EFFECTS OF FIVE DIFFERENT LEVELS OF MILK FAT ON THE LIFE SPAN OF BOVINE SPERMATOZOA STORED IN MILK DILUENTS

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EFFECTS OF FIVE DIFFERENT LEVELS OF MILK FAT ON THE LIFE SPAN OF BOVINE SPERMATOZOA STORED IN MILK DILUENTS

INTRODUCTION

Dilution of semen in milk media has been put into practice in many countries of the world. A milk medium is easy to handle and economical; moreover, it is easy to sterilize since it contains the protein casein, which gives protection to the sperm capsule. A milk medium has a constant pH, about 6.6, and good buffering capacity. It is less viscous than an egg yolk medium and shows no agglutination of sperm at the end of 24-48 hours. The semen of certain bulls, that does not keep well in egg yolk, survives better in milk (Jacquet, 10, pp. 429-432).

In India, most government dairy farms have buffaloes, especially in the province of Assam. There are also numerous small-scale buffalo dairy farms belonging to private owners.

Buffaloes comprise 30% of all milk animals and account for 56% of the total milk production (Shahani, 28, p. 868). The percentage of fat in milk from buffaloes ranges from 7 to 11. In India, egg yolk is used as an extender of buffalo bull semen. As the fat content of buffalo milk is high, the effect of fat percentage on semen preservation needs to be studied to determine the advisability of using buffalo milk as an extender of semen.

The objective of this study is to determine among five different levels, 0%, 3.5%, 7%, 11%, 15%, of fat in dairy cattle milk media the one which will give the best survival of sperm during storage. The higher levels of fat compare with buffalo milk.

REVIEW OF LITERATURE

Artificial insemination is, as Waddington (32, p. 71) stated, an important biological technique passing into general practice.

Along with the rapidly growing practice of artificial insemination, the problems of dilution of semen in different media and in different ways are under investigation to find out scientific ways and means to assist the artificial insemination organizations.

Some research indicates a tendency for semen with lower fertility to be improved by using a milk diluent (Perkins, 22, p. 156). The semen of bulls with low non-return rates when yolk-phosphate was used, tended to give the greater additional response when milk diluent was used (Dreher, 7, p. 677). The non-return rate was three per cent (3%) higher when milk was used than when the yolk-citrate was used to dilute semen from this type of bull (Flerchinger, 9, p. 1019).

Spermatozoal livability was slightly higher during the first ten days of storage when a boiled homogenized milk diluent was used than when yolk citrate was used, but after 10 days motility was retained longer in the yolk citrate diluter (Thacker, 30, p. 178).

Jacqlet investigated numerous semen diluents, already tried by different workers. For reasons such as expense or difficulty of preparing large quantities, all diluents were rejected with the exception of milk, which is easy to sterilize and which gives protection

to the sperm capsule since it contains casein and other proteins. It has a constant pH, about 6.6, and the necessary buffering capacity. It is less viscous than egg yolk, and there is no agglutination of sperm in it at the end of 24-48 hours. The semen of certain bulls which does not keep well in egg yolk, survives better in milk (Jacqlet, 10, p. 429-432).

In a study by Perkins in which homogenized milk and yolk citrate were compared as diluents for bull semen, the former was found to be the better diluent for semen stored for two days and the latter for semen stored for three days (Perkins, 22, p. 156).

By use of the split-sample technique, Thacker compared the fertility of bovine semen diluted with boiled homogenized milk with that of semen diluted with an equal quantity of egg yolk-citrate containing penicillin and streptomycin. The 60-90 day non-return averaged 72.7% for 2,381 first service cows inseminated with the boiled milk diluent, and 71.4% for 2,620 animals inseminated with the egg yolk-citrate diluent. From this result, it was concluded that both were suitable diluents for bovine semen (Thacker, 30, p. 179).

Danish "export cream" (9 per cent butterfat, homogenized and sterilized) was as effective a diluent for bull semen as was pasteurized skim milk and could safely be used as a substitute for egg yolk-citrate (Adler and Rasbech, 1, pp. 497-503). Using boiled milk as the semen diluent, the influence of dilution rate and storage time on the conception rate was investigated. The results were in

good agreement with those obtained by others using egg yolk-phosphate diluent.

Raps et al. (24, pp. 933-938) stated that spermatozoon viability is limited to a narrow pH range and has emphasized the need for the consideration of hydrogen-ion concentration in semen studied. Webster (33, pp. 199-222) first reported that the hydrogen-ion concentration of bull semen varied from pH 7.0 to 7.5.

Milovanov's early observation, quoted by Anderson (4, p. 4) showed bull semen to be slightly acid, with the pH usually ranging from 6.5 to 6.8, but in one case as low as 5.5. He further stated that the presence of abnormal amounts of accessory gland secretion may be responsible for an alkaline reaction (pH 7.0 to 7.5). Davis (6, pp. 246-253) found that collection techniques modified the hydrogen-ion concentration of resultant samples to a marked degree. Rectal massage of the ampulla and the accessory organs yielded semen of pH 7.5 to 8.0, while the use of the artificial vagina produced semen whose pH usually was below 7.0. Anderson (4, p. 4), from a study of 11 fertile bulls, reported the mean pH of semen to be 6.73 ± 0.02. He further observed that, in conditions involving pathology of the genitalia, the reaction was characteristically alkaline. Schneerson (26, pp. 419-420) showed that sperm concentration up to 1.8 billion per milliliter existed in ejaculates having a pH of 5.8 to 6.0 while samples at pH 7.0 contained an average of only 0.6 billion per milliliter. Anderson (4, p. 4) reported a similar

observation.

