Animal Reproduction

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Male reproductive physiology and sperm technology

Early acrosome reaction in heat-shocked bovine sperm is associated with changes in Ca2+/calmodulin-dependent protein kinase II (CaMKII) signaling

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Resumo

Heat shock (HS) during bovine sperm capacitation affects its quality, resulting in low fertility. Capacitation is a biochemical process that renders the sperm competent to fertilize, resulting in a cell prone to undergo acrosome reaction. It is known that CaMKII is an important regulator of this event, in which CaMKII activity during sperm capacitation inhibits early acrosome reaction. Thus, the present work aimed to evaluate the effect of HS on acrosome reaction and phosphorylated CaMKII (pCaMKII) localization during bovine sperm capacitation. For that, bovine sperm were processed immediately after thawing (0h) or incubated in capacitating medium (1x106 cells/mL) at 38.5 °C (control) or 41 °C (HS) for 1h, 2h, 3h, and 4h. Were performed 3 replicates, in which 100 spermatozoa were evaluated per group/replicate. pCaMKII was analyzed by immunofluorescence using antibody anti-CaMKII phosphorylated at T286 (1:100). The DNA was stained with Hoescht 33342 (5 µg/mL) and the acrosome was labeled with Pisum sativum agglutinin (FITC-PSA; 100 µg/mL). The sperm were evaluated by fluorescence microscopy and the data were analyzed by two-way ANOVA followed by the post-hoc Tukey's test with a significant difference when $p \leq 0.05$. No interaction between variables (temperature x time) was observed. The acrosome integrity was negatively affected by 4h of in vitro capacitation. This negative effect on acrosome integrity was more pronounced in spermatozoa incubated at 41 °C (28.5%) compared to 38.5 °C (49.75%), in which HS increased the early acrosome reaction. Similar results were observed on pCaMKII localization. In post-thaw semen, pCaMKII was observed in the apical region of the acrosome (90%). In vitro capacitation gradually decreased the percentage of spermatozoa with pCaMKII localized at the acrosomal region. However, this effect on pCaMKII localization was higher during 4h of in vitro capacitation at 41 °C (27.75%) compared to 38.5 °C (45.75%), evidencing the negative effect of HS on pCaMKII acrosomal localization. The HS effect on pCaMKII localization was associated with early acrosome reaction during in vitro capacitation. In addition, HS affected the pCaMKII localization in sperm with intact acrosome (35.75%) compared to the control group at 38.5 °C (78.25%), showing that the lack of pCaMKII at the acrosomal region precedes the early acrosome reaction in HS sperm. In conclusion, the data presented here suggest that early acrosome reaction induced by HS may be at least in part through the HS effect on CaMKII signaling.