Effect of Fenbendazole on Turkey Semen Quality

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Primary Audience: Veterinarians, Flock Managers, Poultry Scientists

SUMMARY

Fenbendazole (FBZ) is an anthelmintic recently approved to treat and control nematode infections in growing turkeys. When administered to growing turkeys there are no detrimental side effects. However, when we used FBZ to treat nematodes in mature breeder toms, we observed a decrease in semen quality and a subsequent precipitous decline in fertility to less than 20% within 6 wk of administration. An experiment was designed to determine the impact of FBZ administration on aspects of spermatogenesis and semen quality. We discovered that although sperm viability and concentration was not significantly affected by FBZ, this drug significantly reduced sperm mobility. However, normal mobility resumed within 6 wk after FBZ administration. Fenbendazole binds to tubulin and interferes with microtubule assembly. Based on testes histology and the immunocytochemical localization of tubulin in spermatids and mature sperm, FBZ had no affect on any aspect of spermatogenesis. We suggest that FBZ may be affecting sperm mobility by some molecular alteration of the sperm tail axoneme or midpiece, both of which are tubulin-containing structures. If a breeding flock of toms become infected with nematode parasites, a flock manager has the following options: do not treat the toms, find an alternative to FBZ, treat the toms with FBZ and increase the sperm number and frequency of artificial inseminations, or treat the toms with FBZ and secure semen from an uninfected tom flock.

Key words: fenbendazole, sperm, semen, fertility, mobility

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DESCRIPTION OF PROBLEM

Fenbendazole (**FBZ**) is a benzimidazole antihelmintic used in domesticated animals for the treatment and control of nematode parasites and has recently been approved and labeled for growing turkeys. Apparently, FBZ is poorly absorbed by the host animal and selectively absorbed by the nematode parasite. When internalized by the nematode parasite, FBZ binds to the tubulin molecule and consequently interferes with the polymerization of tubulin into microtubules [1].

Faced with a roundworm outbreak in our turkey breeder farm, FBZ was administered at the approved dose rate by the resident veterinarian at the dose recommended by the manufacturer. Within a 2-wk period, sperm mobility was noticeably impaired, and true fertility declined precipitously from 83% the week of FBZ administration to 55% the second week and to 16% by 7 wk later. The hatchability of fertile eggs remained unchanged.

Nothing was found in the literature on the effects of FBZ administration on breeder toms. In contrast, FBZ has been shown to have no adverse effects on semen quality or fertility in cattle, sheep, or swine [2]. In the following communication, we describe results of a study in

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which FBZ has a rapid, long-term, but transitory effect on semen quality when administered to mature toms.

MATERIALS AND METHODS

Hybrid turkey toms (n = 40) were raised and maintained under management conditions recommended by the Hybrid breeder manual [3]. Semen was collected from all toms for 2 wk (28 to 30 wk) and collected twice weekly thereafter. Twenty males were randomly chosen from the tom flock and given FBZ at the dosage recommended by the resident veterinarian (8 g of FBZ 20% premix /kg of feed, fed for 6 d). Semen collection continued unabated for the next 6 wk. Fourteen toms from the control and FBZ treatment groups were killed at intervals during the 6-wk period for tissue collection. Semen samples from individual control (n = 6) and FBZ-treated toms (n = 6) were evaluated separately for sperm concentration, membrane integrity, and sperm mobility. Sperm concentration was determined using an IMV Microreader photometer (optical density of 597 nm) [4] to estimate the optical density of a 1:200 dilution of neat semen in a 3% (wt/vol) sodium citrate solution. Sperm viability was determined using the SYBR-14–propridium iodide live-dead stain combination as evaluated by flow cytometry [5]. Sperm mobility was assessed as previously described by Froman and McLean [6] with the minor modifications suggested by Long and Kulkarni [7]. Briefly, a sperm suspension (1×10^9 sperm/mL of mobility buffer) was overlaid onto a 6% Accudenz solution and incubated for 5 min at 41°C. The optical density was measured by an IMV Microreader photometer (optical density of 597 nm) after 1 min of equilibration; sperm mobility values are presented as absorbance units.

