FERTILITY STUDIES OF SEMEN PRESERVED IN COCONUT MILK EXTENDER

BY

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "FERTILITY STUDIES OF SEMEN PRESERVED IN COCONUT MILK EXTENDER" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

P.K. GEORGE.

Mannuthy,
29 -7-1978.

CERTIFICATE

Certified that the thesis entitled "FERTILITY STUDIES OF SEMEN PRESERVED IN COCONUT MILK EXTENDER" is a record of research work done independently by Sri. P.K. George, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship, or fellowship to him.

Dr. C.P. Neelakanta Iyer,
Associate Professor
(Chairman, Advisory Committee).

Mannuthy,

29 -7-1978.

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This work is dedicated to my wife, daughter and parents.

P.K. GEORGE,

C O N T E N T S

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INTRODUCTION

Artificial insemination of livestock is one of the most sophisticated techniques of advanced husbandry and has made considerable impact in our country. The main emphasis has lately been on cross breeding of indigenous cattle with exotic breeds, as this is the only programme to increase the production potential of our cattle in a short period. With the use of artificial insemination to cover large areas in our country, particularly through Intensive Cattle Development Projects and other developmental schemes, the dissemination of exotic germplasm is going on at a faster pace.

To meet the objectives of any artificial insemination programme, optimum fertilizing capacity of spermatozoa must be preserved for a longer period after collection. This can be achieved by refrigeration, room temperature preservation or deep freezing of semen in suitable extenders.

The difficulties that arise from the lack of refrigeration facilities for storage of fluid semen in conventional extenders need no elaboration. Nevertheless, because of highly skilled labour and cost involved in the preparation, storage and transport of deep frozen semen, in spite of its many inherent advantages, it may not be possible in the immediate future to adopt it in our country at village level

where 90 per cent of the cattle population is located. Formulation of a suitable extender for preservation of semen at room temperature will go a long way to solve these problems.

The advent of Illini Variable Temperature diluent (IVT) for room temperature preservation of semen by Van Demark and Sharma (1957) marked a turning point in the development in the techniques of preservation of semen. Several modifications of this extender were tried by various workers (Dunn and Foote, 1958; Corrias and Molinari, 1963). Though fairly good fertility was obtained on initial studies, subsequent trials with this extender did not yield uniformly promising results.

Norman et al. (1958a) evolved a new diluent using coconut water (Coconut Milk Extender - CME) in which spermatozoa could be preserved for sufficiently longer periods at room temperature with good motility. The potentialities of this diluent was further investigated by various workers from India and abroad (Norman and Goldberg, 1959; Norman et al. 1958b,c; Norman et al. 1959b; Venkataswami and Hanumantha Rao, 1961 and Saxena et al. 1973) who reported that semen could be preserved in CME at room temperature for seven days without substantial loss in motility. Preliminary studies carried

out in the Department of Animal Reproduction, College of Veterinary and Animal Sciences, indicated that bull semen extended in CME maintained fairly good motility upto five days of storage (Gopinatha Pillai, 1969). Since room temperature preservation offers greater advantages over the conventional diluents with respect to storage and transportation, CME is being used extensively for cross breeding programme under White revolution in Kerala State.

It is a well established fact that there is a drop in the fertility of semen stored in conventional diluents under refrigeration temperature beyond 48 hours of storage (Bawa et al. 1968; Mathai et al. 1970). This would be all the more pronounced in CME in which the semen is preserved at room temperature. There is dearth of information on the fertility of semen extended in CME at different periods of storage. It is essential that a detailed study on this aspect is carried out in order to assess how long bull semen could be preserved in CME without substantial loss of fertility. This work has therefore been taken up with a view to evaluate the fertility of semen extended in CME and to assess its utility for extensive field application.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Since the advent of egg yolk phosphate as a medium for dilution of semen by Phillips (1939), much progress has been achieved in the field of preservation of sperm cells. Willett et al. (1940) obtained an overall conception rate of 56.6 per cent using semen extended in EYC and stored for five days at 4 to 5°C. Salisbury et al. (1941) demonstrated the use of sodium citrate in place of phosphate buffer in the yolk diluent and reported good fertility results. Bratton et al. (1949) in a comparative study with phosphate and citrate diluents reported identical conception rates. Swanson (1949) using essentially isotonic solutions of sodium citrate found that the optimum level of the yolk ranged from 12.5 to 20 per cent of the final mixture for protection against cold shock and for optimum livability at 5°C. Holt (1952) reduced the proportion of egg yolk to citrate buffer from 1:1 to 1:4 and found no significant effect on the conception rate. Degroot and Bekedam (1957) in a study of the age of the sperm and the dilution rate on the percentage of non-returns reported no significant effect in the case of diluted semen stored up to four days.

Van Demark and Couturier (1958) developed a flow dialysis technique to prolong the life of bull sperm at room

temperature. Samples of undiluted semen in dialysis sacs were suspended in constantly flowing media of egg yolk citrate, milk and blood products. They reported that undiluted semen controls in test tubes at room temperature lived for about 24 hours, whereas portions of the same samples undiluted in dialysis sacs survived for a week with yolk citrate media constantly passing the sacs.

The earliest report on the preservation of semen at room temperature was by Van Demark and Sharma (1957) viz., Illini Variable Temperature extender. Dunn and Foote (1958) reported 38 per cent fertility rate on a 60 to 90 days non-return basis (NR) using one to six day old semen extended in IVT. The fertility of semen dropped from 69 per cent on the first day of storage to 21 per cent on the sixth day. Using IVT as an extender, Roslanowski (1959) reported conception rates of 53.0 per cent, 51.4 per cent, 43.4 per cent, 39.7 per cent and 43.5 per cent for semen of 24, 48, 72, 96 and 120 hours of age respectively with an overall conception rate of 47.3 per cent. Sharma (1960) carried out fertility trials on 111 cows inseminated with semen preserved at 60 to 80°F in the IVT diluent and reported an overall non-return rate of 74.8 per cent after one to seven days of storage. Hogset (1963) in a comparative study reported a conception rate of 77.2 per cent for IVT against 72.7 per cent for EYC

diluents, in terms of 90 to 140 day non-returns rate. Several modifications of this extender were tried subsequently (Jaskowski et al. 1962; Lunca and Peredean, 1965). However, further trials using IVT extender did not yield good results (Ulaganathan, 1970).

Abdu (1968) reported that bull semen could be diluted and stored in a glucose-egg yolk-citrate-EDTA (Ethylen diamine tetra acetic acid) diluent at 18 to 25°C for one to six days. He also reported a conception rate of 88 per cent (75 to 90 day NR) using three to six day old semen.

Norman et al. (1958a,b) reported that diluents containing coconut water lengthened the lifespan of bovine spermatozoa at room temperature for several days. In this diluent, the metabolic activity of spermatozoa gradually declined as the acidity of the diluent increased. But resuspension of the spermatozoa in fresh alkaline diluent or the addition of calcium carbonate without renewing the diluent restored appreciable metabolic activity after six days of storage at 23 to 27°C. Norman et al. (1958c) reported that spermatozoa were metabolically less active in a coconut water and sodium citrate diluent than the one with the addition of calcium carbonate. This indicated the superiority of the former diluent as the lifespan of the spermatozoa is generally

inversely proportional to their activity. It was also observed that the need for reactivation by alkali could be obviated by using an appreciable initial concentration of the sodium citrate buffer.