The more acid the semen was on collection, the better was the motility retained on storage. Semen which retained its motility well became increasingly acid during storage. A change in the pH in the alkaline direction during storage had an adverse effect on motility. The more acid the pH on collection, the less likely was a decrease in acidity during storage (Anderson, 2, pp. 298-307).

The pH of bull semen was 6.74 during storage (bull and ram semen shows a shift towards acidity), there being a considerable amount of lactic acid formed (Sergin, 27, p. 270).

The spermatozoa are much more sensitive to acidity than to alkalinity. Below a pH of about 5.8, they are immobile and die rapidly, whereas at a pH of 9.5 to 10.0, they remain motile and survive for several hours, although with depressed motility (Emmes, 8, pp. 471).

In a pH range of 5.50 to 5.80, the sperm appeared to have a comparatively insignificant loss of viability. Re-suspending the auto-inhibited spermatozoa in fresh, relatively alkaline coconut milk diluents produced an appreciable resumption of metabolic activity after six days of room temperature storage. The evidence strongly suggests that reversible acid inhibition can serve as a means of extending the life span of sperm for several days at room temperature (Norman et al., 21, p. 1803).

The best results for live-dead sperm differentiation were

obtained with staining mixtures possessing pH values between 6.8 and 7.8. Above pH 8.5, the heads and tails of many of the spermatozoa were separated. Mayer et al. (19, p. 230) and Lasley et al. (15, p. 167) introduced an opal blue-eosin staining mixture for differentiation of dead and live ram sperm. This stain was applied to human spermatozoa by Macleod in 1942 (17, p. 573) and modified by Lasley and Bogart in 1943 for use with bull semen (14, Mo. Agric. Exp. Sta. Res. Bull. No. 376). Rao reported (23, pp. 186-187) satisfactory results with a variety of live and dead differential stains.

In 1951 Swanson and Bearden (29, pp. 981-987) found that the most suitable concentration of the dyes was 1% eosin B and 5% nigrosin.

No papers have been found directly bearing on the effect of different fat levels of milk media on preserving spermatozoa. Thus, the literature provides little evidence directly or indirectly on the effect of different fat levels of milk media for the preservation of spermatozoa other than those of skim and ordinary whole milk.

MATERIALS AND EXPERIMENTAL PROCEDURE

It was the purpose of this experiment to study the life span of spermatozoa in milk of five different fat levels. Storage was at 4°-5° C. for ten days.

Experimental Animals, Feeding and Management

Semen was collected from three dairy bulls belonging to the Oregon Agricultural Experiment Station dairy herd, Corvallis, Oregon.

The Bulls

Name	Tattoo No.	Breed	
(A) Oregon Challenger Baron, registration no. 1308796	H-33B3	Holstein-Friesian	2 yr. (born 9-14-57)
(B) Oregon Ormsby Jess Buccaneer, registration no. 1373663	H-34B2	Holstein-Friesian	2 yr. (born 10-2-57)
(C) Marlu Commando Citation, registration no. 571446	GX-1	Jersey	4 yr. (born 1-6-56)

The bulls were kept in the dairy barn at Oregon State College and were in good condition throughout the period of study. Each Holstein bull received 8 pounds of concentrate a day, the Jersey 5 pounds a day, together with 20-25 pounds of good quality grass hay and 5 pounds of alfalfa hay. They were fed at 7:00 a.m. and again at 4:00 p.m. Well water was supplied by means of automatic drinking cups. They were kept in individual stalls and got sufficient

exercise by free movement in front of the stalls where an open space was provided.

The feed concentrates consisted of the following:

Ground barley	- 300 pounds
Ground oats	- 400 pounds
Mill run	- 300 pounds
Salt	- 10 pounds
Bone meal	- 10 pounds
	1000 pounds

Sanitation of Equipment

All glassware used in handling the semen was washed with Calgonite, rinsed with hot tap water and sterilized by dry heat in an electric oven at 121° C. for a period of not less than three hours. After each collection of semen, the inner rubber tube and cone of the artificial vagina were rinsed with tap water, immersed in Roccal solution (1 oz. of 10% Roccal solution in 1 gallon of water) for about one hour, washed with a high quality powdered detergent (Dreft) and rinsed with hot tap water. All the rubber materials were hung in an electric oven for quick drying.

Collection of Semen

The area around the sheath of the bull was washed with Roccal solution before the collection of semen. The semen was collected

from each bull twice a week, Thursday morning and Friday afternoon. The semen was collected by means of an artificial vagina according to the method described by Lambert and McKenzie (12, pp. 39-43).

In the first experiment, semen was collected twice a week for 10 weeks (May 15 to August 1, 1959) from the three bulls. The ejaculates of any one day of the three bulls were pooled. In all, 20 pooled semen samples were used, each being subjected to 20 treatments.

In the second experiment, semen was collected April 5 and 12, 1960, and each ejaculate was processed and observed separately (6 samples in all, each receiving 20 treatments).

Handling of Semen

Immediately after collection, semen was transferred to the processing laboratory. After the volume of the semen and general appearance were recorded, the semen was examined to determine the motility.

Drops of semen were placed on a clean glass slide, then were examined microscopically (100 X) on a warm stage at 37.75° C. Only the semen which had a motility rating of 5 was used for the preservation. The observed motility was designated as 0 to 5 inclusive, 5 denoting maximum motility.

