Relative sperm volume shift, aquaporin 7 mRNA abundance and bull fertility estimates
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Integrity of sperm plasma membrane is important for sperm function. Mammalian sperm experience a natural osmotic decrease during male to female reproductive tract transition. This hypoosmotic exposure not only activates sperm motility, but also poses potential harm to sperm structure and function when improper swelling of spermatozoa occurs. Aquaporins (AQP) are a family of membrane channel proteins implicated in sperm osmoregulation. There are a variety of assays testing the membrane integrity of spermatozoa including the hypoosmotic swelling test (HOST). However, HOST has shown varied correlation with fertility. Alternatively, sperm volumetric parameters evaluate not only swollen cells, but also the degree of swelling. Given the varied correlation between HOST and fertility, our hypothesis is sperm volumetric measurement will better predict fertility than HOST and aquaporin7 mRNA abundance will putatively play a role in osmoregulation. The objective was to determine the association among relative sperm volume shift, % HOST, sperm AQP7 mRNA abundance and sire conception rate (SCR; fertility index) in Holstein bulls. The SCR estimate for the study bulls (N=34) was based on at least 500 services, (725±13 services/sire) and for each full point SCR score (from −4 to +4), 3 to 5 sires were included. Samples of frozen thawed sperm from a single collection from these commercial Holstein bulls were used to evaluate relative mRNA expression of AQP7 in sperm. Sperm cell volumetric measurements (using Image J1.42q and Quantity One 4.6 software) and % HOST (using microscope) were determined for 400 sperm after incubating the samples in isoosmotic (300 mOsm/kg) and hypoosmotic (100 mOsm/kg) solutions for 15, 30 or 60 min. The relative sperm volume shift ($V_r=V_{hypo}/V_{iso}$) was used as a measure of volume regulation in response to hypoosmotic conditions. Data were analyzed using SAS (Version 9.3 for Windows, SAS Institute, Cary, NC). Coefficient correlations were estimated using PROC CORR to determine the association of CT (threshold) values (dCT) for AQP7, % HOST and relative volume shift with individual bull SCR-scores. The RT-PCR data, $2^{ΔΔCt}$ values for mRNA expression of AQP7, and relative sperm volume shift data were analyzed by ANOVA to ascertain statistical significances. There was no correlation between %HOST and SCR ($r=0.28$, $P>0.1$). There was a significant positive correlation between relative sperm volume shift and SCR ($r=0.43$ to 0.65, $P<0.05$). The AQP7 mRNA abundance was positively correlated to relative volume shift ($r=0.64$ to 0.73; $P<0.05$) and SCR ($r=0.67$; $P<0.05$). The mRNA expression of AQP7 and relative sperm volume shift differed among low (<2 SCR), average (-2 to +2) and high (>2) fertility sire groups ($P<0.05$). In conclusion, bulls with higher SCR had a greater quantity of AQP7 mRNA in frozen thawed sperm. This may have contributed to greater regulation of sperm volume shift which protected the sperm from detrimental swelling and impaired functions.

Keywords: Holstein bull, sire conception rate, sperm volume, HOST, aquaporin