Studies on The Comparative Efficacy of Different Semen Diluents in Respect of Livability and Morphology of Buffalo Spermatozoa

Thesis

SUBMITTED TO THE FACULTY OF VETERINARY SCIENCE,

Rajendra Agricultural University, Bihar

IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE (VETERINARY)

1971

BY

Brajesh Kumar Prasad Sinha

B. V. Sc. & A. H. (Ran.)

Post-Graduate Department of Gynaecology and Obstetrics

BIHAR VETERINARY COLLEGE,

P A T N A.

Dr. S. M. Ishaque

G. B. 'O'. V. C. (Pat.), P. G. (Germany), F. R. V. C. S, (Sweden), Professor of Gynaecology and head, Post-graduate Department of Gynaecology, Ex-Dean, Faculty of Veterinary Science & Principal, Bihar Veterinary College, and Director, Animal Husbandry Education & Research Rajendra Agricultural University.

Bihar Veterinary College,
PATNA

CERTIFICATE

This is to Certify that **Dr. Brajesh Kumar Prasad Sinha** *has worked under my supervision and guidance for his Thesis entitled* "**Studies on the Comparative Efficacy of Different Semen Diluents in Respect of Livability and Morphology of Buffalo Spermatozoa**" *for the degree of M.Sc. (Vet.) in Gynaecology, Obstetrics and Artificial Insemination of Rajendra Agricultural University. The material incorporated in this Thesis is his own work and I have checked up his results from time to time during the course of Academic year 1970-71.*

S. M. Ishaque 10-3-72

PATNA, the 10th March, 1972.

(**S M. Ishaque**)

A C K N O W L E D G E M E N T

The author feels immense pleasure in expressing his sincerest and deepest sense of gratitude to his revered teacher, Dr. S.M. Ishaque, G.B. 'O' V.C. (Pat.), P.G. (Germany), F.R.V.C.S. (Sweden), Post-graduate Professor of Gynaecology, Obstetrics and A.I., Ex-Principal, Bihar Veterinary College and Director, Veterinary Education and Research, Rajendra Agricultural University, Bihar, Patna for suggesting the problem invaluable advice in planning, conducting and interpretating the results reported in this study and competant review of the manuscript. He not only initiated and directed the work but also was a constant source of encouragement to me throughout the course of the present investigation. All that may be of merit to this thesis owes much to his inspiring guidance and constructive criticism.

Thanks are due to Dr. R.C.P. Yadav, M.S., Ph.D. (Mich., U.S.A.), Principal, Bihar Veterinary College and Director, Livestock Research Station, Bihar, Patna for providing necessary facilities during the period of present work.

The author is indebted to Shri R.J. Prasad, B.V.Sc. & A.H., P.G. (Statis.), Assistant Professor for his valuable suggestions and guidance on statistical analysis of the experimental data. Besides, the author is thankful to Shri Vijaya Kumar Sinha, Statistical Assistant, Animal Husbandry Department, Bihar for his help in the statistical analyses of the results.

The author can not check his feelings without expressing his thanks to Dr. Sachida Nand Sinha, M.Sc. (Vet.), Junior Assistant Research Officer, L.R.S., Bihar, Patna for his sustained interest and valuable guidance.

Sincere thanks are due to Dr. S. Bhagat, Artificial Insemination Officer, Semen Bank, Patna and other staff for their help and cooperation in completion of work.

Thanks are also due to the members of the staff of the Gynaecology Department, Bihar Veterinary College, Patna for their cooperation and help in my day to day routine work.

Special gratitudes go to my parents under whose kind shelter the author could carry out his study smoothly.

(Author)

C_O_N_T_E_N_T_S

			Page No.
1.	INTRODUCTION	...	1 - 4
2.	REVIEW OF LITERATURE	...	5 - 37
	A general review of semen diluent	...	5
	Diluents used for the preservation of buffalo spermatozoa	...	17
	Antibacterial agents	...	27
	Measurements of spermatozoa	...	34
3.	MATERIALS AND METHODS	...	38 - 48
	Preparation of dilutors	...	39
	Dilution of the semen	...	43
	preservation of the semen	...	44
	Percentage of live and dead spermatozoa	...	45
	Measurement of head length and head breadth of spermatozoa	...	46
	Statistical analysis	...	47
4.	RESULTS	...	49 - 78
	Effect on live percentage of spermatozoa	...	49
	Head length	...	52
	Head breadth	...	55
5.	DISCUSSION	...	79 - 86
	Live percentage of spermatozoa	...	79
	Morphology of spermatozoa	...	83
6.	SUMMARY	...	87 - 89
7.	BIBLIOGRAPHY	...	90 -104

LIST OF PHOTOGRAPHS AND FIGURES

MICRO-PHOTOGRAPHS

Plates

1	Measurement of head length of spermatozoa
2	Fresh buffalo bull spermatozoa
3	Spermatozoa diluted with whole milk showing fat globules
4	Buffalo bull spermatozoa diluted and preserved in EYGB diluent upto 96 hours showing no abnormality in the head.

Figures

1	Histogram showing variation in live spermatozoa per cent in different diluents at different hours of preservation.
2	Histogram showing variation in the measurement of head length of spermatozoa in different diluents at different hours of preservation.
3	Histogram showing variation in the measurement of head breadth of spermatozoa in different diluents at different hours of preservation.

LIST OF TABLES

Table No.		Page No.
1.	Showing mean values of live spermatozoa (per cent) in fresh and preserved semen at zero hour ...	58
2.	Analysis of variance of live spermatozoa (per cent) in fresh and preserved semen at zero hour ...	58
3.	Showing mean values of live spermatozoa (per cent) in preserved semen at 24 hours ...	59
4.	Analysis of variance of live spermatozoa (per cent) of preserved semen at 24 hours ...	59
5.	Showing mean values of live spermatozoa (per cent) in preserved semen at 48 hours ...	60
6.	Analysis of variance of live spermatozoa (per cent) of preserved semen at 48 hours...	60
7.	Showing critical difference in live per cent of spermatozoa at 48 hours of preservation ...	61
8.	Showing mean values of live spermatozoa (per cent) in preserved semen at 72 hours...	62
9.	Analysis of variance of live spermatozoa (per cent) in preserved semen at 72 hours...	62
10.	Showing critical difference in live per cent of spermatozoa at 72 hours of preservation ...	63
11.	Showing mean values of live spermatozoa per cent in preserved semen at 96 hours ...	64

Table No.		Page No.
12.	Analysis of variance of live spermatozoa per cent of preserved semen at 96 hours ...	64
13.	Showing critical difference in live percentage of spermatozoa at 96 hours of preservation ...	65
14.	Showing mean values of head length (in microns) of fresh and preserved spermatozoa at zero hour ...	66
15.	Analysis of variance of head length of fresh and preserved spermatozoa at zero hour ...	66
16.	Showing mean values of head length (in microns) of preserved spermatozoa at 24 hours ...	67
17.	Analysis of variance of head length of preserved spermatozoa at 24 hours ...	67
18.	Showing mean values of head length (in microns) of preserved spermatozoa at 48 hours ...	68
19.	Analysis of variance of head length of preserved spermatozoa at 48 hours ...	68
20.	Showing mean values of head length (in microns) of preserved spermatozoa at 72 hours ...	69
21.	Analysis of variance of head length of preserved spermatozoa at 72 hours ...	69
22.	Showing mean values of head length (in microns) of preserved spermatozoa at 96 hours ...	70

Table No.		Page No.
23.	Analysis of variance of head length of preserved spermatozoa at 96 hours ...	70
24.	Showing critical difference within diluents at 96 hours of preservation ...	71
25.	Showing mean values of head length (in microns) of spermatozoa at different hours of preservation ...	72
26.	Analysis of variance of head length of spermatozoa at different hours of preservation ...	72
27.	Showing critical difference in head length at different hours of preservation ...	73
28.	Showing mean value for head breadth (in microns) of fresh and preserved spermatozoa at zero hour ...	74
29.	Analysis of variance of head breadth of fresh and preserved spermatozoa of zero hour ...	74
30.	Showing mean values for head breadth (in microns) of preserved spermatozoa at 24 hours ...	75
31.	Analysis of variance of head breadth of preserved spermatozoa at 24 hours ...	75
32.	Showing mean values for head breadth (in microns) of preserved spermatozoa at 48 hours ...	76
33.	Analysis of variance of head breadth of preserved spermatozoa at 48 hours ...	76
34.	Showing mean values of head breadth (in microns) of preserved spermatozoa a5 72 hours ...	77

Table No.		Page No.
35.	Analysis of variance of head breadth of preserved spermatozoa at 72 hours ...	77
36.	Showing mean values for head breadth (in microns) of preserved spermatozoa at 96 hours ...	78
37.	Analysis of variance of head breadth of preserved spermatozoa at 96 hours ...	78

****

LIST OF ABBREVIATIONS

AM	-	Antimeridiem
C	-	Centigrade
CR	-	Conception Rate
CM^3	-	Cubic Centimeter
CV	-	Coefficient of Variation
df	-	Degree of Freedom
EYCBGF	-	Egg yolk-citrate-potassium bicarbonate-glucose-fructose
EYCGGly	=	Egg yolk-citrate-glucose-glycine
EYCGGlyCy	-	Egg yolk-citrate-glucose-glycine-cysteine
EYGB	-	Egg yolk-glucose-bicarbonate
F	-	"F" test value
gm	-	Gram.
hr	-	Hour
hrs	-	Hours
mg	-	Milligram
ml	-	Millilitre
MS	-	Mean sum of squares
NS	-	Non-significant
pH	-	Hydrogen ion concentration
SE	-	Standard error
t	-	Temperature
ug	-	Microgram
µ	-	Micron
WM	-	Whole milk
*	-	Significant at 5 % level
**	-	Significant at 1 % level
%	-	Percentage

