Reprinted from the JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, Vol. 215, No. 9 Pages 1317.1319.

Measurements of reproductive function in stallions treated with trimethoprim-sulfamethoxazole and pyrimethamine

Sylvia J. Bedford, LV, MS, DACT, and Sue M. McDonnell, PhD

Objective-To evaluate the effects of trimethoprim-sulfamethoxazole and pyrimethamine treatment on various measures of reproductive function in healthy pony stallions.

Design-Randomized complete block study.

Animals-12 healthy, mature pony stallions.

Procedure-Stallions were assigned to treatment and control groups balanced for age and various characteristics of reproductive function. The treated group received trimethoprim-sulfamethoxazole and pyrimethamine for 90 days during summer and fall; the control group was not treated. Semen characteristics, sexual behavior, testicular volume, and sperm production efficiency were evaluated before treatment started and at 30-day intervals until 60 days after treatment ended.

Results-Effects of treatment were not detected for semen characteristics, testicular volume, sperm production efficiency, libido, erection, and quantitative measures of ejaculatory efficiency. At 30, 60, and 90 days, 4 of 6 treated stallions had unsteadiness upon mounting, clumsy or weak thrusting, failure to flex the back, and thready or inapparent ejaculatory pulses that resulted in dribbling of semen rather than forceful expulsion.

Conclusions and Clinical Relevance-Although treatment with trimethoprim-sulfamethoxazole and pyrimethamine may not affect semen quality, testicular volume, sperm production efficiency, erection, or libido of healthy stallions, treatment may induce changes in copulatory form and agility and alter the pattern and strength of ejaculation. Stallions that develop neurologic signs during treatment should be used with caution for breeding. *(J Am Vet Med Assoc 1999;215:1317-1319)*

Equine protozoal myeloencephalitis (EPM) is a progressive neurologic disease caused by migration of the protozoon *Sarcocystis falcatula* into the CNS. The parasite causes inflammation and necrosis of the spinal cord and caudal portion of the brain stem. Clinical signs vary and may include hind limb as well as forelimb neurologic deficits (ataxia, circumduction, tetraparesis, knuckling, and crossing over), multifocal muscle atrophy, or cranial nerve deficits (head tilt, facial paralysis, circling, nystagmus, dysphagia, and blindness). Signs result from diffuse, asymmetric CNS involvement.[1,2] Diagnosis may be supported by western blot detection of antibodies in serum and CSF.[2] The parasite is not always detected in CSF; therefore, results of polymerase chain reaction analyses are not consistently positive in samples from infected horses. A definitive antemortem diagnostic test is not presently available.[3]

At this time, recommended treatment of EPM includes concurrent administration of pyrimethamine and trimethoprim-sulfamethoxazole or sulfadiazine. These drugs inhibit folic acid synthesis in the host and parasite; adverse effects may result from folic acid deficiency and can include anemia, leukopenia, or thrombocytopenia. These effects may be alleviated by supplementation with folic acid, which cannot be utilized by the parasite.[2]

In male rats and mice, treatment with pyrimethamine has reduced testicular and epididymal weights, testicular and epididymal sperm counts, and fertility.[4,5] In breeding stallions treated for EPM, there has been anecdotal suggestion of adverse effects of trimethoprim-sulfamethoxazole and pyrimethamine on semen quality, sexual behavior, and ejaculatory function. Objective data are not available to determine whether such treatment contributes to clinical signs in stallions treated for EPM or whether clinical signs are caused solely by the disease. This question is particularly important because of the present difficulties in attaining a definitive diagnosis of EPM. Decisions on treatment of potentially infected horses and prophylactic treatment of valuable breeding animals depend on understanding potential adverse effects of treatment.

The objectives of the study reported here included evaluation of possible effects of treatment with trimethoprim-sulfamethoxazole and pyrimethamine on semen characteristics, testicular volume, sperm production efficiency, and several aspects of sexual behavior in healthy pony stallions.

Materials and Methods

Study design-Twelve mature pony stallions (3 to 18 years old; body weight, 125 to 300 kg [275 to 660 lb]) were used. Stallions were kept together on grass pasture throughout the study. On the basis of results of preliminary trials, 2 balanced groups were formed by ranking stallions by age and index measurements of 3 factors: semen quality, indicated by the total number of progressively motile, morphologically normal sperm in an ejaculate collected 1 hour after a previous ejaculate; libido, indicated by erection latency and mount readiness latency; and ejaculatory efficiency, indicated by ejaculation latency, number of mounts to achieve ejacula-

From the Section of Medicine and Reproduction, Department of Clinical Studies, School of Veterinary Medicine, New Bolton Center, University of Pennsylvania, Kennett Square, PA 19348. Dr. Bedford's present address is Department of Veterinary and Animal Sciences, Paige Laboratory, University of Massachusetts, Amherst, MA 01003.

Supported by the Dorothy Russell Havemeyer Foundation.

