

THE INFLUENCE OF MANAGEMENT AND VETERINARY PRACTICES ON REPRODUCTIVE PERFORMANCE IN THE THOROUGHBRED MARE

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The objective of this study was to examine the effects of management and veterinary practices on reproductive performance of Thoroughbred mares in central Kentucky. Using a prospective cohort design, breeding records were collected from 13 farms in central Kentucky during the 2004 breeding season, and 12 farms in 2005. Factors influencing pregnancy outcomes on day 15 and pregnancy loss between days 15 and 40 were analyzed using multiple logistic regression for clustered data with SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA).

In 2004, data was available from 1091 mares bred on 1718 cycles, with 38.1% of the mares being bred more than one time during the season. In 2005, 894 mares were bred on 1390 cycles, with 37.8% of the mares being bred more than one time. The characteristics of age, status, and average number of cycles bred were similar between the years.

The days 15 and 40 pregnancy rates and pregnancy loss from days 15 to 40 both per cycle and per season were similar between the 2 years. The average pregnancy rates per cycle on days 15 and 40 were 62.0% and 55.8%, respectively. Pregnancy loss from days 15 to 40 per cycle was 9.9%. The average pregnancy rates per season on days 15 and 40 were 92.0% and 89.3%, respectively. Pregnancy loss from days 15 to 40 per season was 3.0%.

Although the overall per cycle pregnancy and pregnancy loss rates were similar between the 2 years, there were significant differences in per cycle pregnancy and pregnancy loss rates between the years on individual farms. On one farm the day 15 per cycle pregnancy rate was 14.7% greater in 2005 than 2004.

In the 2005 foaling season, the live foal rates per cycle and per season were 50.8% and 78.4%, respectively. Pregnancy loss from day 40 to foaling was 12.3%.

The following significantly increased the odds of the mare being pregnant on day 15: mares bred to a stallion with a large (>110 mares) book size, and mares given progesterone supplementation between breeding and the day 15 pregnancy check. As mare age increased, the odds of being pregnant on day 15 decreased significantly. Several farm characteristics were tested, but were not significant in the multivariable model (e.g., boarding mares for clients, regular ultrasound use pre-breeding,

record keeping system, farm size, and presence of a culling program). However, there were significant differences in the day 15 pregnancy outcomes between farms even after controlling for factors listed above. This emphasizes more work is needed to determine farm level characteristics influencing pregnancy rates on day 15.

The following significantly increased the odds of losing a pregnancy between days 15 and 40: increasing mare age, and being a foaling mare at the start of the season. There were not significant differences in pregnancy loss between the farms. Due to the variability of pregnancy and pregnancy loss rates within farms by year it is important to collect data over a multi-year period when studying factors that influence pregnancy rates and pregnancy loss.

Keywords: Mare; Pregnancy; Reproductive performance

UTILITY OF THE SPERM CHROMATIN STRUCTURE ASSAY (SCSA) FOR INCREASED REPRODUCTIVE EFFICIENCY IN LARGE ANIMALS

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The DNA fragmentation index (DFI), as derived by the Sperm Chromatin Structure Assay (SCSA), is a measure of sperm DNA fragmentation in humans and animals. While a semen evaluation provides information regarding the external physical characteristics of sperm, it does not address sperm DNA fragmentation, which has been shown repeatedly by various assays to have a significant negative effect on pregnancy outcome in animals and humans.

Frozen/thawed semen was treated for 30 s with pH 1.20/detergent buffer, stained with acridine orange and measured by flow cytometry as previously detailed.