INTRODUCTION

INTRODUCTION

The domestic buffalo and domestic cattle have similar utility to man kind, yet the former has received, much less attention. Among the Indian farm animals, buffaloes are relatively of greater importance than any other species for the production of milk. In our country, the percentage of milking buffalo population is lesser than milking cow, yet their share of contribution towards total milk production is more. Amble et al.(1965) estimated per lactation milk yield of cows and buffaloes in India to be 173 kg and 491 kg respectively. Buffalo milk contains about twice as much butter fat as the cows milk under average conditions of management. Buffaloes are also valuable as a source of work, meat and leather and recognised as most docile. In view of the importance of buffalo in Indian form economy, the efforts to improve the quality of buffaloes for high production of milk and to augment their work potential will be a material contribution to the progress of the nation.

proper breeding of the livestock is one of the most important ways of bringing about improvement in domesticated animals as it is the best means for improving the genetic make up at a short span of time. Selective breeding of the buffalo for milk production has been shown to be highly profitable in India, which is evident from the establishment of milk colonies at Aarey, Bombay, Haringhata and remarkable productivity is being achieved by the Kaira District Cooperative, Gujrat.

Whatever may be the method of breeding, it is rather impossible to implement improvement programme with natural mating. The number of disease free bulls of improved genetic material is very meagre. The dearth of improved bulls needed for breeding large bovine population is to a greater extent fulfilled when artificial insemination is used as a tool. With the use of this zootechnique, bulls of superior genetic constitution are served to larger and widely spread population within a shorter period of time.

The objective of artificial insemination programme will be fulfilled only when optimum fertilizing power of spermatozoa is preserved for a certain period of time after collection. Optimum fertilizing capacity might be maintained by inhibition (by physical or chemical means) of all but the minimum cellular activity or by providing all of the essential ions, nutrients, enzymes, co-enzymes, vitamins and by continuous withdrawal of all end products which limit survival. Because of the relative ease, with which metabolic processes can be supressed by lowering the temperature, it is now almost universally employed to preserve spermatozoa. Thus, the semen is diluted and the diluent should provide utilisable non-toxic substrates for metabolism, substances protective against toxic end products and protection against cold-shock. Dilution is absolute necessary because the efficiency of semen as such is not much to maintain the longivity of spermatozoas due to insufficient concentration of organic metabolites and the buffering capacity of undiluted semen is not efficient to maintain it for longer period.

The semen diluents not only help in spermatozoal preservation, but maintain its fertility for longer periods. Diluted semen permits the full utilization of a proven sire for bringing conception in a large number of female. Dilution protects the spermatozoa from sudden temperature change, maintains the proper pH, controls bacterial growth and fascilitates semen transhipment.

The techniques followed in artificial insemination of buffalo are the same as in cattle but one of the major problems confronting the efficient utilization of buffalo semen is the difficulty of its '*in-vitro*' preservation over long period. The phenomenal success of artificial insemination in cattle is in no small measure due to the development of efficient semen diluents. Diluents used for the preservation of buffalo semen revealed that the survivability and metabolism of buffalo spermatozoa in common bovine diluents differed from bull spermatozoa.

Because of the discouraging results with egg yolk phosphate (Lardy and Phillips, 1939) and egg-yolk-citrate (Salisbury *et al.*, 1941) for storage of the dilution of buffalo semen, a group of investigators (Roy *et al.*, 1955; Srivastava and Prabhu, 1956; Gokhale, 1958; Singh and Tomar, 1959; Mahajan and Sharma, 1961; Tomar and Desai, 1961a, 1961b; 1961c, 1961d, 1961e; Kale, 1963; Sharma and Sengar, 1965; Kumar, 1966; Singh *et al.*, 1970 etc.) tried other dilutors. The observations made by various workers as to the suitability of diluents for buffalo semen, show great variations as to the relative merits under varied conditions and it is still a dielemma as how to evolve

REVIEW OF LITERATURE

A GENERAL REVIEW OF SEMEN DILUENT

The semen diluents available prior to 1939 served primarily as vehicles for increasing the volume of the ejaculate for immediate service and were not effective in preserving the fertilizing capacity for extended periods. They consisted primarily of sodium and potassium salts of phosphoric, sulfuric or tartaric acids, glucose and sometimes peptone or gelatin. Such diluents were of practical use where groups of females were assembled in one place and many were to be bred each day. The fertility level obtained with the then known diluents varied from 25 % to 45 % of inseminations resulting in conception (Salisbury and VanDemark, 1961).

The discovery of Lardy and Phillips (1939) of the value of hen's egg yolk against cold shock in bull semen diluents laid the foundation for the successful commercial expansion of artificial insemination. The active principle in yolk, responsible for the protective action against cold shock is now thought to be lecithin or a similar phospholipoid occurring either free or in combination with protein. In addition to this protective action against cold shock, egg yolk provides a variety of other vital substances like glucose, aromatic L-amino acids, vitamins and enzymes to sperm which support spermatozoal metabolism (Maule, 1962).

The original yolk diluent, described by Lardy and

Phillips (1939) consisted of equal volumes of egg yolk and a phosphate buffer (2.0 gm Na_2HPO_4 $12H_2O$, 0.2 gm KH_2PO_4 in 100 ml distilled water).

Salisbury (1941) considered the optimum storage temperature to be 5°C. Storage after dilution with the yolk-phosphate diluent gave better results than storage of undiluted sperm or dilution with the Russian diluent SCC-2. One important disadvantage of the yolk phosphate diluent was the presence of large fat globules, which made it impossible to see the individual spermatozoa under the microscope.

Yolk-citrate diluent was originally described by Salisbury et al. (1941), by giving a clear field, it permits the microscopic assessment of the motility of spermatozoa and had no adverse effect on semen metabolism. Equal volumes of egg yolk and of either 3.6 or 2.9 % solution of sodium citrate (Dihydrate) were used. Highly significant difference in motility was observed after longer periods of storage of semen with a mixture containing equal parts of an M/15 solution of sodium citrate or an M/15 solution of potassium hydrogen phosphate and of egg yolk. Actual insemination tests revealed no significant difference between spermatozoa stored in the citrate and in the phosphate diluents. They suggested that if the field tests were extended over a longer storage period, results of inseminations might establish the superiority of the citrate diluent as real.

Knoop (1941) suggested a semen diluent containing

2.14 gm of gelatin (Knox), which maintained some sperm motility for an average of 21.5 days.

Significance of phospholipids as a source of energy to spermatozoa was noted by Lardy and Phillips (1941). Phospholipids obtained from eggs, rat liver and soybean preserved the motility of spermatozoa under aerobic conditions.

The factor in egg yolk affecting the resistance of storage potentialities and fertilizing capacity of spermatozoa was studied by Mayer and Lasley (1945). An active fraction was obtained by successive extraction of dried egg yolk, which enabled 65 - 70 % of bull sperm to resist cold shock ($0^{\circ}C$ for 10 minutes), while whole egg yolk could only protect 50 - 55 %.

Romijn (1947) recommended dilution of 1 part semen to 3 parts of a diluting agent consisting of 1 part fresh egg yolk to 3 parts phosphate buffer. He also recommended the inclusion of small quantities of egg white in the buffer mixture, since it has a stimulating effect on sperm activity. The egg yolk of turkey and hen were found equally satisfactory, for the dilution of semen, by Basu and Berry (1948).

Almquist (1951) reported that the differences in the fertility percentages were non-significant when egg yolk-sodium citrate in the ratio 1:1, 1:3, 1:5 and 1:7 was used to extend the semen. Kok (1953) suggested that lipoprotien of egg yolk is also perishable and of variable quality. In addition, it may under certain circumstances possess spermicidal

properties and can cause an increase in sperm agglutination and dilution shock.

Salisbury (1957) reported that yolk-phosphate diluents depressing effect on spermatozoal motility and survival could adversely effect the fertility of highly diluted semen.

Duck's egg yolk was stated to be as satisfactory as hen's egg yolk, both for bull and buffalo semen by Venkataswami and Hanumantha Rao (1961). Lainck-Vissing (1961) concluded that the filtered whole egg spermasol T diluent (i.e. 60 parts whole egg and 100 parts spermasol T) maintains motility and length of survival time as well as the previously well tried egg yolk-spermasol T diluent (i.e. 20 parts egg yolk and 100 parts spermasol T), and, while not saving anything in cost, obviates the need for separating yolks and whites.

Nedyalkov and Stoyanova (1966) reported improved conception rate of cows when egg yolk was omitted from a glucose-citrate semen diluent. Intradermal testing showed that 14 of 22 infertile cows and heifers were allergic to egg yolk. Bratanov et al. (1968) also noted increased antigenic activity by the addition of egg yolk to semen and suggested that it may be related to some cases of sterility.

