TOXICITY TO BULL SPERMATOZOA OF VARIOUS SALTS, BRANDS AND LOTS OF PENICILLIN, STREPTOMYcin, AUREOMYCIN AND CHLOROMYCETIN

JAMES G. SYKES AND JOHN P. MIXNER
New Jersey Agricultural Experiment Station, Sussex

The control of bacterial growth in diluted bull semen to be used for artificial insemination is a matter of major concern, since such control may result in increased fertility levels in some bulls. Considerations relative to the choice of antibiotics, manufacturers' brands, antibiotic salt or complex and dosages to be employed are of great importance especially in regard to semen toxicity, a major criterion of the possible usefulness of a new material.

Foote and Salisbury (6), in 1948, reported that one of two manufacturers’ brands of penicillin G was toxic to bull spermatozoa even in small amounts, emphasizing the need for a current evaluation of progress in the manufacturing field. Almquist et al. (1, 2, 3) have reported on the semen toxicity of sodium penicillin G (Pfizer) and on streptomycin sulfate (Pfizer) each alone and in combination, but information is lacking on the potassium salt of penicillin G or on other salts of streptomycin, including dihydrostreptomycin. Welch et al. (12) showed that when penicillin G is combined with various cations, the cation contributes substantially to the toxicity of the preparation as measured in mice. Reporting in 1944, Welch et al. (13) found wide variation in the acute toxicity to mice of different manufacturers’ brands of penicillin.

Molitor (10) reported in 1946 that the toxicity of a streptomycin in various animals was greatly affected by traces of impurities present.

Myers et al. (9), working with aureomycin hydrochloride, reported that all levels studied had significant toxic effects on bull spermatozoa motility. Foote and Bratton (7), also working with aureomycin hydrochloride in bull semen, reported that to avoid spermicidal effects the dosage should not exceed 1007 per milliliter of diluted semen.

Chloromycetin (Parke-Davis) is an antibiotic which is similar to aureomycin in its effectiveness against a wide range of gram positive and negative organisms and against some of the large viruses (4, 8, 14).

METHODS AND MATERIALS

Two lots each of four manufacturers’ brands of penicillin G (including both the Na and K salts) were studied as to their relative semen toxicity. Similarly, studies on two lots each of three brands of streptomycin (including the sulfate, hydrochloride and calcium chloride complexes) were made. One lot of dihydrostreptomycin sulfate, both the base and hydrochloride of aureomycin and chloromycetin also were studied as to their semen toxicity reactions (table 1).

Received for publication Nov. 5, 1950.

1 Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Department of Dairy Industry.
Six dairy bull semen samples were used in each toxicity study and the semen was diluted 1:20 with an egg yolk-citrate diluter consisting of 1 part egg yolk and 3 parts of 3 per cent sodium citrate dihydrate to which the antibiotic was added. One-ml. portions of diluted semen were stored at approximately 5° C. in 1.5-ml. stoppered tubes. The diluted semen samples were examined on storage days 5, 10 and 15 and the percentages of motile spermatozoa estimated. The data were analyzed by the analysis of variance method (11).

**RESULTS**

*Penicillin.* A summary of the spermatozoa motility data on penicillin is presented in table 2. These data are presented in an abbreviated form since an analysis of the original data indicated that no significant differences existed between the contrasted means for brands, salts or lots of penicillin. The dosage effects were highly significant, and thus, 4,000 units of penicillin per milliliter of diluted semen may be considered a non-toxic level, while the 8,000 unit level is only mildly toxic.

*Streptomycin.* No significant differences in spermatozoa motility could be attributed to the brands, salts or lots of streptomycin used. Accordingly, only dosage and time effects are presented in the data summary of table 3. A highly
significant dosage effect was found, and an examination of the mean motility
data indicated that a beneficial effect was exerted on motility characteristics of
the stored semen by certain streptomycin dosage levels. The observation of
Easterbrooks et al. (5) that the CaCl₂ complex of streptomycin forms a precipi-
tate on dilution and storing at 5° C. was confirmed.

Dihydrostreptomycin sulfate. One lot of dihydrostreptomycin sulfate was
studied as to its semen toxicity properties (table 4). Analysis of variance showed

no dosage effect upon the percentage of motility, and dosages as high as 8.0 mg.
per milliliter of diluted semen are judged to be non-toxic, being similar to strep-
tomycin in this respect.

Aureomycin. Comparison of aureomycin base and the hydrochloride showed
no differential effects on semen motility. The initial toxic level was found to be
0.125 mg. per milliliter of either the base or hydrochloride, the 0.0625-mg. level
being non-toxic (table 5).
Chloromycetin. One lot of chloromycetin was available for study. Analysis of the data (table 6) showed highly significant dosage effects, which were most pronounced at 10 and 15 days’ storage. The initial toxic level was judged to be 1.0 mg. per milliliter. On this basis, chloromycetin is somewhat less toxic to spermatozoa than aureomycin but more toxic than penicillin, streptomycin or dihydrostreptomycin.

DISCUSSION

This limited survey of manufacturers’ brands, salts and lots of penicillin and streptomycin relative to semen toxicity seems to indicate a rather satisfactory general condition, both from the standpoint of purity (low toxicity) and of comparative toxicity of the various salts or complexes of these antibiotics. If direct comparison could be made with the earlier toxicity work of Almquist and co-workers with both penicillin and streptomycin (this may not be strictly valid) it would seem that initial toxic levels in both instances were raised considerably in the present study, indicating perhaps better manufacturing procedures in purification of the antibiotics. Differential toxicity due to the various salts or complexes of the antibiotics could not be demonstrated in this study.

The toxicity data for aureomycin agree well with those reported by Myers et al. (9) and Foote and Bratton (7). The relatively low toxicity of chloromycetin seems to warrant further consideration of this antibiotic for inclusion in the antibiotic mixture of diluted semen.

SUMMARY

A limited survey was made of the toxicity to bull spermatozoa of several antibiotics including four brands and two salts of penicillin, three brands and three salts of streptomycin, one lot of dihydrostreptomycin, two salts of aureomycin and one lot of chloromycetin. Differences in spermatozoa motility were not attributed to brands, lots or salts of either penicillin or streptomycin. Dihydrostreptomycin sulfate was not toxic to spermatozoa at levels as high as 8 mg. per milliliter of diluted semen. The initial toxic level of aureomycin was found to be above 0.0625 mg. per milliliter while that of chloromycetin was found to be above 0.5 mg. per milliliter of diluted semen.
REFERENCES