Source of Milk

Milk was received from the Dairy Products Laboratory, Withycombe Hall, Oregon State College. Thirty-five per cent fat (cream) and 3.5 per cent fat milk were changed into 7 per cent fat, 11 per cent fat, and 15 per cent fat by "Pearson square method" (Wilster, G. H., 34, p. 144) at the Dairy Products Laboratory and pasteurized and homogenized in the dairy plant with the help of the dairy plant technician, Mr. Larry McBee. Milk containing 3.5 per cent fat and skim milk were used as such for the milk diluents of 3.5 and 0 per cent fat.

Preparation of Milk

After receiving the pasteurized and homogenized milk of different fat levels from the Dairy Products Laboratory, the milk was heated to 92° C. for ten minutes (Thacker, 30, p. 179) in five flasks containing 200 ml. of each of the different fat levels of milk. The flasks were put in a bigger container of water so that the inner flask might not get direct heat from the fire. The milk was heated 12 hours ahead of collection and allowed to cool down to room temperature.

Antibiotics: Crystalline penicillin sodium G., 1000 units in aqueous solution, was added per milliliter of diluter (Thacker, 30,

p. 177, and Wolberg, 35, p. 14).

Experimental Design

Two separate experiments were conducted; the results in the first experiment were appraised entirely on motility rating of the semen during storage, while the results in the second experiment were appraised on motility, pH, and percentage of live sperm during storage.

In both experiments, semen was diluted 1 to 100, using milk diluents of 0, 3.5, 7, 11 and 15 per cent fat after it had been processed as described above. Semen was examined in both experiments on the first, third, sixth and tenth days.

In the second experiment, semen from each of the three bulls was processed and observed separately. The design was as follows:

Semen from 3 bulls.

Milk of 0, 3.5, 7, 11 and 15 per cent fat as separate diluents for semen from each bull.

Examination of semen on days 1, 3, 6 and 10 for motility, pH, and percentage of live sperm.

The entire study was replicated from semen collected one week later.

Estimation of Motility

Motility was examined at 9:00 a.m. the day it was processed and

after 12, 144, and 240 hours of storage by examining under a microscope at 400 X after the semen had been warmed by a warm stage at 37.5° C. The rating scale used for motility was as follows:

5 = Most spermatozoa were very active, motile and progressive.

4 = Most spermatozoa were active, motile and progressive.

3 = Many spermatozoa were active, motile and progressive.

2 = Some spermatozoa were motile but only slowly progressive.

1 = Some spermatozoa were motile but only weakly progressive.

0 = No motile spermatozoa could be found.

Two to three slides from each specimen were examined to be certain about the motility estimate.

Determining pH

The pH of semen was determined on all samples of semen after each of the storage times in the second experiment. The pH was determined using a Beckman pH meter.

Percentage of live spermatozoa

Slides were made using fast green-eosin from which a count was made of non-stained and stained sperm to determine percentage of live sperm on all samples at each of the storage times in the second experiment. The method of preparing the fast green-eosin and of making the slides and doing the counting described by Mayer et al. was followed. The non-stained sperm were considered to be live sperm while

the stained sperm were considered as dead sperm.

Analysis of data

The data in the first experiment were analyzed by analysis of variance considering the design to be a completely randomized block using the method described by Li (16, p. 178-179).

The data in the second experiment were analyzed by analysis of variance considering the design to be a split-plot. Separate analyses were made for motility, pH, and percentage of live sperm. Effects of replications and bulls were determined by using the bull x replication interaction term. Effects due to fat percentage of the diluter and interaction of bull x fat percentage of diluter were determined by use of the day x fat percentage of diluter interaction term. The effects due to day and the interactions of day x bull and day x fat percentage of diluter were tested by use of the three-way interaction, day x bull x fat percentage of diluter.

The correlations among motility, pH, and percentage of live sperm were determined using the error (e) term.

The motility of the diluted semen was examined immediately after preservation for further confirmation, and recorded. Then the samples were put into different 5 ml. vials of milk of different fat levels. The vials containing the diluted semen were placed in the refrigerator, which was maintained at 4°-5° C.

EXPERIMENTAL FINDINGS

First Experiment

It was found that the layer of fat comes to the surface starting on the 3rd day and continuing to the 10th day in the containers of milk media of all four fat levels, but it was also found that the fat layer became thicker as the fat percentage of the milk media became greater.

It was necessary to know how homogenized and non-homogenized milk of different fat levels act in the refrigerator at a temperature of 4°-5° C.

After two or three trials, it was found that the non-homogenized milk fat came up more quickly and developed a thicker layer than that of the homogenized milk. From these results, it was decided that the homogenized milk was better than the non-homogenized milk in terms of equal concentration of fat for the preservation of semen.

Sperm Motility

The semen for these experiments received the maximum motility rating of 5 on the day of collection for all three bulls. The motility observed subsequently was based on the relative motility of the first day. From these experiments, it was found that the milk diluter containing 3.5 per cent fat was significantly different statistically from the media of 0 per cent fat medium, 7 per cent

fat, 11 per cent fat, and the 15 per cent fat ($P<0.01$). However, on the 10th day (final day of the experimental period), the motility of the sperm in the milk diluter containing 3.5 per cent fat and the sperm in the diluter containing 7 per cent fat was the same, as can be observed in Table 1, p. 17. The relative motility of the sperm in the diluter containing 7 per cent fat was significantly different statistically from media of 0 per cent fat, 11 per cent fat and 15 per cent fat.