Chieffi and Masotti (1959) diluted semen with a mixture of egg yolk and coconut water and reported that the percentage motility after storage for five days at 2 to 5°C averaged 65 Vs 42 for controls diluted with yolk citrate. It was later confirmed by Norman (1961), Norman and Goldberg (1959), Norman et al. (1959b) and Gopinatha Pillai (1969) that a diluent containing 15 per cent fresh boiled coconut water, 2.20 per cent sodium citrate and antibiotics maintained the required number of viable spermatozoa at room temperature for seven to ten days. In a preliminary trial, Norman (1961) reported conception rate of 61.6 per cent using 0 to 6 day old semen suspended in coconut milk citrate medium. This suggested that the normal fertilizing capacity of bovine sperm suspended in coconut milk-sodium citrate extender remained unimpaired during 0 to 6 days storage at room temperature.

Norman and Goldberg (1959) and Norman et al. (1959a) suggested that oxygen damage, temperature shock and visible light might have detrimental effect on the fertilizing capacity of the diluted semen. Dunn et al. (1960) showed that

addition of catalase gave effective protection against oxygen damage and 0.5 to 1 per cent fresh egg yolk protected against temperature shock during transit. Coconut milk-citrate diluent was modified by the addition of 0.5 per cent catalase and 0.5 per cent egg yolk (Norman et al. 1960; Norman, 1961) and semen was stored in the dark in vials filled completely to avoid any air space. Using such semen at 18 to 30°C they reported a conception rate of 77.9 per cent and 71.2 per cent for semen stored for two days and six days respectively. Venkataswami and Rao (1961) observed that addition of coconut water to duck egg yolk and citrate diluent significantly improved the average percentage of motile spermatozoa for the first four days of storage at 5°C. Norman et al. (1962) confirmed their earlier findings, by reporting that semen stored two to four days at 5°C in coconut milk citrate with five per cent egg yolk effected non-return rate of 68 per cent.

Foote et al. (1962) passed coconut water through a column of activated charcoal and separated two fractions, 'neutral' and 'active'. From this work it was shown that the beneficial effect on sperm preservation was entirely due to the neutral fraction which contained various anions and cations, free sugars (largely glucose), sugar alcohol (mainly sorbitol) and inositols. The effect of this fraction was reduced but not eliminated by removal of all anions and

cations. They concluded that coconut water does not contain any unknown substance with special properties.

Norman (1964) reported that semen diluted with coconut water was sent by air under non-refrigerated conditions to seven countries. Using three to fifteen days old semen stored in the dark at room temperature he reported conception rate from 39 per cent to 61.5 per cent in these countries. In Uganda a conception rate of 67 per cent was obtained in 200 cows with semen supplied once weekly and 69 per cent in 718 cows with twice weekly issue. He also reported that when bovine semen diluted in CME was stored for a week during which the temperature varied from 8 to 30°C the percentage of motile spermatozoa fell from 65 per cent to 35 per cent.

Smith (1964) reported 51.2 per cent conception rate with CME extended semen, air lifted to Kenya from U.S.A. and stored under non-refrigerated conditions for 8 to 12 days. Later trials at Central Artificial Insemination Station, Kabete, Kenya, using semen extended and stored in Norman's coconut milk citrate medium for a period of 5 days achieved, a conception rate of 50 per cent on first insemination (Grove, 1965).

From the report of Norman et al. (1968) it is found that the functional lifespan of buffaloe spermatozoa could

be increased to 10 days if they were kept at ambient temperature in CME. They also confirmed 89 pregnancies (62%) out of 144 buffaloe cows given first insemination with one to five day old semen. Grove (1968) diluted semen in CME and milk by split sample technique and stored them at room temperature and 4°C respectively. Using these samples stored for four days, he obtained a non-return rate of 71 per cent and 88 per cent respectively for CME and milk extended semen. Norman and Rao (1972) reported that both cattle and buffaloe sperm, preserved at ambient temperature for four days in CME, maintained accepted levels of conception. Among cattle they reported an overall conception percentage of 45.0, 56.4, 47.5 and 50.1, for local breeds, 46.1, 55.7, 48.0 and 51.4 for Jersey and 44.7, 56.6, 47.2 and 47.1 for 1, 2, 3 and 4 days old semen respectively. In buffaloe, the rate of conception obtained was 49.7, 51.4, 50.7 and 43.7 for 1, 2, 3 and 4 day old semen respectively. Malmberg and Israelsson (1972) in a complimentary trial, used semen extended in CME for seven consecutive days and showed a marked drop in the non-return rate for semen stored for more than four days. El-Wishy (1976) in a comparative study with frozen semen reported the conception rate for 1, 2, 3, 4, 5, 6 and 7 day old CME semen as 54.0, 49.3, 52.2, 49.5, 49.2, 31.0 and 31.3 per cent respectively as against 69.1 per cent for first insemination with frozen semen.

Norman (1964) formulated two diluents viz., NJ-1 and NJ-2. These extenders were modifications of CME and made by replacing coconut water with chlorides, sulphates and nitrates of calcium, magnesium and potassium. Using bovine semen preserved in NJ-1 of 0 to 6 days age, he reported a conception rate of 73 per cent based on 90 to 120 day non-return rate. He further reported that rabbit spermatozoa could be preserved in NJ-2 and obtained 78.5 per cent conception rate with 2 to 144 hours old spermatozoa, centrifuged and resuspended in NJ-1. Freund and Wiederman (1964) reported that human spermatozoa preserved well in NJ-1 and NJ-2 diluents. Reynolds et al. (1964) found NJ-2 to be very satisfactory for preservation of boar semen, the fertility being 46 per cent after storage for 2 to 96 hours.

MATERIALS AND METHODS

MATERIALS AND METHODS

Semen samples for the study were collected from the bulls maintained at the Artificial Insemination Centre attached to the College of Veterinary and Animal Sciences, Mannuthy, Trichur. Three breeding bulls (Nos. HT 166, 359 and AJ 204) in the age group of two to six years maintained under identical conditions of feeding and management were selected. The semen samples were collected by means of artificial vagina and were subjected to routine evaluation. Samples with good initial motility alone were used for the study. Altogether 42 samples were collected from the three bulls.

Using split sample technique, the semen was extended in egg yolk citrate (EYC- control) diluent and coconut milk extender (CME - experimental) at the ratio 1:25 to 1:30 and 1:50 to 1:80 respectively. The samples extended in EYC were gradually cooled and stored in a refrigerator at 5°C. All the samples diluted with CME were filled in 1 ml black plastic vials, tightly capped and stored in a dark box at room temperature. Both samples were preserved upto 96 hours. Samples extended in EYC served as the control for the experiment. All the samples were examined for motility at intervals of

24 hours and those sample having progressive motility of more than 40 per cent alone were used for insemination.

A quantity of 1 ml semen, either diluted with EYC or CME was used for each insemination. In all, 804 cows and 308 heifers were inseminated at the Artificial Insemination Centres at Mannuthy and Trichur, attached to Kerala Agricultural University during the period from July 1977 to February 1978. The cows inseminated were in the age groups of three to eight years and were not bred since last calving. The age of the heifers varied from two to four and half years and were all unbred. The animals chosen for the study were healthy and free from any apparent genital diseases. All the animals were inseminated at mid to late heat. Inseminations were done in alternate animals with CME or EYC extended semen observing strict principles of hygiene and sterilization.

All the animals were followed up between 60 to 90 days after insemination and pregnancy was confirmed by rectal examination. Data collected were tabulated and analysed according to Snedecor and Cochran (1967).

EYC diluent was prepared as per Roberts (1971) and CME was prepared according to Norman (1964).

