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## TOXICITY TO BULL SPERMATOZOA OF TERRAMYCIN HYDROCHLORIDE AND ITS USE AS AN ANTIBACTERIAL AGENT IN SEMEN EXTENDERS<sup>1</sup>

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The control of bacteria in extended bull semen is a matter of major concern because such control may result in increased fertility levels in the semen of some dairy bulls (1). Considerations relative to the choice of antibiotic, antibiotic salt, or complex, and dosages to be employed are of great importance, especially in regard to semen toxicity, which is a major criterion of the possible usefulness of a material.

Finlay *et al.* (2) have reported that terramycin has a low degree of toxicity in animals, insofar as its use in intravenous injections is concerned. They further reported that this material is bacteriostatic to many Gram-negative enteric organisms, aerobic spore formers, and Gram-positive cocci. There are no data in the literature concerning the use of this material in semen extenders or its relative toxicity to bull spermatozoa.

*Methods and Materials.* Terramycin hydrochloride (Pfizer) was used in this investigation. The potency of the material was of the order of 890 mcg. of terramycin per mgm. This compound is stable over long periods of time in aqueous solutions at pH 2.0 to 5.0 at room temperature.

To make a simultaneous comparison of the effects of the antibiotic on the motility of spermatozoa and upon bacterial control, bovine semen samples were divided into as many equal portions as there were levels of the antibiotic chosen to be tested plus one sample to be used as a control. Each portion of fresh semen was extended with 3.0 per cent citrate-egg yolk extender to which the terramycin had been added. The average dilution rate was 1 part of semen to 40 parts extender. The extender semen was then stored at 5° C in 9-ml test tubes filled to capacity. Daily microscopic examinations of the extended semen were made in order to estimate the effects of the antibiotic on the motility of the spermatozoa therein.

The number of viable bacteria was determined by the plate count method. Control plates were made to check the sterility of the water used for dilutions, of the agar, and of the atmosphere in the laboratory during the plating procedure. All plates were incubated at 37° C. for 48 hours before counting.

*Results.* Eighteen semen samples were stored at 5° C in citrate-egg yolk extender containing 0, 20, 25, 30, and 40 mgm per 100 ml of diluted semen, the sample containing no antibiotic serving as a control. The effects of the different levels of the antibiotic on the motility of the spermatozoa after 48 hours' storage, the number of hours' duration of a motility rating of 30 per cent, and the numbers of bacteria present after 48 hours' storage are presented in Table 1.

These data indicate that the 20-mgm. level of terramycin is definitely bactericidal but not spermicidal in action, compared with the control sample. The 25-mgm. level was found to give better bacterial control than the 20-mgm level, but it had a spermicidal action both on the numbers of progressively motile spermatozoa after 48 hours of storage and on the duration of motility. The 30- and 40-mgm. levels were both bactericidal and spermicidal.

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TABLE 1. Motility of Spermatozoa and Numbers of Bacteria in Extended Semen Containing Terramycin Hydrochloride: Average of 18 ejaculates.

Subject	Mgm. extender per 100 ml diluted semen				
	0	20	25	30	40
Average motility after 48 hours' storage	58.6	58.8	47.4	32.6	17.6
Average number of hours during which 30% motility was maintained	180.0	176.0	144	81.3	38.8
Average number of bacteria per ml of extended semen	7,106	1,936	1,020	943	422

*Summary.* The effect of terramycin hydrochloride on the motility of bovine spermatozoa and upon the control of bacterial growth in bovine semen extended with citrate-buffered egg yolk and stored at 5° C. was investigated. It was found that 20 mgm. of terramycin hydrochloride could be added per 100 ml. of extender without spermicidal effects. This level of the antibiotic was highly bacteriostatic and/or bactericidal.

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