Fertility is assessed in stallions by a breeding soundness exam, usually including a physical exam and a routine semen analysis. Significantly lower DFI and a higher seasonal pregnancy rate (SPR) were found in fertile stallions in comparison to the sub fertile group. Correlations between DFI and motility/morphology showed significant but biologically weak relationships which are in agreement with human data. Medications have been shown to negatively affect sperm DNA fragmentation in humans. Likewise stallions and bulls exposed to hormonal or therapeutic

medication had elevated DFI values, e.g., 48% which following removal of the medication decreased over a period of 2 months to 10%. For boars, a heterospermic trial was conducted to assess fertility. The boars were divided into two groups consisting of three boars of higher fertility and three of lower fertility. Boars siring more piglets than statistically expected had significantly lower %DFI than those that had fewer piglets. In another study, a significant negative correlation was found between average total number of pigs born per litter versus %DFI suggesting that sperm with fragmented DNA fertilized the eggs but the embryos died in utero due to sperm deficient DNA. Bulls with the best heterospermic performance had significantly ($r = 0.94$, $p < 0.01$) lower %DFI. In other bull fertility studies, SCSA data were significantly correlated (-0.65 , $p < 0.01$) with non-return rates.

Many cases have been seen where different species of males have been considered fertile due to an acceptable semen analysis. However, when such males failed to produce offspring, the SCSA has often provided the likely etiology, namely, the presence of live, normal appearing sperm that have fragmented DNA leading to early embryo death.

Keywords: Sperm DNA fragmentation; SCSA; Infertility

CRYOPRESERVATION OF STALLION SEMEN TREATED WITH CHOLESTEROL-LOADED-CYCLODEXTRINS

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Cholesterol-loaded-cyclodextrins (CLCs) were added to stallion sperm in an attempt to improve cryosurvival. Eight stallions were collected three times a week for 5 weeks. Each ejaculate was diluted to 50×10^6 sperm/mL in a modified Tyrode's Medium and split into two equal fractions. One fraction was treated with 1.5 mg/mL CLCs while the other was not treated. Samples were held at 22 °C for 15 min, then centrifuged at 2400 rpm for 11 min, and the sperm pellets suspended to 400×10^6 sperm/mL in a EZ-Freezin "LE" (Animal Reproduction Systems (ARS), Chico, CA, USA). The sperm were packaged into 0.5 mL straws, frozen in liquid nitrogen vapor, and stored in liquid nitrogen for >48 h prior to thawing. Straws were thawed in 37 °C water for 30 s, the sperm diluted to 40×10^6 sperm/mL

in EZ-Mixin' CST (ARS) and maintained at 37 °C for 5 min prior to motility analysis using a computer-assisted sperm analysis system (IVOS, Hamilton Thorne Biosciences, Beverly, MA, USA). Treating sperm, with CLCs prior to freezing, improved cryosurvival of sperm for all stallions and ejaculates within stallions. Samples treated with CLCs exhibited higher percentages of total motile sperm (51%) than the control sperm (37%; S.E.M. = 3; $P < 0.05$). Similarly, progressive motility for CLC-treated sperm was higher than the control sperm (33% versus 22%, respectively, S.E.M. = 2; $P < 0.05$). In conclusion, addition of CLCs to stallion sperm prior to freezing maintained higher total and progressive motility of equine sperm after cryopreservation. In addition, this procedure can easily be incorporated into routine processing of stallion sperm for cryopreservation.

Keywords: Cholesterol; Semen; Equine; Cryopreservation; Motility

EFFECT OF CENTRIFUGATION ON EQUINE SEMEN QUALITY OVER TIME DURING COOL-STORAGE INCUBATION

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The objective of this study was to determine the effect of centrifugation over time on equine semen quality during cool-storage incubation. We hypothesized that the removal of seminal plasma would result in higher semen quality over time than non-centrifuged semen samples.

Twenty ejaculates were collected from eight stallions of known fertility. Immediately after collection, concentration, motility, membrane integrity (HOST; swelling test), and viability (eosin–nigrosin) were determined. Samples from each ejaculate were extended with one of five commercially available semen extenders (four skim-milk based and one egg-yolk based) to a final concentration of 50×10^6 mL⁻¹. An aliquot of these samples were centrifuged at $400 \times g$ for 10 min and resuspended to 50×10^6 mL⁻¹ with its respective extender. All samples were stored at 5 °C and analyzed at 24 h and 48 h. Data were statistically analyzed utilizing a repeated measurements design with a 2×5 treatment structure (SAS Institute Inc., Cary, NC). Results, summarized in Table 1, were expressed as