In addition, fertility in terms of conception rate was studied from the field inseminations using semen extended in CME from bulls maintained at Bull Station, Dhoni. In all, data on 18245 first inseminations performed in 55 artificial insemination centres in Kottayam district during the period from March 1974 to November 1976 were analysed for the purpose. The semen samples collected from three Jersey bulls (Nos. 170, 99 and 96) and extended in CME and shipped by post from Bull Station, Dhoni, were utilized upto 96 hours of storage. The follow up results were arranged according to season, periods of storage and analysed. The data were subjected to analysis according to Snedecor and Cochran (1967).

RESULTS

RESULTS

Results of the study conducted to evaluate the fertility of semen extended in CME, for various periods of storage, to assess its utility for extensive field application are presented in Tables 1 to 15 and Figures 1 to 4.

Data regarding the overall conception rate of all animals inseminated with semen extended in CME and EYC are presented in Table 1. It could be seen from the table that out of 525 animals inseminated with semen extended in CME and stored upto 96 hours (experimental) 221 conceived at first insemination (42.09%) and out of 587 animals inseminated with semen extended in EYC (control) 288 (49.06%) conceived. The variation in the conception rate between the two diluents was not statistically significant.

The conception rate in cows inseminated with semen extended in CME and EYC and stored upto 96 hours are separately presented in Table 2. Perusal of data revealed that conception rates of 43.73 per cent and 50.34 per cent were obtained for cows inseminated with semen extended in CME and EYC respectively. The variation in the conception rate was not found to be statistically significant. Similarly the variation in the conception rate in heifers between

CME (38.0%) and EYC (45.56%) was also not statistically significant (Table 3).

Comparison of the conception rate between cows and heifers using CME is presented in Table 4. Out of the 375 cows inseminated 164 (43.73%) conceived while 57 heifers out of 150 (38.0%) conceived in the latter group. The variation in the conception rate betwen the two groups (Table 4) was found to be highly significant ($P < 0.01$). Similarly, significant difference was observed in the conception rate between cows (50.34%) and heifers (45.56%) with the use of EYC ($P < 0.01$) also (Tables 5 and 10).

The overall percentage of conception using semen diluted in CME and stored upto 24, 48, 72 and 96 hours of storage were 47.36, 47.25, 33.92 and 26.66 respectively. The corresponding values for EYC were 56.22 per cent, 50.48 per cent, 40.67 per cent and 29.54 per cent respectively (Table 6a and Fig. 1). Statistical analysis revealed that there is no significant difference in conception rate between CME and EYC for the same periods of storage.

Data on comparison between the overall conception rate with CME at different periods of storage is presented in Table 6b. It may be observed that there is significant difference in the conception rate between 0 to 24 hours and

73 to 96 hours (M.D. = 134.0692*) and 25 to 48 hours and 73 to 96 hours (M.D. = 139.76**) of storage. But no significant difference was observed between 0 to 24 hours and 25 to 48 hours (M.D. = 5.6974), 0 to 24 hours and 49 to 72 hours (M.D. = 79.09), 25 to 48 hours and 49 to 72 hours (M.D. = 84.78) and 49 to 72 hours and 73 to 96 hours (M.D. = 54.97) of storage of semen.

Statistical analysis of overall conception rate using EYC between different time interval of storage has revealed that there is significant difference in the conception rate between 0 to 24 hours and 49 to 72 hours (M.D. = 101.7739*), 0 to 24 hours and 73 to 96 hours (M.D. = 185.5114**) and 25 to 48 hours and 73 to 96 hours (M.D. = 172.4321**) old semen. There was no significant difference between 0 to 24 and 25 to 48 hours (M.D. = 13.0793), 25 to 48 hours and 49 to 72 hours (M.D. = 88.6946) and 49 to 72 hours and 73 to 96 hours (M.D. = 83.73) of storage (Table 6c).

The percentage conception in cows with CME diluted semen upto 24, 48, 72 and 96 hours of storage were 49.12, 47.76, 36.58 and 31.11 respectively. The corresponding values for EYC were 58.64, 50.33, 41.37 and 31.03 (Table 7 and Fig. 2). Statistical analysis revealed that there is no significant difference between conception rate in cows inseminated with semen extended in CME, EYC at the same time

interval of storage.

The conception rate in heifers with CME diluted semen upto 24, 48, 72 and 96 hours of storage were 43.85 per cent, 45.83 per cent, 26.66 per cent and 13.33 per cent and values for EYC were 49.09 per cent, 50.87 per cent, 38.70 per cent and 26.66 per cent respectively (Table 8 and Fig. 3). Analysis of the data revealed no significant difference between conception rate using CME and EYC at the same time interval of storage, in heifers.

Altogether 412 inseminations with the semen of bull No. HT 166; 356 inseminations with the semen of bull No.359; and 344 inseminations with the semen of bull No. AJ 204 were done. The number of animals conceived were 180 (43.68%), 172 (48.31%) and 157 (45.63%) respectively. The variation in the fertility rate between the bulls was not found to be significant (Tables 9 and 10).

The rate of conception for field inseminations with CME semen is presented in Table 11. Out of a total 18245 cattle inseminated 6096 conceived (33.41%). On comparison of this conception rate with the conception rate for experimental insemination (Table 1) it was found that the former was significantly lower ($P < 0.01$).

The overall percentage conception using semen extended in CME at Mannuthy (experimental) upto 24, 48, 72 and 96 hours of storage were 47.36, 47.25, 33.92 and 2666 respectively. The corresponding data from the field were 47.26 per cent, 39.18 per cent, 33.17 per cent and 18.28 per cent respectively (Table 12a). Statistical analysis revealed that there is highly significant difference (P $\angle$0.01) in conception rate between field and experimental data on CME diluted semen at the same time interval of storage (Tables 12a and 15).

Statistical analysis of the overall conception rate at different periods of storage in the field (Table 12b) revealed that there was significant difference in the conception rate between 0 to 24 hours and 49 to 72 hours (M.D. = 308.9201*), 0 to 24 hours and 73 to 96 hours (M.D. = 335.5654*), 25 to 48 hours and 73 to 96 hours (M.D. = 586.8603**) and 49 to 72 hours and 73 to 96 hours (M.D. = 644.4855**) of storage. There was no significant difference between 0 to 24 hours and 25 to 48 hours (M.D. = 251.2949) and 25 to 48 hours and 49 to 72 hours (M.D. = 57.6252) of storage.

The conception rate with CME extended semen between seasons was compared and are presented in Table 13 and figure 4. The overall conception rate during summer (February to May) rainy season (June to October) and winter

(November to January) was 34.16 per cent, 34.19 per cent and 31.12 per cent respectively. The variation in the conception rate between summer and rainy seasons was not significant. However, between summer and winter, and rainy season and winter, the variation in the conception rate was significant ($P < 0.01$).

Analysis of the conception rate obtained using CME extended semen from bulls (Nos. 170, 99 and 96) showed that the fertility rate of the bulls was 34.24 per cent, 32.54 per cent and 32.63 per cent respectively. The variation in the fertility rate between the three bulls was observed to be highly significant ($P < 0.01$) (Tables 14 and 15).

Table 1. Comparison of overall conception rate using semen extended in EYC and CME and stored for 96 hours.

Extender used	Number inseminated	Number pregnant	Percentage conception
CME	525	221	42.09
EYC	587	288	49.06

C.D. = 50.30 (at 5% level) Mean difference 23.56

* Will denote significant difference.
** Will denote highly significant difference.

Table 2. Comparison of conception rate in cows using semen extended in CME and EYC and stored for 96 hours.

