

Evaluation of the effects of topical permethrin insecticide on bull semen quality

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Abstract

A number of different chemicals, including synthetic pyrethroids are used to treat and prevent ectoparasitic infestations in cattle. Permethrin is a type I pyrethroid insecticide that is commonly used to treat cattle for lice and flies via topical application. Concerns have recently been raised as to the effects of pyrethroid insecticides, including permethrin, on male fertility and semen quality. However, no controlled studies have been conducted to evaluate the effects of type I pyrethroids on beef bull semen quality. To this end, 15 purebred beef bulls were assigned to either a permethrin treatment group (PER; n=8) or control group (CON; n=7). Bulls in the PER group were treated with Permethrin 1% (Control Solutions Inc., Pasadena, TX) at 150% label dose on d 0 and d 14. Sperm motility and morphology were analyzed via light microscopy on d 0, 7, 14, 21, 35, 49, 63, and 84. Dermal application of a permethrin insecticide did not affect the proportion of normal sperm cells even at 150% label dose. Thus, it was concluded that permethrin parasiticide labeled for livestock use did not increase the risk of producing morphologically abnormal sperm cells.

Keywords: Permethrin, pyrethroid, insecticide, bull fertility, bull breeding soundness

Introduction

Pyrethrins and their synthetic derivatives, pyrethroids, have been widely used as insecticides for over 40 years and currently account for over 25% of worldwide insecticide use.¹ These chemicals target voltage gated sodium and chloride channels to kill external parasites, but are not considered a toxicological threat to humans or wildlife due to their high insect to mammal toxicity ratio.^{2,3} Nevertheless, concerns have been raised that pyrethroid insecticides may act as potentially harmful endocrine disrupters and cause reproductive dysfunction by altering hormonal pathways. However, *in vivo* experimental findings as to the endocrine effects of pyrethroid chemicals have been largely inconclusive.^{4,5} The toxic effects of pyrethroids on mammals, including potential reproductive effects, were reviewed by Tsuji et al.⁶ The majority of studies conducted thus far on the effects of pyrethroids on fertility use rodent models and relatively large, highly concentrated, oral doses of pesticide. Thus, these studies do not accurately model a livestock production scenario of pyrethroid exposure. In one recent study, researchers examined the effects of cyfluthrin and beta-cyfluthrin (type II pyrethroids) pour-on and impregnated eartags at label dosages on bull fertility. There were no significant differences in sperm motility, morphology, and testosterone concentration between pyrethroid exposed and control bulls.⁷

Synthetic pyrethroids have been implicated as the cause of transient male infertility in many livestock species. In case reports, drastic increases in secondary sperm abnormalities, particularly distal midpiece reflexes, were observed in bulls and rams mere days after initial pyrethroid exposure and persisted for up to four weeks after initial insecticide contact.^{8,9} However, these observations have not been validated in any livestock species in a controlled experimental setting. Permethrin is a type I pyrethroid insecticide that is commonly used to treat cattle for ectoparasites, particularly lice and flies, via dermal application. As most currently published studies on permethrin altered fertility focus on teratogenicity and the female reproductive system, there has been much debate as to the effects of permethrin on the male reproductive system. Oral administration of permethrin was reported to have had antiandrogenic effects in male rats.⁵ In another study, sub-chronic dermal exposure of pubescent male rats to permethrin resulted in arrested spermatogenesis and altered testosterone concentrations, however both interactions were dependent on dosage and duration of exposure.¹⁰

Bull fertility and breeding readiness is commonly evaluated by breeding soundness examinations (BSE). The Society for Theriogenology (SFT) has published guidelines designed to assess bull reproductive performance. These standards set thresholds for scrotal circumference (SC), percent of

morphologically normal sperm, and percent of progressively motile sperm and are used to identify whether or not a bull is a satisfactory potential breeder, physiologically capable of impregnating ≥ 25 normally cycling females in a 65 to 70 d breeding season.^{11,12} Thus, bull BSEs may be used to identify subfertile or transiently infertile males under traditional cattle production scenarios.

The objective of the present experiment was to determine the effects of a topical permethrin insecticide on bull semen quality. As pyrethroid chemicals have been used for ectoparasite control in breeding males with no obvious adverse effects for decades, it was hypothesized that dermal application of permethrin would not have a significant deleterious effect on the semen quality and fertility of breeding age beef bulls.