Glucose and Fructose Addition to the Diluent:

Salisbury and VanDemark (1945) demonstrated that the addition of glucose prolonged the life of spermatozoa

in-vitro, and stimulated the rate of glycolysis even when there was no shortage of glucose in the storage medium. Valerani (1948) confirmed slight increase in vitality and found no adverse effect on fertility with glucose addition.

Kampschmidt et al. (1951) concluded that metabolisable sugars were most satisfactory for replacing solutions of buffer salts in the diluting medium. However, reducing the proportion of buffer salt in the medium was beneficial only if a sufficient quantity remained to maintain an optimum pH level during the entire storage period. A diluting medium composed of one part of egg yolk and five parts of an isotonic mixture of one part $NaHCO_3$ solution (1.3 per cent) plus four parts glucose solution (5 per cent) gave better results than any dilutor studied. Kok (1952) observed superior results by adding fructose to the egg yolk citrate dilutor. Again, Kampschmidt et al. (1953) stressed the importance of glucose in diluting medium and stated that osmotic balance was maintained by a proportionate increase of glucose. These observations on spermatozoal survival was confirmed by Smith et al. (1954). Melrose and Stewart (1956) found in-vitro better survival of spermatozoa in glucose bicarbonate egg yolk buffer but there was a significant decrease in conception rate when compared with the standard yolk citrate buffer. They had also reported negative results with the addition of fructose (0.05 gm per 100 ml) to yolk citrate.

Sharma and Mahajan (1961a) observed that modifications of IVT diluent containing 0.9 % or 1.2 % glucose were significantly superior to those containing 0.3 % or 0.6 % glucose when the dilution rate was 1:20.

Martin and Emmens (1961) reported that the addition of fructose significantly improved motility percentage when deep frozen semen was thawed. Dimitropoulos (1961) noted that an egg yolk-citrate-glucose-fructose diluent (CGF) resulted in 5.5 % more conceptions than C.U.E. diluent. When CGF was compared to D11, which was composed of 2 parts egg yolk, 3 parts glucose + fructose and 5 parts citrate, they were found to be identical as regards survival and temperature resistance, but D11 averaged 2 % more non-returns to 1st insemination.

Andersen and Rottensten (1961) observed no difference between sodium citrate, C.U.E., or C.U.-16 diluent in spermatozoal viability upto 8 days of storage. The addition of glucose or fructose did not affect spermatozoal viability in any of the diluents.

Maule (1962) concluded that addition of glucose and/or fructose has not clearly shown to improve conception rates, although there is a beneficial effect on motility under certain conditions. The evidence indicated that the physical effects of the addition of these sugars on the maintenance of the correct osmotic tension and electrolyte balance is more important than their possible action by

increasing the carbohydrate available for utilisation by the spermatozoa.

Saxena and Singh (1967), investigating with Hariana bull semen, recorded least decrease in motility and live spermatozoa per cent in EYC, followed by CUE, SMY and EYGB.

Marczewski (1967) added fructose to a milk yolk glycerol diluent at the rates of 1000, 500, 750 and 1000 mg % respectively and noted improved over all conception rate. Better results were obtained with the addition of 500 and 750 mg % fructose than with 1000 mg % and it was suggested that the optimum amount of fructose required for the semen of individual bulls should be investigated.

Papov (1968) suggested that a diluent should contain 0.3 gm glucose, 2.21 gm sodium citrate, 0.25 gm ethylenediamine tetra-acetic acid, 0.32 gm argenine, 100,000 I.U. Penicillin, 100,000 I.U. streptomycin, 0.3 gm streptocid and 10 mg egg yolk in 100 ml distilled water.

Milk Diluents:

A German investigator, Kolliker was the first to publish (in 1856) on the use of milk as a bull semen diluent. At that time interest in the subject was limited, and since he studied the effect of a large number of substances and pursued none with an objective of prolonging sperm-cell life, his observations were not widely known.

In 1942, Underbjerg et al. compared the fertility of semen diluted with antoclaved milk, egg yolk phosphate and non-diluted bull spermatozoa. Comparable levels of fertility was found for each system of storage with a limited number of cows after storage of 24 - 123 hours at about 5°C. Fresh semen used without dilution gave higher conception rates than did the semen in either of the two diluents. The authors concluded that the diluents had no beneficial effect on fertility.

Michajilov (1950) reported that boiled, filtered milk gave highly satisfactory results at extension ratios upto 1:25. The boiling not only sterilized the milk, but also changed the lactose to glucose and galactose, both of which were more readily available for energy production of the sperm. In 1951, Thacker and Almquist observed motile spermatozoa in the boiled pasteurized, homogenized milk for periods approximately equal to those for spermatozoa in the egg yolk citrate control. However, motility was maintained only for 1 to 3 days, in the unboiled milks. Boiling of milk and skim milk for 10 minutes appeared to be adequate although no marked differences in sperm livability were obtained between intervals of 1, 5, 10 and 30 minutes or between boiled milks and yolk-citrate.

Almquist and Thacker (1952) reported that the semen diluted in boiled, homogenized milk with antibiotics averaged 4 % more conceptions than the same milk without antibiotics

and 7 % higher than the egg yolk-citrate control. It was found that addition of 1 % cooking cornstarch would increase the viscocity of milk so that it was easier to control during insemination process. Corn starch was non-toxic to spermatozoan motility survival and did not affect fertility significantly. Flerchinger et al. (1953) also found boiled homagenized milk to be significantly superior than egg yolk citrate diluent. In 1953, Thacker and Almquist obtained optimum spermatozoan livability when the milk was heated to 95°C for 1 to 10 minutes. Gentle boiling yielded better motility survival results than did vigorous boiling. Boiling milk in an open vessel for more than 10 minutes reduced the duration of motility, but heating as long as one hour in a covered double boiler was not harmful to livability. Reaching the proper temperature (92°C minimum) appeared to be a more critical consideration in obtaining maximum spermatozoan livability than the length of the heating period.

Collins (1953) compared evaporated milk with boiled homogenized whole milk and yolk citrate extender for bull semen dilutor. The mean per cent of motile spermatozoa, after 120 hours storage, was 32.9 for homogenized milk, 32 for yolk citrate and 29.5 for evaporated milk. The use of evaporated milk extender resulted in approximately 15 % lower fertility than that found with the other two extenders.

A comparison was made of yolk-phosphate and milk diluted semen by Dreher and Webb (1953). The milk diluted

semen resulted in a 69.8 per cent non-return whereas yolk phosphate diluted semen, resulted in a 59.2 % non-return. Based on the fertility results, Almquist et al. (1954) concluded that fresh, heated homogenized milk and fresh heated skim milk can be used as practical diluting fluids for bull semen. The addition of corn-starch to increase the viscocity of milk dilutor did not improve fertility and was found to be unnecessary.

The factor in unheated skim milk, which is toxic to spermatozoa, was identified by Thacker et al. (1954). The toxicity was associated with albumen containing fractions, which was eliminated by heating at 92°C for 10 minutes. Flipse et al. (1954) described Lactenin, an antistreptococcal substance of milk, to be highly toxic to bovine spermatozoa. This substance is destroyed after heating for 10 minutes at 92°C.

Johnson et al. (1954) stated that the addition of cysteine hydrochloride to pasteurized skim milk produced livability results equivalent to those obtained by heating pasteurized skim milk at 92°C for 10 minutes. It was postulated that cysteine hydrochloride and other sulfhydral compounds, may act by lowering the oxidation reduction potential of the milk, thus inactivating lactenin, an apparently spermicidal substance found in milk. Again, Johnson et al. (1955) reported that 1 mg of cysteine hydrochloride per millilitre of unheated skim milk resulted in a semen diluent which maintained

the livability of bull spermatozoa equally as skim milk heated at 92°C for 10 minutes. Gluthathione was also shown to be effective in supporting spermatozoan livability in unheated skim milk, but methionine was found to be ineffective indicating that the beneficial effect was due to the sulfhydral groups. Pickett and Cowan (1961) studied that 10 % egg yolk was nearly as effective in inhibiting the spermicidal factor in unheated skim milk as egg yolk + heat treatment.

The efficacy of milk as semen dilutor was compared and evaluated by many workers. Lunca (1961) reported that the spermatozoal survival averaged 83.1, 72 and 84 hours in diluents containing citrate, yolk-glucose-citrate and milk respectively. Sevinc and Hafs (1961) diluted the semen with the C.U.E., heated homogenized whole milk (H.M.), C.U.E. + H.M., C.U.E. + catalase, H.M. + catalase and C.U.E. + H.M. + Catalase. Highest over all average motility and motility at 21 days, was obtained with C.U.E. + H.M. + catalase and the lowest with C.U.E. alone. C.U.E. + H.M. was significantly superior to C.U.E. or H.M. alone. A comparison between whole homogenized milk and C.U.E. as extenders for bull semen was also made by Pickett et al. (1962). They reported significantly better non-return percentages for the milk diluents. Ehlers and Mikota (1962) further reported significant differences in percentages of non-returns for age of semen, month, year and breed, when the semen was diluted in milk.

Nikolaidou-Varella (1966) studied the fertility of

bull semen diluted with milk + 5 % egg yolk to be 7 % higher than semen diluted with 3 % sodium citrate + 2 % egg yolk and 9 % higher than that diluted with milk + 5 % egg yolk + 10 % glycerine.

