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®),