Extender used	Number inseminated	Number pregnant	Percentage conception
CME	375	164	43.73
EYC	429	216	50.34
C.D. = 71.14 (at 5% level)		Mean difference	27.99

Table 3. Comparison of conception rate in heifers using semen extended in CME and EYC and stored for 96 hours.

Extender used	Number inseminated	Number pregnant	Percentage conception
CME	150	57	38.00
EYC	158	72	45.56

C.D. = 71.14 (at 5% level) Mean difference 19.12

Table 4. Comparison of conception rate with CMB between cows and heifers.

Group	Number inseminated	Number pregnant	Percentage conception
Cows	375	164	43.73
Heifers	150	57	38.00
C.D. = 95.05 (at 1% level)		Mean difference	99.59**

Table 5. Comparison of conception rate with EYC between cows and heifers.

Group	Number inseminated	Number pregnant	Percentage conception
Cows	429	216	50.34
Heifers	158	72	45.56
C.D. = 95.05 (at 1% level)		Mean difference 108.47**	

Table 6 (a). Comparison of overall conception rate in cattle with CME and EYC within the same periods of storage.

Extender	Age of semen in hours											
	0 - 24			25 - 48			49 - 72			73 - 96		
	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception
CME	171	81	47.36	182	86	47.25	112	38	33.92	60	16	26.66
EYC	217	122	56.22	208	105	50.48	118	48	40.67	44	13	29.54
Mean difference			46.7867			28.0100			24.1035			4.6555

C.D. = 100.6238 (at 5% level).

Fig. 1. Comparison of Overall Conception rate in Cattle with C.M.E. and E.Y.C within the Same periods of Storage.

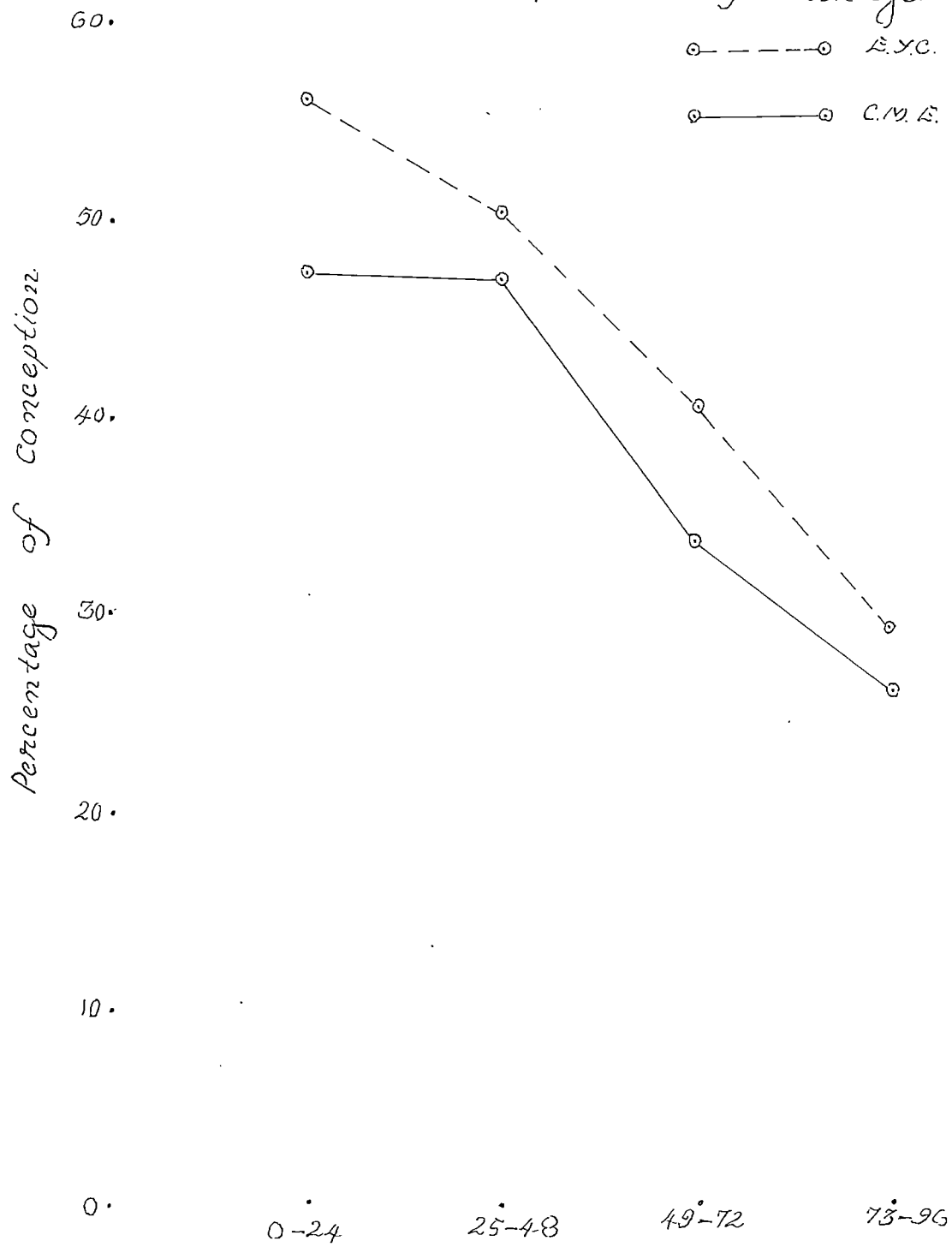

Table 6 (b). Comparison of the mean difference (M.D.) of overall conception rate with CMB between different periods of storage.

Age of semen in hours	0 - 24	25 - 48	49 - 72	73 - 96
0 - 24		5.6974	79.09	134.0692*
25 - 48			84.78	139.76**
49 - 72				54.97
73 - 96				

C.D. = 100.6238 (at 5% level).
C.D. = 134.42 (at 1% level).

Table 6 (c). Comparison of the mean difference of overall conception rate with EYC between different periods of storage.

Age of semen in hours	0 - 24	25 - 48	49 - 72	73 - 96
0 - 24		13.0793	101.7739*	185.5114**
25 - 48			88.6946	172.4321**
49 - 72				83.73
73 - 96				

C.D. = 100.6238 (at 5% level).
C.D. = 134.42 (at 1% level).

Table 7. Comparison of conception rate in cows with CME and EYC within the same periods of storage.

Extender	Age of semen in hours											
	0 - 24			25 - 48			49 - 72			73 - 96		
	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception
CME	114	56	49.12	134	64	47.76	82	30	36.58	45	14	31.11
EYC	162	95	58.64	151	76	50.33	87	36	41.37	29	9	31.03
Mean difference			90.0891			28.9218			23.0397			30.0628

C.D. = 142.30 (at 5% level).

Fig. 2. Comparison of Conception rate in Cows with C.M.E. and E.Y.C. within the same periods of Storage.

