Scientific Research Publishing

Advances in Reproductive Sciences, 2019, 7, 7-15
http://www.scirp.org/journal/arsci
ISSN Online: 2330-0752
ISSN Print: 2330-0744

Ergot Alkaloid Effects on Bovine Sperm Motility *In Vitro*

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How to cite this paper: Page, R., Lester, T., Rorie, R. and Rosenkrans Jr., C. (2019) Ergot Alkaloid Effects on Bovine Sperm Motility *In Vitro*. *Advances in Reproductive Sciences*, **7**, 7-15.
https://doi.org/10.4236/arsci.2019.71002

Received: December 18, 2018
Accepted: January 14, 2019
Published: January 17, 2019

Abstract

Cattle in some parts of the world graze pastures that consist of tall fescue that may contain ergot alkaloid contamination. Those ergot alkaloids are associated with reduced reproductive rates in cattle. Our objective was to determine if ergot alkaloids [dihydroergotamine (DHET), ergonovine (EN), and ergotamine (ET)] directly affect bovine sperm characteristics. Spermatozoa were collected from mature Angus (n = 2) and Balancer (n = 4) bulls. Within bull, treatments were structured as a 3 × 5 factorial with three alkaloids (DHET, EN, and ET) and five concentrations of each alkaloid (0, 33, 66, 100, or 200 μM). Spermatozoa (25×10^6) were incubated (39˚C) in 1 mL of modified sperm medium. Sperm motility characteristics were evaluated using CASA (Hamiliton Thorne IVOS, Beverly, MA) at 0, 3, and 6 h after initial alkaloid exposure. Initial sperm motility was (69% ± 1.1%) and declined ($P = 0.01$) to (35% ± 2.6%) at 6 h. Sperm motility decreased ($P < 0.05$) with increasing concentrations of DHET and ET, but not EN. As concentration of ET or DHET increased all CASA sperm characteristics were altered. The interaction of alkaloid concentration and incubation length affected sperm velocity and head size; exposure to 200 μM of ET or DHET for six hours decreased ($P < 0.05$) both characteristics. Our results demonstrate that ergot alkaloids (ET and DHET) can directly alter bovine sperm motility and morphology, which adds to our understanding of how ergot alkaloids may hinder cattle reproductive rates.

Keywords

CASA, Bull Spermatozoa, Toxins

1. Introduction

Cattle consuming tall fescue (*Lolium arundinaceum* = *Schedonorus arundinaceus* = *Festuca arundinacea*) may suffer from numerous physiological conse-

quences that result in significant economic losses [1]. Most tall fescue pastures are infested with wild-type endophyte *Epichloë coenophiala* (=*Neotyphodium coenophialum* = *Acremonium coenophialum*; see [2]). Ergot alkaloids synthesized and released by that endophytic fungus have been associated with toxic effects on livestock (see [3]). More specifically, they have been linked to depressed reproductive performance [4]. Much of the previous research has focused on reproductive biology of females, such as altered endocrine profiles [5], changes in the estrous cycle [6] and decreased progesterone concentrations [7].

While there have been fewer whole animal studies evaluating ergot alkaloid effects on males, biological effects have been reported. Angus bulls consuming diets supplemented with toxic fescue seed had increased scrotal temperatures and decreased scrotal circumference [8]. Brahman-influenced bulls grazing toxic tall fescue pastures had decreased sperm motility during summer months (July and August; [9]). Schuenemann *et al*. [10] found that bulls fed an ergot alkaloid had spermatozoa with reduced fertilizing capabilities *in vitro*. Those whole animal trials suggest that toxic fescue can alter sperm function and fertilizing capabilities; however, they cannot rule out environmental interactions on male gamete development and function. Previously, we have demonstrated that ergot alkaloids use specific signaling pathways to interact with bull spermatozoa [11]. Our objective was to characterize the effects of ergot alkaloids on bovine sperm motility using computer assisted sperm analysis (CASA).

2. Materials and Methods

2.1. Semen Collection and Preparation

For 60 days prior to semen collection, retired mature-breeding bulls [Angus (n = 2) and Balancer (n = 4)] were maintained in a dry lot where they had free access to mixed grass hay, water, and mineral supplement. On the morning of sample days, bulls were electro-ejaculated (Electroejac IV; Neogen Corporation, Lansing, MI) and semen collected in 15-mL conical centrifuge tubes. Ejaculates were transported to the lab in a water bath (39˚C) where they were centrifuged at 750 ×g for 10 min. Following centrifugation, seminal plasma was removed and spermatozoa were washed once and re-suspended in modified sperm TALP (mSPTL, pH = 7.4 and osmolarity = 300 mOsm.; [12]). Spermatozoa were diluted 25:1 in mSPTL, counted using an integrated visual optical system [(IVOS) Hamilton-Thorne Biosciences, Beverly, MA], and placed in experimental treatments at a final concentration of 25×10^6 spermatozoa/mL of mSPTL.

