

The effects of heat stress on sperm plasma membrane integrity, mitochondrial membrane potential and acrosome reaction in bulls

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Effects of elevated temperatures on spermatogenesis have been well documented for mammals and consist of impaired efficiency of spermatogenesis, as reflected by increased sperm abnormalities and DNA integrity. Until now there are no investigations on other disturbances of sperm function associated with testicular hyperthermia. The aim of this study was to examine the effects of local heating of bull testes on plasma membrane integrity, mitochondrial membrane potential and acrosome reaction of sperm.

Testes of 4 bulls were exposed to scrotal surface temperature of 34 to 36 °C induced by scrotal insulation for 48 hours. Semen was collected 3 times a week from week –1 to week 9 and once a week from week 10 to week 12 (week –1 and week 1= one week before and after scrotal insulation). After semen collection the sperm number per ejaculate (SNE) was determined photometrically and diluted at a concentration of 20×10^6 spermatozoa/mL. Using flow cytometry the proportions of sperm with following parameters were examined: intact plasma membranes (PMI) after staining with SYBR 14 and PI, high mitochondrial membrane potentials (HMP) after staining with JC1 and acrosome reaction (AR) after staining with PNA-FITC and PI. In all bulls characteristic, time depended changes in SNE, PMI, HMP and AR could be noticed. SNE decreased ($P < 0.05$) between week 1 and 4 from 7.13 to 1.81×10^9 and increased ($P < 0.05$) between week 7 and 12 from 2.26 to 7.78×10^9 . PMI and HMP fell ($P < 0.05$) from week 1 to 4 (PMI: from 77.5% to 19.6%; HMP: from 78.0% to 23.1%) and augmented ($P < 0.05$) subsequently until week 6 (PMI = 64.4%; HMP = 68.1%). Conversely AR increased ($P < 0.05$) between week 1 and 3 from 14.1% to 31.4% followed by a decline between week 4 and 7 from 30.4% to 14.7% ($P < 0.05$). From week 7 on PMI, HMP and AR did not differ ($P > 0.05$) from the values before hyperthermia. PMI and HMP were positively related ($r = 0.97$, $P < 0.0001$), while negative relationships occurred between AR and PMI ($r = -0.73$, $P < 0.0001$) and between PMI and HMP ($r = -0.73$, $P < 0.0001$).

The results of this study show that hyperthermia of testes induces defects in sperm plasma membrane integrity, mitochondrial membrane potential and acrosome reaction. These defects occur in a characteristic, time depended pattern. As alterations could already be detected in the first week after hyperthermia, it could be concluded that changes in these examined parameters are not only caused by disturbances in spermatogenesis, but also by effects of elevated temperatures on epididymal sperm.

Keywords: heat stress, sperm, plasma membrane, mitochondria, acrosome