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Original Research

An Evaluation of Equine Sperm Chromatin After Exposure to Ambient Heat Stress

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ABSTRACT

Several studies have evaluated the effects of excessive heat stress on chromatin damage; however, DNA damage due to ambient seasonal heat stress has yet to be evaluated. The objective of the present study was to evaluate the effects of natural heat stress on equine sperm chromatin structure by means of two tests: the sperm chromatin structure assay (SCSA) and Sperm-Halomax kit (Halo). Stallion semen samples were collected after a period of excessive heat load (31.05°C) and were compared with samples collected at lower ambient temperatures (14.26°C) to evaluate chromatin damage caused by ambient heat stress produced in the testes. The data indicate that there is no correlation between the SCSA and Halo assays ($r = 0.18$ and $F = 0.48$). Additionally, no correlation for ambient heat stress was observed for any of the applied techniques—SCSA, Halo, and live:dead staining ($P = .2682$, .4628, and .0377, respectively). These results suggest that ambient heat load has little effect on stallion chromatin damage. Other elements, such as relative humidity and heat index, were outside the realm of this study and should be assessed in future studies with a larger sample size.

1. Introduction

In the performance equine industry, stallions are generally selected for excellent pedigree, past performance, and performance of offspring. In contrast to other livestock, breeding stallions are not generally selected for reproductive soundness. However, reproductive efficiency increases the value of the stallion for commercial purposes, and extensive reproductive soundness examinations are used before the use of the stallion for breeding. Stallion Breeding Soundness Examinations (BSEs) involve elements of structural soundness, overall health, testicular measurements, cultures of preejaculation and postejaculation to test for infections, and a complete semen analysis [1]. A complete stallion BSE is important to predict the potential fertility. A traditional semen analysis evaluates parameters such as volume, concentration, progressive motility, viability, and morphology [2]. The use of newer technology, such as the computer-assisted semen analysis (CASA), provides additional breakdown of progressive motility, which is useful when assessing the BSE [3]. Although these parameters serve as predictors for fertility, a stallion may still exhibit decreased fertility rates, or be classified as infertile, despite acceptable values in these parameters [4]. Furthermore, parameter may rapidly decrease with increased collection rates due to stallion popularity.

Sperm chromatin, found within the head of the spermatozoa cells, is comprised of densely packed DNA and heterogeneous nucleoproteins [5]. A number of factors have been shown to compromise sperm chromatin leading to decreased sperm viability, including an excessive heat load on the testes [6,7].

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Heat can cause an animal's body to become stressed in various ways. In the case of reproduction, increased scrotal temperatures can have detrimental effects on spermatozoa cells that may impair fertilization [6]. One study in Holstein bulls examined excess heat on sperm chromatin by the insulation of the scrotum to induce heat buildup [8]. Insulation sacks were fastened around the bulls' entire scrotum up to the abdominal wall and remained on the bulls for 48 hours, after which semen samples were collected and evaluated for standard semen parameters and chromatin damage. The study determined that increased scrotal temperatures adversely affected semen motility and resulted in reduced fertility rates [8]. Furthermore, it was demonstrated that insulating the scrotal sacks unfavorably affected the epididymal and testicular sperm chromatin [8]. Similar results were seen in bulls [6] and stallions [7]. In all cases, there were more cells with damaged chromatin during periods of induced heat stress which reversed when the stress was removed and the testes given time to recover [6,7]. Previous research has consistently demonstrated heat loads cause loss of cell function associated with chromatin damage. However, two recent studies [9,10] dispute the association. These conflicting findings raise doubt as to the utility of measuring chromatin damage as a measure of stallion fertility and if such damage could be caused by ambient temperatures. Another method of assessment in the use of DNA damage as an indication of fertility is the sperm chromatin structure assay (SCSA) itself [11]. This method is based on flow cytometry [5,12,13], making it impractical for rapid use in most settings. Recently, a relatively new assay, the Sperm-Halomax kit (Halotech DNA SL; Madrid, Spain), was developed for the detection of DNA damage in sperm cells [14]. Through the use of bright field microscopy and a protein depletion treatment, the heads of spermatozoa cells are observed for the presence of a halo. The presence of a small and compact halo is indicative of native DNA, whereas the presence of large and spotty halos represents damaged DNA [14].