2.2. Preparation of Alkaloid Treatments and Experimental Procedures

Final concentrations of all alkaloids were prepared directly prior to incubation with spermatozoa. Methanol (100%) was used as the solvent to prepare stock (1 mM) solutions of each alkaloid [dihydroergotamine (DHET), ergonovine (EN), and ergotamine (ET)]. Stock solutions of each alkaloid were aliquoted into final

experimental concentrations (0, 33, 66, 100, 200 μM) in sterile flat-bottom 24-well tissue culture plates, evaporated to dryness, and resuspended in mSPTL (1 mL). Plates containing spermatozoa (25×10^6/mL) were incubated (39˚C) in an atmosphere of humidified air. Sperm motility characteristics (Table 1) were evaluated at 0, 3, and 6 h of incubation using an integrated visual optical system (IVOS, Hamilton-Thorne Biosciences, Beverly, MA) and Animal Motility Software (version 12.1).

2.3. Statistical Analysis

Sperm motility characteristics were analyzed using mixed model procedures. Experimental design was a randomized complete block with bull serving as the block. Experimental unit was the concentration within alkaloid, and time was the repeated measure. Treatments were structured as a 3 × 5 factorial with three alkaloids (DHET, EN, and ET) and five concentrations of each alkaloid (0, 33, 66, 100, and 200 μM). Following initial analyses, a reduced statistical model was used to evaluate CASA motility characteristics. That model was a 2 × 5 factorial

Table 1. Sperm motility characteristics determined by CASA (IVOS, Hamilton-Thorne Biosciences, Beverly, MA).

CASA Variable	Description
Motile	Percentage of total sperm moving at a track velocity ≥30 μm/sec and progressive velocity ≥15 μm/sec
Progressive	Percentage of total sperm moving at a track velocity ≥50 μm/sec and straightness ≥70%
Rapid	Percentage of Progressive % with path velocity >50 μm/sec
Medium	Percentage of Progressive % with path velocity <50 μm/sec but >30 μm/sec
Slow	Percentage of Progressive % with path velocity <30 μm/sec and progressive velocity <15 μm/sec
Static	Sperm with no movement at all
Path Velocity (VAP)	Average velocity of the smoothed cell path (μm/sec)
Progressive Velocity (VSL)	Average velocity measured in a straight line from the beginning to the end of the track
Track Speed (VCL)	Average velocity measured over the point-to-point track
Lateral Amplitude (ALH)	Mean width of the head oscillation as the sperm swims
Beat Frequency (BCF)	Frequency of sperm head crossing the sperm average path in either direction
Straightness (STR)	Measures departure of average sperm path from straight line (ratio of VSL/VAP)
Linearity (LIN)	Measures departure of actual sperm path from straight line (ratio of VSL/VCL)
Elongation (ELONG)	Ratio of head width to head length
Sperm Head Size (AREA)	Average size in square micrometers of all sperm heads

with two alkaloids (DHET vs. ET) and five concentrations of each alkaloid. When F-tests were significant ($P < 0.05$), means were separated using multiple t-tests with Tukey's adjustment.

3. Results

Sperm motility was inhibited by a three-way interaction between hour, alkaloid, and concentration (Table 2). Both ET and DHET reduced ($P < 0.05$) sperm motility in a concentration and time dependent manner. In contrast, EN did not reduce ($P > 0.9$) sperm motility or other CASA characteristics (data not shown); therefore, we reduced the statistical model. With the reduced model, percentage of motile spermatozoa was affected by main effects of alkaloid (33.8 ± 1.34 vs. 29.4 ± 1.34; DHET vs. ET, respectively; $P < 0.03$) and hour of incubation (36.5 ± 1.34 vs. 26.7 ± 1.34; 3 vs. 6 h, respectively; $P < 0.0001$).

Based on our reduced statistical model, CASA sperm characteristics were affected ($P < 0.08$) by concentration of alkaloids used during incubation (Table 3). Progressive and rapid sperm motility decreased ($P < 0.0001$) as alkaloid concentration increased; conversely, static spermatozoa increased as alkaloid concentration increased (Table 3). Percentage of static spermatozoa was greater ($P < 0.01$) for spermatozoa incubated with ET when compared to DHET (51.2 ± 1.57 vs. 45.3 ± 1.57, respectively).