Glycine Containing Diluents:

Knoop and Karuss (1944) reported an improved survival with the addition of 1.09 % glycine to the phosphate-yolk diluent, when compared with yolk gelatin and yolk-phosphate diluents. In 1952, Tyler and Tanabe noted prolonged spermatozoal survival with the use of a glycine containing salt diluent with storage at 38°C, but not at 4°C, when compared with the yolk-citrate diluent, Later, Roy and Bishop (1954) reported that in equal volumes of egg yolk and 4 % glycine solution in distilled water there was longer spermatozoal survival at 5°C than in the yolk phosphate or yolk citrate diluents. But, Willett and Ohms (1956) stated that addition of glycine or versene to yolk citrate did not improve viability of spermatozoa when compared with viability in yolk citrate alone. Adler and Rasbech (1956) in an experiment with semen diluted in yolk citrate obtained 66.2 % non-returns for 4410 inseminations and with glycine-fructose-yolk the non-return rate was 66.0 % for 4392 inseminations. The semen was used within 12 hours of collection.

Saha and Singh (1958) reported the average length of time that a + 3 motility was maintained in egg yolk-

citrate-glycine, egg yolk-glycine and egg yolk-citrate, was 6.8, 8.8 and 3.5 days respectively for buffalo sperm. They noticed better visibility of sperm in microscopic preparations in egg yolk-sodium citrate-glycine mixture.

Albright et al. (1958) observed that 2.9 % glycine enhanced the ability of sperm to maintain progressive motility under storage at 5°C when used with whole milk, skim milk or egg yolk-citrate. In 1966, Sinha reported highly significant better motility of spermatozoa in 4 % egg yolk glycine than in 3 % egg yolk-citrate or 1.3 % egg yolk-citrate diluent. The methylene blue reduction time was lower in glycine diluent than 3 % egg yolk-citrate diluent. Marshall et al. (1968) also opined that glycine dilutors prolong the motile life of spermatozoa but adversely affect the fertility of semen.

DILUENTS USED FOR THE PRESERVATION OF BUFFALO SPERMATOZOA

Little scientific investigations have been carried on buffaloes as they are found mostly in developing or underdeveloped countries. The difficulty of semen preservation in artificial insemination operation of buffalo necessiated extensive research for better semen diluent. As large percentage of world population of buffalo is bred in India, most of the literatures on preservation of buffalo semen have been contributed from here.

Srivastava et al. (1953) preserved buffalo bulls'

semen at 4°C in the active principle of egg yolk and found that the maximum duration of survival of spermatozoa was 21 days whereas it was 12 days for control samples kept in egg yolk phosphate.

Roy et al. (1955) diluted the buffalo bull semen in an egg yolk-citrate diluent and noted a rapid decline in motility whereas, buffalo semen diluted 1:5 with glycine-egg yolk showed a high motility for 2 weeks, and the total life span of spermatozoa was markedly prolonged. Fertility tests with buffalo semen were not done but bull semen in a glycine-egg yolk diluent gave slightly better conception rates than in the citrate diluent.

Madatov (1956) observed motility for 4 days in buffalo semen diluted in yolk-citrate-glucose and stored at 0°C.

Roy et al. (1956) observed that buffalo semen diluted and preserved in glycine-egg yolk upto 72 hours, could be successfully deep-frozen. They recorded recovery of 80 % of the initially motile spermatozoa when frozen samples were thawed at room temperature.

Srivastava and Prabhu (1956) compared the efficiency of six diluents, viz., Active Principle (AP), egg-yolk-phosphate (EYP), egg yolk-citrate (EYC), autoclaved milk (AM), spermasol (S) and egg yolk-glucose-bicarbonate (EYGB) with Sulphamezathine (ICI). On the 8th day of storage the highest percentage of live sperm was obtained in EYGB diluent. The

next best diluent was spermasol, followed by EYC.

Gokhale (1958) obtained better preservability of buffalo semen in egg yolk-glucose-bicarbonate dilutor containing Sulphamezathine (ICI) and recorded 52.29 % conception with glucose dilutor, 40.8 % with EYC dilutor, 41.0 % with EYP dilutor, at the rate of 90 - 120 days non-return.

Singh and Tomar (1959) experimented with a dilutor consisting of 10 parts of 1.3 % sodium bicarbonate, 40 parts of 5 % glucose, 25 parts of 5 % fructose and 25 parts of egg yolk. This dilutor could preserve semen upto + 3 motility for a longer period than egg yolk-sodium citrate and egg yolk-glycine. It could be used for insemination purpose for 6.25 days, whereas the egg yolk-sodium citrate and egg yolk-glycine could be used for 3.5 and 4.25 days respectively. The pH value upto + 3 motility remained almost the same.

At NDRI (Bhatia, 1960), Banglore, it was observed from the preliminary work done on the keeping quality of buffalo bull semen that 4 % glycine mixed with equal part of egg yolk increased the preservation time.

Sharma and Mahajan (1961b) obtained the following results in a series of experiments designed to develop a superior semen diluent for buffalo semen:

(i) When semen was stored in the conventional yolk-phosphate, yolk-citrate, yolk-glycine, yolk-milk and yolk-glucose-bicarbonate diluents at 1:10 and 1:50 levels of

dilution, the motility was best preserved in the yolk-glucose-bicarbonate at the 1:10 level of dilution.

(ii) When semen was diluted at different dilution levels from 1:5 to 1:100, it was found that dilutions upto 1:25 did not significantly effect motility but higher levels were harmful.

(iii) In separate experiments with several modifications of the yolk-glucose-bicarbonate diluent, in which the concentration of yolk varied from 0 to 50 %, a minimum of 20 % yolk in the medium appeared essential for maximum sperm survival.

Kalev et al. (1961) found glucose-yolk-citrate as a suitable buffalo semen diluent for storage at 0° to + 5°C.

Sharma and Mahajan (1961c) reported that the motility and livability of buffalo spermatozoa improved significantly with the addition of increasing volumes of seminal plasma to the diluted semen.

Mahajan and Sharma (1961) designed an experiment to study the effect of various levels of glucose and sodium bicarbonate on the preservation of buffalo semen. Ten different modifications of glucose bicarbonate buffer were tested. 95 parts of isotonic sodium bicarbomate solution and 5 parts of isotonic sodium bicarbonate solution was superior to all other modifications studied.

Tomar and Desai (1961a) used whole egg (egg yolk

and egg-albumen) with glucose, fructose, sodium citrate and sodium bicarbonate for the preservation of buffalo bull semen. The whole egg-glucose-fructose (40:40:20) preserved the buffalo bull semen for longer periods (+ 3 motility for 4.9 days, whole life for 27.95 days). It was concluded that the alkaline pH of the whole egg dilutors (7.2 to 7.6) was disadvantageous for maintaining the progressive motility of the spermatozoa.

Tomar and Desai (1961b) made a comparative study of egg yolk-bicarbonate dilutors in various combinations with glucose, fructose, glycine and sodium citrate for the preservation of buffalo bull semen. The egg yolk-glucose-fructose-sodium bicarbonate and egg yolk-glucose-fructose-sodium citrate-potassium bicarbonate dilutors proved superior and could maintain the + 3 motility for 6.15 days and 7 days and the livability for 23.2 days and 23.85 days respectively. Presence of extra potassium ions in the diluting medium was advantageous, when the dilution rate was moderate or higher.

Tomar and Desai (1961c) found that the incorporation of glucose and fructose into the egg yolk-glycine medium has a prompting action on the motility of the spermatozoa, which seems to utilize these sugars in the glycine medium under partial aerobic and anaerobic conditions. The presence of electrolyte, however, in the egg yolk glycine medium had an adverse effect on the livability of spermatozoa.

Tomar and Desai (1961d) compared the efficiency of

egg yolk-sodium citrate, egg yolk-glucose-sodium citrate, egg yolk-glucose-fructose-sodium citrate, egg yolk-glucose-glycine-sodium citrate and egg yolk-glycine-sodium citrate, for the in-vitro preservation of buffalo bull semen. Egg yolk-glucose-glycine-sodium citrate and the egg-yolk-glucose-fructose-sodium citrate maintained + 3 motility for 6.45 days and 5.8 days, and livability of spermatozoa for 23.65 and 22.15 days respectively.

Tomar and Desai (1961e) used heated skim milk, egg yolk-glycine-heated skim milk (25:25:50) and egg yolk-fructose-heated skim milk (20:20:60) and reported that egg yolk-glycine-heated skim milk and the egg yolk fructose-heated skim milk diluents maintained + 3 or higher motility of the spermatozoa for an average period of 8.1 days and 7.65 days respectively. They concluded that the addition of glycine or fructose to the skim milk medium equipped that with the readily available energy sources for the spermatozoa.

Nayudu (1961) observed the superiority of egg yolk-glucose-bicarbonate diluent (EYGB) over egg yolk-citrate (EYC), egg yolk-glycine (EY-Gly) and egg yolk-Illini Variable temperature (EYIVT) diluents for preservation of buffalo semen at 4 $\pm$ 1^{o}C.

Mahajan and Sharma (1962) did not obtain an improvement in the viability of spermatozoa after removal of yolk sediment by centrifugation from the yolk-glucose-bicarbonate dilutor.

Kale (1963) preserved buffalo semen, using varying proportions of whole milk, egg yolk, glucose, sodium bicarbonate and sodium citrate dihydrate. Milk diluents, in general, were able to maintain a + 3 motility of spermatozoa over three days. GMC and GMY were found to be superior than others and maintained desirable motility of spermatozoa over five days (5.42 and 5.17 days respectively).

