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

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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

the fluid. On abdominal ultrasonography, there was no free abdominal fluid observed. Ultrasonography of the penis and urethra revealed a hyperechoic structure proximal to the glans penis, and the urethra was intact. The penis could not be extended for examination due to nonseparation of the penis from the prepuce as a result of early castration. The urine pH was 6.0 and specific gravity was 1.028. On microscopic examination of the urine sample urate crystals were observed. Serum chemistry had an elevated BUN (52.3 mg/dl; normal range 11 - 30), creatinine (7.6 mg/dl; normal range 1-2.3), AST (79 U/L) and serum iron (22 µg/dl). The diagnosis was obstructive urolithiasis. A tube cystotomy surgery was done in a 'cushed' position using the following intravenous anesthetic protocol: detomidine (0.03 mg/kg), torbugesic (0.06 mg/kg) and ketamine (2.5 mg/kg). The bladder was accessed with a blind stick using a scalpel blade. A Foley catheter with a stylet was inserted through the incision and the bladder was flushed with saline solution. The tube was sutured in place to allow for urine expression. Antibiotic therapy with ceftiofur crystalline free acid (6.6 mg/kg subcutaneous, daily) and sulfadimethoxine (55 mg/kg initial dose, 27.5 mg/kg subsequent doses, intravenously, daily) and an antiinflammatory (flunixin meglumine 1.1 mg/kg, intravenously, once every 12 hours) was started. Acepromazine (20 mg intravenously) to cause urinary tract relaxation and fluid therapy (5 liter bolus, once every 12 hours) were done. There was a continuous and steady urine dripping from the Foley catheter after the surgery and during the next 3 days, so the treatment plan was continued. However, on the fourth day after surgery, the animal was seen posturing to urinate and the bladder was lavaged with 5 liters of saline solution, during which bloody drops were noted in the prepuce. Urine had a pH of 7 and struvite crystals were seen. Hence, a total of 120 ml of Walpol's solution was placed in the bladder. Then, 30 minutes later, another 5 liters lavage was done. The next day, the Foley catheter had become occluded due to fibrin deposition. The animal was posturing more frequently, although the bladder was small on ultrasound. Animal was sedated in accordance with the previous surgery and an epidural was given with 3 ml of lidocaine. A perineal urethrostomy and penile amputation was performed while the animal was in a cushed position. The urethra was then spatulated at the level of the skin incision and a catheter was placed. The camel continued to urinate successfully, and treatment was discontinued. The animal was sent home on allopurinol to be given every other day to aid in reduction of uric acid and was instructed to receive a low grain diet. Early castration in camelids can predispose to a narrowed urethra that coupled with a high grain diet may lead to urolithiasis.

Keywords: Camelid, urogenital system, bladder, urethra

Evaluation of ovarian response to PG600 in alpacas

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The hormonal preparation PG600, a combination of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG), is commonly used for swine estrus synchronization. However, it is often used off-label in sheep and goats to replace the eCG formulation not available in the US. In alpacas, eCG has been used alone or in combination with FSH to induce superovulation, with variable results. The aim of this preliminary trial was to investigate the effect of PG600 on follicular dynamics in alpacas. We hypothesized that a single treatment of PG600 (800 IU eCG and 400 IU hCG in 5 ml) 2 days after ovulation results in ovarian follicular superstimulation in alpacas. Adult multiparous alpacas (n = 9) were used in the experiment. Ovarian follicular activity was monitored by transrectal ultrasonography. Ovulation was induced with GnRH (100 µg) given intramuscularly when a dominant follicle reached at least 8 mm in diameter and uterine tone and edema were present. All females received 5 ml PG600 intramuscularly 2 days after induction of ovulation. Ovarian follicular response was assessed by transrectal ultrasonography on day 7 after PG600 treatment and the follicles were recorded and counted by 2 clinicians. Ovulation was induced with GnRH (100 µg) given intramuscularly. The mean number of follicles between 7 and 12 mm in diameter present in the ovaries after the treatment was 14.9 ± 13.8 (mean ± SD). There was a large variation among females in the number of follicles, which ranged from 1 to 38. The ovulation rate (number of corpora lutea) following induction was very low 1.1 ± 1.6 (mean ± SD). The maximum ovulation rate (5) was observed in a female that had 6 follicles after stimulation. All females presented anovulatory hemorrhagic follicles 2 days after induction of ovulation (10.0 ± 11.5, mean ± SD). In conclusion, PG600 induced ovarian follicular stimulation (> 2 mature follicles) in 7 out 9 alpacas. However, the follicular response had large individual variability. The ovulation rate after ovarian superstimulation with PG600 was poor. However, this poor ovulatory response has been also observed with eCG and FSH. This preliminary trial suggests that PG600 may not be appropriate for ovarian superstimulation in multiple ovulation embryo transfer programs in alpacas but could be used for oocyte recovery within other advanced reproductive techniques.

Keywords: Ovulation, superovulation, camelids, anovulatory follicles