Testicular histology was conducted on 3 to 4 males per week from 34 wk of age (2 wk before FBZ treatment) through 44 wk of age (8 wk after FBZ treatment). Toms were euthanized by cervical dislocation, and the left testes were removed and fixed in 6% formaldehyde in PBS. After fixation for 24 h, the specimens were prepared for paraffin embedding, sectioned at 6 to 7 μm, and mounted on Fisher Superfrost Plus slides [8]. Sections were stained with hematoxylin and eosin for general histology and immunocytochemically stained for tubulin localization. After dewaxing, the sections were stained with fluorescein-isothiocyanate (**FITC**)-conjugated anti-tubulin and examined with a Zeiss Axiomat. For immunocytochemistry, sections were permeablized with 0.1% Triton X-100 for 18 h at 4°C and then washed for 2 h at room temperature in 1% BSA in 0.4% polyvinylpyrrolidone in buffer. Sections were then flooded with mouse anti-α-tubulin or β-tubulin at 1:1,000 and 1:500, respectively, for 12 h at 4°C and washed in buffer for 4 h. Sections were then flooded with goat anti-mouse IgG-conjugated FITC overnight at 4°C and washed in buffer for 4 h. To visualize nuclei, sections were stained with *bis*-benzimide (final concentration of 5 μg/mL of buffer) for 10 min at ambient temperature, rinsed in distilled water, air-dried, and cover-slipped with Vectashield mounting medium.

Statistical Analysis

Three statistical analyses were performed on the data collected. Percentage of sperm viability was arcsine transformed [$z = \sin^{-1}\sqrt{(y/100)}$, where y is the data value measured as a percentage, and z is the transformed variable used in the analysis] to stabilize the variance and satisfy ANOVA assumptions. Sperm concentration and mobility were not transformed. For each tom, 3 wk of pretreatment values were averaged, transformed if they represented percentages (sperm viability), and used as covariates to adjust the subsequent values for each tom. A mixed model ANOVA was performed for each of the 3 dependent variables, using PROC MIXED in SAS [9]. The independent variables in the model were the covariate, a treatment effect, a week (time) effect, and a week-by-treatment interaction. The time series dependencies resulting from repeatedly measuring each tom were modeled using an autoregressive (1) covariance structure. Degrees of freedom were computed using the Kenwood-Rogers estimate [9]. Least squares means and their standard errors were also calculated by the PROC MIXED SAS subroutine. For percentage of viable sperm, an approximate 95% confidence interval on each mean was calculated on the transformed scale. The means and 95% confidence intervals were then back-transformed to the percentage scale for ease of interpretation. If a significant treatment effect was observed, we

also expected a treatment by week interaction, because the effect of the FBZ administration on semen quality should diminish over time.

RESULTS AND DISCUSSION

After administration of FBZ, semen quality decreased within 2 wk. Sperm mobility significantly decreased ($F_{1, 20.5} = 33.77$, $P < 0.01$) the second week after FBZ administration and remained quite low for the next 2 wk. There was a significant treatment-by-week interaction ($F_{6,52.4} = 2.54$, $P = 0.03$) for mobility, interpreted as the treatment effect gradually wearing off following the FBZ administration (Figure 1a) because sperm mobility in the treatment group increased with time since treatment. Control sperm mobility declined slightly over the course of the experiment. The overall week effect was not significant ($F_{6, 52.4} = 0.86$, $P = 0.53$). [Note that the week effect does not test for this decline because it pools both control (for which mobility gradually declined) and treatment (for which mobility gradually increased after the initial decline) groups.] A pretreatment mobility mean for a tom was a significant predictor of subsequent mobility scores ($F_{1, 21.1} = 14.56$, $P < 0.01$).

There was no detectable effect of the FBZ administration on any other predictor variable for sperm viability (Figure 1b) and concentration (Figure 1c), including the pretreatment means, used as covariates. Thus, for these variables, the pretreatment mean of a tom was not a good predictor of subsequent scores. There was an overall slight (but not significant) decrease in sperm concentration over time.

Histologically, the seminiferous epithelium and the interstitial spaces in tissue sections from FBZ treated and control toms revealed no noticeable variation in appearance. Given its negative impact on sperm mobility and that FBZ binds to the tubulin protein and interferes with polymerization of tubulin into microtubules [1], we focused our attention on 2 tubulin-containing cell structures: the manchette of tubules [10] found in elongating spermatids and the axoneme found in the sperm tail. The immunocytochemical localization of tubulin revealed a strong positive reaction product around the elongating nuclei in spermatids and to a lesser extent, around the forming spermatid tails regardless of treatment (Figure 2). In dual fluorescent stain preparations the anti-tubulin-positive manchette appeared to surround the *bis*-benzimide stained elongating spermatid nuclei. In anti-tubulin

