

EFFECT OF VASCULAR DEGENERATION ON UTERO-OVARIAN BLOOD FLOW AND PERFUSION IN THE CYCLIC MARE

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Adequate blood supply to the uterus is necessary for normal function during reproductive cyclicity and gestation in the mare. Hormonal distribution, signaling, uterine contraction, placentation and early and late gestation are vascular-dependent events. Infertile/subfertile mares with histories of chronic uterine infection and uterine biopsies of Grade III present moderate to severe vascular degeneration throughout the uterus (12/16); this presumably has a negative effect on uterine perfusion and subsequently on normal reproductive function. Our objective was to examine the effect of vascular degeneration on utero-ovarian blood flow and perfusion. Uterine blood perfusion was determined using fluorescent microspheres. When 15 μ m microspheres are injected into the left ventricle of the heart, blood perfusion to specific target organs is proportional to the number of microspheres sequestered in that organ. Mares ($n = 5$) were anesthetized and 40×10^6 fluorescent microspheres were injected directly into the left ventricle of the heart via a cardiac infusion catheter at a calibrated rate, while four different blood samples were also withdrawn from the carotid, facial, and dorsal metatarsal arteries. Mares were then euthanized and the reproductive tract was removed. Uteri and ovaries were sectioned into small pieces. Blood samples and sections were incubated in 4 M KOH for the digestion of the tissue and recovery of microspheres. The fluorescent dye was extracted with 2 mL of Cellosolve acetate and each sample was then read with a spectrofluorometer to determine fluorescent intensity and rate of blood perfusion. Degree of angiopathy and endometriosis was evaluated in full thickness sections of the uterine wall and classified for vascular degeneration depending on the severity and extent of the lesions. Four mares were injected with microspheres during diestrus. The uteruses of two mares were categorized as having a Grade I biopsy classification (Kenney classification) and normal vasculature; two other mares had moderate to severe elastosis of the vasculature and Grade III

biopsy. Uterine blood perfusion values showed a large difference between the two diestrous groups, 11.91 mL/min/100 g (control mares) versus 7.15 mL/min/100 g (mares with elastosis). This represents a difference of 40%, which appeared to be distributed throughout the uterus with maximal differences (up to 57.7%) in the distal uterine body. A uterine blood perfusion value of 7.93 mL/min/100 g was found in a single mare with normal vasculature during estrus. Blood perfusion values for the ovaries were similar in the diestrous groups, with a higher blood perfusion in the ipsilateral versus the contralateral ovary (57.5 mL/min/100 g versus 7.3 mL/min/100 g). Results suggested that blood perfusion of the uterus was decreased in mares with elastosis of the uterine vasculature and is cycle-dependent. This compromised blood flow may be responsible for some unknown causes of subfertility/infertility. Further studies are needed to confirm these findings and to assess differences in perfusion during estrus, fertility rates and treatment strategies.

Keywords: Uterus; Mare; Perfusion; Infertility; Elastosis

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ANALYSIS OF EQUINE SEMEN FOR EQUINE HERPESVIRUS 1 USING TAQMAN PCR

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Equine herpesvirus 1 (EHV-1) has been shown to be present and active in the testes, epididymides and accessory sex glands of stallions under both experimental and natural conditions. In other species, herpes viruses are known to be present and transmitted via semen and to cause infertility and abnormalities in the ejaculate. The objective of this study was to analyze equine semen for the presence of EHV-1 DNA, using a previously validated real-time Taqman PCR assay. Sperm cell morphology and cellular composition of the samples collected were concurrently evaluated.

Semen samples were collected from stallions involved in breeding programmes at local stud farms, as well as from stallions presented to the Equine Reproduction Service at UC Davis. Stallions in this

latter group were presented for a variety of reasons, most commonly breeding soundness examination or investigation of sub-fertility. Samples were collected from 50 stallions of various breeds (age range, 3–25 years) using a Missouri model artificial vagina with an in-line gel filter. Volume and sperm cell concentration were measured, and the total number of sperm in the ejaculate calculated. A fixed sample and a stained smear were obtained for sperm morphologic evaluation and cellular analysis respectively. Raw semen (0.13 mL) was added to 1 mL of PBS, and centrifuged (1500 rpm for 10 min). Following aspiration of the supernatant, the sperm pellet was re-suspended in 0.5 mL of lysis buffer to minimize sample deterioration prior to testing. Samples were placed in a 96-well plate and stored at -20°C until testing. Samples were analyzed for EHV-1 DNA using real-time Taqman PCR. The primers used in this assay target the highly conserved glycoprotein B gene on EHV-1 viral DNA. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for quality control.

