Effects of ergot alkaloids on bovine sperm motility

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Story in Brief

Toxic tall fescue grass has been associated with reduced reproductive rates in cattle. This study was conducted to determine the direct effects of the ergot alkaloids [ergonovine (EN), ergotamine (ET), and dihydroergotamine (DHET)] on motility of bovine spermatozoa. Spermatozoa were collected from mature Angus (n = 2) and Balancer (n = 4) bulls, washed once, and resuspended in modified sperm medium (mSPTL). The experimental design was a randomized complete block, with bull serving as the block. Treatments were structured as a 3 × 5 factorial with three alkaloids (EN, ET, DHET) and five concentrations of each drug (0, 33, 66, 100, and 200 μM). Spermatozoa (25×10^6) were incubated in 1 mL of mSPTL with treatment at 39 °C. Sperm motility characteristics were evaluated at 0, 3, and 6 h using CASA (Hamiliton Thorne IVOS, Beverly, Mass.). Initial sperm motility was (69 + 1.7%) and declined to (35 + 2.6%) at 6 h. Percent motile spermatozoa was affected ($P = 0.015$) by a three way interaction between time, concentration, and alkaloid. Sperm motility decreased ($P < 0.01$) over time and with increased concentrations of alkaloids with the exception of EN. The number of static spermatozoa also was affected ($P < 0.01$) by a three way interaction and increased as ET and DHET concentrations increased. Percentages of progressively motile and rapidly motile spermatozoa decreased ($P < 0.01$) in a two way interaction between alkaloid and concentration. Overall sperm motility was decreased by ET and DHET; furthermore, the qualities of motility were decreased by those alkaloids. Ergot alkaloids commonly found in toxic tall fescue are detrimental to bovine spermatozoa.

Introduction

Cattle consuming toxic tall fescue (E+) may suffer from numerous detrimental effects, resulting in significant economic losses for cattle producers annually. Three-fourths of all tall fescue pastures in the Unites States are infested with endophyte at a level of at least 60%. Ergot alkaloids located within the endophytic fungus have been linked to depressed reproductive performance.

Bulls consuming E+ and ergot alkaloid may suffer from altered scrotal temperatures, decreased scrotal circumference (Jones et al., 2004), declines in sperm motility (Looper et al., 2009), and reduced fertilizing capabilities (Schuenemann et al., 2005). Collectively, results from those in vivo studies suggest that toxic fescue can alter sperm function and fertilizing capabilities; however, they do not illustrate what specific effects ergot alkaloids may have directly on spermatozoa. Wang et al. (2009) showed that certain ergot alkaloids use specific signaling pathways to interact with spermatozoa. Our objective was to investigate the direct effects ergot alkaloids on bovine specific sperm motility characteristics by using computer assisted sperm analysis.

Materials and Methods

Semen Collection and Preparation. Semen was collected from mature bulls (n = 6) via electro-ejaculation (Electro-ejac IV) at 0700 h and placed in a 15 mL conical centrifuge tube. Ejaculates were transported to the lab in a 39 °C water bath where spermatozoa were centrifuged at 750 × g for 10 min. Seminal plasma was removed, and spermatozoa were washed once and re-suspended in modified sperm TALP (mSPTL). The mSPTL was prepared prior to collection and consisted of: NaCL (49.5 mM), KCL (1.5 mM), NaH_2PO_4 (0.17 mM), $CaCL_2{\cdot}2H_2O$ (0.10 mM), $MgCL_2{\cdot}2H_2O$ (0.055 mM), 5.25% $NaHCO_3$ (0.16 ml), HEPES (10 mM), Na-pyruvate (1 mM), 60% Na-lactate syrup (21.6 mM), gentamicin (0.05 mg), EGTA (2 mM), and PVA (0.05 mg) with pH adjusted to 7.4 and an osmolarity of ~300 mOsm. Spermatozoa were diluted 25:1, counted using an integrated visual optical system (IVOS; Hamilton-Thorne Biosciences, Beverly, Mass.), and placed in experimental treatments (25×10^6 sperm/ml).

Preparation of Alkaloid Treatments. All alkaloids were prepared directly prior to incubation with spermatozoa. Methanol (100%) was used as the solvent to prepare each alkaloid [ergonovine (EN), ergotamine (ET), dihydroergotamine (DHET)]. Stock solutions were aliquoted at experimental concentrations into the wells of sterile flat-bottom 24-well tissue culture plates and methanol was allowed go evaporate. Alkaloids were then re-suspended in mSPTL.