As the ability of long-term, naturally occurring, ambient heat loads to disrupt and damage DNA has not been proven, the aim of the present study was to assess equine sperm chromatin as a direct result of extreme ambient heat stress. The study assessed stallions in three different states (Texas, New Mexico, and Oklahoma) during the warmest and coolest seasons of the year at each location to determine difference in DNA fragmentation using both the SCSA and the Sperm-Halomax kit. An additional aim of the study was to compare the two methods to assess damage of stallion chromatin.

2. Materials and Methods

2.1. Study Design

Semen from 12 registered Quarter Horse and Thoroughbred stallions were collected in October 2011 from three differing southern states including Texas, New Mexico, and Oklahoma. All stallions were actively involved in breeding programs at commercial equine facilities, and each had passed a breeding soundness examination at the beginning of the previous season. All stallions had previously sired foals. The stallions were housed individually in non–climate-controlled stalls, and each stallion had daily turnout, weather permitting.

Given that the goal of the study to compare sperm DNA damage during periods of ambient heat stress and the well-documented fact that spermatogenesis in the stallion takes approximately 54 days with varying storage time in the epididymis [15], collections were coordinated to maximize or minimize heat loads over a minimum of 90 days. Heat-stressed samples were collected during mid-October 2011 after the hottest 90-day period of heat at all four collection sites (mean temperature across sites was 33.6°C; Table 1). The second collections took place during the normal equine breeding season for all the three states in March 2012, after the coolest period of the year. Initially, 12 stallions used in commercial operations were recruited for this study. However, because of circumstances beyond the control of the researchers, several stallions were relocated between sites or sold after the October collection, thus preventing a spring collection from the same stallions at the same stallion stations. Therefore, only eight of the original 12 Quarter Horse and Thoroughbred stallions were available for a second collection in the spring. Although the location of several animals changed, the fact that this occurred during the cooler period of time and outside the 90-day temperature exposure window suggests it would have little to no impact on temperature load on the testes.

To maintain the integrity of the experiment, none of the stallions were exposed to artificial heat stress of the testes and stallions were only exposed to ambient heat. To protect the identities of the stallions, all stallions were randomly assigned identification numbers.

Table 1
Average monthly temperature (°C) for location.

Date	Lubbock, Texas	Hondo, New Mexico	Wynnewood, Oklahoma	Pilot Point, Texas
July 2011[a]	22.78, 37.31, 37.31	15.56, 24.87, 27.78	26.11, 39.87, 40	26.11, 38.42, 38.33
August 2011[a]	22.78, 37.31, 37.31	15.56, 24.87, 27.78	26.11, 39.87, 40	26.67, 39.68, 39.44
September 2011[a]	13.89, 30.09, 30	11.11, 23.74, 23.89	16.11, 31.15, 30.56	17.22, 33, 32.78
October 2011[a]	8.89, 24.94, 25	6.11, 18.74, 18.89	11.67, 24.44, 25	11.67, 25.52, 25.56
December 2011[b]	−1.67, 8.33, 8.33	−4.44, 4.66, 4.44	1.67, 11.63, 11.11	2.22, 12.17, 12.22
January 2012[b]	−2.22, 16.04, 16.11	−1.11, 10.47, 10.56	1.67, 14.71, 15	1.67, 15.29, 15.56
February 2012[b]	−1.11, 14.1, 13.89	−2.78, 8.7, 8.89	3.89, 14.04, 15.56	3.89, 15.19, 15
March 2012[b]	5.56, 23.03, 23.33	2.22, 15.03, 15	6.67, 22.13, 18.33	11.11, 22.56, 22.78

[a] Fall collection.
[b] Spring collection.