Likewise, it was found that as the percentage of fat increased, the motility of the sperm decreased. However, in the diluter at 0 per cent fat, the sperm motility decreased more sharply than in any of the diluters containing fat during the first three days, but on the sixth and tenth days, the motility of the sperm in the diluter at 0 per cent fat was greater than motility of the sperm in the diluter at 15 per cent fat (Figure 1, p. 18).

From the analysis of variance, it was found in this study that the diluter with 3.5 per cent fat was better than all the other diluters throughout the experimental period (10 days).

The diluter with 7 per cent fat was second until the 6th day, but on the 10th day it was the same as the diluter with 3.5 per cent fat (Table 2).

The diluter with 11 per cent fat medium ranked 3rd, but the motility of the sperm decreased more sharply starting from the 3rd day and continuing to the 10th day, than that in diluters between 3.5

Table 1. Means of motility values of sperm (Measured 0-5 scale) preserved in five different levels of fat in milk media and stored at 4° C. to 5° C. for 0 day, 3 days, 6 days and 10 days.

Storage Time	Percentage of fat in diluter 0%	3.5%	7%	11%	15%	Day means
1st day	5.00	5.00	5.00	5.00	5.00	5.00
3rd day	2.10	3.50	3.20	2.90	2.60	2.86
6th day	1.75	2.80	2.50	2.10	1.90	2.21
10th day	1.35	2.10	2.10	1.40	0.95	1.58
Treatment Means	2.55	3.35^{a}	3.20^{b}	2.85^{c}	2.61^{d}	

a = significantly different from 0%, 7%, 11%, 15% fat ($P<0.01$)

b = significantly different from 0%, 11%, 15% fat ($P<0.01$)

c = significantly different from 0%, 15% fat ($P<0.01$)

d = significantly different from 0% fat ($P<0.01$)

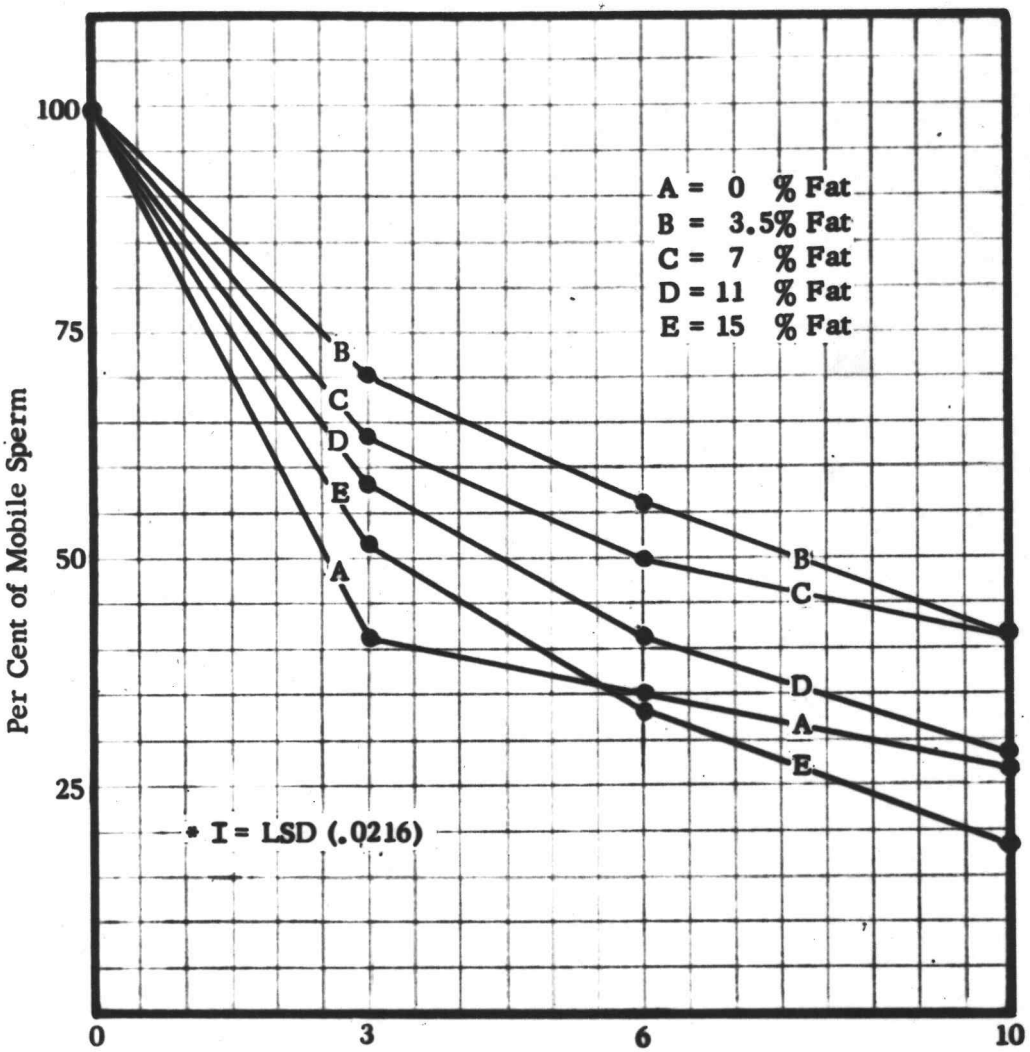

Figure 1.

Comparative sperm survival of five different levels of fat in milk medium and stored at 4° C to 5° C. (Means of 20 samples of each different fat level. Ejaculates from three dairy bulls.)

