programs. Thus, the purpose of this study was to characterized pig zonadhesin protein under three different physiological conditions: noncapacitated (NCS), selected after a one-hour sperm capacitation via *swim-up* (CS1), and acrosome reacted (ARS1). The sperm-rich fraction was obtained from mature fertile tested Piétran boars (n=7). Sperm were capacitated by swim-up and the acrosome reaction was induced by calcium ionophore A23187. In each physiological condition, the immunofluorescence of the ZAN and acrosome status was determined using confocal microscopy. Negatives controls were performed. The analysis of zonadhesin distribution revealed six different staining patterns. The first pattern (P1) was significantly observed in noncapacitated spermatozoa with fluorescence only in the periacrosomal region (NCS vs CS1 $p < 0.05$; NCS vs ARS1 $p < 0.01$). Pattern two (P2), also observed mainly in NCS, showed fluorescence in the periacrosomal region as well as in the postacrosomal region. However, the dominant pattern in NCS was pattern 3, with fluorescence in the periacrosomal, acrosomal and equatorial region (NCS vs CS1 $p < 0.01$; NCS vs ARS1 $p < 0.001$). Once the sperm are capacitated by selection (CS1), P1, P2 and P3 patterns decrease leaving a new dominant pattern named P4 (CS1 vs NCS $p < 0.01$). This fourth pattern displays fluorescence in the acrosomal and postacrosomal regions. Finally, after inducing the acrosomal reaction, fluorescence is mostly observed only in the postacrosomal region (P5; ARS1 vs NCS $p < 0.001$; ARS1 vs CS1 $p < 0.01$). A small percentage of spermatozoa in all conditions were unresponsive to anti-ZAN antibodies, resulting in pattern 6. In contrast to the literature, our results demonstrate the presence of ZAN both in the acrosome and in the postacrosomal region. This might be because our antibody targets a sequence located in the MAM domain (meprin/ A5 antigen/ mu receptor tyrosine phosphatase). Since the main function described to update of zonadhesin is the specific recognition of the zona pellucida, the presence of this protein in the equatorial and postacrosomal regions could indicate that this ZAN is presumably involved in various events of fertilization. Hence, further studies are needed to determine this new function.

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