The authors thank Anita Ruducha, Georgina Russell, Elizabeth Torello, Jaime Miller, and Amy Hinze for technical assistance.

tion, number of thrusts in the ejaculatory mount, and number of palpable ejaculatory pulses. The balanced groups were then randomly assigned to treatment (n = 6) or control (6) conditions.

For all stallions, semen quality, sexual behavior, testicular volume, and sperm production efficiency were evaluated in standard teasing and semen collection trials immediately before treatment, at 30-day intervals during treatment, and 30 and 60 days after treatment ended. Handling of stallions and all measurements were conducted blind to treatment status of subjects.

Treatment-For 90 days beginning in June (breeding season), treated stallions received trimethoprim-sulfamethoxazole[a] (20 mg/kg [9.1 mg/lb] of body weight, PO, q 12 h) and pyrimethamine[b] (1 mg/kg [0.05 mg/lb], PO, q 24 h) in 50 to 100 g of grain by hand-feeding. One stallion that occasionally refused treatment when mixed with grain was administered treatment as a paste by oral dose syringe and then fed grain. Control stallions were similarly fed grain but not treated.

Semen evaluation-At each evaluation time, 2 ejaculates were collected (1 hour apart); values determined for the second ejaculate were used as an estimation of semen characteristics and daily sperm output according to methods of Kenney et al.[6] Key measures for comparison were gel-free volume, sperm concentration, number of sperm, percentage progressively motile sperm, percentage morphologically normal sperm, and pH. An ovariectomized, estrogen-primed pony mare was used as a stimulus mare. All semen collections were done by manual stimulation with stallions mounted on a dummy mount.[7]

Testicular volume and sperm production efficiency-Testicle measurements (caliper width, height, and length) were taken on each semen collection day. Ellipsoid testicular volume and sperm production efficiency (estimated daily sperm output [total number of sperm]/estimated testicular parenchymal volume [cm^3]) were estimated according to the method of Love et al.[8]

Sexual behavior-Semen collection trials were videotaped for subsequent detailed analysis of precopulatory and copulatory behavior. Specific measurements of libido included erection latency (time [seconds] from entering the breeding shed to first full erection) and mount readiness latency (time [seconds] from entering the breeding shed until readiness to mount with full erection minus time [seconds] elapsed during penis washing and application of a semen collection bag).

Measurements of ejaculatory efficiency included ejaculation latency (time [seconds] from entering the breeding shed to ejaculation), number of mounts to achieve ejaculation, number of thrusts on the ejaculatory mount, number of palpable ejaculatory pulses, and assessment of copulatory form and agility (descriptive notation of performance during each mount). The technician performing the manual stimulation also recorded assessments of pattern and rigidity of erectile tumescence, maintenance of erection during thrusting, detumescence following ejaculation, and rate, strength, and rhythmic pattern of copulatory thrusts and ejaculatory pulses.

Health monitoring and statistical analyses-General health of each stallion was monitored twice daily throughout the study by physical examination. Blood samples were obtained on each semen collection day to assess effects of treatment on RBC and WBC counts. For all measurements, repeated measures ANOVA was used to evaluate within- and between-subject differences attributable to treatment. Differences were considered significant at $P < 0.05$.

Results

Semen evaluation-Before treatment started, control stallions had significantly greater percentage of progressively motile sperm (67%) than did treated stallions (53%); similarly, at 60 days of treatment, control stallions had significantly greater percentage of progressively motile sperm (73%) than did treated stallions (53%). Differences within each group were not detected among values obtained at the 6 evaluation times. For all other measurements of semen characteristics, significant differences between groups or within each group at each evaluation time were not detected.

Testicular volume and sperm production efficiency-Testicular volume for control and untreated groups decreased over time from baseline values, reflecting normal seasonal changes. Testicular volume was not significantly different between groups or within each group at different evaluation times. Sperm production efficiency was similar for both groups except at 60 days of treatment, when treated stallions had significantly higher values than did control stallions.

Sexual behavior-For the semen collection performed before treatment, control stallions had significantly lower mount readiness latency than did treatment stallions. During treatment, significant differences between treated and control stallions in erection latency and mount readiness latency were not detected, and all values were within reference ranges for our laboratory. For all stallions, pattern and penile rigidity of tumescence, maintenance of erection during thrusting, and detumescence following ejaculation remained normal.

Effects attributable to treatment were not detected for ejaculation latency, number of mounts to achieve ejaculation, number of thrusts on the ejaculatory mount, or number of palpable ejaculatory pulses; all quantitative measures of ejaculatory efficiency were within reference ranges for our laboratory.