Materials and methods

All procedures were approved by Mississippi State University Institutional Animal Care and Use Committee (Protocol #13-031). This project was conducted in the spring of 2013 at H. H. Leveck Animal Research Center at Mississippi State University. Early spring was chosen to initiate this study in attempt to avoid confounded results due to heat and insect stress.

Animals and experimental design

Purebred *Bos taurus* beef bulls (n=15) aged 14 months to 4 years were stratified by scrotal circumference (SC) and assigned to either a permethrin treatment group (PER; n=8) or control group (CON; n=7). Bulls in the PER group were treated with Permethrin 1% (Control Solutions Inc., Pasadena, TX) at 150% label dose (225 mL) based on body weight on d 0 and d 14. All bulls received a complete BSE¹¹ on d 0 including SC measurement, transrectal palpation of accessory sex glands, and semen evaluation. On d 0, 7, 14, 21, 35, 49, 63, and 84, semen was collected via electroejaculation and sperm gross motility and morphology were analyzed using light microscopy. For morphological analysis, 100 sperm cells were assessed. All samples were analyzed by the same veterinarian who was blinded to treatment group assignments.

Statistical analyses

SAS for Windows 9.3 (SAS Institute Inc., Cary, NC) was used for all statistical analyses. Based on a histogram generated with the UNIVARIATE procedure, the data for initial SC were deemed approximately normal. Therefore, the TTEST procedure was used to compare initial (d 0) mean SC between the two treatment groups. The proportion of normal sperm cells on d 0 in each of the two groups was compared with logistic regression using the GLIMMIX procedure. The GLIMMIX procedure was also used to analyze dependent variables repeated over time. Multivariable repeated measures logistic regression was used to analyze the effects of treatment, time, and their interactions on the proportion of normal sperm cells, primary abnormalities, and secondary abnormalities. Events/trials syntax was used in the logistic regression model statements. The residual option and a first-order autoregressive covariance structure, in the case of repeated measures, were specified in the random statements. P-values less than 0.05 were considered significant. Data are presented as means standard deviation, unless otherwise noted.

Results

Initial fertility

Average SC on d 0 was 36.1 ± 3.45 cm and 35.5 ± 3.07 cm for PER and CON bulls, respectively, and was not significantly different between groups ($p=0.55$). Furthermore, there was no significant difference in proportions of morphologically normal sperm on d 0 ($p=0.54$). Thus, initial fertility between bulls in the CON and PER groups was regarded as not different. Additionally, all bulls were body condition score (BCS) 6 at d 0 of the study. Table 1 provides descriptive information on the bulls used in the study. After d 35, three bulls (two PER and one CON) were removed from the study in order to be used for breeding purposes, thus semen quality data from those individuals were not available for inclusion in d 49, 63, and 84 analyses.

Table 1. Breed composition, age, body condition score (BCS), and initial fertility metrics for permethrin treated (PER) and control (CON) bulls.

Group	n	#Angus	#Charolais	#Hereford	Age (mo)*	BCS	SC (cm)	%Normal**
PER	8	7	1	0	17.2	6	36.1±3.45	79.3±10.99
CON	7	4	2	1	16.7	6	35.5±3.07	75.4±12.35

*Median age in months

**Mean (±standard deviation) percentage morphologically normal sperm on d 0

Critical period for permethrin induced infertility

Days 0, 14, and 35 were chosen as critical points *a priori* for sperm morphological analysis. Clinically observed pyrethroid-induced fertility changes had rapid onset and persisted for up to four weeks.^{8,9} If treatment with topical permethrin were to have a deleterious effect on fertility, it should have been clearly evident on both d 14 (14 d after initial application) and d 35 of the study (35 d after the first application and 21 d after the second parasiticide treatment). The results of repeated measures logistic regression for the effects of treatment, day, and treatment x day interactions on sperm morphology measures are presented in Table 2.

Table 2. Results of multivariable logistic regression for d 0, 14, 35 for treatment, day, and treatment*day interactions on proportions of morphologically normal sperm and sperm cells with primary or secondary defects from permethrin treated bulls (PER) and control bulls (CON).

