QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES
DIVISION OF ANIMAL INDUSTRY BULLETIN No. 142

COMPARISON OF STORAGE MOTILITY AND FERTILITY OF CHILLED BOVINE SEMEN EXTENDED IN DIFFERENT DILUENTS

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SUMMARY

Motility assessments of spermatozoa extended in two 20% egg yolk-glycine diluents (CUE and CU-16), skim-milk 10% glycerol (MG) and 50% egg yolk citrate (EYC) were compared over 12 days' storage at 5°C. CUE and CU-16 were not significantly different and both were superior to EYC and MG ($P < 0.001$). EYC was also superior to MG ($P < 0.05$).

Inseminations with semen extended in CUE on the third day of storage gave a significantly higher 60–90 day non-return percentage (64·3) than was obtained with semen extended in EYC and stored for a similar period (46·9) ($P < 0.02$).

I. INTRODUCTION

The egg yolk citrate diluent (EYC) for chilled semen developed by Salisbury, Fuller, and Willett (1941) was used routinely for 4 years at the Artificial Insemination Centre, Wacol, Queensland. With this diluent it was observed that first insemination non-return percentages were considerably higher for semen of less than 2 days' storage than for semen stored for longer periods. A significant improvement in storage motility was reported by Foote and Bratton (1960), using two 20% egg yolk-glycine diluents (CUE and CU-16). In a further trial, Foote *et al.* (1960) compared insemination non-return rates obtained with EYC, CUE and CU-16 and concluded that the last two diluents resulted in improved fertility.

The addition of glycerol to semen diluents was first reported to improve fertility by Polge and Rowson (1952) and this was later substantiated by Holt (1953). Williams, Green, and Dombroske (1957) and Almquist and Wickersham (1962) indicated that 10% of glycerol in milk diluents was optimal for improving both storage motility and fertility.

This study was designed to compare the motility of semen extended in EYC, CUE, CU-16 and milk-10% glycerol (MG) after various periods of storage. The first service non-return rate to inseminations with semen extended in EYC and CUE was also compared.

II. MATERIALS AND METHODS

Twenty first ejaculates collected, using an artificial vagina, from 20 different bulls (10 Jersey and 10 Australian Illawarra Shorthorn) held at the Artificial Insemination Centre, Wacol, Queensland, were used for the comparison of motility after storage. Sperm density was assessed by the spectrophotometric method described by Salisbury *et al.* (1943), using a Bausch and Lomb Spectronic 20. The semen was diluted in each of the four diluents under test to a constant density of 50×10^6 spermatozoa per ml, using the split ejaculate technique.

The composition of the three egg yolk diluents is shown in Table 1. Semen was diluted directly into these extenders at 32°C and cooled to the storage temperature of 5°C over a period of 6 hr. The milk-extended semen was diluted initially to 100×10^6 spermatozoa per ml in reconstituted skim-milk which had been preheated to 93°C for 10 min and cooled to 32°C. This diluted semen (fraction A) was cooled to 5°C over 6 hr. An equal volume of preheated skim-milk containing 20% glycerol by volume was prepared and cooled to 5°C (fraction B). Fraction B was added to fraction A in three equal portions at intervals of 10 min. Streptomycin sulphate (1,000μg/ml) and crystalline penicillin G (1,000 i.u./ml) were included in both fractions A and B.

TABLE 1

COMPOSITION OF EYC, CUE AND CU–16 DILUENTS

—	EYC	CUE	CU–16
Buffer (g/l)—			
Sodium citrate dihydrate ..	30·0	14·5	14·5
Sodium bicarbonate ..	—	2·1	—
Potassium chloride ..	—	0·4	—
Glucose	—	3·0	12·5
Glycine	—	9·37	9·37
Sulphanilamide	—	3·0	3·0
Citric acid	—	0·87	—
Extender—			
Buffer (% by vol.)	50	80	80
Egg yolk (% by vol.) ..	50	20	20
Crystalline penicillin G (i.u./ml)	500	1,000	1,000
Streptomycin sulphate (μg/ml)	500	1,000	1,000