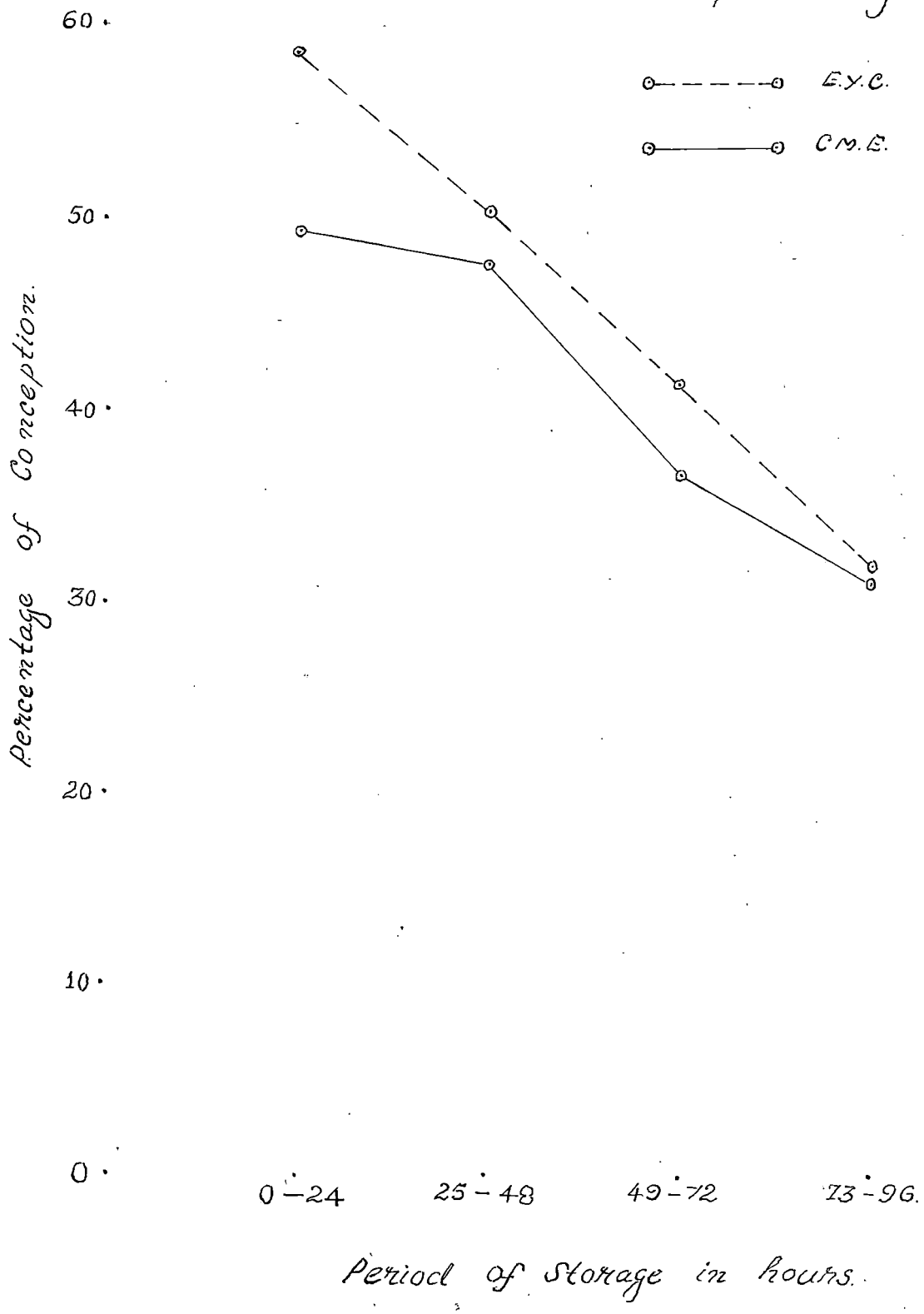

Table 8. Comparison of conception rate in heifers with CME and EYC within the same periods of storage.

Extender	Age of semen in hours											
	0 - 24			25 - 48			49 - 72			73 - 96		
	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception
CME	57	25	43.85	48	22	45.83	30	8	26.66	15	2	13.33
EYC	55	27	49.09	57	29	50.87	31	12	38.70	15	4	26.66
Mean difference			3.4843			27.0980			25.1672			20.7517

C.D. = 142.30 (at 5% level).

Fig 3 Comparison of Conception rat
in heifers with C.M.E. and E.Y.C.
within the same periods of Stor

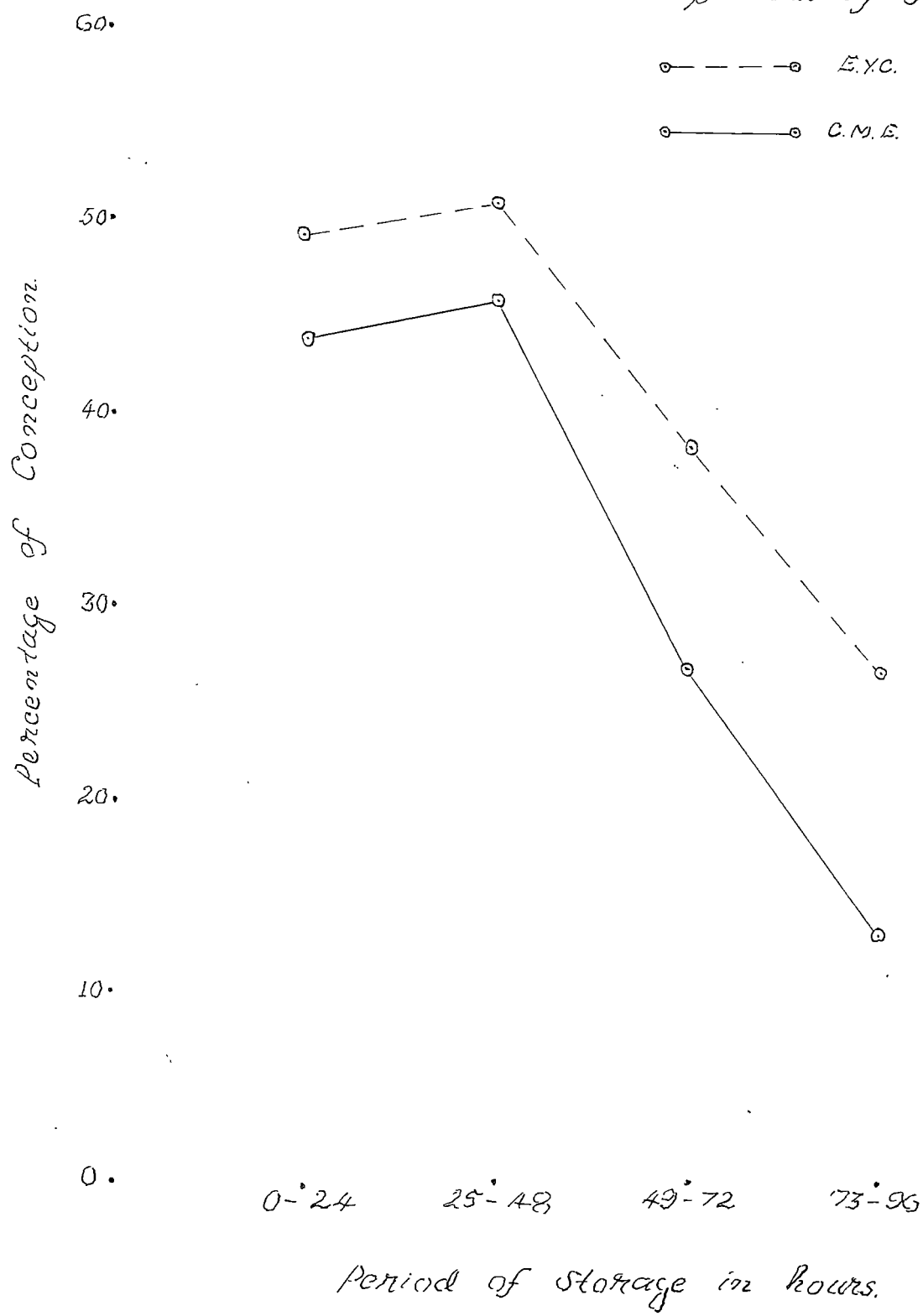

Table 9. Comparison of fertility rate between bulls.

