Injectable trace minerals increase mRNA expression of some Toll like receptors in testes of bulls with heat induced testicular degeneration

Mohammad Ibrahim,[a] Maria Ferrer,[a] Roberto Palomares,[b] Annie Bullington,[a]
Alejandro Hoyos-Jaramillo,[b] João Bittar[b]
[a]Department of Large Animal Medicine, [b]Department of Population Health
College of Veterinary Medicine, University of Georgia, Athens, GA

Activation of Toll like receptors (TLRs) initiates an innate immune response. These receptors recognize pathogen-associated ligands in microorganisms and endogenous damage-associated molecular patterns (DAMPs) released by damaged cells. Treatment with 1 dose of injectable trace minerals (ITM; 1 ml/150 lb, Multimin 90, Multimin North America, Fort Collins, CO) resulted in a more pronounced decrease in semen quality in bulls with testicular degeneration. Release of DAMPs by degenerating germ cells may exacerbate testicular dysfunction via activation of testicular TLRs. Since ITM can upregulate immune function in cattle, we hypothesized that TLRs are expressed in bovine testis, and that TLR mRNA transcript levels are affected by TMs in bulls with testicular degeneration. Objectives were to characterize mRNA expression of TLRs in bulls with testicular degeneration and after treatment with ITM. Testicular degeneration was induced by scrotal insulation for on days 0 - 8.
Bulls (n = 9) were assigned to 3 groups; C: control without ITM; TM1: received ITM on day -21; and TM2: received ITM on days -21 and 8. Testicular biopsies were collected from 3 bulls in each group on day 110. Testicular samples were frozen at -80ºC until RNA extraction. Samples were cryopulvurized and total RNA was extracted using Qiagen RNeasy Mini Kit. Total RNA was subjected to reverse transcription and qPCR. Level of gene transcription was estimated in comparison with standard curves generated from 10 fold serial dilutions of amplicons. Expression values were normalized against GAPDH as a housekeeping gene. Expression of TLRs 1 - 10 was confirmed in testis of bulls with testicular degeneration. Bulls in group TM1 had higher mRNA expression of TLR1 (mean ± SEM:
C 0.032 ± 0.005, TM1 0.056 ± 0.006, TM2 0.04 ± 0.006) ($p = 0.022$, ANOVA) than group C, but not TM2. A similar tendency was seen for increased transcripts levels of TLR4 ($p = 0.057$), TLR6 ($p = 0.075$) and TLR10 ($p = 0.05$) in group TM1. Bulls in group TM1 also had a tendency for increased transcript levels of TLR1 ($p = 0.089$), 3 ($p = 0.079$), 4 ($p = 0.076$), 7 ($p = 0.066$) and 9 ($p = 0.068$) compared to group TM2. There were no differences in mRNA expression between C and TM2. There was moderate to strong correlation ($R^2 > 0.3$) between certain TLRs, which indicated that TLRs were expressed cooperatively to provide a robust innate immune response within bovine testes. We concluded that bovine testicular tissue expressed TLRs at the mRNA level and expression of some TLRs was upregulated by a single dose of ITM in bulls with testicular degeneration.

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