Comparative efficiency of yolk phosphate, yolk citrate, yolk boiled milk, yolk glycine and yolk glucose bicarbonate, was observed by Mahajan and Sharma (1963). Yolk-glucose-bicarbonate diluent was found to be significantly superior to the other diluents compared in this experiment. The average number of days for which the sperm survived in this diluent were 16 and 12 days at dilution rates of 1:10 and 1:50. The average number of spermatozoa surviving after 10 days of storage were 22 and 8 per cent at low and high dilution levels respectively. It was concluded from this investigation that buffalo sperm can possibly be preserved in yolk-glucose-bicarbonate diluent for at least 3 - 4 days with desired motility rating for A.I. use, provided the dilution rate of semen is not high.

Barr et al. (1964) diluted buffalo bull semen at 1:10 or 1:15 levels and noted average motility for 4.9, 5, 7.1, 5 and 8 days in egg yolk citrate glycerol, egg-yolk-phosphate-glycerol, spermasol T-glycerol, spermasol-milk-glycerol, and skim milk + antibiotics respectively.

Basirov (1964) using yolk-citrate and yolk-cows milk diluents and storing diluted semen at 0°, obtained 79 - 86 % conception rates.

Kouser (1965) reported the use of six dilutors containing skim milk for the preservation of buffalo semen at 4 ± 1°C for 96 hours. Motility was best maintained in skim milk-yolk-sodium bicarbonate (MYS) followed by skim milk-yolk-glucose (MYD), skim milk-yolk (MY), skim milk-yolk-citrate (MYC), skim milk-yolk-glycerol (MYG) and skim milk-glycerol (MG). Highest percentage of dead spermatozoa were recorded in MG, followed by MYG, MYC, MYS, MYD and MY.

Sharma and Sengar (1965) diluted buffalo semen in (i) Egg-Yolk-Phosphate (EYP), (ii) Egg-Yolk-Citrate (EYC), (iii) Egg-yolk-glucose-bicarbonate (EYGB), (iv) Egg-yolk-glycine (EYGly), (v) Egg yolk-IVT (EYIVT) and (vi) Egg yolk-cornell university extender (EYCUE). The egg yolk always formed 25 % of the diluent. The highest over all total motility upto 7 days preservation was obtained in EYGB; EYGB, EYC and EYIVT gave significantly better preservation than EYCUE, which was significantly better than EYGly and EYP upto 72 hours storage.

Kumar (1966) carried out investigation to evaluate the livability of buffalo spermatozoa in respect of motility per cent and unstained spermatozoa per cent, upto storage of 144 hours in four series of diluents, viz., egg yolk-citrate

series, skim milk series, tomato juice series and coconut milk series. The results were compared with egg yolk-glucose-bi-carbonate diluent. A gradual decline was recorded in the motility per cent and unstained spermatozoa per cent in all diluents with an increase in storage upto 144 hours. The least decline was noted in EYGB diluent, while the motility and unstained spermatozoa per cent decreased maximum in coconut milk.

Sharma and Mahajan (1966) diluted buffalo bull semen in the ratio of 1:15 to 1:25 in IVT and stored at 4° - 6°, 20° to 30° (in air conditioned room) or 18° - 30°C (room temperature). The percentage of motile spermatozoa after 3 days of storage was 58, 49 and 60 at the three temperature ranges respectively, and after 7 days of storage, 16, 25 and 35.

Milk of various species (cow, buffalo, goat) were used as diluent for buffalo semen by Joshi et al. (1967). The diluted semen samples were stored at 5° - 7°C. The percentage of motile sperms in EYC was significantly less when compared to cow milk after six hours of storage. The next in order of efficacy were goat, buffalo and skim milk. The superiority of cow milk was maintained even after 54 hours of storage (3rd day). The percentage decline in livability in cow milk at this storage period was 30 per cent as compared to 40 per cent in buffalo milk, goat milk and EYC diluents. The maximum percentage decline (55.7 %) was recorded in the skim milk and it was, therefore, rated inferior even to EYC.

Srivastava et al. (1968) opined that the sulphamezathine in glucose-egg yolk-bicarbonate diluent depressed fructolysis. This was considered to be a factor in the better maintenance of motility in this medium than in other media investigated.

Norman et al. (1968) noticed that the functional life span of buffalo spermatozoa could be increased to 10 days if they were kept at ambient tempperature in coconut milk extender (CME). The 89 confirmed pregnancies out of 144 buffalo cows given 1st inseminations with 1 to 10 day old semen in CME indicated a significant advance in the conservation of buffalo semen.

Joshi et al. (1968) noted that four per cent solution of amino-acids-alanine and serine with 25 per cent egg yolk maintains the motility of buffalo bull semen equal to 4 % glycine. CUE was found to be a satisfactory diluent for buffalo semen. Whole milk of cow, buffalo and goat and skim milk of buffalo, in general, and cow milk in particular were satisfactory for preservation of buffalo semen. Milk of goat was inferior to that of cow and buffalo after 54 hours of preservation. Addition of glycine, serine, alanine, succinate and egg yolk to the whole milk diluent was not advantageous.

Singh et al. (1970) observed buffalo semen extended in 6 diluents, viz., egg yolk-glucose-bicarbonate (EYGB), Egg yolk-skim milk (EYSM), egg yolk-tomato juice-glucose-fructose-bicarbonate (EYTJGFB), egg-yolk-coconut milk glucose bicarbonate

(EYCMGB), egg yolk-glucose-glycine (EYGGly) and egg yolk-skim milk-cysteine (EYSMCY). Motility and live-sperm per cent were considered as criteria for livability and oxygen uptake, and MBRT for metabolism of buffalo spermatozoa. Buffalo spermatozoa remained active and viable in all the semen diluents except in a diluent containing tomato juice (EYTJGFB), and gave optimum fertility. Best results were obtained in EYSmCy diluent. Satisfactory results were also noted with EYGB and EYSm diluents.

Ability of 10 different dilutors for reviving motility was recorded by Tomar, Sharma and Singh (1970). 0.2 ml of non-motile buffalo semen was added to 1 ml of each media and the motility was rated after 3 - 4 minutes. Egg yolk-citrate and the glycine-citrate-glucose-yolk buffer were better for reviving motility than was the glucose-fructose-bicarbonate-yolk buffer.

Sengupta and Choubey (1971) studied the fertility of buffalo semen extended in a cysteine containing diluent (1:15) and stored for 18 - 50 hours at 1 - 2°C. The over all conception rate was 57 % whereas age of semen-wise break-up was 60 % (18 - 26 hours old semen) and 53.5 % (42 to 50 hours old semen) respectively. Fertility of individual bulls varied rather widely between 38.9 and 76.5 %.

ANTIBACTERIAL AGENTS

Antibacterial agents are added in the semen becuase of (a) the possible transmission from the bull to many females,

via the semen, of disease causing organisms, (b) the possibility of direct or indirect bacterial or microbial effect on the sperm cells, resulting in their reduced ability to fertilize ova, and (c) bacterial or microbial competition for vital substances, resulting in reduced livability of stored spermatozoa.

Salisbury et al. (1939) first called the attention of the workers in the field of artificial insemination to the problem of bacterial contaminants in semen and the importance of bacterial control. He suggested that the growth of microflora in semen could be checked by the addition of suitable agents.

Knodt and Salisbury (1946) incorporated sulphanilamide in levels of 10 - 1000 mg per 100 ml and observed that sulphanilamide aided motility, but less at 500 and 1000 mg than at lower levels. Livability of spermatozoa was lowered by all levels of sulphanilamide added to fresh bull semen diluted with yolk citrate; 100 to 500 mg was the optimum quantity and livability was not as good at the 1000 mg as at lower levels. The rate of motility was optimal when 300 mg sulphanilamide was added to ejaculates diluted 1:9 or 1:49 with yolk citrate and stored for 20 days at 5°C. Glycolysis was stimulated progressively with each increase in sulphanilamide. Oxygen utilisation of washed spermatozoa decreased progressively as the level of sulphanilamide increased.

Hennaux et al. (1947) observed that streptomycin

added to bull semen at the rate of 10 mg per 10 cm^3. Semen diluted with egg yolk-citrate and stored for 9 days at 5°C did not influence the percentage of sperm with progressive motion or motility grade but 30 mg streptomycin in 10 cm^3 diluted semen increased the motility grade. Streptomycin also inhibited the development of Brucella abortus and Bacterium Coli in the semen. Again in 1948, Hennaux et al. noted decreased motility on the 3rd day when 4,000-10,000 units, on the 4th day when 2000; and on the 5th day when 50 - 1000 units Penicillin per cm^3 were added to bull semen.

Highest percentage of motile spermatozoa was recorded by Foote and Bratton (1949) from the semen sample in which sulphanilamide was added. Based on 60 to 90 days non-return of 5769 first service and 2749 second service cow, the fertility level of sulphanilamide containing diluent was reported to be 6.5 % higher.

Almquist et al. (1949) reported effective control of bacterial growth in diluted semen with the addition of various levels of 100 - 1000 units penicillin + 100 - 1000 units streptomycin per ml diluted semen, stored for 20 days at 4.5°C.