*** Least significant difference at 1% level of probability.**

Table 2. Analysis of variance on motility values of sperm preserved in five different fat levels of milk.

Source of variation	Degrees of freedom	Mean square	F value
Total	399		
Treatment	4	9.98	21.22[a]
Day	3	22.10	47.02[a]
Day x treatment	12	1.58	3.36[a]
Error	380	.47	

a = significant at the 0.01 level of probability.

per cent fat and 7 per cent fat.

The diluter with 15 per cent fat stood 4th in its ability to preserve semen. The diluter with no fat showed a decreased motility on the 3rd day, but it was similar to the diluter with 15 per cent fat on the 6th day, and on the 10th day it approached the diluter with 11 per cent fat. In other words, the diluter with no fat was generally better than the diluter with 15 per cent fat on the 6th and 10th days of storage.

Second Experiment

The original data on motility, pH, and percentage of live sperm are shown in Appendix Tables 5 and 6. The motility rating decreased as storage time progressed (Table 3). There was a significant difference between replications in motility. The percentage of fat in the diluter influenced motility significantly (Table 3).

Table 3. Motility rating of sperm during storage in milk diluters varying in fat percentage.

Storage Time	Percentage of fat in diluter				
	0%	3.5%	7%	11%	15%
1st day	5.00	5.00	5.00	5.00	5.00
3rd day	2.00	2.91	3.33	2.75	3.00
6th day	2.00	2.59	2.91	2.09	2.75
10th day	0.00	1.50	1.33	1.00	1.59

The highest motility at the third day of storage was obtained in

Table 4. Analysis of Variance of Motility Rating

Source of Variation	D. F.	Sum of Squares	Mean Square	F
Total	119	274.5813	—	—
Rep	1	1.7521	1.7521	33.63 *
Bull	2	.0875	.0438	.84
Error (a)	2	.1042	.0521	—
Fat	4	13.0501	3.2625	42.32 **
Bull x Fat	8	.2249	.0281	.36
Error (b)	12	.9250	.0771	—
Day	3	236.7730	78.9243	276.54 **
Day x Bull	6	.5958	.0993	.35
Day x Fat	12	6.7999	.5667	1.99 *
Day x Bull x Fat	24	1.4251	.0594	.21
Error (c)	45	12.8417	.2854	—

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

the diluter containing 7 per cent fat and the lowest motility at this time was in milk containing no fat. In fact, the non-fat milk was inferior to all others throughout the storage trials. At the sixth day of storage, the diluter containing 7 per cent fat was highest in motility, followed by diluters containing 15 and 3.5 per cent fat, in that order. At the tenth day of storage, the diluters at 3.5 and 15 per cent fat were superior to the ones at 7 and 11 per cent fat. No motility was present in semen stored in non-fat milk on the tenth day of storage.

Analysis of variance (Table 4) revealed that there was a significant effect of fat in the diluting medium, of days of storage, of replications, and of interaction of days of storage with fat percentage of diluter.

The percentage of live sperm is shown in Table 5 by percentage of fat in the diluter and by days of storage since the analysis of variance did not show a significant difference between bulls or replications.

Table 5. Percentage of live sperm during storage in milk diluters varying in fat percentage.

Storage Time	Percentage of fat in diluter				
	0%	3.5%	7%	11%	15%
1st day	91.5	86.5	86.2	87.5	86.2
3rd day	84.2	79.5	81.5	76.2	81.7
6th day	76.7	73.3	75.7	69.7	75.5
10th day	53.3	47.2	63.5	44.7	51.7

There was a steady decline in percentage of live sperm as storage time progressed. All samples showed a high percentage of live sperm at the third day of storage, but the diluter containing 11 per cent fat was below that in all the other diluters. In fact, the diluter containing 11 per cent of fat showed the lowest per cent of live sperm all during the storage period even though it was equal initially to all other diluters containing fat (Table 5). On the sixth day of storage, there was generally a high percentage of live sperm. In fact, all diluters appear satisfactory for storage of bull semen up to 6 days of storage if percentage of live sperm is used as the criterion for appraisal (Table 5).

On the tenth day of storage, the diluter containing 7 per cent fat was superior to all the other diluters. The diluter containing 11 per cent fat was inferior and little difference among the other diluters was noted in percentage of live sperm.

The analysis of variance (Table 6) showed a significant difference between diluters and days of storage with no significant interactions.

The pH of the diluted semen is shown in Table 7 by percentage of fat in the diluter and by days of storage. The analysis of variance (Table 8) did not show a significant difference between bulls or replications.

Table 6. Analysis of Variance of Percentage of Live Sperm

Source of Variation	D. F.	Sum of Squares	Mean Square	F
Total	119	28418.80	—	—
Rep	1	240.83	240.83	.77
Bull	2	375.80	187.90	.60
Error (a)	2	628.27	314.14	—
Fat	4	919.88	229.97	4.93 *
Bull x Fat	8	147.87	18.49	.40
Error (b)	12	559.65	46.64	—
Day	3	21242.20	7080.73	137.92 **
Day x Bull	6	142.00	23.67	.46
Day x Fat	12	871.39	72.62	1.41
Day x Fat x Bull	24	980.66	40.86	.80
Error (c)	45	2310.25	51.34	—

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

Table 7. The pH of diluted semen during storage in milk diluters varying in fat percentage.