Bulls used	Number inseminated	Number pregnant	Percentage conception
HT 166	412	180	43.68
359	356	172	48.31
AJ 204	344	157	45.63

C.D. = 61.61 (at 5% level)

Comparison:

HT 166 Vs 359 = 6.5124
HT 166 Vs AJ204 = 13.4865
359 Vs AJ204 = 6.98

Table 10. Analysis of variance table of the percentage conception pertaining to CME and EYC.

Source	df	SS	MSS	F
Between bulls	2	1455.9589	727.97	0.1591
Between extenders within bulls	3	13720.7982	4573.59	0.2039
Between cow and heifer within extender within bulls	6	134580.2184	22430.03	3.0700*
Error	36	263022.4527	7306.17	
Total	47	412779.4282		

Table 11. Comparison of overall conception rate with semen extended in CME between experimental and field data.

Group	Number inseminated	Number pregnant	Percentage conception
Experimental	525	221	42.09
Field	18245	6096	33.41
C.D. = 747.2822 (at 1% level)		Mean difference	1057.9089**

Table 12 (a). Comparison of conception rate with CME extended semen at Mannuthy (Experimental data) and Kottayam District (Field data) within the same periods of storage.

Centres	Age of semen in hours											
	0 - 24			25 - 48			49 - 72			73 - 96		
	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception	Number inseminated	Number pregnant	Percentage conception
Mannuthy (Exptl.)	171	81	47.36	182	86	47.25	112	38	33.92	60	16	26.66
Kottayam (Field)	2744	1297	47.26	4938	1935	39.18	6260	2077	33.17	4303	787	18.28
Mean difference			929.6793**			1170.9224**			1349.3208**			781.7114**

C.D. = 362.7459 (at 1% level).

Table 12 (b). Comparison of the mean difference of overall conception rate with CME between different periods of storage (Field data).

Age of semen in hours	0 - 24	25 - 48	49 - 72	73 - 96
0 - 24		251.2949	308.9201*	335.5654*
25 - 48			57.6252	586.8603**
49 - 72				644.4855**
73 - 96				

C.D. = 262.3045 (at 5% level).
C.D. = 362.7459 (at 1% level).

Table 13. Comparison of the conception rate with CME extended semen between seasons (Field data - 1974-76).

Season	Number inseminated	Number pregnant	Percentage conception
Summer (February-May)	8694	2970	34.16
Rainy season (June-October)	4986	1705	34.19
Winter (November-January)	4565	1421	31.12
Total	18245	6096	33.41

Chisquare = 10.020 (2 df) There is significant difference between the three groups ($P < 0.01$).

Comparison:

Summer	Vs	Rainy season	Chisquare = 0.002 There is no significant difference.
Summer	Vs	Winter	Chisquare = 8.734 There is significant difference ($P < 0.01$).
Rainy season	Vs	Winter	Chisquare = 7.034 There is significant difference ($P < 0.01$).

Fig. 4. Comparison of Conception rate with C.M.E. extended Semen between Seasons.

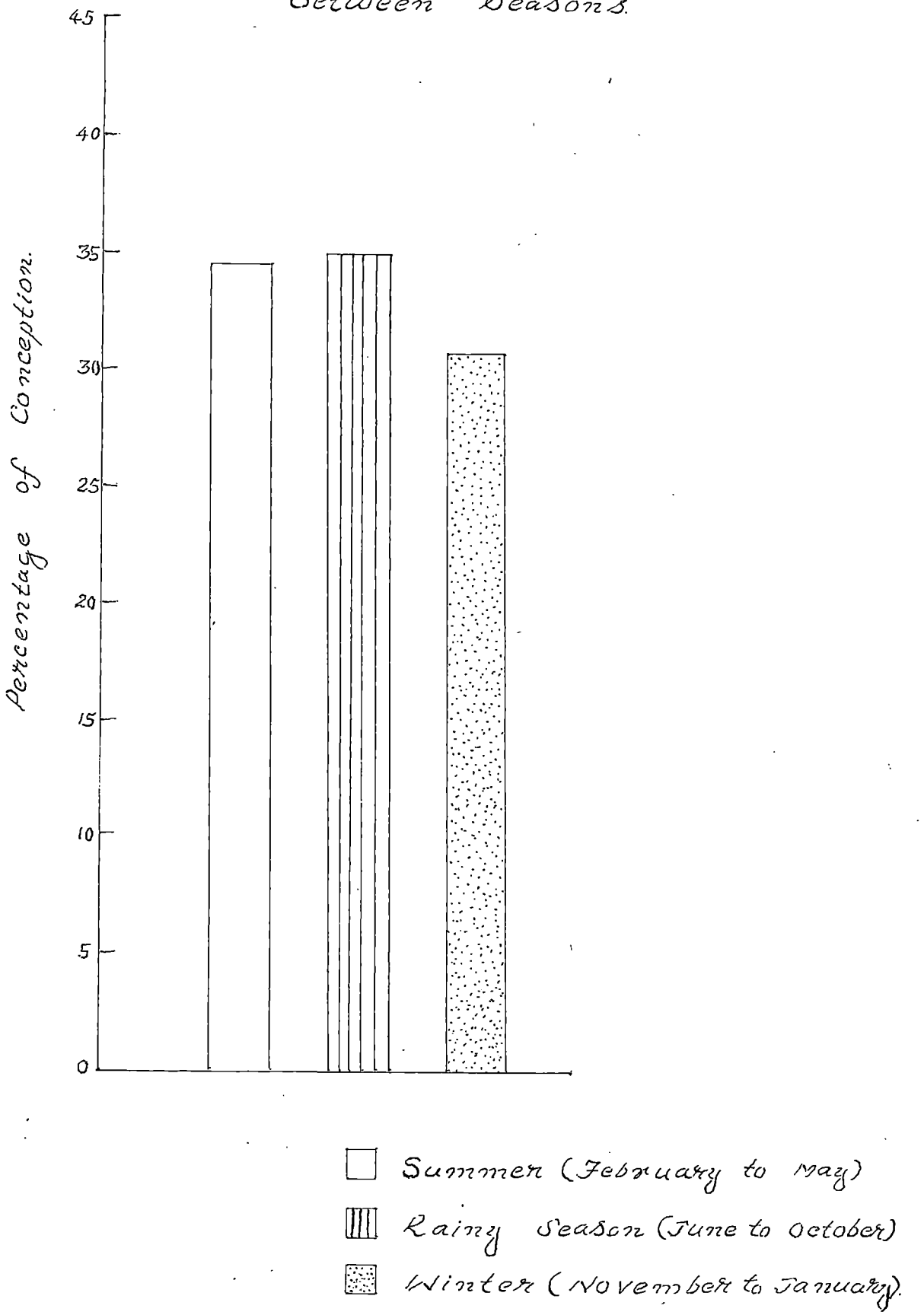

Table 14. Comparison of fertility rate between bulls (Field data).

Bulls used	Number inseminated	Number pregnant	Percentage conception
170	9182	3144	34.24
99	6636	2160	32.54
96	2427	792	32.63

C.D. = 227.1624 (at 5% level).
C.D. = 314.1472 (at 1% level).

Comparison:

170	Vs	99	=	298.0128*
170	Vs	96	=	863.8733**
99	Vs	96	=	565.8605**

Table 15. Analysis of variance table of percentage conception pertaining to CME (Field data) and CME (Experimental data).