Sperm velocity (VAP, VSL, and VCL), BCF, and STR decreased ($P < 0.0001$) as alkaloid concentration increased (Table 3). Velocity defined as VAP, VCL, and STR were affected ($P < 0.05$) by an interaction of hours of incubation and concentration of alkaloids (Table 4). During the first three hours of incubation, alkaloid concentration did not affect VAP or VCL; however, by the end of six hours of incubation, alkaloid concentrations of 100 and 200 μM decreased both of those velocity characteristics. After six hours of incubation, STR was decreased

Table 2. Interactive effects of incubation time, alkaloid, and concentration on the percentage of motile bovine spermatozoa.

Time (h)	Conc. (μM)	ET	DHET	EN
3	0	61.8^{a}	60.7^{a}	60.3^{a}
3	33	46.7^{ab}	55.2^{ab}	57.5^{a}
3	66	32.7^{bc}	36.3^{bc}	58.5^{a}
3	100	18.2^{c}	24.0^{c}	56.3^{a}
3	200	14.3^{c}	15.0^{cd}	55.2^{a}
6	0	50.3^{ab}	53.8^{a}	52.7^{a}
6	33	39.5^{bc}	44.8^{ac}	55.8^{a}
6	66	19.3^{bc}	27.8^{c}	53.2^{a}
6	100	9.0^{c}	16.3^{cd}	48.8^{a}
6	200	2.5^{c}	3.8^{d}	43.8^{a}

^{a,b,c,d}Within an alkaloid, least-squares means (SE = 4.18) without a common superscript differ ($P < 0.05$). Sperm motility percentage at time 0 was 69.0 ± 1.08.

Table 3. Alkaloid[1] concentration effects on sperm motility characteristics.

Sperm	Alkaloid Concentration, μM					SEM	P-value
Characteristic[2]	0	33	66	100	200		
Motility, %	56.7^{a}	46.5^{b}	29.1^{c}	16.9^{d}	8.9^{d}	2.12	0.0001
Progressive, %	38.6^{a}	33.1^{a}	19.9^{b}	9.9^{c}	4.1^{c}	1.77	0.0001
Rapid, %	49.1^{a}	39.6^{ab}	24.6^{bc}	13.4^{c}	14.4^{c}	3.96	0.0001
Static, %	25.8^{c}	32.8^{c}	50.5^{b}	61.8^{a}	70.4^{a}	2.47	0.0001
VAP, μm/s	98.7^{a}	94.4^{a}	88.6^{ab}	73.8^{bc}	58.0^{c}	4.39	0.0001
VSL, μm/s	81.9^{a}	80.7^{a}	74.1^{ab}	59.9^{bc}	47.4^{c}	4.43	0.0001
VCL, μm/s	161.5^{a}	155.1^{ab}	149.0^{ab}	128.0^{b}	95.0^{c}	6.73	0.0001
ALH, μm	6.1	6.0	5.7	5.7	4.6	0.4	0.08
BCF, Hz	31.4^{a}	33.5^{a}	29.7^{ab}	24.3^{b}	20.5^{c}	1.75	0.0001
STR, %	81.1^{a}	82.8^{a}	81.4^{a}	75.8^{ab}	65.4^{b}	3.17	0.0001
LIN, %	52.9	53.5	52.0	47.4	42.7	2.77	0.04
ELONG, %	47.5^{a}	42.9^{ab}	42.0^{ab}	39.8^{bc}	34.2^{c}	1.92	0.0004
AREA, μm^{2}	5.9^{a}	5.9^{a}	5.8^{a}	5.3^{a}	4.5^{b}	0.21	0.0001

^{a,b,c,d}Within sperm motility characteristic, least-squares means without a common superscript differ ($P < 0.05$). [1]least-squares means represent the main effects of alkaloid concentration across both DHET and ET. [2]See Table 1 for definitions.

Table 4. Interactive effects of incubation time and alkaloid concentration on select bovine sperm motility characteristics.

Time (h)	Conc.[1] (μM)	VAP	VCL	STR	Area
3	0	102.3^{a}	168.5^{a}	80.9^{a}	6.0^{a}
3	33	98.4^{a}	157.4^{ab}	83.8^{a}	5.9^{a}
3	66	93.3^{ab}	157.5^{ab}	80.5^{a}	5.8^{a}
3	100	80.4^{ab}	136.8^{ab}	78.6^{a}	5.4^{a}
3	200	81.2^{ab}	132.8^{ab}	77.2^{a}	5.5^{a}
6	0	95.0^{ab}	154.5^{ab}	81.3^{a}	5.9^{a}
6	33	90.4^{ab}	152.7^{ab}	81.8^{a}	5.9^{a}
6	66	83.9^{ab}	140.5^{ab}	82.3^{a}	5.8^{a}
6	100	67.2^{b}	119.3^{b}	72.9^{ab}	5.2^{a}
6	200	34.9^{c}	57.1^{c}	53.7^{b}	3.6^{b}
SEM	-	6.21	9.52	4.49	0.3
P value	-	0.02	0.004	0.05	0.02