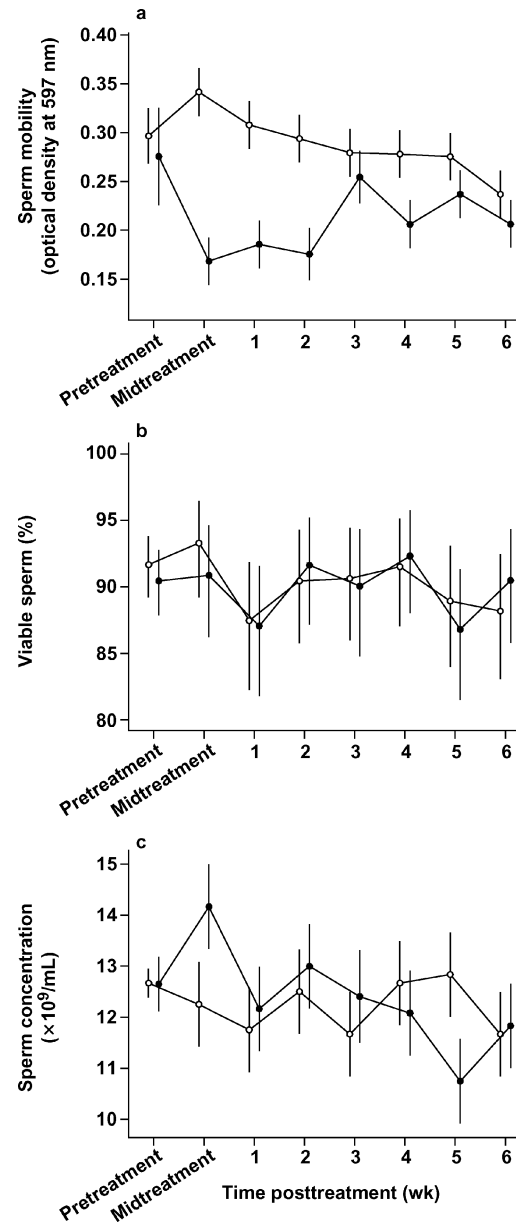

Figure 1. Panel A: least squares means of sperm mobility for control toms (open circles, n = 6) and toms treated with fenbendazole (closed circles, n = 6) over time. Bars represent one standard error of the mean in each direction. Panel B: least squares means of the percentage of viable sperm. Bars represent 95% confidence intervals on the means. Panel C: least squares means of sperm concentration. Bars represent one standard error of the mean in each direction.

