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PHENYLBUTAZONE TREATMENT IN BREEDING STALLIONS: PRELIMINARY EVIDENCE FOR NO EFFECT ON SEMEN OR TESTICULAR SIZE

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ABSTRACT

Effects of phenylbutazone treatment on semen and testicular size were evaluated using 6 mature stallions. Three stallions were given 1 g phenylbutazone orally in feed twice daily for 4 weeks, and 3 stallions remained untreated. Semen samples were obtained daily for 7 consecutive days once during a 2-week baseline period (Week 2), once after 4 weeks of treatment (Week 6), and once following 1 complete spermatogenic cycle (60 days) after treatment was discontinued (Week 16). During the remaining weeks (Weeks 1,3,4,5,7,8 and 9), semen was collected twice weekly. Semen samples were evaluated using traditional and computer-assisted techniques at the time of collection and at 24 and 48 hours of storage at 4°C. Testicular volume was estimated using ultrasonography once during each period of the study. Repeated measures analysis of variance revealed no significant measurable effects of phenylbutazone treatment on semen at the time of collection or after storage at 4°C for 24 or 48 hours. Similarly, no effect on testicular volume was found.

Key words: stallion, semen, phenylbutazone, testicle

INTRODUCTION

Phenylbutazone is a synthetic, nonsteroidal, anti-inflammatory and antipyretic compound with demonstrated effectiveness in treating chronic painful musculoskeletal conditions in horses (1). Phenylbutazone is commonly used in the

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management of hind limb or back pain in breeding stallions that, if untreated, could interfere with mounting and thrusting. Data are not available on the possible side effects of phenylbutazone on semen or fertility. Phenylbutazone actively inhibits prostaglandin synthesis, and would be expected to alter prostaglandin constituents of seminal fluid. Larsen and co-workers (2) have demonstrated lowered concentrations of prostaglandin F-2alpha (PGF-2alpha) metabolites in the seminal plasma of 3 stallions treated with phenylbutazone. Although the role of prostaglandins in semen is not well defined, it is reasonable to expect a role in viability, motility or fertility of the spermatozoa. Without critical data, it is difficult to clinically recommend phenylbutazone treatment for breeding stallions. This preliminary study was conducted to evaluate possible effects of phenylbutazone treatment on the semen of stallions. Total number of spermatozoa, sperm concentration, total and progressive motility and morphological characteristics of semen of phenylbutazone-treated and control stallions were compared immediately after ejaculation using standard and computer-assisted semen analysis techniques. Also, motility of spermatozoa of phenylbutazone-treated stallions and control stallions was compared at 24 and 48 hours after cooling and storage in an industry-standard, slow-cooling (4°C), short-term (48 hours) semen storage system.[a] Testicular volumes for phenylbutazone-treated and control stallions were also compared.

MATERIALS AND METHODS

General Design

Six mature stallions (2 Standardbred, 4 Thoroughbred; ages 7 to 20 years), each with two scrotal testicles of normal size and consistency, and with semen evaluation results within the range of normal, were used in the study. The animals were maintained in individual stalls with daily paddock exercise. Stallions were randomly assigned to the phenylbutazone treatment group (1 g orally in grain twice daily for 4 weeks; n=3)[b] or to the control group (no treatment, n=3). The study was conducted for 16 consecutive weeks from July through October. Characteristics of fresh and stored (4°C) semen as well as testicular volumes of treated and control stallions were compared during a 2-week pretreatment (baseline) period, a 4-week treatment period, and a lo-week post-treatment period. Semen samples were obtained and evaluated daily for 7 consecutive days (in order to reach levels of daily sperm output) once during the baseline period (Week 2), once after 4 weeks of treatment (Week 6), and once following one complete spermatogenic cycle (60 days) after treatment was discontinued (Week 16). During the remaining weeks (Weeks 1,3,4,5,7,8 and 9), semen was collected twice weekly.

a Equitainer, Hamilton-Thorn Research, Danvers, MA.
b Coopers Animal Health, Inc., Kansas City, KS.