TABLE 2

MEAN ± STANDARD ERROR OF THE PERCENTAGE MOTILE SPERMATOZOA IN EYC, MG, CUE, and CU–16 STORED AT 5°C FOR 12 DAYS

Diluent	Days of Storage											
	1	2	3	4	5	6	7	8	9	10	11	12
EYC ..	71·7±1·84	66·0±1·25	60·0±1·66	53·5±1·85	47·5±1·97	40·0±3·91	33·3±4·29	33·0±3·03	31·5±1·50	26·7±2·50	18·6±2·25	13·6±2·84
MG ..	71·5±1·74	65·5±1·16	59·4±1·94	47·5±3·05	42·7±3·27	35·8±7·34	31·8±6·10	27·2±3·25	21·5±2·24	17·3±2·56	11·8±1·59	9·4±1·57
CUE ..	72·0±1·79	69·5±1·74	66·1±1·62	62·0±1·59	57·5±1·75	53·9±3·61	47·0±3·81	46·0±2·91	39·5±3·37	35·5±4·52	28·3±3·29	21·8±3·04
CU–16	72·4±1·80	68·5±1·83	61·1±1·54	60·3±1·93	56·8±1·89	49·3±3·59	46·1±3·89	45·0±2·78	40·0±3·07	36·6±3·13	28·9±2·72	23·1±2·61

All samples of semen extended by the four diluents were stored at 5°C in 5 ml stoppered test-tubes each containing 4 ml of extended semen. At daily intervals for 12 days the semen in the test-tubes was mixed, a sample was mounted on a slide with a coverslip warmed to 32°C by a stage incubator and the percentage of motile spermatozoa was estimated microscopically at X320 magnification.

Semen from 8 bulls (4 Jersey and 4 Australian Illawarra Shorthorn) was used for insemination of cows to compare the fertility of semen extended in EYC and CUE. The semen was diluted in the same manner as outlined for the motility storage trial, using the split ejaculate design. A total of 32 batches was used and inseminations were made on the first, second and third day of storage with each batch. Two technicians performed the inseminations; each used semen extended in the two diluents for alternate cows. Extended semen was coded so that its identity was unknown to the technicians.

III. RESULTS

The mean motility estimations on semen extended in the four different diluents and from each of the 20 bulls, for each day of storage, are shown as percentages in Table 2. The average percentages of motile spermatozoa for the 12 days of storage in each diluent were as follows: CUE 49·9; CU-16 49·0; EYC 41·3; and MG 36·8. Analysis of data showed no significant difference between CUE and CU-16 ($P > 0{\cdot}05$) but both these diluents were superior in preserving spermatozoal motility to EYC and MG ($P < 0{\cdot}001$). EYC was further shown to be more efficient than MG ($P < 0{\cdot}05$).

The 60–90 day non-return data for first inseminations with split ejaculate batches diluted in EYC and CUE are shown in Table 3. The difference in the percentage of non-returns was not significant for inseminations with semen stored for 1 or 2 days ($P > 0{\cdot}05$), but the difference with third day semen was significant ($P < 0{\cdot}02$) in favour of CUE. The difference in the percentage of non-returns to all services was not significant ($P > 0{\cdot}05$).

TABLE 3

NUMBER OF FIRST INSEMINATIONS AND THE 60–90 DAY NON-RETURN PERCENTAGES FOR FIRST, SECOND AND THIRD DAY SEMEN DILUTED WITH EYC AND CUE

Age of Semen Used for Insemination	EYC		CUE	
	Number of First Inseminations	60–90 Day Non-returns (%)	Number of First Inseminations	60–90 Day Non-returns (%)
First day	93	72·0	112	73·2
Second day ..	103	69·9	86	72·1
Third day ..	83	46·9	101	64·3
Total/average	279	63·8	299	69·9

IV. DISCUSSION

The motility storage trial clearly indicated the superiority of CUE and CU-16 when compared with EYC and MG. The results obtained with MG showed a more rapid decline in motility than was reported by Almquist (1962) and Almquist and Wickersham (1962). Because the viscosity of a milk extender is lower than that of an egg yolk extender, visual motility estimations on a drop of extended semen might easily be biased in favour of milk. In this experiment, it is considered, this bias was largely eliminated by examining the sample under a coverslip to obtain a thin film and by using high magnification. The difference in average motility over 12 days of storage at 5°C was not significant between CUE and CU-16. This result is in agreement with the findings of Foote and Bratton (1960), who found no significant difference between the two extenders over 12 days of storage ($P > 0{\cdot}05$).

In the fertility trial, first inseminations with semen diluted in CUE averaged a higher percentage of non-returns than first inseminations with semen diluted in EYC at all storage periods tested. The differences, however, were only significant at the 0·02 level for inseminations performed with third day semen. This finding is in agreement with that of Foote *et al.* (1960), with the reservation that they could not establish the differences to be significant at any stage, though their trials strongly indicated an improvement in non-return rate in favour of CUE.

V. ACKNOWLEDGEMENTS

The assistance received from Messrs. J. Edmunds, W. B. Hornbuckle, G. W. Legg and R. Neiper of the Department of Primary Industries, Brisbane, in the collection and processing of semen and in the insemination of cows is gratefully acknowledged.

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(Received for publication January 24, 1969)

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