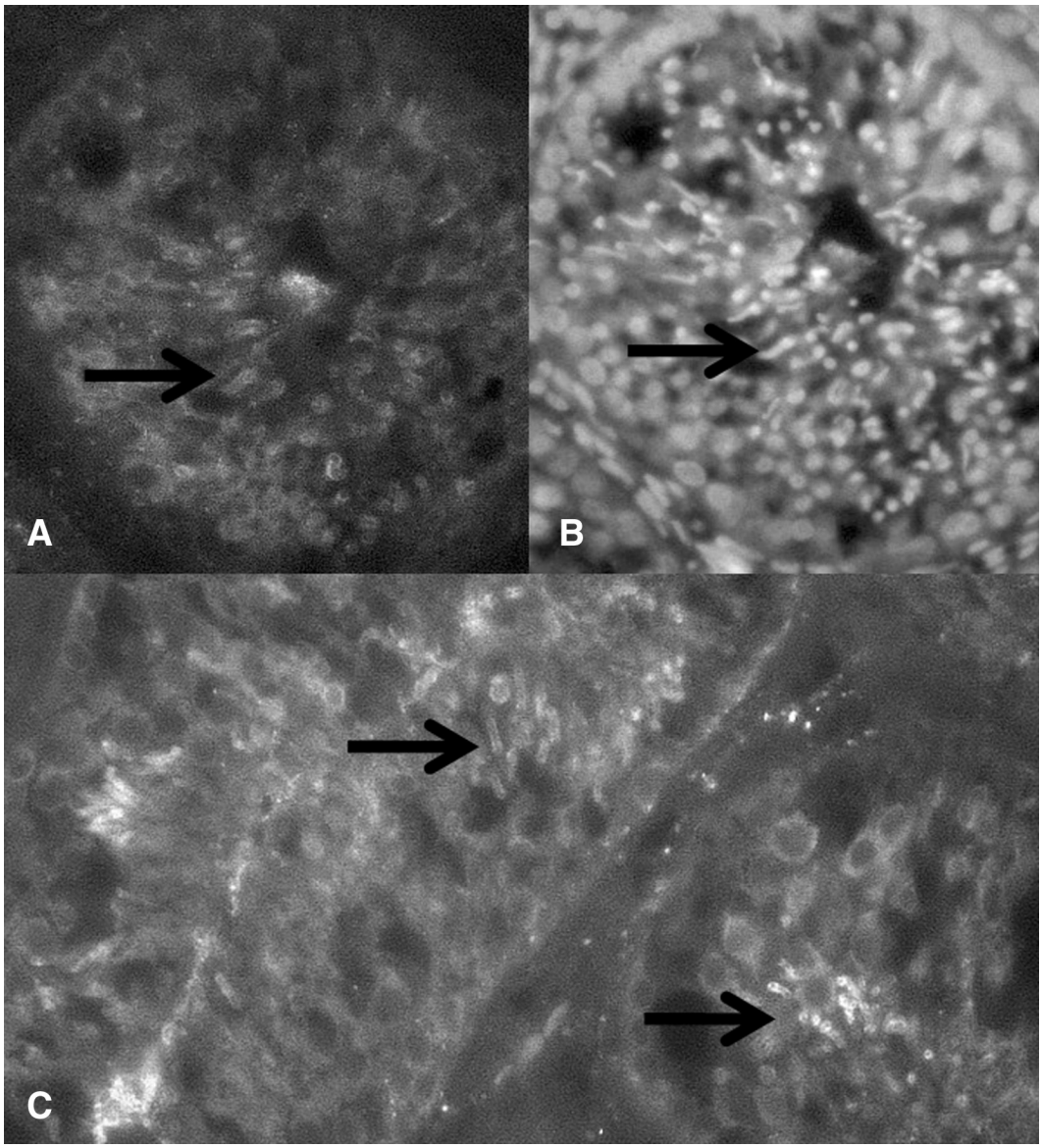

Figure 2. Panel A: a section showing a seminiferous tubule from a tom 4 wk after treatment with fenbenzadole. Anti-tubulin fluorescence is apparent with the manchette tubules in the elongating spermatids (arrow). Panel B: the same section as A, showing fluorescing nuclei following staining with the nuclear fluorescent stain *bis*-benzimide. The arrow highlights the elongating nucleus that is enveloped by the manchette tubules observed at the arrow in A. Panel C: tubulin associated with the manchette tubules (arrows) characteristic of the elongating spermatids is highlighted in this section (control tom). One can visualize the tube-like investment of the manchette enveloping the elongating nucleus in longitudinal section (upper arrow) and in cross section (lower arrow).

preparations the slight curvature of the manchette was observed in longitudinal sections while its tubular organization was observed in cross sections (Figure 2c).

Sperm viability and concentration appeared to be insensitive to FBZ but also did not seem to differ in a systematic way from among toms (the pretreatment score of a tom did not predict subsequent values, even for controls). These variables may be inherently less valuable for detecting the effects of medication like FBZ, or there may be sufficient measurement error or

other noise to obscure treatment effects. In contrast, sperm mobility differences were consistent between toms, and sperm mobility showed a clear decrease for those turkeys given this medication.

The recovery of mobility of sperm as the time since treatment increased probably reflected the gradual replacement of the incapacitated sperm with normal sperm. Figure 1a suggests that this process is essentially complete by wk 6 after FBZ administration.

If FBZ had an effect on the microtubules comprising the manchette and sperm tail axoneme, it was not evident with the immunocytochemistry procedure used in this study. Most likely, given that the mobility measurement is based on the ability of sperm to penetrate a viscous medium (Accudenz), the impact of the FBZ is possibly reflected in the sperm tail beat frequency or amplitude. That fertility and not hatchability (data not shown) was affected suggests that FBZ acted on the tubulin forming the axoneme and not the manchette tubules that are associated with the elongating, condensing nucleus. Dysfunctional manchette tubules would produce abnormal condensation of the sperm chromatin that would negatively impact sperm nuclear decondensation and syngamy at the time of fertilization.

CONCLUSIONS AND APPLICATIONS

1. In the doses recommended, FBZ reduced sperm mobility and, based on our preliminary observations, subsequently depressed the percentage of fertile eggs laid by hens inseminated with semen from the FBZ treated toms for at least 4 wk following FBZ administration.
2. If a tom in semen production became infected with nematode parasites, a flock manager would have the following options: do not treat the tom; find an alternative to FBZ; treat the tom with FBZ and increase the sperm number and frequency of artificial inseminations; or treat the tom with FBZ and secure semen from an uninfected tom flock.

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