Easterbrooks et al. (1950a) diluted bull semen in citrate-sulphanilamide-yolk and added streptomycin sulphate at the rate of 100 per ml to half of the diluted semen. 61.6 % of the 2340 cows inseminated with the diluted semen did not

return for service within 90 days whereas 69.8 % of the 2379 cows inseminated with diluted semen to which streptomycin sulphate was added, returned for service. Easterbrooks et al. (1950b) also noticed that streptomycin calcium chloride complex was incompatible with phosphate buffers.

Rozsa (1950) found penicillin to be most effective against gram positive and streptomycin against gram negative bacteria in bull semen. The best results were obtained using a combination of the 2 antibiotics, namely, 30 to 50 units of penicillin + 0.02 to 0.04 mg streptomycin per 1 cm^3 egg yolk-citrate diluted semen. The bactericidal action was effective even 12 days after storage at + 4°C. Larger doses of the antibiotics were found to be harmful to sperm motility and metabolism. Foote and Bratton (1950) concluded that the addition of penicillin, streptomycin or a combination of these plus polymyxin and sulphanilamide to bull semen diluted in citrate-yolk increased the fertility level.

Sykes and Mixner (1951) reported no significant difference in spermatozoal motility due to differences in the brands, salts or lots of penicillin or streptomycin or betwen aureomycin base and hydrochloride. Initial toxic levels indicated chloromycetin to be relatively low toxic.

Myers and Almquist (1951) observed that Aureomycin levels of 100 - 1000 µg were about equally as effective in controlling bacterial growth as 1000 units of penicillin, 1000 µg

of streptomycin or a combination of 1000 units of penicillin + 1000 µg of streptomycin per ml of diluted semen, but aureomycin at a concentration of 1000 µg/ml of diluted semen was highly significantly more toxic to spermatozoa than either penicillin and/or streptomycin at the above mentioned levels.

Stewart (1951) added 1000 µg of streptomycin calcium chloride complex to one ml of normal semen diluent and obtained 67.5 % conception rate in the streptomycin treated group vs. 57.4 in the untreated group.

Almquist (1951) stated that penicillin and streptomycin, either singly or together, offer a valuable means for improving the fertility of semen from certain bulls of low fertility in artificial breeding.

Easterbrooks (1951) observed 4 % fewer 60 to 90 days non-returns to first service when aureomycin was used, 3.7 % fewer when chloromycetin was added, and 0.1 % fewer when terramycin was added, in comparison to the addition of streptomycin.

Old et al. (1951) found a trend for antibiotics to improve the fertility of the semen of low fertility bulls.

Dunn, Bratton and Henderson (1953) concluded that penicillin and streptomycin, together, have similar effects on fertility when they are added either to the yolk or the whole egg formula.

Branton and Prather (1954) found that streptomycin and penicillin either singly or in combination, had beneficial effects on spermatozoan livability, whereas sulphanilamide apparently had a detrimental effect on viability of the spermatozoa. Sulphanilamide, streptomycin and penicillin each depressed the utilization of total reducing substances and the fructose and the production of lactic acid by the spermatozoa. Sulphanilamide gave the most marked metabolic effects. A combination of streptomycin and penicillin in the egg yolk citrate diluent depressed spermatozoan metabolism almost as much as sulphanilamide and gave the best livability in comparison with the other five diluents. Semen diluents containing citrate or phosphate buffers and all combinations of sulphanilamide, streptomycin and penicillin were compared by Campbell and Edward (1955).

Zvereva and Jablonsjkii (1961) observed that addition of streptomycin, penicillin or white streptocide to washed, incubated spermatozoa acted as a depressant to respiration.

Beneficial effect of penicillin and streptomycin on sperm survival in diluted semen was noticed by Marinov (1962). Kersulis (1963) tested 12 antibiotics and reported that phenoxy methyl penicillin and erythromycin were the most suitable.

Literatures are scantly regarding the addition of chemotherapeutic agents or antibiotics for the preservation of buffalo semen. Gokhale (1958) added penicillin (500 to

1000 I.U./ml) and dihydrostreptomycin sulphate (500 - 1000 µg/ml) to buffalo semen extended in glucose-bicarbonate-sulphamezathine buffer, but, no beneficial effect on the survival time of spermatozoa was noticed. The diluting media with antibiotics, however, maintained a motility of + 3 or above for somewhat longer periods (18.2 hours). The use of antibiotics or their omission did not give any significant difference in the fertility rate.

Penicillin 500 international unit per ml and streptomycin 0.01 mg per ml of dilutor was added for the preservation of bull semen at the N.D.R.I., Bangalore (Bhatia, 1960).

Sharma, Singh and Desai (1962) reported that addition of penicillin and dihydrostreptomycin to buffalo semen increased the half life (+ 3 motility) and full life of spermatozoa as compared to that of control samples without antibiotics. Aureomycin and terramycin did not increase half life but were found toxic to spermatozoa. When dihydrostreptomycin and penicillin were added to EYC diluted buffalo semen, the conception rate was 43.3 % whereas it was 35 % for controls, inseminated with semen without additions.

Singh (1965) recommended the use of penicillin (1000 I.U./ml of diluted semen) streptomycin (1000 µg/ml of diluted semen) and sulphanilamide (0.3 gm/200 ml) in semen diluents as a routine for preservation of bull and buffalo semen.

Saxena (1970) diluted buffalo semen with egg yolk-glucose-sodium bicarbonate diluent. Three sulpha drugs, 5 antibiotics, and three combination of these, were added in different doses to diluted samples and the diluted semen was stored at 5°C. Dihydrostreptomycin (200 µg/ml) gave the best results for preservation of semen, followed by sulpha tried, Elkosin, Sulphapyridine, Aureomycin, Terramycin, Procain Penicillin and Penicillin G sodium. Among combinations, streptomycin + Penicillin G sodium gave the best results; the maximum life span of spermatozoa averaged 33.0 ± 2.41 days.

MEASUREMENTS OF SPERMATOZOA

Spermatozoan consists of a head, a neck, a middle piece and a tail. The structure of spermatozoa has been described by Mann (1954) in detail. The head for the most part consists of a nucleus, fitted with densely packed chromatin (deoxyribonucleic acid) and its anterior part is covered by the acrosome cap.

The early workers have emphasized the significance of sperm head dimensions in relation to fertility. Savage, Williams and Fowler (1927) studied relationship between head length variability of bovine spermatozoa and fertility. Laferlof (1934) also reported about the sperm head dimensions in relation to fertility.

Pursley and Herman (1950) suspended bull sperm in hypotonic and hypertonic solutions, but no deviation was

recorded in the head dimensions of spermatozoa due to the variation in the pressure of diluting media.

Mukherjee and Dott (1960) studied the head length and head breadth of Dexter and Friesian bull spermatozoa, preserved in egg yolk-glycine for 72 hours and reported significant decrease in size, while no significant change was noted in these dimensions during storage in EYC diluent. Mukherjee (1961) also obtained the same results with egg-yolk-glycine dilutor.

Head length and head breadth of ram spermatozoa were recorded by Rathore and Mukherjee (1961) before and after 72 hours of preservation in egg yolk-phosphate, milk and egg yolk-boric acid-sodium-bicarbonate dilutors. The head length and head breadth of preserved spermatozoa were significantly less than those without preservation.

Pande, Mukherjee and Bhattacharya (1961) found significant decrease in head breadth of Hariana bull spermatozoa after preservation in egg yolk-glycine for 72 hours.

Mayudu (1961) reported no significant change in the dimensions of head when semen of Murrah bulls were preserved for 72 hours at 4 $\pm$ 1^{o}C in egg yolk-glucose-bicarbonate, egg yolk-citrate, egg yolk-glycine and egg yolk-illini variable temperature diluents.

Rathore and Mukherjee (1965) preserved ram semen in egg yolk-citrate and egg yolk-glycine dilutor for 72 hours

and noticed that head length and head breadth of spermatozoa were significantly less than those before preservation.

Krishnayya (1966) demonstrated the effect of glucose-sodium bicarbonate-sulphamezathine-yolk and skim milk-yolk dilutor on the biometrics of buffalo spermatozoa at intervals of 24, 48 and 72 hours of preservation. A highly significant drop in head length and head breadth was noticed between fresh and preserved spermatozoa at 24, 48 and 72 hours. The skim milk yolk dilutor had shown a marked decrease in head length of spermatozoa at 24 hours of preservation but the lost decrease was regained subsequently at 48 and 72 hours of preservation. With glucose-sodium bicarbonates-sulphamerathine dilutor, the decrease in head length of preserved spermatozoa had increased as the interval increased from 24 to 48 to 72 hours.

Kumar (1966) investigated the effect of four series of diluents, viz., egg yolk-citrate series, skim milk series, tomato juice series and coconut milk series on measurement of head length and head breadth of buffalo spermatozoa. Variable measurements of head length and head breadth were recorded in different diluents upto 144 hours storage, but their differences were not significant.

Saxena and Singh (1967), with Hariana bull semen diluted in skim milk-yolk, egg yolk-glucose-bicarbonate, egg yolk-citrate and C.U.E. observed a significant decrease only in head length of spermatozoa preserved in skim milk-yolk.

Cimpeanu et al. (1968) noted structural changes, particularly in the acrosome due to dilution of spermatozoa.