Outcome	Variable	Comparison	Odds Ratio	95% CI of OR*	P-value
Normal	Treatment	PER vs. CON	1.41	0.80, 2.48	0.208
	Day				0.098
		d 35 vs. d 0	1.5049	1.070, 2.0921	
		d 14 vs. d 0	1.07	0.80, 1.43	
	Treatment*Day				0.264
	Primary	Treatment	PER vs. CON	0.68	0.34, 1.35
Day					0.152
		d 35 vs. d 0	0.67	0.44, 1.01	
		d 14 vs. d 0	0.84	0.63, 1.11	
Treatment*Day					0.034
		PER vs. CON d 35	0.38		0.030
	PER vs. CON d 14	0.70		0.326	
	PER vs. CON d 0	1.16		0.663	
Secondary	Treatment	PER vs. CON	0.77	0.44, 1.33	0.315
	Day				0.106
		d 35 vs. d 0	0.74	0.49, 1.12	
		d 14 vs. d 0	1.07	0.78, 1.45	
	Treatment*Day				0.144

*95% Confidence interval of the odds ratio

Proportion of normal sperm cells did not decrease over time as a result of permethrin exposure, and there were no significant differences in sperm morphology between groups. No detrimental effects on semen quality were observed in bulls treated with permethrin. There was a significant treatment x day interaction on the incidence of primary abnormalities due to the apparent protective effect of permethrin against primary defects on d 35 of the study.

Sperm morphology over time

Bulls in both groups increased the mean percent of morphologically normal sperm as the study progressed. Consequently the proportion of sperm cells with abnormal morphology tended to decrease over time. The results of sperm morphology analyses for d 0-84 are illustrated in Figures 1-3. The incidence of sperm cells displaying a primary defect is shown in Figure 2. As previous studies observed an increase in the number of sperm cells with defects of epididymal origin (i.e. secondary abnormalities), the proportion of secondary defects over time was of particular interest and is shown in Figure 3. There was no significant difference in the incidence of secondary abnormalities between PER and CON during the critical period, and a graph of secondary abnormalities between groups reveals a slight, gradual decrease over time for bulls in both groups.

