Effects of Ponazuril on Reproductive Function of Stallions

Steven P. Brinsko, DVM, MS, PhD, Diplomate ACT; Terry L. Blanchard, DVM, MS, Diplomate ACT; Charles C. Love, DVM, PhD, Diplomate ACT; Dickson D. Varner, DVM, MS, Diplomate ACT; Sherri L. Rigby, DVM, PhD, Diplomate ACT; Janet F. Roser, PhD; Wendell L. Davis, DVM; and Thomas J. Kennedy, PhD

Stallions (n = 4) treated for the recommended duration (28 days) at twice the recommended oral dosage (10 mg/kg) with ponazuril (Marquis™) showed no adverse affects on semen parameters or reproductive hormones. Authors' addresses: Department of Large Animal Medicine and Surgery (Brinsko, Blanchard, Rigby, Varner) and Department of Physiology and Pharmacology (Love), College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4475; Department of Animal Science, University of California, Davis, CA 95616 (Roser); Bayer Animal Health, Veterinary Services, P.O. Box 390, Shawnee Mission, KS 66201 (Davis, Kennedy).© 2002 AAEP.

1. Introduction

With the increased diagnosis and treatment of equine protozoal myeloencephalitis (EPM) in recent years, the use of trimethoprim-sulfa drugs in combination with pyrimethamine in the horse has escalated dramatically. Recommendations for EPM therapy are to use high-dose combinations of the folic acid-inhibiting drugs sulfamethoxazole or sulfadiazine in conjunction with pyrimethamine for a minimum of 5–6 mo.¹ Numerous side effects, including reproductive problems, have been reported using these drug regimens.¹

Dr. Rigby’s current address is Bayer Animal Health, Veterinary Services, P.O. Box 390, Shawnee Mission, KS 66201.
Tranzuril, have shown promise for the treatment of EPM. Ponazuril (toltrazuril) has been investigated for the treatment of EPM. These drugs have minimal side effects in the horse, and their bioavailability and pharmacokinetics should dramatically shorten the required time of treatment.

Recently, the FDA has approved the use of ponazuril for the treatment of EPM. Ponazuril (toltrazuril sulfone) is the active metabolite of toltrazuril. The recommended treatment for EPM with ponazuril is at a dosage of 5 mg/kg for 28 days. The objective of this study was to evaluate the effects of ponazuril on semen quality, daily sperm production, and reproductive hormone levels in normal stallions.

2. Materials and Methods

Eight mature (aged 4–20 yr) stallions of Quarter Horse and Thoroughbred breeding (400–600 kg) were used. All stallions had an acceptable health examination and two normally sized testes that were fully descended into the scrotum, and had passed a breeding soundness examination to ensure normal semen quality. Before the onset of the study and after a period of acclimation and training, semen was collected from stallions for 10 consecutive days. Mean numbers of sperm obtained on days 8–10 were used to establish daily sperm output (DSO) for each stallion. Stallions were then randomly assigned to treatment groups, which were balanced for age and daily sperm output. Identical syringes, containing ponazuril or placebo, were labeled for each individual horse by the manufacturer, thereby blinding all personnel directly involved in the study to the treatment groups. Treated stallions (n = 4) were administered ponazuril 15% paste orally at twice the recommended dose (10 mg/kg) once daily for 28 days, whereas control stallions were administered the carrier paste containing talc once daily for 28 days (March 26–April 22). Data were obtained before, during, and through 65 days after cessation of treatment. Variables measured included physical examinations, evaluation of blood parameters, semen quality, and daily observations for clinical health. Semen quality assessments included total sperm numbers, sperm morphology, sperm motility, and sperm chromatin structure assay. Percentages of motile sperm and progressively motile sperm in ejaculates at DSO were analyzed using computer-assisted semen analysis. Throughout the study period (March–June), semen was collected twice per week and for 10 consecutive days at monthly intervals for DSO determinations. Blood samples were collected once per month for hematology, clinical chemistry, and reproductive hormone analysis. Reproductive hormone assays were performed on aliquots of plasma pooled from four blood samples obtained at hourly intervals (7:00–10:00 a.m.). Within 48 h of the last semen collection for DSO (day 65 post-treatment), all stallions were castrated and their testes were sectioned and fixed in 2% glutaraldehyde. After fixation, tissues were homogenized for determination of daily sperm production per gram of tissue. Semen and hormone data were examined by two-way repeated measures analysis of variance procedures to identify effects of treatment, time, and treatment × time interactions. Daily sperm production between treatment groups was compared using Student’s t test. Differences were considered significant at a probability level of p < 0.05.

3. Results

Total sperm numbers, percentages of morphologically normal sperm, motile sperm, and progressively motile sperm were not affected by treatment (p > 0.1; Table 1). Treatment had no effect on the stability of sperm chromatin (COMPα), determined by chromatin structure assays (p = 1.00; Table 1). Circulating levels of reproductive hormones (luteinizing hormone, follicle-stimulating hormone, testosterone, estradiol, and inhibin) were not affected by treatment (p > 0.1). Within the control group, estradiol and inhibin differed across time.