Source	df	SS	MSS	F
Between places	1	6715027.5499	6715027.5499	17.4055*
Between bulls within places	4	1543194.2304	385798.5576	16.9756**
Between periods	3	570416.4698	190138.8232	8.3663**
Error	15	340900.4159	22726.6943	
Total	23	9169538.6660		

DISCUSSION

DISCUSSION

Egg yolk-citrate diluent is one of the most widely used extenders for dilution and storage of semen. Coconut milk extender has an advantage over egg yolk-citrate diluent, in that, the semen can be preserved under room temperature for an extended period of time without substantial reduction in motility. This potentiality can be exploited for wider application of artificial insemination programme, under the rural conditions existing in our country, provided a fairly high fertility of semen is assured. Though there are ample data on the motility of semen extended in CME, there is dearth of information on the fertility of semen at various intervals of storage. Hence the present work was undertaken to fill up this void.

Perusal of the data (Table 1) revealed that the overall conception rate (CR) of animals inseminated with CME semen stored for 96 hours was 42.09 per cent compared to 49.06 per cent with EYC. Thus the conception rate for CME semen was apparently lower by 6.97 per cent than that for EYC semen. This apparent reduction was noticed both in heifers and cows, when the fertility was separately assessed (Tables 2 and 3). However, on statistical analysis, the difference in the conception rate between CME and EYC was found to be not significant.

The results, presently observed with CME, are almost in agreement with the findings of earlier workers (Norman, 1964 and Norman et al. 1962 and 1968). Smith (1964) reported a conception rate of 51.2 per cent with CME extended semen, air lifted to Kenya from U.S.A. and stored for 8 to 12 days. Grove (1965) recorded 50 per cent conception rate for CME semen stored upto five days, and this is comparable with the present findings. However, certain reports with high non-return rate for inseminations with CME semen are also on record (Norman, 1961; Grove, 1968 and Norman et al. 1960). Literature on conception rate with semen extended in CME and EYC using split sample technique is found wanting. In the present study, it is observed that semen extended in CME gives comparable conception rate with that extended in EYC. Earlier published reports on the fertility of CME semen are mostly based on non-return rate. The reason for a comparatively low conception rate in the present work than many of the published reports may be due to the fact that the assessment is based on actual pregnancy.

The conception rate varied in cows and heifers in both extenders. In CME, out of 375 cows inseminated, 164 (43.73%) conceived as against 57 in 150 (38%) heifers. The variation in the conception rate between the two groups (Tables 4 and 10)

was found to be significant (P $<$0.01). Similarly, there was significant difference (P $<$0.01) in the conception rate between cows (50.34%) and heifers (45.56%) with the use of EYC (Tables 5 and 10). This is in keeping with the earlier reports of Ahmed and Tantowi (1959), Hafez (1968) and Roberts (1971) who have observed higher number of services per conception in heifers than in cows. Morrow (1969), Morrow et al. (1970) and Namboodiripad and Raja (1976) reported higher occurrence of ovulatory disturbances in heifers. Donaldson (1976) also reported higher conception rate in cows than in heifers. Erb and Holtz (1958) reported a lower fertility in heifers due to higher percentage of early embryonic loss. The difference in the conception rate currently observed in these two groups might be due to the above factors.

The present study revealed no significant difference in the conception rate between CME and EYC, between same time interval of storage upto 96 hours (Tables 6a, 7 and 8). However, for each 24 hours interval of storage, higher conception rate was observed in EYC. El-Wishy (1976) reported higher conception rate for frozen semen when compared with CME of different days of storage. Literature on conception rate of CME in comparison with EYC at different time interval of storage is scanty.

The comparison of conception rate between CME and EYC shows no significant difference between 0 to 24 hours and 25 to 48 hours; between 25 to 48 hours and 49 to 72 hours and between 49 to 72 hours and 73 to 96 hours of storage. In CME there was no significant difference in conception rate between 0 to 24 hours and 49 to 72 hours while significant difference was observed in EYC. In both CME and EYC there was difference in the conception rate between 0 to 24 hours and 73 to 96 hours and between 25 to 48 hours and 73 to 96 hours of storage (Tables 6b and c). This shows a definite fall in the conception rate in CME after 72 hours and in EYC after 48 hours of storage of semen. The rate of fall in the conception rate was even in EYC. In CME, the conception rate in 0 to 24 hours and 25 to 48 hours was found to be the same. After 48 hours, it decreased at a faster rate. The maximum rate of fall in the conception rate in CME was observed between 25 to 48 hours (Tables 6a, 7 and 8). After 72 hours, the rate of fall was found to be decreased in CME unlike in EYC in which the rate was increasing from 0 to 24 to 73 to 96 hours of storage in a more or less steady rate. At 73 to 96 hours of storage interval, the conception rate of CME decreased upto 26.66 per cent. The present finding is not in agreement with the reports of Norman and Rao (1972) and El-Wishy (1976) who have reported higher conception rate upto

four days and seven days respectively. Malmberg and Israelsson (1972) observed marked drop in the conception rate after four days of storage.

In heifers, a rise in the conception rate was observed in CME and EYC at 25 to 48 hours compared to 0 to 24 hours of storage (Table 8). Sakala and Turecek (1957) and Bawa et al. (1968) reported higher conception rate on second day than on first day in EYC. Salisbury (1968) reported a rise in conception rate in cattle inseminated with two day old semen and attributed this to an in vitro conditioning or maturation reaction which temporarily improved the ability of the sperm to effect syngamy. Norman and Rao (1972) reported higher conception rate on the second day of storage of CME semen. The present findings are in general agreement with Salisbury (1968) and Norman and Rao (1972).

Bonadonna (1951) and Diazoyarzun (1955) reported significant difference in conception rate on semen, of different periods of storage and observed better results with semen stored for about 24 hours. Campbell (1953) and Fryer et al. (1958) reported that the average rate of fall in the conception rate for each day of storage of semen upto four days varied from 3.4 to 8.3 per cent and 4 to 8 per cent units respectively. Mathai et al. (1970) observed that the fertility of semen declined on an average by 3.68 per cent for every

ten hours of storage upto 80 hours. In the present study the average reduction in conception rate per unit time of 24 hours of storage is 6.9 per cent in CME. The present findings compare favourably with these reports.

A difference in the fertility rate of bulls was observed in the present study but was not significant statistically. This difference in the fertility between the bulls might be attributed to the variation in the ability of their spermatozoa to withstand storage. This is in agreement with the findings of Roslanowski (1966).

Analysis of the results of a sizable number of field inseminations with CME semen (Table 11) revealed that the conception rate was 33.41 per cent compared to 42.09 per cent with experimental insemination. Statistical analysis showed significant difference ($P < 0.01$) in the fertility between the two groups (Table 11). Significant difference ($P < 0.01$) was observed between experimental and field data at the same time interval of storage upto 96 hours (Tables 12a and 15). For each 24 hours interval, experimental data recorded a higher conception rate. Norman and Rao (1972), Malmberg and Israelsson (1972) and El-Wishy (1976) reported higher conception rate with CME than in the present work. The reason for obtaining comparatively low conception rate in the field

might be due to sperm damage during transport and poor technical skill of lay inseminators.

Comparison of experimental and field data between different time interval of storage (Tables 6b and 12b), revealed no significant difference in the conception rate between 0 to 24 hours and 25 to 48 hours, and between 25 to 48 hours and 49 to 72 hours. However, field data showed a significant difference between 49 to 72 hours and 73 to 96 hours of storage. This was not observed in the experimental data. Conception rate was significantly different in both, when comparison was made between 0 to 24 hours and 73 to 96 hours and between 25 to 48 hours and 73 to 96 hours. Significant difference in the conception rate was observed between 0 to 24 hours and 49 to 72 hours of storage in the field data unlike the experimental data. While the maximum rate of fall in the experimental data was noticed between 25 to 48 hours and 49 to 72 hours of storage (13.33%), this was observed in the field data between 49 to 72 hours and 73 to 96 hours of storage (14.89%). Norman and Rao (1972) and Malmberg and Israelsson (1972) reported that the conception rate in CME dropped markedly after four days of storage. Norman et al. (1962) reported that the fertilizing ability of CME semen diminished with age abruptly after third day. The result observed from the present work would support this view.

