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Motility of Spermatazoa and Control of Bacteria in Bovine Semen Extenders Containing Sulfanilamide, Aureomycin, Terramycin, Bacitracin, Penicillin, and Strepromycin

H. K. McCartney
University of Arkansas, Fayetteville

O. T. Stallcup
University of Arkansas, Fayetteville

MOTILITY OF SPERMATOZOA AND CONTROL OF BACTERIA IN BOVINE SEMEN EXTENDERS CONTAINING SULFANILAMIDE, AUREOMYCIN, TERRAMYCIN, BACITRACIN, PENICILLIN, AND STREPTOMYCIN*

H. K. McCARTNEY and O. T. STALLCUP[1]

University of Arkansas

The importance of controlling bacterial populations in bovine semen used for artificial insemination has been pointed out by Salisbury *et al.* (15). A significant relationship has been found between the number of bacteria in diluted semen and its fertility when all types of bacteria were considered (4). The growth of many organisms commonly found in bovine semen is reportedly inhibited by the addition of sulfonamides (6, 9, 10, 13, 16), of penicillin (1, 8, 9, 18), of streptomycin (2, 18), of streptomycin plus penicillin (3, 12), of polymyxins (6), and of aureomycin (6, 11, 18), without significant toxic effects on the motility of spermatozoa. However, no data have been reported on the control of bacterial growth in semen by the use of bacitracin or terramycin.

Finlay *et al.* (5) have reported that terramycin is bacteriostatic to many Gram-negative enteric organisms, aerobic spore formers, and Gram-positive cocci. It also has antirickettsial activity and appears to inhibit certain influenza viruses.

Rose (14) has reported that bacitracin is effective against most strains of hemolytic streptococci, nonhemolytic streptococci, coagulase positive staphylococci, pneumococci, gonococci, anaerobic cocci in general, certain bacilli, certain spirochetes, actinomycetes and some protozoa. It shows little action against the large group of aerobic Gram-negative nonsporeforming bacilli. In view of the reported high bactericidal activity of terramycin and bacitracin, it seemed desirable to study the control of bacterial growth and effects on motility of the spermatozoa by these compounds as compared to previously used bactericidal agents.

Materials and Methods. The antibacterial agents used in these investigations are shown in Table 1.

TABLE 1. Levels of Antibacterial Agents Used per 100 ml of Citrate-yolk Extender

Extender	Sulfanilamide (mgm)	Penicillin G sodium (mgm)	Aureomycin hydrochloride (mgm)	Bacitracin (55 units per mgm)	Dihydrostreptomycin (mgm)	Terramycin hydrochloride (mgm)
Control - - - -	0	0	0	0	0	0
A - - - - - - -	300	30	10	10	100	10
B - - - - - - -	---	--	--	--	---	--
C - - - - - - -	---	--	--	10	---	--
D - - - - - - -	300	30	--	--	100	--
E - - - - - - -	---	--	10	--	---	--

Extender D was used for comparison because of the common usage of these antibacterial agents in extenders. Extender A was used to determine if a combination of all the antibiotics studies was desirable. To make a simultaneous comparison of the effects of the different antibiotics on the motility of sperm-

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atozoa and upon bacterial control, semen samples obtained from the Arkansas Artificial Breeders' Association of Fayetteville, Arkansas, were divided into as many equal portions as there were antibacterial agents to be tested. Each portion of semen was mixed with 3 per cent citrate-yolk extender to which the antibiotics, or antibiotics plus sulfanilamide, had been added. The average dilution rate was one part of semen to 40 parts of citrate-yolk extender. The extended semen then was divided into equal portions and placed in 9-ml test tubes filled to capacity. One portion was stored in a refrigerator at 5° C while the other portion was exposed to a temperature of 30° C ± 2° C. Daily microscopic examinations of the diluted semen afforded an estimate of the effects of the antibacterial agents on the motility of the spermatozoa. Average motility readings, after 48 hours of storage, are presented as this time interval and probably represent a near maximum storage time during which semen is used by most artificial breeding associations in this country. The samples were read until the motility reading fell below 30 per cent and then discarded. Statistical significance between means was tested by analysis of variance (17).

The number of living bacteria was determined by the plate count method. After incubation at 37° C for 48 hours, the number of colonies on each plate was counted and the number of bacteria that would have been present in an entire milliliter of the diluted semen was calculated. 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.

Results. A summary of the data concerning the spermatozoa motility, the duration of motility, and the number of bacteria in extended semen containing the antibacterial agents studied is presented in Table 2. After 48 hours of storage at 5° C, bacterial growth was not excessive in any of the extended semen samples. The combination of all antibacterial agents usually inhibited bacterial growth completely. Bacitracin and aureomycin completely inhibited bacterial growth in 50 per cent of the samples, this difference in bactericidal action apparently resulting from the predominating types of bacteria in different samples.