Experimental Design. Experimental design was a randomized complete block, with bull serving as the block. Treatments were structured as a 3 × 5 factorial with three alkaloids (EN, ET, DHET) and five concentrations of each alkaloid (0, 33, 66, 100, 200 μM). Spermatozoa (25×10^6) were incubated in 1 mL of mSPTL with each treatment at 39 °C in an atmosphere of humidified air. Sperm motility characteristics were evaluated at 0, 3, and 6 h of incubation. Spermatozoa were evaluated by placing them on a warm slide and assessed using an IVOS and utilizing Animal Motility Software, version 12.1.

Statistical Analysis. Sperm motility characteristics were analyzed using mixed model procedure (SAS Institute, Inc, Cary N.C.). Bull served as the block, experimental unit was the concentration within alkaloid, and time was repeated measure. If F-test were significant ($P < 0.05$), means were separated using multiple *t*-tests.

Results and Discussion

Sperm motility was inhibited by a three-way interaction between hour (time), alkaloid, and concentration. Both ET and DHET re-

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duced sperm motility in a concentration and time dependent manner ($P < 0.05$). When compared to control spermatozoa (0 μM alkaloid), spermatozoa exposed to ET (≥33 μM) for 3 h and (≥66 μM) for 6 h were less motile ($P < 0.05$; Fig. 1). Similar affects were observed for DHET. Concentrations of 66 μM and above reduced ($P < 0.05$) sperm motility (Fig. 2). Ergonovine had minimal effects on sperm motility until the 6 h observation at 200 μM ($P < 0.05$). Similarly, static spermatozoa also were affected by a three-way interaction between alkaloid, concentration, and time. As ET and DHET concentrations increased, the number of static spermatozoa increased. For progressive and rapid spermatozoa, a two-way interaction was observed. Both ET and DHET reduced ($P < 0.05$) the percentage of progressive (Fig. 3) and rapid (Fig. 4) spermatozoa when concentrations reached 66 μM and greater.

There has been conflicting data published showing the effects of toxic agents found in E+ on male gametes, but our results demonstrate that ergot alkaloids can directly affect bovine sperm motility. More specifically, ET and DHET reduced motile, progressive, rapid, and static spermatozoa and altered multiple other sperm characteristics associated with sperm viability. These data provide a possible explanation for decreased conception rates and reproductive performance amongst cattle grazing toxic tall fescue.

The altered sperm parameters observed in our study were similar to Wang et al. (2009); however, their method of evaluating sperm motility utilized subjective measures. According to Farrell et al. (1998), the repeatability and consistency within each sperm evaluation is likely to be more accurate using CASA rather than subjective measures. In fact, Farrell's group reported a repeatability of 0.99 when using CASA. This could also provide a valid explanation for the contrasting results encountered with many other trials. For example, with the use of visual optics, Schuenemann et al. (2005) documented that sperm motility and morphology was not affected when bulls were supplemented with ET in their diet.

The use of CASA allowed us to evaluate both quality and quantity of sperm motility. Although sperm movement is important, it is not the only criterion necessary for a sperm to fertilize an oocyte. The ability of the sperm to progress forward into the reproductive tract in an efficient manner is critical to achieve conception. Results, in this report, showed declines in overall motility and reductions in progressive and rapid spermatozoa. These results confirm our earlier work where the average velocity of the smoothed sperm path as well as the average velocity measured over the actual point to point track became slower with elevated temperatures (Looper et al., 2009). We also demonstrated that the percentage of static spermatozoa increased due to the effects of ET and DHET, which exasperate the intracellular energy of the sperm, and thereby accelerate the pace at which sperm undergo apoptosis. We observed morphological changes in the size and shape of the sperm head as exposure time and alkaloid concentration increased.

Eliminating subjective measures for sperm evaluation is not the only methodology that differs in our study as compared to others. Multiple trials have been performed showing that ergot alkaloids can indirectly affect sperm function. The previously mentioned alterations in scrotal temperature and scrotal circumference along with changes in prolactin concentrations are physiological changes that occur after ingestion of E+ (Jones et al., 2004), and these changes may be partially responsible for reducing sperm viability under normal physiological conditions. It is known that thermal regulation of the testis and prolactin levels are both important factors that can regulate the development of sperm.

In two of the more current whole animal studies, both Looper et al. (2009) and Jones et al. (2004) observed a small decline in sperm motility. Although it is important to understand how cattle react to toxic agents under normal grazing conditions, it is important to note that breed type and exposure period may have affected their results. Looper's trial utilized Brahman-influenced bulls which are known for their heat tolerance; therefore, it may be that Brahman-influenced bulls withstand the toxic effects of E+ better than other breed types. Even though sperm motility was not greatly affected over the entire length of the study, Jones' article did state that motility decreased during the final two weeks of the 60 d trial. Perhaps bulls with a longer exposure period may begin to respond differently. It is also known that elevated environmental temperatures can magnify the effects of toxic fescue. Ultimately, there are many factors that could possibly influence whole animal trials such as breed type, exposure period, toxin concentrations, temperature, and body weight. By taking an in vitro approach to this study we were able to determine if ergot alkaloids directly interact with spermatozoa. As we try to discover different alternatives to combat fescue toxicosis, it is important that we understand all mechanisms and specific alkaloids that possibly reduce the animals reproductive capabilities.