For the analysis of the data from the field, the year was divided into three seasons as per Singh et al. (1965) viz., Summer (February to May), Rainy season (June to October) and Winter (November to January). On analysis, significant difference was observed in the conception rate between the three seasons (Table 13). Maximum conception rate was obtained in rainy season (34.19%) and minimum (31.12%) in winter. The results observed in the present study is in conformity with that of earlier workers (Maule, 1962; Cicardi and Magnani, 1963; Kale, 1963; Kirjusina, 1966; Roslanowski, 1966 and Bawa et al. 1968). In summer and winter, the fluctuation in the ambient temperature at different time intervals is very high unlike in rainy season. This fluctuation in the atmospheric temperature may cause greater damage to spermatozoa. The availability of green grass during rainy season may improve the reproductive efficiency of animals. The higher conception rate observed in the present study during rainy season might thus be explained.

Statistical analysis revealed significant difference ($P < 0.01$) in the fertility of bulls used in the field (Tables 14 and 15). This observation is in agreement with the reports of Salisbury et al. (1952), Maule (1962) and Grove (1965) who have reported significant difference in the fertility of breeding bulls used for artificial insemination.

In conclusion, it might be stated that the conception rate obtained using CME semen stored upto 96 hours is comparable with EYC. Since a substantial drop in the conception rate has been recorded beyond 72 hours of storage, it is advisable to use CME semen within that period of storage.

Analyses of the data obtained from the field revealed that both season and individual bull influence conception rate. The comparative low conception rate observed in the field might be due to increased spermatozoal damage in transit and poor technical skill of the lay inseminators.

S U M M A R Y

SUMMARY

In the present investigation an attempt has been made to study the fertility of bull semen extended in CME at different periods of storage, and to assess its utility for extensive field application.

Semen samples were collected from three breeding bulls of two to six years of age, maintained at the Artificial Insemination Centre attached to the College of Veterinary and Animal Sciences, Mannuthy. The semen was extended in CME and EYC at the ratio 1:50 to 1:80 and 1:25 to 1:30 respectively using split sample technique. While the semen extended in EYC was preserved at 5°C, the semen diluted with CME was kept under room temperature in a dark place. Semen samples upto 96 hours of age were used at a dose of 1 ml each per insemination.

A total of 804 cows and 308 heifers of breedable age brought to the Artificial Insemination Centres at Mannuthy and Trichur were inseminated alternately with CME and EYC extended semen. Out of these, 375 cows and 150 heifers were given first insemination with CME after confirming their sexual health status. The remaining 429 cows and 158 heifers were inseminated with semen extended in EYC. Pregnancy was confirmed by rectal examination between 60 to

90 days after insemination. To supplement the data, particulars of 18,245 inseminations from 55 artificial insemination centres in the field using CME semen stored upto 96 hours were also collected and analysed.

Out of 525 animals inseminated with semen extended in CME, 221 conceived at first insemination (42.09%) and out of 587 animals inseminated with semen extended in EYC, 288 (49.06%) conceived. The variation in the overall conception rate (6.97%) between the two was not significant.

Conception rates of 43.73 per cent and 50.34 per cent were obtained for cows inseminated with CME and EYC respectively. The percentage conception obtained for heifers with CME and EYC were 38 and 45.56 respectively. In both cows and heifers the difference in conception rate between CME and EYC was not found to be significant. Higher conception rate was recorded in cows than in heifers using both CME and EYC.

The over-all percentage of conception with semen diluted in CME at 24, 48, 72 and 96 hours of storage were 47.36, 47.25, 33.92 and 26.66 and the corresponding values for EYC were 56.22, 50.48, 40.67 and 29.54 respectively. Statistical analysis revealed no significant difference

between CME and EYC at similar intervals of storage. Similarly, no significant difference in the conception rate between CME and EYC was noticed at similar intervals of storage in cows and heifers assessed separately. In CME, no significant difference was observed in the conception rate upto 72 hours, but in EYC after 48 hours of storage, the difference in conception rate was found to be significant.

The fertility rate of bulls HT 166, 359 and AJ 204 was 43.68, 48.31 and 45.63 per cent respectively. The variation in the fertility rate between the bulls was not found to be significant.

Comparison of conception rate obtained for experimental inseminations (42.09%) and field inseminations (33.41%) using CME semen stored upto 96 hours revealed significant reduction in the latter.

The overall percentage conception using CME at Mannuthy (experimental) within 24, 48, 72 and 96 hours of storage were 47.36, 47.25, 33.92 and 26.66 respectively and the corresponding data from the field were 47.26, 39.18, 33.17 and 18.28 respectively. Statistical analysis revealed that there is highly significant difference in the conception rate between field and experimental data on CME extended semen

at the same time interval of storage. Analysis of the field data also revealed a significant fall in the conception rate after 48 hours of storage, while in the experimental data this was observed only after 72 hours.

The conception rate during summer (February to May), rainy season (June to October) and winter (November to January) was 34.16, 34.19 and 31.12 per cent respectively. The variation in the conception rate between summer and rainy season was not found to be significant but significant variation was observed between summer and winter and between rainy and winter seasons. Thus the conception rate for CME semen in the present study was found to be maximum in rainy season and minimum during winter season.

The fertility rate of the bulls Nos. 170, 99 and 96 used in the field during the study were 34.24 per cent, 32.54 per cent and 32.63 per cent respectively. This, on analysis, was found to be significantly variant.

To conclude, it may be stated that semen extended in CME can be used upto 72 hours of storage without substantial loss in the fertility.

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FERTILITY STUDIES OF SEMEN PRESERVED IN COCONUT MILK EXTENDER

BY

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ABSTRACT OF A THESIS

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ABSTRACT

The object of the study was to assess the fertility of semen extended in CME at various periods of storage in comparison with semen extended in EYC. Semen samples collected from three breeding bulls maintained at the Artificial Insemination Centre, Mannuthy were diluted with CME and EYC using split sample technique and stored at room temperature and at 5°C respectively. Both the samples were preserved upto 96 hours and utilized for the study. A total number of 1112 animals were utilized for the fertility evaluation. Alternate animals were inseminated with semen extended in CME or EYC. Conception rates were estimated on the basis of rectal examination. Data from the field comprising of 18,245 first inseminations with CME from 55 artificial insemination centres in Kottayam district were also incorporated in the study.

The overall conception rate did not vary significantly between CME and EYC extended semen. The conception rate in cows was significantly higher than in heifers both in CME and EYC. Within the same period of storage, conception rate between CME and EYC was not found significant. In EYC, a significant fall in the conception rate was observed after

48 hours of storage but in CME this was noticed only after 72 hours of storage. The variation in the fertility rate between the bulls was not found to be significant.

Higher conception rate was observed in the experimental animals than in the field. Significant fall in the conception rate was observed in the field after 48 hours of storage. Seasonal variation in the conception rate was observed, with higher percentage conception in rainy season (June to October) and lower in winter (November to January). Highly significant difference in the fertility of bulls used in the field was also observed.

In conclusion, it may be stated that semen preserved in CME can be used upto 72 hours of storage time without substantial reduction in the fertility.