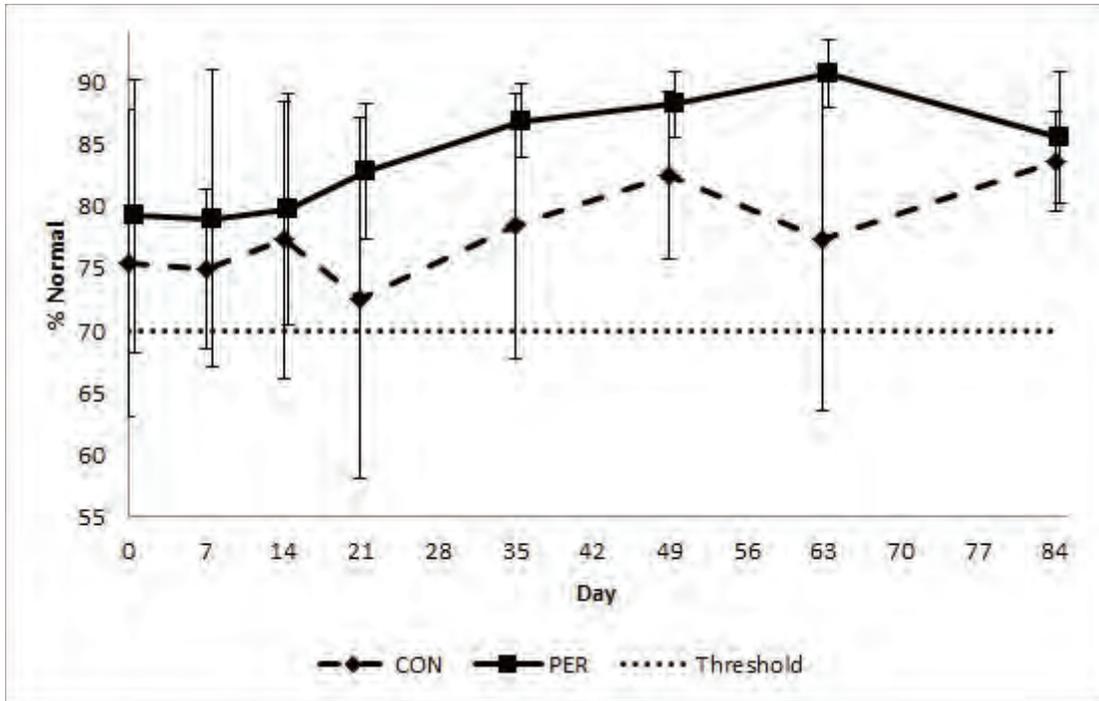


Figure 1. Mean proportions (\pm standard deviation) of morphologically normal sperm from d 0-84 from permethrin treated bulls (PER) and control bulls (CON). The threshold line represents the minimum percent normal sperm cells (70%) in order to achieve satisfactory potential breeder status per SFT standards.

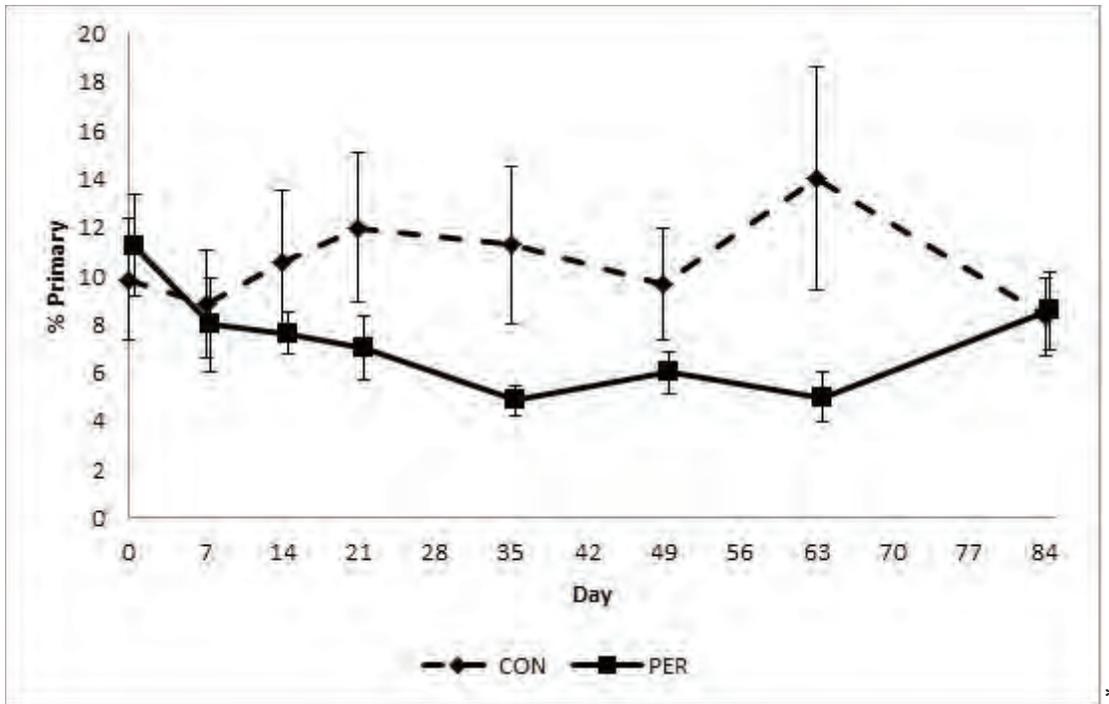


Figure 2. Mean proportions (\pm standard deviation) of sperm with primary defects from d 0-84 from permethrin treated bulls (PER) and control bulls (CON).