Storage Time	Percentage of fat in diluters 0%	3.5%	7%	11%	15%
1st day	6.64	6.63	6.63	6.63	6.63
3rd day	6.78	6.77	6.77	6.72	6.76
6th day	6.73	6.73	6.73	6.68	6.70
10th day	6.82	6.79	6.84	6.73	6.79

There was generally a progressive increase in pH with storage, indicating that the diluted semen was becoming less acid. The change in pH during storage was not definitely related to storage time because the pH readings were lower on the sixth day of storage than on either the third or the tenth day.

The diluter with 11 per cent fat generally showed less change in pH during storage than did any of the other diluters. There was no difference between the diluters with 3.5 and 15 per cent fat in their pH changes during storage. The diluters with 0 and 7 per cent fat showed greater pH changes during storage than did the other diluters.

The analysis of variance (Table 8) showed significant effects of different diluters and of storage time only. There were no significant interactions.

It is of interest to note that there were no differences among the three bulls in motility, percentage of live sperm or in pH. All three of the bulls responded similarly to type of diluter (Tables 9,

Table 8. Analysis of Variance of pH of Diluted Semen.

Source of Variation	D. F.	Sum of Squares	Mean Square	F
Total	119	1.6351	—	—
Rep	1	.1936	.1936	12.41
Bull	2	.0204	.0102	.65
Error (a)	2	.0313	.0156	—
Fat	4	.0519	.0130	4.33 *
Bull x Fat	8	.0127	.0016	.53
Error (b)	12	.0364	.0030	—
Day	3	.4411	.1470	11.40 **
Day x Bull	6	.1567	.0261	2.02
Day x Fat	12	.0233	.0019	.15
Day x Fat x Bull	24	.0890	.0037	.29
Error (c)	45	.5787	.0129	—

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

Table 9. Mean Values for Bull A of Motility, Percentage of Live Sperm, and pH.

Storage Time	Percentage of fat in diluter														
	0%			3.5%			7%			11%			15%		
	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH
1st day	5.00	95	6.67	5.00	86	6.64	5.00	93	6.63	5.00	92	6.63	5.00	90	6.64
3rd day	2.00	86	6.83	2.75	78	6.81	3.50	85	6.81	3.00	78	6.77	3.00	85	6.80
6th day	2.00	77	6.81	2.25	73	6.76	2.75	80	6.77	2.25	70	6.72	2.75	77	6.72
10th day	0.00	61	6.75	1.75	58	6.77	1.50	68	6.79	1.00	48	6.71	1.50	47	6.87

10 and 11), and their sperm showed about the same abilities to survive during storage.

The correlation coefficient between percentage of live sperm and motility rating of +0.75 is highly significant. This indicates that the two methods of appraising semen during storage are in good agreement. On the other hand, the correlations of pH with percentage of live sperm of -0.15 and with motility of -0.27 both lacked significance. Thus pH change was not closely associated with changes in either motility or in percentage of live sperm.

Table 10. Mean Values for Bull B of Motility, Percentage of Live Sperm, and pH.

Storage Time	Percentage of fat in diluter														
	0%			3.5%			7%			11%			15%		
	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH
1st day	5.00	92	6.64	5.00	88	6.64	5.00	81	6.63	5.00	84	6.63	5.00	86	6.64
3rd day	2.00	84	6.78	3.00	79	6.77	3.33	80	6.76	2.80	76	6.72	3.00	83	6.76
6th day	2.00	77	6.75	2.50	73	6.74	3.00	75	6.73	2.00	69	6.68	2.80	77	6.73
10th day	0.00	45	6.69	1.80	43	6.75	1.50	59	6.85	1.00	49	6.66	1.80	47	6.72

Table 11. Mean Values for Bull C of Motility, Percentage of Live Sperm, and pH

Storage Time	Percentage of fat in diluter														
	0%			3.5%			7%			11%			15%		
	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH
1st day	5.00	88	6.63	5.00	87	6.63	5.00	85	6.62	5.00	87	6.62	5.00	83	6.62
3rd day	2.00	84	6.74	3.00	82	6.73	3.33	80	6.74	2.50	75	6.67	3.00	78	6.72
6th day	2.00	76	6.64	3.00	71	6.69	3.00	72	6.70	2.00	70	6.63	2.80	73	6.67
10th day	0.00	54	7.01	1.00	41	6.87	1.00	64	6.90	1.00	38	6.81	1.50	62	6.78

DISCUSSION

The survival of sperm during storage in milk diluters containing 0.0, 3.5, 7.0, 11.0 and 15.0 per cent fat was appraised at 3, 6 and 10 days by motility rating in two experiments and by percentage of live sperm and pH in one experiment. In general, the milk diluters with 3.5 and 7.0 per cent fat were superior to milk diluters with no fat or those with 11 or 15 per cent fat. Motility rating in the first experiment tended to favor the diluter with 3.5 per cent fat over diluters with higher or lower fat percentages while in the second experiment the diluter with 3.5 per cent fat was one of the best; diluters with a greater fat percentage were also satisfactory. This is also borne out in the first experiment in which the diluter with 7 per cent fat was quite satisfactory, particularly with longer storage time. However, in the second experiment the diluter containing 15 per cent fat was satisfactory.

When survival during storage was appraised by percentage of live sperm, the milk diluter containing 7 per cent fat was superior to all others throughout the storage period of 10 days.

Considering both experiments and both methods of appraising survival of sperm (motility rating and percentage live sperm), one would consider the milk diluters containing 3.5 or 7 per cent fat to be superior to all others.

