PRODUCTION ANIMAL SESSION

Preliminary report on bovine prenatal sex determination using PCR in maternal peripheral blood

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Fetal sex identification at pregnancy diagnosis may prove beneficial to beef producers as they could make herd management decisions in an organized manner well in advance. Realtime ultrasonography is an ideal tool to obtain immediate information about the pregnancy status and fetal sex; however, the method is time-consuming, needs expertise, and lacks accuracy beyond 90 days of pregnancy. Alternatively, sex-chromosome-specific genes of the fetal DNA circulating in maternal peripheral blood can be targeted using polymerase chain reaction (PCR), but the technique needs further validation before its commercialization. Therefore, the objective of the present study was to identify an appropriate PCR methodology to target sex-chromosome-specific genes to detect fetal DNA in maternal plasma for determining fetal sex in pregnant cows. We hypothesized that both bovine amelogenin (bAML) and Y-chromosome specific genes are targets via PCR to predict fetal sex in cattle. In this initial experiment, we enrolled dairy cows (n = 5; 4 - 5 months pregnant) carrying male fetuses (confirmed by ultrasonography) from the Rayner Dairy Research and Teaching Centre at the University of Saskatchewan, Saskatoon, Canada. We collected 18 ml blood from each cow in K2 EDTA vials to harvest plasma. Fresh plasma and frozen aliquots were processed for DNA extraction using DNeasy Blood and Tissue, MagMAX cfDNA isolation, KAPA express extract, Nucleomag cfDNA isolation, QIAamp DNA Blood Midi, and QIAamp DSP Virus Kits. In addition, blood cells from bulls (n = 5) and nonpregnant heifers (n = 5) were processed for DNA isolation and subjected to PCR to validate primers and identify the PCR conditions. Isolated DNA from the plasma of pregnant cows was used as a template for bAML and Y-specific gene PCR to predict fetal sex. No statistical comparisons were carried out due to the small sample size. The experiment results indicated that when frozen plasma samples were processed for DNA isolation (DNeasy Blood and Tissue Kit, MagMAX cfDNA isolation Kit), PCR failed to predict fetal sex. However, PCR on DNA isolated from fresh maternal plasma using the QIAamp DSP Virus, DNeasy Blood and Tissue, Nucleomag cfDNA isolation, and MagMAX cfDNA isolation Kits correctly predicted the presence of male fetii in 3/5, 2/5, 2/5, and 2/5 cows, respectively. However, PCR on the DNA obtained from both QIAamp DNA Midi and KAPA express Kits failed to predict male fetus in all 5 cows. In conclusion, the DNA isolation methods compared so far had variation in their ability to isolate fetal DNA from the maternal plasma of pregnant cows. We are pursuing spiking experiments with fetal DNA from aborted fetuses to further validate DNA extraction methodology for cell-free DNA from maternal plasma.

Keywords: Bovine amelogenin, fetal DNA, Y-chromosome specific gene, fetal sex, pregnant cattle

Melatonin and l-arginine mitigate heat stress-induced reductions in quality of frozen-thawed ram sperm

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Adding melatonin or l-arginine to semen extender enhanced postthaw sperm quality and protected against cryopreservation-induced oxidative stress.[1,2] The objective was to determine the effects of melatonin or l-arginine on quality of frozen-thawed sperm from rams subjected to heat stress (HS). We hypothesized that addition of melatonin or l-arginine mitigates the effects of heat stress on frozen-thawed ram sperm. Ten Dorset rams with good semen quality were group-housed indoors (~ 18˚C), randomly allocated into 2 equal groups and subjected to either whole-scrotum insulation for 96 hours or placed in a warm room (28˚C, 30 - 34% relative humidity) for 8 hours per day for 4 consecutive days. Semen was collected weekly for 1 - 5 weeks after HS, extended (Steridyl CSS One Step®) and divided into 5 aliquots: no additives (control) or 0.5 or 1 mM of either melatonin or l-arginine. For cryopreservation, semen was refrigerated for 2 - 3 hours, cooled to 5˚C, then loaded into 0.5 ml straws that were placed in straw racks and held horizontally in a styrofoam box, 5 cm above liquid N_2 for 10 minutes and then plunged into liquid N_2. Straws were subsequently thawed at 37˚C for 35 seconds and immediately evaluated for postthaw motility using CASA (Sperm Vision®),