It is still unknown exactly what mechanisms ergot alkaloids use to inhibit sperm motility. The chemical structures of the three alkaloids used in this study could possibly explain the observation differences amongst the alkaloids. Ergonovine, the smallest structure of the three, is a simple lysergic acid amide that doesn't contain a peptide group. Ergonovine decreased sperm motility when exposure occurred during cryopreservation, and another demonstrated that EN increased rate of sperm transport when placed in the vagina of ewes. Even though we observed a reduction in sperm motility when exposed to large amounts of EN (200 μM), overall, the data from this current study showed EN to have a minimal effect on bovine sperm motility. Both ET and DHET are classified as ergopeptines. Inhibitory effects of DHET were slightly less intense than ET, which was not expected since DHET was originally synthesized to be a more stable version of ET for use in human pharmacology. Ergot alkaloids are lipid soluble and presumably can permeate sperm membranes. Sperm motility is dependent on many cellular functions including cAMP and calcium concentrations. It is plausible that ergot alkaloids can directly affect sperm motility by altering cAMP and calcium levels within the germ cell. Fertilizing capacity and motility also may be compromised by ergot alkaloid interaction with plasma membrane receptors on spermatozoa (Wang et al., 2009).

It is unlikely that producers will eradicate all of their E+ stands due to its persistence and agronomic benefits, so establishing a method to help overcome the negative consequences toxic fescue has on reproductive performance seems to be the more feasible option. This study provides a better understanding of the effects ergot alkaloids have on male reproduction. Unfortunately, ergot alkaloid concentrations are not known under normal physiological conditions, but with the knowledge that these toxic agents can directly hinder sperm motility, we can address the mechanisms used to inhibit sperm function.

Implications

Understanding the mechanisms by which ergot alkaloids and toxic tall fescue reduce male reproduction may lead to management tools that will result in increased overall livestock reproductive success.

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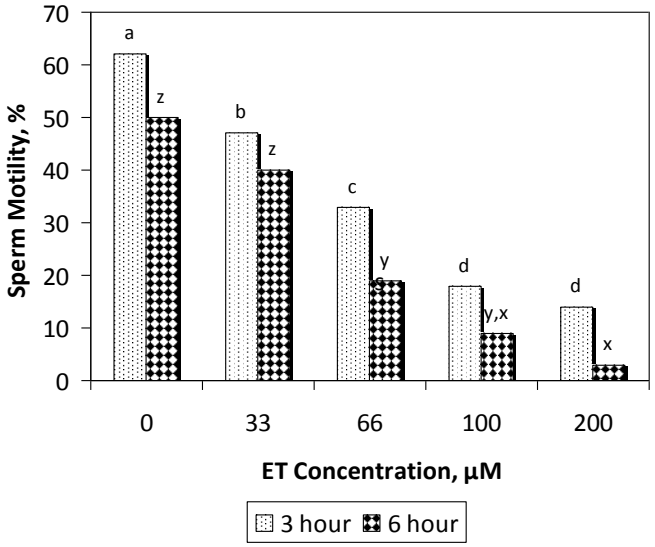

Fig. 1. Effects of ergotamine tartrate (ET) concentration and incubation time on bovine sperm motility. Spermatozoa (25×10^6 sperm/ml) were incubated with ET at various concentrations (0-200 μM) in modified sperm TALP (mSPTL). Sperm motility characteristics were evaluated at 0, 3, and 6 h. Initial motility was 68% and SE = 4.1. Superscripts a,b,c,d are designated to 3 h columns and superscripts z,y,x are designated to 6 h columns. Values without a common superscript within evaluation time differ ($P < 0.05$).