Semen Collection and Analysis

Semen samples were obtained using a live ovariectomized stimulus mare, a dummy mount mare and a Missouri style artificial vagina.[c] For 1 stallion (control group), semen was collected by manual stimulation while the stallion was standing on the ground, as described by Crump and Crump (3). Each semen sample was evaluated immediately after collection according to Society for Theriogenology recommendations (4). Traditional measures at the time of collection included the pH, gel-free semen volume, gel volume, concentration of spermatozoa, total number of spermatozoa, percentages of specific morphological defects as well as visually estimated total and progressive motilities. In addition, a motility analyzer (Hamilton-Thorn Motility Analyzer HTM-2030)[d] was used to obtain measures of the percentage of motile cells, percentage of rapid cells, percentage of moderate cells, percentage of slow cells, and percentage of static cells as well as mean linearity, mean path velocity, and mean progressive velocity of motile cells. For this procedure, semen in a quantity containing an estimated 400 million spermatozoa was added to 20 ml of skim milk glucose extender (Kenney) without an antibiotic (4) in a 50-cc Whirlpak bag[c] for a target dilution rate of 20 million spermatozoa to 1 ml of extender. Each sample was evaluated based on 3 fields of each of 2 chambers. Six frames per field were scanned at the rate of 19 frames/second. Chamber temperature, set at 35°C, varied from 35 to 39°C. Additional gate size and luminosity settings used throughout the study are shown below (Table 1).

Table 1. Motility Analyzer Settings

Minimum contrast	6	Critical path velocity	35
Minimum size	5	Critical linear index	25
Low size gate	0.3	Default intensity	85
High size gate	2.0	Slow velocity gate	20
Low intensity gate	0.4	Illumination	Dark Field
High intensity gate	2.0		

Twenty millimeters of diluted semen was then cooled to 4°C and stored for 48 hours in an industry standard slow cooling 48 hour storage container (5).[e] Motility characteristics were assessed at 24 and 48 hours, using the motility analyzer, as described above.

[c] Nasco, Fort Atkinson, WI 53538.
[d] Hamilton-Thorn Motility Analyzer HTM-2030, Hamilton Thorn Research, Danvers, MA
[e] Equitainer Hamilton Thorn Research, Danvers, MA.

Table 2. Traditional and HTMA[a] semen results as well as testicular volume measures for phenylbutazone-treated and control stallions during baseline, treatment, and post-treatment periods. Values represent group means with standard deviations in parentheses. Group means for semen measures are based on each stallion's mean of Days 5, 6, and 7 of a 7-day daily collection sequence.

	Baseline		Treatment		Post-treatment	
Traditional Measures at 0 hours	Control	Phenylbutazone	Control	Phenylbutazone	Control	Phenylbutazone
Gel-free Volume (ml)	66.1 (11.10)	58.9 (24.06)	58.3 (10.41)	56.1 (25.62)	40.4 (8.67)	21.7 (7.17)
Gel volume (ml)	17.2 (9.48)	7.2 (11.10)	15.0 (13.64)	13.3 (14.53)	0	0
Concentration (million per ml)	96.7 (27.14)	141.6 (82.51)	122.9 (48.13)	233.9 (216.30)	154.3 (53.35)	296.1 (136.4)
Total Sperm (billion)	5.778 (1.07)	6.737 (2.79)	5.862 (1.64	7.896 (3.05)	5.807 (0.56)	5.5 19 (1.75)
% Morphologically normal spermatozoa	76.8 (8.85)	80.4 (4.53)	74.0 (9.87)	74.6 (3.33)	76.9 (11.03)	78.9 (5.17)
% Total motile spermatozoa	62.2 (23.42)	66.1 (28.69)	68.9 (18.43)	7 1.67 (25.22)	80.0 (10.14)	86.11 (6.74)
% Progressively motile spermatozoa	5 1.1 (29.45)	58.9 (30.84)	60.0 (22.42)	62.8 (27.76)	74.4 (11.10)	81.1 (6.74)
Total no. of normal, progressively motile spermatozoa (billion)	2.158 (1.0)	3.404 (2.5)	2.891 (1.7)	4.039 (3.0)	3.406 (1.1)	3.619 (1.3)
PH	7.3 (0.03)	7.3 (0.03)	7.4 (0.06)	7.4 (0.07)	7.5 (0.02)	7.5 (0.01)
HTMA Measures at 0 hours						
% static cells	24.4 (4.0)	18.5 (8.2)	24.4 (8.0)	23.7 (2.1)	21.0 (3.9)	14.2 (5.3)
% motile cells	75.6 (4.0)	81.5 (8.2)	75.6 (8.0)	76.2 (2.1)	79.0 (3.8)	85.8 (5.3)
% rapid	61.4 (6.6)	67.4 (9.0)	60.0 (6.3)	61.0 (7.1)	54.7 (4.8)	69.2 (5.7)
% moderate	3.1 (1.1)	3.4 (0.4)	3.6 (1.4)	3.7 (2.2)	4.4 (0.5)	4.1 (0.9)
% slow	11.0 (2.4)	11.0 (2.5)	12.0 (2.4)	12.0 (3.4)	20.0 (1.2)	12.0 (1.2)
Mean linearity (%)	77.4 (3.0)	82.0 (2.6)	78.0 (4.7)	80.0 (1.0)	74.0 (3.5)	75.0 (2.1)
Mean path velocity (mic per second)	194.0 (0.8)	189.0 (15.8)	189.1 (5.1)	178.4 (25.8)	166.0 (5.5)	171.2 (15.0)
Mean progressive velocity (mic per second)	175.0 (2.5)	176.8 (15.3)	171.5 (9.2)	165.0 (23.7)	149.7 (4.9)	154.3 (14.7)

