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## JOURNAL ARTICLE

# Chromatin structural changes in sperm after scrotal insulation of Holstein bulls

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The reported effects on semen quality ascribed to testicular heat stress generally relate to traits impacting sperm transport and fertilizing ability but not to the genetic material contained by the sperm. To characterize the effects of testicular heat stress on sperm chromatin, susceptibility of DNA in sperm nuclear chromatin to in situ acid denaturation was measured by flow cytometry after staining with acridine orange using the sperm chromatin structure assay (SCSA). Semen was collected from Holstein bulls at 3-day intervals, before and after 48-hour scrotal insulation, until the morphologically abnormal sperm content in raw semen exceeded 50%. After cryopreservation in egg yolk-citrate extender, semen was thawed and sampled during incubation in vitro at 38.5 degrees C. Overall, SCSA results showed that chromatin susceptibility to denaturation was increased for sperm collected post- vs. preinsulation and was more pronounced for sperm presumably in the testes during insulation than for those sperm presumably in the epididymides. Increased susceptibility was detected as early as the first collection postinsulation; however, chromatin of sperm presumably in the proximal epididymis during insulation did not appear to have been detrimentally affected. Chromatin susceptibility to denaturation increased with increased incubation time in vitro, but the rate of change in susceptibility during incubation did not differ among pre- vs. postinsulation specimens. We conclude that elevated scrotal temperatures adversely affect both epididymal and testicular sperm by reducing sperm chromatin stability. The effects of heat stress on the chromatin of epididymal sperm were more subtle than those exhibited by testicular sperm but detectable within close proximity to the heat stress event.

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