EFFECTS OF MILK PROTEINS AND EXTRACELLULAR CALCIUM CONCENTRATION ON THE INTRACELLULAR CALCIUM CONCENTRATION OF STALLION SPERM

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Capacitated sperm bind to the zona pellucida and undergo the acrosome reaction before penetrating and fertilizing the oocyte. Sperm capacitation is poorly understood, but includes increased intracellular calcium. We reported that milk proteins during gamete interaction greatly enhanced sperm binding to the zona pellucida (Proceedings of the 6th international symposium on equine embryo transfer; 2004. p. 24). The objectives of the present study were to determine the effects of milk proteins and calcium concentrations in the incubation media on the intracellular calcium concentration of stallion sperm. Single ejaculates from four stallions were used for each experiment. Semen was centrifuged, resuspended in modified Tyrode's medium (TALP; 2 mM $CaCl_2$) and stained with Fura-2/AM (1 μM) for 30 min at 37 °C. After incubation, sperm were washed twice, resuspended in TALP (25×10^6 sperm/mL), and incubated at 37 °C for 30 min. For intracellular calcium measurements, 2.7 mL of sperm suspension were placed in a cuvette with a microstirrer and analyzed using a spectrofluorometer. The 340/380 nm emission ratios were recorded, and the percentage of change from baseline was compared by ANOVA (and Tukey's hsd test). Regression analysis was performed for samples evaluated over a 3-h interval. In Exp. I, samples containing 2 or 4 mM $CaCl_2$ were evaluated for 150 s (baseline) and then challenged with 500 nM ionomycin to induce calcium influx into sperm at 0 and 3 h. In addition, intracellular calcium concentration was evaluated every 15 min for 3 h. In Exp. II, samples were treated with TALP (2 mM $CaCl_2$, control), native phosphocaseinate (NPPC; 1 mg/mL) or sodium caseinate (SC; 1 mg/mL) for 150 s, and then challenged with 500 nM ionomycin to induce calcium influx into sperm at 0 and 3 h. Samples were evaluated over a 3 h-period (as described above). In Exp. I, a greater increase in intracellular calcium concentration was observed after ionomycin treatment in TALP containing 4 mM versus 2 mM calcium (0 h: 17.4% versus 11.5%; 3 h: 15.0% versus 8.9%, respectively). A slow increase in intracellular calcium occurred during a 3 h-period ($p < 0.01$); however, this increase in calcium was similar between groups. In Exp. II, the increase in intracellular calcium after ionomycin treatment was similar for TALP, NPPC and SC treatments (0 h: 10.5, 7.8 and 9.2%; 3 h: 8.9, 8.3 and 8.6%, respectively). Intracellular calcium tended ($p < 0.1$) to slowly increase during a 3 h-period; however, this increase was similar among groups. In conclusion, a higher increase in intracellular calcium was observed in stallion sperm after treatment with ionomycin when TALP contained 4 mM versus 2 mM calcium. Milk proteins did not affect intracellular calcium concentration in equine sperm. Overall, intracellular calcium increased slowly over a 3 h-period, but this increase was not affected by extracellular concentrations of calcium or milk proteins.

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