Table 2. • continued

	Baseline		Treatment		Post-treatment	
HTMA Measures at 24 hours	Control	Phenylbutazone	Control	Phenylbutazone	Control	Phenylbutazone
% static cells	24.5 (4.1)	36.5 (4.1)	36.5 (9.2)	33.8 (12.5)	31.2 (9.0)	22.7 (6.7)
% motile cells	75.5 (4.1)	63.5 (4.1)	63.5 (9.2)	66.2 (12.5)	68.8 (9.0)	77.4 (6.7)
% rapid	58.2 (7.6)	44.4 (7.6)	50.0 (12.5)	54.9 (16.5)	56.6 (9.2)	65.9 (7.5)
% moderate	3.6 (0.7)	5.2 (0.7)	2.6 (0.7)	2.7 (0.6)	2.8 (0.2)	3.0 (0.4)
% slow	13.6 (4.1)	13.8 (4.1)	11.0 (3.6	8.6 (3.9)	9.4 (0.6)	8.5 (1.9)
Mean linearity (%)	76.6 (2.8)	79.5 (2.8)	75.6 (6.4)	77.1 (6.2)	76.3 (3.4)	80.4 (1.5)
Mean path velocity (mic per second)	159.2 (4.2)	113.3 (4.2)	171.1 (9.6)	15 1.7 (36.6)	155.5 (10.2)	148.1 (27.9)
Mean progressive velocity (mic per second)	144.0 (8.6)	106.8 (8.6)	152.3 (7.6)	139.2 (30.9)	142.8 (9.6)	140.2 (24.5
HTMA Measures at 48 hours						
% static cells	35.0 (7.1)	51.1 (22.5)	44.2 (12.3)	43.2 (13.3)	34.7 (9.1)	29.6 (8.5)
% motile cells	65.0 (7.1)	48.9 (22.5)	55.8 (13.3)	56.8 (23.3)	65.3 (9.1)	70.4 (8.5)
% rapid	49.0 (8.9)	31.0 (26.0)	42.8 (10.2)	45.4 (18.0)	55.0 (9.1)	59.3 (13.5)
% moderate	4.4 (1.1)	4.5 (1.2)	2.6 (0.4)	2.4 (0.4)	2.1 (0.7)	2.8 (0.8)
% slow	11.8 (1.1)	13.5 (3.2)	10.4 (2.7)	8.9 (4.5)	8.6 (0.5)	8.3 (4.5)
Mean linearity (%)	71.2 (9.9)	69.2 (11.0)	75.3 (7.2)	78.2 (5.8)	70.7 (6.8)	71.0 (5.1)
Mean path velocity (mic per second)	134.0 (6.5)	78.0 (26.6)	159.0 (20.6)	125.0 (43.8)	141.0 (11.0)	119.0 (22.0)
Mean progressive velocity (mic per second)	117.1 (14.4)	70.2 (27.1)	142.8 (19.2)	114.2 (35.5)	127.2 (11.3)	110.2 (21.5)
Testicular Measures						
Left volume (cubic centimeters)	135.2 (14.28)	116.0 (33.04)	122.4 (15.57)	118.6 (47.72)	123.0 (26.77)	109.7 (53.75)
Right volume (cubic centimeters)	149.1 (38.21)	123.3 (29.03)	109.2 (31.24)	131.1 (26.56)	118.63 (40.83)	135.0 (36.72)
Total volume (cubic centimeters)	284.2 (41.34)	239.2 (62.07)	231.6 (43.81)	249.7 (73.65)	241.5 (63.76)	244.7 (87.52)