Marshall et al. (1968) studied the effect of glycine and other dilutors on acrosome alteration and concluded that glycine treatment increased the rate of acrosomal alteration and lowered the fertility adversely.

MATERIALS AND METHODS

MATERIALS AND METHODS

The studies were made with five adult Murrah buffalo bulls of different age groups, maintained at Semen Bank, Patna. These bulls were maintained under the same environment and identical conditions of farm management. They were given exercise in the early hours in the morning. The rations of the bulls consisted of hay, green fodder, wheat bran and groundnut cake. Fifteen lbs of hay was constantly given everyday throughout the experimental period but the green fodder was changed according to its availability. The concentrates were divided into two equal parts and given in morning and evening. One ounce each of mineral mixture and common salt was also supplied. The bulls were allowed to drink water ad-libidum. Water bath was given twice a day; morning bath was given about an hour earlier before the schedule of semen collection programme.

An anestrous buffalo cow was used as dummy for collection of semen from bulls. As suggested by Branton et al. (1952), every bull was allowed one false mount before collecting the first ejaculate for a good sample of semen. Fifty semen collections (10 collections from each bull) were studied. These collections were taken in the forenoon between 8-30 and 11-30 A.M. Collections were taken under identical conditions maintaining all possible sterile methods. The temperature of Artificial Vagina was maintained between 40°

to 45°C. The semen sample, soon after collection, was brought in the laboratory and kept in a beaker containing water at approximately 32°C. Two to three ml of neat semen sample from each bull was brought to the laboratory in sterilised test-tubes, which were carried in a thermoflask containing ice. The semen was processed as quickly as possible after its evaluation. Dilution, ampouling, labelling and sealing of ampoules were done without any loss of time.

The four diluents, viz., Egg -yolk-citrate-potassium bicarbonate-glucose-fructose, Egg yolk-citrate-glucose-glycine, Egg yolk-citrate-glucose-glycine-cysteine hydrochloride and whole milk were taken for the study along with Egg yolk-glucose-bicarbonate as a control. Identical semen samples were diluted and stored in each of the five diluents, including the control. Diluted semen samples were then examined at intervals of zero hour, 24 hours, 48 hours, 72 hours and 96 hours for counting the percentage of live sperms present and measuring the head length and head breadth.

PREPARATION OF DILUTORS

In the present experiment five dilutors, including the control were tried. The 'Analar' quality reagents were utilised for the preparation of different buffer solution with glass redistilled water. The composition and method of preparation of diluents are detailed below:

1. Egg yolk-citrate-potassium bicarbonate-glucose-fructose dilutor:

i) Potassium bicarbonate	0.25 gm
ii) Sodium citrate dihydrate	11.222 gm
iii) Glucose anhydrous	18.750 gm
iv) Fructose	5.125 gm
v) Glass distilled water	Added to one litre.

The pH of the buffer solution ranged between 6.4 to 6.6. Firstly the potassium-bicarbonate and sodium citrate solution was prepared and autoclaved at 15 lbs pressure for 15 minutes. Other ingredients of the buffer were added after the solution was cooled and then it was preserved in the refrigerator for further use.

Eighty ml of buffer solution was mixed with 20 ml of Egg yolk to make 100 ml of the dilutor. One lakh I.U. of penicilline G sodium and 100 mg of dihydro-streptomycine were added to 100 ml of prepared extender.

2. Egg yolk-citrate-glucose-glycine dilutor:

i) Sodium citrate dihydrate	9.375 gm
ii) Glucose anhydrous	18.750 gm
iii) Glycine	12.500 gm
iv) Glass distilled water	Added to one litre

The pH of the buffer solution varied from 6.4 to 6.6. The sodium citrate solution was first made and autoclaved at

15 lbs pressure for 15 minutes. Other chemicals were added afterwards and the buffer, thus, prepared was kept in the refrigerator for longer use.

Buffer solution and egg yolk were mixed in the ratio of 80:20 respectively by volume. One lakh I.U. of penicilline G sodium, and 100 mg of dihydro-streptomycine were added to per 100 ml of the prepared extender.

3. Egg yolk-citrate-glucose-glycine-cysteine dilutor:

i)	Sodium citrate dihydrate	1.40 gm
ii)	Glucose anhydrous	1.25 gm
iii)	Glycine	0.93 gm
iv)	Cysteine hydrochloride	0.125 gm
v)	Glass distilled water	100 ml

pH of the buffer solution ranged between 6.6 to 6.8. The sodium citrate solution was first made and then it was autoclaved at 15 lbs pressure for 15 minutes. All other chemicals were mixed after the solution cooled down and then it was kept in the refrigerator.

The dilutor was made after mixing 4 parts of buffer solution with 1 part of egg-yolk. One lakh I.U. of penicilline G sodium and 100 mg of dihydrostreptomycine were added to 100 ml of the prepared extender.

4. Whole milk:

Cow's milk, obtained from a local milkman, was used

throughout the experiment. It was procured one day before its use. The milk was taken directly from the teat into the container. The milk was heated in a water bath at the temperature of 92° - 95°C for 10 minutes. Over heating and under heating was avoided. The heated milk was allowed to cool down to room temperature, and then kept over night in the refrigerator. The milk was filtered through a thin layer of cotton, next morning. The thick mass of fat which had collected at the surface did not pass through the filter, giving thereby a milk with smaller fat globules. One lakh I.U. of penicilline G sodium and 100 mg of dihydro streptomycine were added to every 100 ml of the milk and then it was used for diluting semen.

5. Control:

Egg yolk-glucose-bicarbonate dilutor was used as control. The compositions and procedure are detailed below:

(A) Solution

i) Sodium bicarbonate	1.3	gm
ii) Glass distilled water	100	ml

(B) Solution

i) Glucose anhydrous	5	gm
ii) Glass distilled water	100	ml

(C) Solution

One part of (A) and 4 parts of (B) were mixed to make the buffer. pH of the buffer solution was recorded between 6.6 to 6.8.

Solution (A) was autoclaved at 15 lbs pressure for 15 minutes. Glucose solution was made with autoclaved distilled water. One part of egg-yolk was mixed with 2 parts of (C) to make the control dilutor.

One lakh I.U. of penicilline G sodium and 100 mg of dihydro-streptomycin were added to 100 ml of the extender to make the control extender for final use.

Egg-yolk:

Only unfertilized fresh hen's eggs were used in the experiment. Before opening, shells of eggs were cleaned and sterilised with a swab of alcohol. The white of the egg was discarded and the yolk was placed on a sterlised filter paper. The vitelline membrane was broken, and the yolk was placed out into a beaker.

DILUTION OF THE SEMEN

The dilutors were prepared, as described earlier, and kept in 10 c.c. capacity test-tubes. The test-tubes containing dilutors were labelled with bull number, date of collection and name of dilutor. These test-tubes were then kept in a water bath at 32°C.

Before dilution, semen smear was made for the determination of dead and live percentage of spermatozoa and measurement of head length and head breadth.

The semen sample from 5 bulls were diluted at the rate of 1:10. Four dilutors with one control were tried. 10 semen samples from one bull were studied. A total of 250 samples (5 bulls x 10 samples x 5 dilutors) were studied during the experiment.

Care was taken that the temperature of dilutor and semen remained at 30°C - 32°C at the time of dilution. The test-tubes were gently rotated between the palms for uniform distribution of sperms in the dilutor. Immediately after dilution of the semen, smears were prepared for observing the live percentage of spermatozoa and measuring the head length and head breadth.

PRESERVATION OF THE SEMEN

20 clean and sterilised test tubes of 3 c.c. capacity each were taken. They were divided into four lots, each having 5 tubes duly labelled to contain five different diluents. One ml of the diluted semen of the corresponding dilutor was placed in each tube, and tightly corked. Separate lots were kept for examination at the end of different intervals of preservation, i.e. 24 hrs 48 hrs, 72 hrs and 96 hrs. The four lots of tubes, each containing 5 tubes were kept in four beakers having 300 ml of water at 25° - 30°C and kept in the refrigerator for gradual cooling. The beakers were also labelled as 24, 48, 72 and 96 hours for early identification. After 2 - 3 hours when the water of the beaker attained 4°C


0
10
20
30
40
50

temperature, it was removed and the tubes were kept in the same empty beakers for preservation at $4^{\circ} \pm 1^{\circ}C$ as suggested by Muller (1962).

On the expiry of every 24 hours, the corresponding beaker was taken out from the refrigerator. The semen tubes were thawed well one by one between the palms till they attained body temperature. Twice or thrice the tubes were moved up and down for homogeniety of spermatozoa in the dilutor. The tubes were then placed in constant temperature water bath at $32^{\circ}C$. Then the samples were examined for live percentage of spermatozoa and measurement of head length and head breadth.

PERCENTAGE OF LIVE AND DEAD SPERMATOZOA

The percentage of live and dead spermatozoa was estimated with Eosin and Nigrosin stain in the strength of 1 % and 5 % respectively, as suggested by Swanson et al. (1951). The compound stain was prepared as follows:

i)	Eosin (Water soluble)	1 gm
ii)	Nigrosin "	5 gms
iii)	Sodium-citrate dihydrate	3 gms
iv)	Distilled water	100 ml

This solution was warmed in a water bath for 30 minutes and filtered after cooling.