^{a,b,c}Within sperm motility characteristic, least-squares means without a common superscript differ ($P < 0.05$). [1]least-squares means represent the interactive effects of time and alkaloid concentration across both DHET and ET.

by 200 μM concentration of alkaloids (Table 4). Physical characteristics (ELONG and AREA) of spermatozoa were greatest for those incubated without alkaloids and larger ($P < 0.05$) than spermatozoa incubated in 200 μM of alkaloids (Table 3). Sperm head size (AREA) was smallest ($P < 0.05$) for sperm incu-

bated for six hours in 200 μM of alkaloids (**Table 4**).

4. Discussion

Development of strategies for prevention or treatment of fescue toxicosis requires that we understand the physiological mechanisms by which specific alkaloids affect animal fecundity. There has been conflicting data published showing the effects of wild-type endophyte-infected (E+) fescue on the male gamete, but the results from this experiment demonstrate that ergot alkaloids can directly affect bovine sperm motility. More specifically, ET and DHET reduced the percentage of spermatozoa that were motile, progressive, and rapid, which are characteristics associated with sperm viability. These data provide a possible explanation for decreased conception rates and reproductive performance for cattle grazing E+ [13]. Chemical structure of the alkaloids used in our study likely explains their effects on sperm motility. Both ET and DHET are ergopeptines [14]. The reduced inhibitory effects of DHET when compared to ET were not expected since DHET was synthesized to be a more stable pharmaceutical form of ET for use in human medicine [15]. Ergonovine, the smallest structure of our three alkaloids tested, is a simple lysergic acid amide that doesn't contain a peptide group [15].

In our study, ET and DHET decreased sperm motility, which is consistent with previous reports of alkaloid effects on sperm motility determined using subjective methods [11]. According to Farrell *et al*. [16], 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.

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 criteria necessary for a sperm to fertilize an oocyte. The ability of the sperm to progress in the reproductive tract in an efficient manner is critical to achieve conception [17]. Our results showed that ET and DHET reduced overall motility, progressive, and rapid spermatozoa. These results confirm our earlier work where we observed the average velocity of the smoothed sperm path (VAP) as well as the average velocity measured over the actual point-to-point track (VCL) became slower with elevated temperatures and increased *in-vivo* exposure to ergot alkaloids [9]. We also demonstrated that the percentages of static spermatozoa were affected by alkaloids. Altered motility patterns and morphological changes suggest that ET and DHET may have been altered by intracellular metabolism of spermatozoa.

Altered scrotal temperature and circumference along with changes in prolactin concentrations are just a few of the physiological changes that occur after ingestion of E+ tall fescue [8] [18]. Those observations may be, in part, responsible for reducing sperm viability under normal physiological conditions. It is known that thermal regulation of the testis and prolactin levels are both important fac-

tors that can regulate the development of sperm [19] [20] [21] [22].

Pratt and Andrae [23] reviewed the effects of in vivo ergot alkaloid exposure on bull fertility traits. Due the paucity of studies and how those studies were conducted makes interpretation difficult, but breed and length of exposure to alkaloids may affect bull fertility characteristics. It is also known that elevated environmental temperatures can magnify the effects of toxic fescue [7]. Ultimately, there are many factors that influence whole animal trials such as breed type, exposure period, toxicity levels, temperature, and body weight. By taking an *in-vitro* approach, we have demonstrated that ergot alkaloids directly impact sperm motility characteristics.

Ergot alkaloids are lipid soluble and presumably can permeate readily across sperm membranes. Sperm motility is dependent on many cellular functions including cAMP and calcium concentrations [24] [25] [26]. It is plausible that ergot alkaloids can directly affect sperm motility by altering intracellular cAMP and calcium concentrations. Fertilizing capacity and motility also may be compromised by ergot alkaloid interaction with sperm plasma membrane receptors [9]. Unfortunately, concentrations of ergot alkaloids in the reproductive tract of cattle are not known, but the concentrations tested in this study are within the range of possible local concentrations.

5. Conclusion

Ergot alkaloids have a long history of impacting reproduction in humans and livestock. Most of the historical research has been related to female fertility, but in recent decades it has been recognized that ergot alkaloids may affect male fecundity. Our research demonstrates that ET and DHET directly decrease sperm motility. Future studies will focus on the functional viability of spermatozoa exposed to ergot alkaloids.

Acknowledgements

This work was supported by the taxpayers of Arkansas through the University of Arkansas System Division of Agriculture, and in part by the USDA National Institute of Food and Agriculture (Hatch project ARK02435).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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