TABLE 2. Motility of Spermatozoa, Duration of Motility, and Numbers of Bacteria in Extended Semen Containing Various Antibacterial Agents

	Storage Temperatures	After 48 hours storage motile spermatozoa[1]	bacteria per ml.[2]	Duration motility rating of 30 per cent[2]
	°C	*per cent*	*Thousands*	*Hours*
Control	5°	39.8	5.0	145.3
	30°	9.6	415.7	28.0
A (Combination of antibacterial agents)	5°	48.7	.3	181.3
	30°	32.8	2.5	85.3
B (Terramycin hydrochloride)	5°	46.0	6.5	154.7
	30°	19.9	5.2	42.7
C (Bacitracin)	5°	48.1	.8	180.0
	30°	7.9	474.7	38.0
D (Penicillin + streptomycin + sulfanilamide)	5°	44.1	5.1	173.3
	30°	33.5	15.2	77.3
E (Aureomycin hydrochloride)	5°	40.6	.7	156.0
	30°	28.0	18.2	58.7

[1]Average of 10 ejaculates.

[2]Average of nine ejaculates.

At 30° C bacteria multiplied rapidly when no antibacterial agent was present as was the case when bacitracin was present, this antibacterial agent apparently being inactivated at the higher temperature. Putrefaction of the egg yolk consistently occurred when bacterial growth was high. The percentage of

samples exhibiting a putrid odor after 48 hour's storage at 30° C was 40, and 30, respectively, for the extenders containing bacitracin and no antibacterial agents, respectively.

The analyses of variance of the percentage of progressively motile spermatozoa after 48 hour's storage is presented in Table 3. At 5° C the percentage of motile spermatozoa was similar in all extenders throughout the 48 hour's storage period. The effects of temperature appear to be significant, all samples showing a lower average motility after 48 hours at 30° C as compared to 5° C, regardless of the diluter used ($P < 0.01$). Similar results for a 72 hour storage interval were reported by Foote and Bratton (7). At 30° C after a 48 hour storage period significant differences between extenders were observed ($P < 0.01$). Aureomycin, penicillin + streptomycin + sulfanilamide, and a combination of antibacterial agents were significantly superior ($P < 0.01$) to no antibacterial agent and bacitracin at this temperature. Bacitracin, terramycin, and no antibacterial agent were not significantly different from each other ($P < 0.05$). This was probably caused by a high bacterial count in samples containing bacitracin, and the low motility of the samples containing terramycin may have resulted from toxicity although no such effects were evident when samples were stored at 5° C.

An analysis of variance of the data concerning the length of time a progressive motility rating of 30 per cent was maintained is presented in Table 4. The temperature effects were again significant, the duration of motility being appreciably lower in samples stored at 30° C as compared to those stored at 5° C. The fact that the extender effect at 30° C is not significant suggests that the criterion of response used here is not as precise as the one used in Table 3. This is probably because the spermatozoa died rapidly in some extenders stored at 30° C, and daily microscopic examination would not detect precisely when 30 per cent of the spermatozoa were motile.

TABLE 3. Analysis of Variance of Percentages of Progressively Motile Spermatozoa after 48 hour's Storage

Factor	S.S.	D.F.	M.S.	F
Total	33,093.5	119	---	---
Extenders at 5° C.	702.95	5	140.59	2.23
Extenders at 30° C.	6,418.55	5	1,283.71	20.39[1]
Temperature	15,322.8	1	15,322.8	22.91[1]
Ejaculates	4,417.0	9	---	---
Remainder (error)	6,232.2	99	62.95	---

[1]Highly significant ($P = < 0.01$).

TABLE 4. Analysis of Variance of the Hours that 30 Per cent of the Spermatozoa Remained Progressively Motile

Factor	S.S.	D.F.	Variance	F
Total	809,263	107	---	---
Extenders at 5° C.	10,221.3	5	2,044.3	---
Extenders at 30° C.	27,232	5	5,446.4	1.85
Temperature	337,345	1	337,345	114.47[1]
Ejaculates	175,099	8	---	---
Remainder	259,365.7	88	2,947	---

[1]Highly significant ($P = < 0.01$).

Summary. The effect of terramycin, bacitracin, aureomycin, penicillin + streptomycin + sulfanilamide, and a combination of the aforementioned antibacterial agents, on the motility of bovine spermatozoa and upon the control of bacterial growth in bovine semen extended with citrate buffered yolk and stored at 5° C. and 30° C, was investigated. It was found that there was no significant difference in the motility of the spermatozoa or in the duration of a 30

per cent motility rating of samples stored at 5° C that could be attributed to differences in the action of extenders. A significant difference was found in the percentage motility after 48 hours storage and the duration of motility between samples stored at 5° C and 30° C, those stored at 30° C being consistently lower. Low motility ratings were observed in extenders containing bacitracin, terramycin, and no antibacterial agent when samples were stored at 30° C. All antibacterial agents studied were highly bacteriostatic and/or bactericidal with the exception of bacitracin when samples containing this antibiotic were stored at 30° C.

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