At 30, 60, and 90 days of treatment and 30 days after treatment was discontinued, changes in copulatory form or ejaculation pattern were detected in 4 of 6 treated stallions. These changes were observed by the semen collection team, independently detected by a clinician who reviewed the videotapes to evaluate the stallions' sexual behavior, and confirmed by a second clinician who reviewed the tapes. All clinicians were experienced with stallion practice and unaware of the treatment status of the stallions and purpose of the study. Clinicians independently characterized the changes as unsteadiness upon mounting the dummy clumsy or weak thrusting, failure to flex the back and wrap closely around the dummy mount, stiff back and forelimbs during copulation, as well as undetectable or spastic ejaculatory tail movements. The semen collection technician also noted thready ejaculatory pulses resulting in dribbling of semen rather than forceful expulsion. The 4 stallions that exhibited these changes returned to normal copulatory form and ejaculation pattern at 60 days after treatment was discontinued. None of the 6 control stallions had changes in copulatory form and ejaculation pattern. Difference between groups in the number of affected stallions, as determined by a Fisher exact test, was significant ($P = 0.03$).

All stallions remained healthy throughout the study. Results of CBC were within reference ranges for our laboratory.

Discussion

Measures of general health, semen quality, testicular volume, sperm production efficiency, libido, and ejaculatory efficiency of healthy stallions were not affected by treatment with trimethoprim-sulfamethoxazole and pyrimethamine at recommended dosages for treatment of EPM. Significant differences in percentage of progressively motile sperm and mount readiness latency at baseline, in percentage of progressively motile sperm and sperm production efficiency at 60 days, and in ejaculation latency at 90 days into treatment, were detected between control and treated stallions. These were not considered a result of lack of balance between groups or treatment effect; with the large number of measures in the present study it was expected that some values may differ, only by chance, at any given time, between the 2 groups of stallions.

In rats and mice,[4,5] pyrimethamine at higher dosages (10 to 400 mg/kg [4.5 to 180 mg/lb], PO, q 24 h) than used in these stallions adversely affected spermatogenesis. Higher dosages, longer treatments, or more sensitive tests to directly evaluate spermatogenesis, such as testicular biopsy, may have revealed adverse effects on sperm production in horses of our study

Four of the 6 treated stallions developed transient changes in copulatory behavior, suggestive of musculoskeletal stiffness across the back and possible neurologic deficits in the hind limbs and ejaculatory apparatus. Interestingly, this same pattern of abnormal copulatory form was observed in 1 treated EPM-seropositive breeding stallion and 3 of 6 prophylactically treated breeding stallions on a well-managed farm in our practice during the 1997 breeding season. In each stallion, signs developed after approximately 5 to 6 weeks of treatment at the same dosage as used in the study reported here and subsided within approximately 3 weeks, although treatment was not discontinued.

Drugs used in our study inhibit folic acid synthesis. In humans, there is evidence that folic acid deficiency may result in neurologic problems referable to the spinal cord." Although RBC folic acid concentration was not measured, and stallions in our study did not have other evidence of folic acid deficiency, we speculate that horses might be quite sensitive to reduced concentrations of circulating folic acid, and that subclinical drug-related folic acid deficiency might provide an explanation for the changes we observed. Accordingly, we recommend caution in continuing to breed stallions that are being treated with these drugs if neurologic signs develop or worsen, especially if EPM has not been confirmed.

[a] Sulfamethoxazole and trimethoprim tablets, Sidmak Laboratories Inc, East Hannover, NJ.

[b] Daraprim (pyrimethamine), courtesy of Burroughs Wellcome Co, Research Triangle Park, NC.

References

1. George LW. Diseases of the nervous system. In: Smith BP, ed. *Large animal internal medicine*. 2nd ed. St Louis: Mosby Year Book Inc, 1996;1046-1049.

2. Reed SM, Moore BR. Equine protozoa1 encephalomyelitis, in *Proceedings*. 14th Am Co11Vet Intern Med Forum 1996;341-342.

3. Bernard WV Equine protozoal myelitis-laboratory tests and interpretation, in *Proceedings*. Intern Equine Neuro1 Conf 1997;7-10.

4. Awoniyi CA, Chandrashekar V, Hurst BS, et al. The effects of chronic administration of pyrimethamine on spermatogenesis and fertility in male rats. *J Androl* 1993;14: 174-179.

5. Consentino MJ, Pakyz RE, Fried J. Pyrimethamine: an approach to the development of a male contraceptive. *Proc Natl Acad Sci* USA 1990;87:1431-1435.

6. Kenney RM, Hurtgen JP, Pierson R, et al. *Clinical fertility evaluation of the stallion*. Hastings, Neb: Society for Theriogenology, 1983.

7. McDonnell SM, Love CC. Manual stimulation collection of semen from stallions: training time, sexual behavior and semen. *Theriogenology* 1990;33:1201-1210.

8. Love CC, Garcia MC, Riera FL, et al. Evaluation of measures taken by ultrasonography and calipers to estimate testicular volume and predict daily sperm output in the stallion. *J Reprod Fert* 1991;44(suppl):91-105.

9. Swain RA, St. Clair L. The role of folic acid in deficiency states and prevention of disease: clinical review. *J Fam Pract* 1997; 44:138-144.