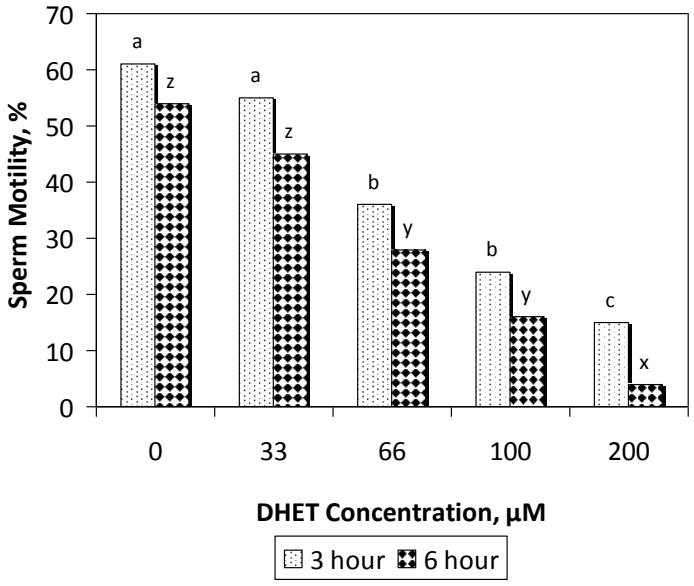

Fig. 2. Effects of dihydroergatamine (DHET) concentration and incubation time on bovine sperm motility. Spermatozoa (25 × 10^6 ml^{-1}) were incubated with DHET at various concentrations (0-200 μM) in modified sperm TALP (mSPTL). Sperm motility characteristics were evaluated at 0, 3, and 6 h. Initial sperm motility was 67% and SE = 4.1. Superscripts a,b,c are designated to 3 h columns and superscripts z,y,x are designated to 6 h columns. Values without a common superscript within an evaluation time differ ($P < 0.05$).

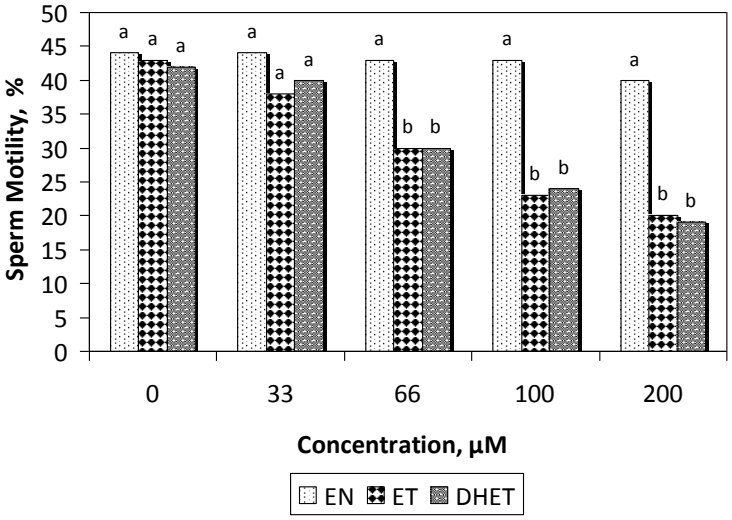

Fig. 3. Effects of alkaloid and concentration interaction on progressive sperm motility. Spermatozoa (25 × 10^6 ml^{-1}) were incubated with alkaloid [ergonovine (EN), ergotamine tartrate (ET), dihydroergotamine (DHET)] at various concentrations (0-200 μM) in modified sperm TALP (mSPTL). SE = 2.1. Values without a common superscript differ ($P < 0.05$).

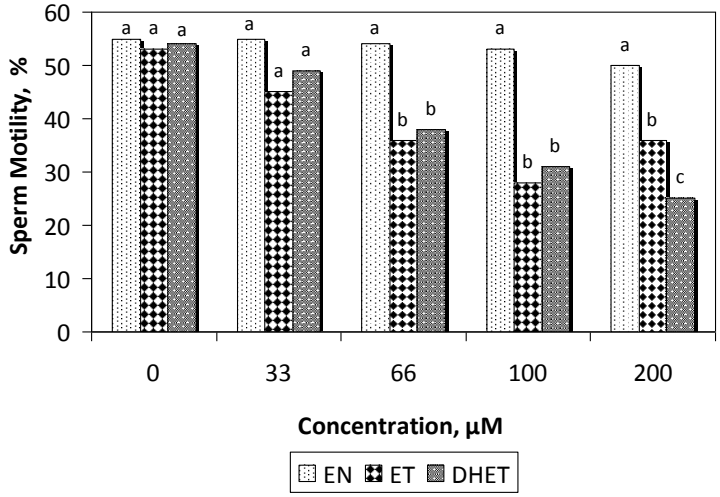

Fig. 4. Effects of alkaloid and concentration interaction on rapid sperm motility. Spermatozoa (25×10^6 ml^{-1}) were incubated with alkaloid [ergonovine (EN), ergotamine (ET), dihydroergotamine (DHET)] at various concentrations (0-200 μM) in modified sperm TALP (mSPTL). SE = 3.5. Values without a common superscript differ ($P < 0.05$).