In general, when one considers both experiments and both methods

of appraising survival of sperm during storage, the non-fat milk was not found to be as desirable as a semen diluent as milk containing fat. The higher percentages of fat (11 and 15 per cent) in semen diluents certainly were not advantageous and may have been detrimental to survival of the sperm.

There was close agreement between motility rating and percentage of live sperm. This suggests that both are good criteria for evaluation of sperm survival during storage. Both methods are more difficult to use as the fat percentage of the milk diluent is increased because of the large number of fat globules. However, increases in fat percentage of the diluent up to 15 per cent do not prevent the use of either method for evaluating sperm survival during storage. Since there was good agreement between the two methods, more confidence can be placed in the value of milk diluters with 3.5 to 7.0 per cent fat as desirable semen diluents for preservation during storage.

The pH increased during storage in diluters with all fat percentages used in this study. This change in pH to less acid was surprising because the glycolytic activity of the sperm should tend to produce lactic acid and thereby lower the pH. It is recognized that milk should have a high buffering capacity and, consequently, should prevent highly acid conditions from developing. There must be some type of change in milk during storage which results in the development of a higher pH. Apparently, this small change towards a higher pH was not particularly harmful to the sperm. There did not

appear to be a direct association between fat percentage of the diluent and change in pH during storage.

It was particularly important to note that the three bulls reacted similarly to the various diluters. Thus, no evidence was obtained that indicated sensitivity of sperm from a particular bull to a higher or a lower percentage of fat in the semen diluent.

There was some interaction between fat percentage of the diluent and storage time. Most of the diluents were satisfactory for a storage period of 3 days; whereas, for longer periods of storage the diluters containing 3.5 and 7 per cent fat were superior to the others.

Why the milk diluters containing fat were superior to the non-fat diluter may reside in the protective effects of the fat preventing entrance of harmful electrolytes into the sperm cell. Secondly, it may be due to the presence of fat globules. The movement of the spermatozoa in diluters containing fat was more restricted than in the non-fat milk.

The results in these studies are in general agreement with the literature in showing that milk diluents are satisfactory for the preservation of bull semen during storage. The present study extends the information by showing that non-fat milk is less satisfactory than milk with 3.5 and 7 per cent fat as diluents for bull semen. Also, the present study indicates that 11 and 15 per cent fat in the milk diluent show no advantage and that they are less satisfactory

for preserving bull semen than milk diluents with 3.5 and 7 per cent fat.

SUMMARY AND CONCLUSIONS

Semen from three bulls was used in two separate experiments to study the effects of fat content of milk diluents on survival of sperm during storage. In the first experiment, semen from three bulls was pooled, from which samples were used for the dilution materials. In the second experiment, the semen from each bull was treated separately.

Survival of sperm during storage was appraised in the first experiment by motility rating while in the second experiment, motility rating, percentage of live sperm, and pH were used to evaluate survival of sperm.

The semen was examined on the first, third, sixth, and tenth days of storage in both experiments.

The results in the first experiment, using motility as the criterion of evaluating survival of sperm during storage, and in the second experiment, using both motility and percentage of live sperm for evaluating survival of sperm, showed that milk diluents of 3.5 and 7 per cent fat were superior to either non-fat milk or to milk diluents with 11 and 15 per cent fat for storage of bull semen.

The semen from the three bulls reacted to the different diluents in the same way, showing that certain bulls are not likely to be sensitive to certain types of milk diluents.

The pH increased in all samples with storage. There was no

relation of pH changes to changes in motility or percentage of live sperm during storage.

There was a close agreement between motility rating and percentage of live sperm, and both methods showed that sperm survival decreased with increased storage time though not so rapidly in milk diluents with 3.5 and 7 per cent fat.

The non-fat milk was not nearly as satisfactory as a diluent for bull semen as milk diluents with 3.5 and 7 per cent fat. In fact, non-fat milk for longer periods of storage was less satisfactory than milk diluents containing the higher fat percentages.

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APPENDIX

Appendix Table 1
First Experiment
1st day, 0 hour
Pooled Semen of Bulls A, B and C
Motility of Bull Sperm in Five Different Levels of Milk Fat

Sample No.	0% fat	3.5% fat	7% fat	11% fat	15% fat
1	5	5	5	5	5
2	5	5	5	5	5
3	5	5	5	5	5
4	5	5	5	5	5
5	5	5	5	5	5
6	5	5	5	5	5
7	5	5	5	5	5
8	5	5	5	5	5
9	5	5	5	5	5
10	5	5	5	5	5
11	5	5	5	5	5
12	5	5	5	5	5
13	5	5	5	5	5
14	5	5	5	5	5
15	5	5	5	5	5
16	5	5	5	5	5
17	5	5	5	5	5
18	5	5	5	5	5
19	5	5	5	5	5
20	5	5	5	5	5
Total	100	100	100	100	100

Appendix Table 2
First Experiment
3rd day, 72 hours
Pooled Semen of Bulls A, B and C
Motility of Bull Sperm in Five Different Levels of Milk Fat

Sample No.	0% fat	3.5% fat	7% fat	11% fat	15% fat
1	1	4	3	3	3
2	0	3	3	2	3
3	3	3	3	3	4
4	3	4	4	3	3
5	2	4	4	4	4
6	2	3	4	3	3
7	2	4	3	4	4
8	4	4	4	4	4
9	2	3	2	3	2
10	2	3	2	3	3
11	0	4	3	2	1
12	3	5	5	3	2
13	2	3	4	2	2
14	2	3	3	3	2
15	2	3	3	2	2
16	2	3	3	2	2
17	3	4	4	3	2
18	3	3	4	3	2
19	2	4	4	3	2
20	2	3	3	3	2
Total	42	70	64	58	52