a HTMA = Hamilton-Thorne Motility Analyzer, Hamilton-Thorn Research, Danvers, MA.

Testicular Volume

Testicular volume was estimated once during each period of the study: Week 2 (baseline), Week 6 (after 4 weeks of treatment) and Week 16 (10 weeks after treatment had stopped). Measures of length and the largest cross-sectional area obtained by ultrasonography[f] were used to estimate the volume of each testis, using Love's formula (6):

$$\text{Volume} = 4/3\ \text{Area}\ (\text{Length}/2)$$

Statistical Analysis

Repeated measures analysis of variance techniques were used to evaluate differences between treatment and control groups by period for each measure. For each semen measure, analysis was performed by using, for each stallion, the mean of Days 5, 6, and 7 for each 7-day semen collection sequence. Levels of $P < 0.05$ were considered significant.

RESULTS

Results of semen evaluation and testicular measurements are summarized in Table 2. In this repeated measures design, a treatment effect would be manifest by an interaction between treatment and period. For only one measure, the percentage of slow cells at Hour 0, was there a significant treatment-by-period interaction. The percentage of slow cells in semen of the control treated stallions during the post-treatment period was greater than that in semen of phenylbutazone-treated stallions during any period, and greater than that in semen of the control stallions during the baseline or treatment periods.

Several main effects of period were noted. PH was higher during the post-treatment period than during the baseline period. Both the gel and gel-free volumes during the post-treatment period were significantly lower than during the baseline or treatment periods. Both the total percentage of motile spermatozoa and the percentage of progressively motile spermatozoa during the baseline period were significantly lower than during the post-treatment period.

DISCUSSION

Lower volumes of gel and gel-free semen were observed in both the treated and control stallions during the post-treatment period, probably due to seasonal changes in these parameters. Baseline and treatment period samples were collected in late July through August, while post-treatment samples were obtained during the first week of November. The lower spermatozoa1 motility measured in all stallions

f Pie Medical 450, Classic Medical Supply, Jupiter, FL.

during the baseline period may have been due to exceptionally hot weather (35 to 37°C) during that period. The effects may have been due to high temperature in the laboratory or to elevated testicular temperature (7).

No evidence of adverse effects of phenylbutazone treatment on semen or testicular volume was found in this study. For some of the semen parameters evaluated there is high inherent variability. Accordingly, the statistical power of our design was low for detecting small to moderate differences due to treatment. Therefore, these data should be considered to be preliminary. Further work is needed to fully evaluate potential effects on fertility that would not be reflected in the semen and testicular measures evaluated in the small number of stallions in this study. A common type of sexual behavior dysfunction in stallions involves chronic or intermittent, slow sexual arousal or ejaculatory failure in association with apparent back or hind limb pain (8,9). These stallions typically exhibit difficulty mounting, coupling and thrusting, which may lead to aggressive behavior or a "sour attitude." Breeding performance in such cases can usually be improved or maintained at acceptable levels by use of pain medication. Clinically, a widely recommended treatment is phenylbutazone. While it remains untested whether fertility is actually affected by treatment, the breeding lives of many horses with chronic hind limb or back pain are extended for many years by phenylbutazone treatment.

Short-term storage and/or shipping of all or portions of ejaculates can in some cases reduce the number of ejaculates necessary to breed a given number of mares. Therefore, longevity of motility of spermatozoa in such a system is an important factor in reproduction of these horses. In the 3 stallions studied, there were no detectable adverse effects of phenylbutazone treatment on the motility of spermatozoa after storage at 4°C for 24 or 48 hours.

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