EHV-1 DNA was not detected in any of the samples analyzed. These results suggested that the incidence of EHV-1 shedding in the semen of normal stallions is either very low or non-existent.

Keywords: Taqman PCR; Equine herpesvirus-1; Semen; Stallion

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Food animal

EFFECTS OF rBST ON PREGNANCY RATES OF BEEF COWS SYNCHRONIZED WITH A CO-SYNCH-CIDR PROTOCOL AND INSEMINATED AT FIXED TIME

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Recombinant bovine somatotropin (rBST, Posilac, Monsanto, St. Louis, MO, USA) increases milk production, but the effects of this hormone on reproductive performance are controversial. We evaluated the effect of rBST on the conception rates of beef cows synchronized with a CoSynch-CIDR protocol and inseminated at fixed time (TAI). High fertility semen from a single bull was used and TAI was performed by the same technician. Multiparous nursing beef cows ($n = 134$) were allocated in two groups paired by age,

days postpartum, and parity. Sixty-nine cows were in the Spring and 65 in the Fall breeding herd, respectively. Treated cows ($T: n = 34$ in the Spring and $n = 33$ in the Fall) received 500 mg of Posilac and Control cows ($C: n = 35$ in the Spring and $n = 32$ in the Fall) received sham treatment every 2 weeks, starting 14 days prior to the onset and during the entire spring and fall breeding seasons (60 days per season). All cows were synchronized using a CoSynch-CIDR and TAI protocol as follows: *Day 1*: an intravaginal progesterone-releasing device (CIDR) inserted and a GnRH injected; *Day 8*: CIDRs were removed and cows were given $\text{PGF}_2\alpha$; *Day 11* (66 h after $\text{PGF}_2\alpha$ injection): TAI and a dose of GnRH. Cows were ≥ 45 days postpartum at TAI. Pregnancy diagnosis was performed using real time ultrasonography using a 5 MHz transducer at 28, 42 and 56 days after TAI. Pregnancy rate and the effect of season and treatment was analyzed using Pearson's Chi-Square Test, whereas days open was analyzed using a Student's *t*-test for independent samples. There were no significant differences in pregnancy rates at 28 days (Spring: $T = 50\%$ versus $C = 43\%$, and Fall: $T = 67\%$ versus 56%), 42 days (Spring: $T = 44\%$ versus $C = 43\%$, and Fall: $T = 67\%$ versus $C = 56\%$) and 56 days after TAI (Spring: $T = 38\%$ versus $C = 40\%$ and Fall: $T = 64\%$ versus $C = 56\%$). However, there was a difference between the mean number of days postpartum and pregnancy rate in the treatment group where cows with fewer days postpartum had higher pregnancy rates (T open = 78.9 days, T pregnant = 72.9 days, $P = 0.019$). Similar results occurred when season was considered; there was a difference between the mean number of days postpartum and pregnancy rate in the fall group with cows with less days postpartum having higher pregnancy rates (Fall open = 77.4 days and Fall pregnant = 71.8 days; $P = 0.011$). Despite the fact that the differences were not statistically significant, a trend for an improvement in pregnancy rates in treated cows was observed, especially in the fall breeding season. Treated cows with shorter postpartum had better pregnancy rates, probably due to increased levels of IGF-I improving follicular development and oocyte maturation. Heat stress, increased milk production and decreased dry matter intake in cows treated during spring/summer might explain this difference. In conclusion, treatment of beef cows with rBST in conjunction with CoSynch-CIDR-TAI did not have detrimental effects on pregnancy rates.

Keywords: Somatotropin; Beef cows; Timed artificial insemination

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