Detection of Abnormal Stallion Sperm Cells by Using the Feulgen Stain

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Feulgen staining is a rapid and easy staining technique that is used to aid in the visualization of stallion sperm cell abnormalities, such as abnormal head shapes, vacuoles, and DNA condensation defects. Author’s address: Dept. of Herd Medicine Theriogenology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon S7N 5B4, Canada. © 1998 AAEP.

1. Introduction
The Feulgen staining procedure has been utilized in many species to detect and image abnormalities in the sperm cell nucleus, such as abnormal head shapes, vacuoles, and chromatin condensation, that might otherwise be difficult to see or detect by conventional staining procedures. This staining procedure is used as an adjunct to a breeding soundness examination or the detailed investigation of a subfertile stallion, or a stallion with unexplained infertility such as a high rate of early embryonic loss. This staining technique may also be used as a measure of sperm cell nuclear chromatin stability in a variety of extenders and as a means of determining which stallions should be evaluated by using other tests of chromatin stability, such as flow cytometry. This paper describes the appearance of stallion sperm cell nuclei when the Feulgen stain is used, the effect of semen handling (dilution with extender, and formalin fixation), and the frequency and variability of the sperm cell head defects and DNA condensation abnormalities in a clinical population of fertile and subfertile or infertile stallions.

2. Methods
Semen is collected via an artificial vagina and filtered. Raw semen and extended semen slides are prepared by smearing out semen on a slide with a two-slide method or a wooden stick. Slides are allowed to air dry or, preferably, dry on a warmer tray. A small amount of semen is fixed in 10% buffered formalin in a ratio of 1:10 (semen:formalin). Current staining techniques would use eosin nigrosin stain in a 1:1 ratio of stain to sperm cells, and a differential spermiogram would be performed by evaluating a minimum of 100 cells under oil immersion. Slides are left overnight. Dried slides from 15 stallions were exposed to 5 N hydrochloric acid in a glass jar for 30 min and then treated with Schiff’s reagent for 60 min; they were then rinsed in water and dried. Cells were examined under phase-contrast microscopy oil immersion (1000×). A yellow filter may be used to heighten the contrast of the image.

3. Results
Normal sperm cell nuclei stain a uniform magenta to deep purple color with the Feulgen stain, although
4. Conclusions

The use of Feulgen staining of stallion sperm cells helps to clearly reveal normal abnormalities such as vacuoles, variations in head shape, and abnormally condensed chromatin. This can lead to more confidence in identifying abnormal sperm cell nuclei and can increase the accuracy and confidence with which sperm cell morphology is performed by practitioners. The staining reaction is reduced by formalin fixation, presumably by influencing the ability of the acid to hydrolyze the DNA or the binding of the Schiff's reagent. The process of acid exposure is a stress to the nuclear chromatin, which normal DNA tolerates. The acid removes the purines in the DNA and exposes the aldehyde groups for Schiff's reagent to bind. Fertile stallions had low numbers of abnormal nuclei and low numbers of cells with chromatin abnormalities. The stability of the chromatin was unaffected by exposure to Kenney's extender in BSE satisfactory stallions. The importance of this staining technique is illustrated by a stallion with an apparently normal BSE, but who had very unstable chromatin, which further destabilized in the extender, and a poor breeding record. Unsatisfactory stallions had higher percentages of abnormal sperm cell heads and nuclei containing abnormal chromatin. Poor semen quality may be associated with sperm cell membrane damage. This may lead to increased susceptibility to acid hydrolysis or extenders, further stressing the sperm cell chromatin. Chromatin abnormalities increased after the addition of Kenney's extender, with variations in percentages of abnormal chromatin condensation between ejaculates in most stallions with fertility problems. A clumping pattern of chromatin most likely represents the most severe defect, followed by the fine granular pattern; the significance of apical clearing is not known. Abnormal sperm cell chromatin is likely a contributing factor in male factor infertility in horses as it is in other species. Milk-based extenders may unmask an intrinsic defect or destabilize sperm cell chromatin in stallions with fertility problems, and they may decrease the chance of success in breeding programs for this group of horses using artificial insemination. Artificial insemination with neat semen or a series of extenders may have to be evaluated to determine the best method for horses with fertility problems.

References and Footnotes


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