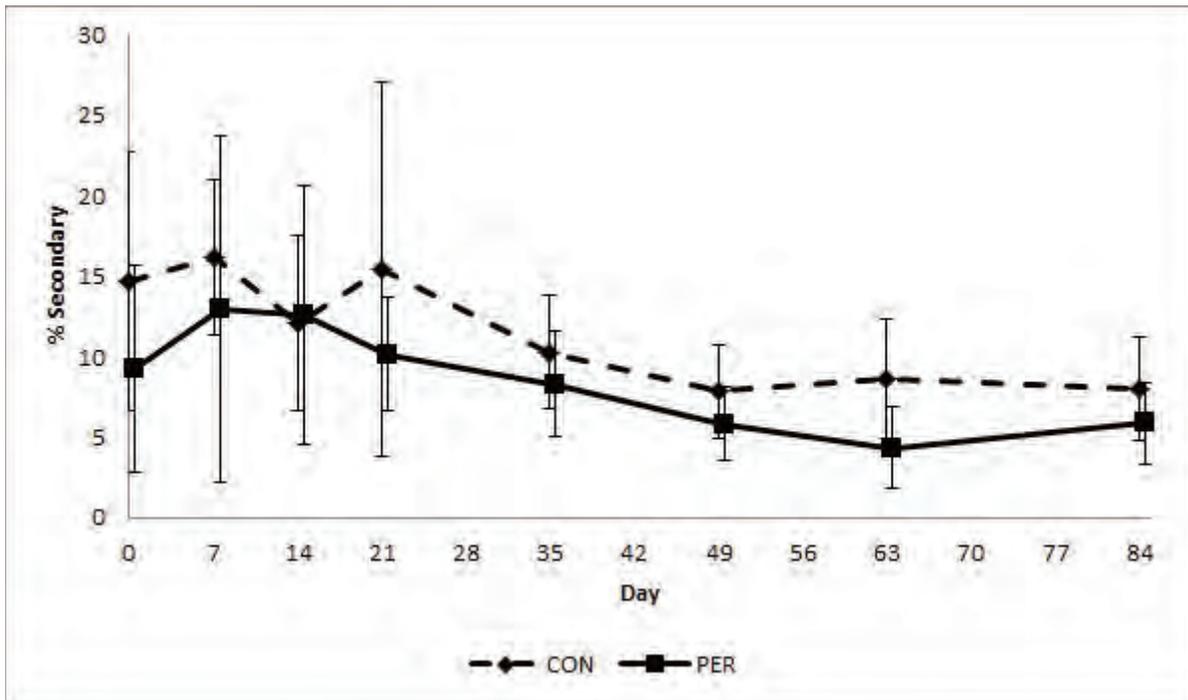


Figure 3. Mean proportions (\pm standard deviation) of sperm with secondary defects from d 0-84 from permethrin treated bulls (PER) and control bulls (CON).

Gross motility

Progressive motility was not compromised by permethrin exposure as all bulls maintained acceptable gross motility (>30% progressively motile, per SFT standards) for the entirety of the study.

Discussion

Results of the present study suggest that topical permethrin does not adversely affect the semen quality of beef bulls. The average proportion of normal sperm cells increased slightly over the course of the study (see Figure 1). Keeping in mind that the median age at the start of the study was 16.8 months this improvement is consistent with earlier findings in which bull semen quality and percent normal sperm morphology improved in the months following attainment of puberty.¹³⁻¹⁵

The results of the present study do not agree with the findings of Issam et al, in which dermal administration of permethrin led to arrest of spermatogenesis in male rats.¹⁰ Our results also do not corroborate clinical observations in which the percentage of morphologically normal sperm in bulls and rams was drastically reduced for weeks after pyrethroid exposure. However, the specific types of pyrethroid insecticide implicated in these instances of transient infertility were not specified in all case reports. In at least one instance, the synthetic pyrethroid bifenthrin was presumed responsible for observed teratospermia.^{8,9} Like permethrin, bifenthrin is a type I pyrethroid.¹⁶ Unlike permethrin, however, bifenthrin is primarily marketed as a premise insect treatment and is not commonly found in parasiticides labeled for animal use.

The results of the present study are, however, consistent with the findings of French et al in which cyfluthrin and beta-cyfluthrin insecticides were found to have no deleterious effects on bull fertility.⁷ However, it should be noted that cyfluthrin and beta-cyfluthrin are type II pyrethroids, whereas permethrin is a type I pyrethroid. Also, unlike the French et al study, the present study applied a greater dose of parasiticide (150% label dose).

It should be noted that the present study measured neither androgen hormone concentrations nor libido. It is possible that a chemically induced alteration in either could detrimentally affect the ability of a bull to service and impregnate the cowherd. There is evidence that permethrin may disrupt hormone pathways and alter testosterone concentrations in male rodents.^{5,10}

Conclusion

In the present study, no detrimental effects on bull semen quality were observed even at 150% of label dose of topical permethrin labeled for livestock use.

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Conflicts of interest

The authors declare no conflicts of interest.

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