Appendix Table 3
First Experiment
6th day, 154 hours
Pooled Semen of Bulls A, B and C
Motility of Bull Sperm in Five Different Levels of Milk Fat

Sample No.	0% fat	3.5% fat	7% fat	11% fat	15% fat
1	1	2	3	3	3
2	0	1	1	1	0
3	2	4	3	3	3
4	1	2	2	2	1
5	3	4	3	3	2
6	2	3	2	2	2
7	1	3	2	2	2
8	2	3	3	2	2
9	1	2	2	2	2
10	3	3	3	2	2
11	2	2	2	2	2
12	2	3	2	2	2
13	3	3	2	2	2
14	2	3	2	2	2
15	2	3	3	2	2
16	1	3	3	3	2
17	1	3	3	2	2
18	2	3	3	2	2
19	2	3	3	2	2
20	2	3	3	2	1
Total	35	56	50	42	38

Appendix Table 4
First Experiment
10th day, 240 hours
Pooled Semen of Bulls A, B and C
Motility of Bull Sperm in Five Different Levels of Milk Fat

Sample No.	0% fat	3.5% fat	7% fat	11% fat	15% fat
1	0	2	3	0	0
2	0	1	1	0	0
3	0	2	2	1	0
4	2	3	2	2	2
5	1	2	2	1	0
6	0	2	2	1	1
7	1	3	2	2	1
8	2	2	2	2	2
9	1	2	2	2	2
10	1	2	2	2	1
11	2	2	2	2	1
12	2	2	2	1	1
13	2	2	2	1	1
14	2	2	2	1	0
15	1	2	2	1	1
16	2	3	2	1	1
17	2	3	3	2	1
18	2	3	3	2	1
19	2	2	2	2	1
20	2	2	2	2	2
Total	27	42	42	28	19

Appendix Table 5
Second Experiment
1st Sample

Motility, Percentage of Live Sperm, and pH of Semen of Bulls A, B and C in Diluters Varying in Fat Content

Day	Bull	0% Fat			3.5% Fat			7% Fat			11% Fat			15% Fat		
		M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH
1st day	A	5	96	6.60	5	80	6.58	5	96	6.58	5	94	6.60	5	94	6.60
4-5-60	B	5	90	6.60	5	90	6.60	5	80	6.60	5	90	6.60	5	92	6.61
	C	5	94	6.60	5	95	6.60	5	90	6.58	5	92	6.59	5	90	6.59
3rd day	A	2	90	6.90	3	80	6.87	3.5	90	6.87	3	85	6.87	3	90	6.85
4-8-60	B	2	88	6.82	3	80	6.80	3	78	6.80	3	82	6.78	3	85	6.78
	C	2	87	6.76	3	92	6.73	3	88	6.72	3	85	6.69	3	85	6.69
6th day	A	2	76	6.90	2	70	6.87	3	82	6.87	2.5	72	6.85	3	78	6.85
4-11-60	B	2	78	6.82	2	65	6.81	3	72	6.80	2	70	6.78	3	80	6.78
	C	2	77	6.75	3	82	6.76	3	80	6.75	2	74	6.73	3	76	6.71
10th day	A	0	62	6.75	1	50	6.75	1	65	6.75	0	45	6.75	1	38	6.88
4-15-60	B	0	38	6.66	1	30	6.78	1	50	6.95	0	50	6.66	1	40	6.67
	C	0	60	7.21	0	30	7.02	1	68	7.08	0	30	7.10	1	65	6.95

Appendix Table 6
Second Experiment
2nd Sample

Motility, Percentage of Live Sperm, and pH of Semen of Bulls A, B and C in Diluters Varying in Fat Content

Day	Bull	0% Fat			3.5% Fat			7% Fat			11% Fat			15% Fat		
		M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH	M	L&D	pH
1st day	A	5	94	6.73	5	91	6.69	5	90	6.68	5	90	6.65	5	85	6.68
4-12-60	B	5	93	6.68	5	85	6.68	5	82	6.66	5	78	6.66	5	80	6.66
	C	5	82	6.65	5	78	6.65	5	79	6.65	5	81	6.65	5	76	6.64
3rd day	A	2	82	6.75	2.5	75	6.75	3.5	80	6.75	3	70	6.67	3	80	6.74
4-15-60	B	2	80	6.73	3	78	6.73	3.5	82	6.72	2.5	70	6.66	3	80	6.73
	C	2	78	6.73	3	72	6.73	3.5	71	6.75	2	65	6.65	3	70	6.75
6th day	A	2	78	6.71	2.5	75	6.65	2.5	78	6.66	2	68	6.58	2.5	75	6.58
4-18-60	B	2	76	6.68	3	80	6.66	3	78	6.65	2	68	6.58	2.5	74	6.67
	C	2	75	6.52	3	68	6.61	3	64	6.65	2	66	6.53	2.5	70	6.62
10th day	A	0	60	6.75	2.5	65	6.78	2	70	6.82	2	50	6.67	2	55	6.85
4-22-60	B	0	52	6.72	2.5	56	6.72	2	68	6.75	2	48	6.65	2.5	54	6.77
	C	0	48	6.81	2	52	6.71	1	60	6.71	2	45	6.52	2	58	6.60