morphology using eosin-nigrosin, and acrosome integrity using FITC-PSA. Data were analyzed using repeated measures, with a post-hoc Bonferroni test. For total and progressive motility, there were effects of group ($p = 0.023$ and $p = 0.0008$, respectively); for total abnormalities, there were effects of group ($p = 0.001$ and a group*week interaction ($p = 0.003$); and for acrosome integrity, there were effects of group ($p = 0.046$) and week ($p = 0.0001$). On all days, all end points were significantly improved for all treatments compared to control. All 4 treatments improved motility, whereas improvements in total abnormalities and acrosomal integrity were dose-dependent (greatest improvement with 1 mM). Total and progressive motility were improved by ~ 5 - 10 percentage points, whereas total abnormalities and intact acrosomes were improved by ~ 7 and 12 percentage points, respectively. Bowed midpiece, ruffled acrosome and distal midpiece reflex were highest in the control group. In summary, exogenous melatonin or l-arginine in semen extender mitigated HS-induced reductions in quality of frozen-thawed ram sperm by improving motility and acrosome integrity and reducing total abnormalities.

Keywords: Ram, sperm, melatonin, l-arginine, Heat stress

References

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An example of incorrect storage of bull semen samples on spermiogram assessment

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Sperm morphology assessment and interpretation is an integral part of bull breeding soundness evaluation. Historically the spermiogram has been classified into primary, secondary, and tertiary; major or minor; compensable or uncompensable abnormalities; and reporting individual sperm defects. Abnormalities that might occur after semen collection (tertiary abnormalities) are often discussed. However, these abnormalities are generally not well defined nor explained. We report tertiary abnormalities that were detected because of incorrect samples submitted for morphological assessment. Samples from a cohort of bulls were collected for morphological assessment as part of breeding soundness examination. The samples were examined grossly, crush side motility was assessed by diluting the sample in isotonic phosphate buffered saline (PBS). An ejaculate aliquot was placed in 10% buffered formol saline (BFS) for morphological assessment via differential interference contrast microscopy at 1000 x magnification. In this case, some of the PBS diluted samples were inadvertently sent for morphological assessment in the first instance. The results appeared aberrant, with a large proportion of loose and detached heads, and abnormal tails. The correctly stored samples were located and subsequently assessed. PBS and BFS samples ($n = 13$) had substantial differences in spermiograms between the storage methods. The BFS samples had 12/13 spermiograms with ≥ 68% morphologically normal sperm. By comparison, 1/13 of the PBS samples had ≥ 68%, with 5/13 having fewer than 20% normal sperm. There were 2 samples in the BFS cohort that had ≥ 19% loose or detached heads, compared to 12 in the PBS cohort that had ≥ 35%, 7 of which had ≥ 55% loose or detached heads. Most of the abnormalities detected in the PBS samples were a combination of loose and detached heads. Interestingly, the tails, particularly the detached tails, were noticeably devoid of the plasma membrane for some or most of the principal piece and other parts of the tail. These were typically not documented in the interpretation of the spermiogram, as most were recorded as detached heads. Particular tertiary abnormalities are not often described in the literature. The inadvertent error of assessing the incorrect samples has given an opportunity to report abnormalities that are most likely due to incorrect storage of samples for morphological assessment of an ejaculate. It is clear from these observations that appropriate collection and storage of samples for morphological assessment is carried out when assessing spermiograms. Incorrect sample preparation and storage should be considered as a reason for an abnormal spermiogram, especially when a large proportion of detached heads, with or without tail plasma membrane abnormalities, are detected in a semen sample.

Keywords: Bull, spermiogram, tertiary morphological abnormalities, detached heads

Obstructive urolithiasis in a dromedary camel

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A 9-year-old, castrated, male dromedary camel was presented with an inability to urinate for 1.5 days. The camel had a history of severe malnutrition and had been castrated prior to onset of puberty. The camel was maintained in a petting zoo and had received an excessive amount of grain prior to his presentation. The animal was bright, alert and responsive with moist and pink mucous membranes, but had mild icteric sclera and was posturing to urinate. There were perineal urethral pulsations accompanied by tail flagging. Severe enlargement of the bladder was diagnosed via transrectal palpation and ultrasonography. The bladder was ~ 12 inches in diameter with a thickened wall and mucus debris within