| Table 1. Mean (±SEM) Values for Total Numbers (TSN), Morphologically Normal (Normal), Motile (TMOT), Progressively Motile (PMOT), and Percentage of Sperm Susceptible to Chromatin Damage (COMPα) in Ejaculates at Daily Sperm Output in Control and Ponazuril-Treated Stallions (n = 4/group) Throughout the Study Period |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Pre-Treatment   | End of Treatment | 1 mo Post-Treatment | 2 mo Post-Treatment |
|                                | Control         | Ponazuril       | Control         | Ponazuril       | Control         | Ponazuril       |
| TSN (×10⁹)                     | 5.2 ± 1.3       | 4.9 ± 1.0       | 7.0 ± 1.6       | 5.4 ± 1.1       | 5.4 ± 0.8       | 5.1 ± 0.5       | 5.7 ± 0.9       | 5.5 ± 0.4       |
| Normal (%)                     | 68.9 ± 4.4      | 64.9 ± 0.1      | 65.6 ± 6.5      | 64.9 ± 2.7      | 67.8 ± 3.9      | 69.3 ± 3.4      | 70.6 ± 5.1      | 69.1 ± 2.6      |
| TMOT (%)                       | 83.8 ± 2.6      | 83.9 ± 2.0      | 83.4 ± 2.6      | 83.9 ± 1.2      | 84.0 ± 2.4      | 84.9 ± 2.6      | 84.3 ± 2.8      | 83.5 ± 1.3      |
| PMOT (%)                       | 76.2 ± 2.9      | 74.4 ± 3.5      | 73.1 ± 1.7      | 73.0 ± 3.3      | 73.8 ± 2.5      | 71.3 ± 5.1      | 73.5 ± 2.8      | 72.3 ± 3.3      |
| COMPα (%)                      | 8.0 ± 1.5       | 9.3 ± 2.3       | 8.0 ± 1.1       | 8.3 ± 1.4       | 9.0 ± 1.9       | 8.5 ± 2.2       | 8.3 ± 1.2       | 7.3 ± 1.2       |

*aStallions treated with ponazuril (10 mg/kg, q 24 h, PO) or placebo for 28 days. Within rows, values do not differ (p > 0.1).*
(p < 0.05), but these differences were not observed in the ponazuril-treated group (Table 2). Although some variables were affected by sampling date, there were no time × treatment interactions (p > 0.1) for any variable measured. Hematology and clinical chemistry values remained within normal limits for all stallions throughout the study, as did physical examination findings. No side effects caused by treatment, including adverse effects on mating behavior, were observed.

4. Discussion

In human medicine, anti-fertility effects of therapeutic drugs are a major concern. Studies have shown the sulfa drug sulfasalazine and its metabolite sulfapyridine to cause infertility in male rats and humans by decreasing sperm motility and/or sperm numbers and increasing morphologic defects. Similar anti-fertility effects have not been documented for sulfamethoxazole and sulfadiazine; the two most commonly used sulfa drugs in the horse. There are, however, clinical reports that suggest a possible detrimental effect of trimethoprim-sulfamethoxazole on the semen quality of human patients.

Another folate inhibitor, pyrimethamine, is often combined with trimethoprim-sulfamethoxazole in the treatment of EPM. Unlike trimethoprim-sulfamethoxazole, pyrimethamine clearly has adverse effects on male fertility. Pyrimethamine does not seem to diminish reproductive hormone levels but significantly reduces testicular size and causes spermatogenic arrest when high doses are given for prolonged periods. It is suggested that the adverse effects on spermatogenesis are caused by the same anti-folate action that makes pyrimethamine an effective antimicrobial. When used in combination with two other antifolate medications (trimethoprim-sulfamethoxazole) for the treatment of EPM, the synergistic action of all three drugs may have the potential to impact stallion fertility. Surprisingly, Bedford and McDonnell did not detect any adverse effects on semen quality of pony stallions treated with trimethoprim-sulfamethoxazole and pyrimethamine. They did, however, report that copulatory and ejaculatory function were adversely affected in four of six treated stallions in their study, and these effects mimicked those observed in treated stallions on a client breeding farm. Side effects and the potential for diminished semen quality warrant that caution be exercised when deciding whether or not to treat breeding stallions with combinations of trimethoprim-sulfamethoxazole and pyrimethamine for prolonged periods.

Many drugs and drug combinations, such as trimethoprim-sulfamethoxazole and pyrimethamine, were developed for use in other domestic animals or humans, and their use in the horse is based on their efficacy and safety in other species. Within each major class of antibiotics, individual agents have been shown to have significant adverse effects on spermatogenesis and spermatocoidal function in mammals. However, because of the cost involved, most drugs do not undergo safety trials, especially not reproductive safety trials, in horses unless the product is developed specifically for the equine. The majority of drugs are considered safe for pregnant mares and breeding stallions unless there are specific contra-indications or precautionary statements to that effect on the label or package insert.

Ponazuril was developed specifically for use in the horse for the treatment of EPM. Previous studies have documented the efficacy and safety of ponazuril in the horse. The present study was performed to determine whether stallions undergoing treatment with ponazuril would experience detrimental effects on semen quality or reproductive performance. Results of the present study indicate that there were no adverse effects of ponazuril treatment in breeding stallions when twice the recommended dose was administered for the recommended treatment duration of 28 days. It seems that this product is a safe alternative to the combination trimethoprim-sulfamethoxazole and pyrimethamine in the treatment of EPM in breeding stallions.

This study was supported by a grant from Bayer Animal Health. The authors thank Lou Solonyka for technical assistance in the study.

References and Footnotes


*aClinatox®, Janssen Pharmaceuticals, Beerse, Belgium.*

*bBaycox®, Bayer Canada, Ontario, Canada.*

*cMarquis™, Bayer Animal Health, Shawnee Mission, KS 66201.*

*dHTM-IVOS Version 10 Spermatozoal Motility Analyzer, Hamilton Thorne Research, Beverly, MA 01915.*