PREPARATION OF SLIDES

The bottle containing stain was kept in a beaker

of warm water at 37°C. One drop of neat or diluted semen was placed on a clean, warm watch glass and a large drop of compound stain was added to it. The semen and the stain drops were thoroughly mixed by gentle blowing through a pipette and allowed to stand for one minute. Thin smears were drawn on two clean, grease free slides and dried quickly in air. The slides were marked with grease pencil indicating bull number, date of collection, neat or diluted semen as the case might be and the name of dilutor. The smear was examined under oil immersion (X 1000).

Live spermatozoa did not take any stain and were colourless. The dead spermatozoa took pink colour due to eosin stain. Those spermatozoa taking partial stain anteriorly or posteriorly were considered dead. The nigrosin stain presented a very homogenous violet background. A total of 150 sperms were counted in a slide (300 spermatozoa from 2 slides) at random under different field of microscope, and the percentage of live spermatozoa was determined.

MEASUREMENT OF HEAD LENGTH AND HEAD BREADTH OF SPERMATOZOA

The measurement was taken by using an ocular micrometer with 10 X eye piece under oil immersion with a magnification of 1000 times. A blue light filter was fitted in the substage ring underneath the microscope condenser. The smears taken for live and dead count were used for measuring the head length and head breadth of spermatozoa. The unstained medium size sperms showing clear boundries were selected at

random for measurement. Five sperms from each slide (10 in a sample) were measured. Fifty spermatozoa were measured (5 diluents x 2 slide x 5 spermatozoa/slide) from one sample of semen at five different hours of interval, i.e. 0 hour, 24 hours, 48 hours, 72 hours and 96 hours.

To measure the head length, posterior length of the head was taken as tangent at straight angles to the main axis of the micrometer and the anterior position of the head was located, and the divisions of the ocular micrometer were noted. The head breadth was also measured in the same way. The measurements were taken at the maximum possible dimensions of the head. The ocular divisions were converted into microns and the means of the biometrics of the spermatozoa were worked out. (Each ocular division = 0.166 division of stage micrometer or 1.66).

STATISTICAL ANALYSIS

The data were statistically analysed according to Snedecor and Cochran (1967) and Pillai and Sinha (1968). The observations in percentage for live spermatozoa were transformed to angles value before the analysis of variance was carried out. The mean, standard error and coefficient of variance were obtained from the mean, S.E. and coefficient of variance of the transformed values.

The mean was calculated with the formula: $\frac{\Sigma x}{n}$

(n = number of observations)

Standard error (S.E.) was calculated after calculating standard deviation (S) with the formula:

$$\sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

$$\text{Standard error} = \sqrt{\frac{S}{n}}$$

Coefficient of variance was calculated with the formula:

$$\frac{S \times 100}{\bar{X}} \qquad (\bar{X} = \text{average})$$

Analysis of variance was run separately for fresh and preserved semen in all the diluents at 0, 24, 48, 72 and 96 hours. When 'F' test was found significant, a further test of all comparisons was performed by computing critical difference value (C.D.) at 1 % and 5 % level, and with this, the all means individually were compared. If the value of mean was found higher than C.D. value, the difference was taken as significant.

RESULTS

RESULT

The experiment was undertaken on five Murrah buffalo bulls, maintained at Semen Bank, Patna. The semen was collected in artificial vagina and the live percentage and morphology of fresh and preserved spermatozoa was studied at different hours of storage, viz., 0 hr, 24 hrs, 48 hrs, 72 hrs and 96 hrs.

EFFECT ON THE LIVE PERCENTAGE OF SPERMATOZOA

Live percentage of spermatozoa were studied in Egg yolk-citrate-potassium-bicarbonate-glucose-fructose (EYCBGF), Egg yolk-citrate-glucose-glycine (EYCGGly), Egg yolk-citrate-glucose-glycine-cysteine hydrochloride (EYCGGlyCy) and whole milk (WM) along with egg yolk-sodium bicarbonate-glucose (EYGB) as a control.

A gradual decline in live percentage of spermatozoa with an increase in storage hours was noted with all the diluents. All the five diluents maintained more than 60 % of live spermatozoa upto 96 hours of storage, and it was best maintained in EYGB. The mean values of live spermatozoa per cent of fresh and preserved semen are presented diagrammatically in Figure 1. The results of live percentage of spermatozoa in the five diluents with increase in storage period upto 96 hours are presented in Tables 1 to 13.

Fresh and Preserved Semen at 0 Hour:

The mean values of live spermatozoa per cent and their over all means ± S.E. and C.V. % of fresh and preserved semen at 0 hour storage are presented in Table 1. Statistical analysis of the data (Table 2) showed no significant variation in live spermatozoa per cent in different diluents.

Preserved Semen at 24 Hours:

The means of live spermatozoa per cent with their over all means ± S.E. and C.V. % are presented in Table 3. Small variation was recorded between the mean values of live spermatozoa per cent in the five semen diluents. The over all means varied from 76.47 to 79.61 %. The data were treated statistically and the results indicated that the variation in live spermatozoa per cent of preserved semen was not significant (Table 4).

Preserved Semen at 48 Hours:

The average of live spermatozoa per cent of individual bull and average live spermatozoa per cent of over all bulls along with its standard error and coefficient of variation were calculated and shown in Table 5. The data were subjected to 'F' test (Table 6). Since 'F' was significant the data were further subjected to critical difference test (Table 7) to observe the significant difference between two diluents. From critical difference test, it was concluded

that dilutors EYCGGly, EYCGGlyCy and EYGB were significantly superior to WM whereas dilutors EYCBGF, EYCGGly and EYCGGlyCy, showed no significant difference between each other.

Preserved Spermatozoa at 72 Hours:

The means of live spermatozoa per cent of individual bull with the average live spermatozoa per cent ± S.E. of over all bulls and C.V. % were calculated and presented in Table 8. The mean percentage of live spermatozoa varied from 73.64 to 66.45. The data were subjected to analysis of variance (Table 9). Highly significant differences were noted due to treatments. The critical difference revealed that the EYCBGF, EYCGGly and EYGB were highly significantly superior and EYCGGlyCy was significantly superior at 5 % level than WM. No significant difference in live percentage of spermatozoa was noted between EYCBGF, EYCGGly, EYCGGlyCy and EYGB diluents (Table 10).

Preserved Spermatozoa at 96 Hours:

The average of live spermatozoa per cent of individual bull and average live spermatozoa per cent ± S.E. of over all bulls and their C.V. % are given in Table 11. The mean live spermatozoa per cent varied from 69.57 to 60.42 %. The EYGB maintained the live spermatozoa per cent best. It was poor in WM, eventhough it was more than 60 %. The results of statistical analysis revealed significant difference in live

percentage of spermatozoa due to diluents (Table 12). The critical difference (Table 13) showed the EYCBGF, EYCGGly, EYCGGlyCy and EYGB to be highly significantly superior than WM. The EYGB was significantly superior at 5 % level than EYCGGly and highly significantly superior than EYCGGlyCy. There was no statistically significant difference in live spermatozoa per cent between EYCBGF and EYCGGly, EYCBGF and EYCGGlyCy, EYCBGF and EYGB and EYCGGly and EYCGlyCy.

EFFECT ON THE MORPHOLOGY OF SPERMATOZOA

A. HEAD LENGTH

The effect of five diluents, viz., egg-yolk-citrate-potassium bicarbonate-glucose-fructose (EYCBGF), egg-yolk-citrate-glucose-glycine (EYCGGly), egg-yolk-citrate-glucose-glycine-cysteine hydrochloride (EYCGGlyCy), whole milk (WM) and egg yolk-glucose-bicarbonate on the head length of spermatozoa were studied. The head length of spermatozoa of fresh and preserved semen at 0 hour, 24, 48, 72 and 96 hours were measured by ocular micrometer (1 ocular micrometer unit = 1.66 μ). The mean values for head length of spermatozoa at 0, 24, 48, 72 and 96 hours of storage are presented diagrammatically in Fig. 2. The measurement of head length of spermatozoa in microns are presented in Tables 14 to 27.

The over all means of head length of spermatozoa at 0, 24, 48, 72 and 96 hours during preservation showed a

decreasing trend in all the diluents. EYGB showed minimum decrease, while maximum decrease was noticed in EYCGGlyCy and EYCGGly diluents at 96 hours of storage.

Fresh and Preserved Semen at Zero Hour:

The mean values of head length of fresh and preserved spermatozoa at 0 hour and their over all means ± S.E. and C.V. % are given in Table 14. Statistical analysis of the data showed no significant difference in head length of spermatozoa due to diluents (Table 15).

Preserved Spermatozoa at 24 Hours:

The means of the measurement of head length at 24 hours of preservation with their over all means ± S.E. and C.V. % are presented in Table 16. The data were statistically analysed and revealed no significant difference in head length of spermatozoa due to diluents (Table 17).

Preserved Spermatozoa at 48 Hours:

The mean values and their over all means ± S.E. and C.V. % of head length of spermatozoa of preserved semen at 48 hours are given in Table 18. The mean of the head length of spermatozoa ranged from 7.085μ to 7.052μ. The data were subjected to statistical analysis which showed no significant difference in the measurement of head length of spermatozoa due to diluents (Table 19).

Preserved Spermatozoa at 72 Hours:

The mean values and their over all means ± S.E. and C.V. % of head length of spermatozoa of preserved semen at 72 hours are given in Table 20. Slight variation in the mean of the head length of spermatozoa was noticed. The mean of head length ranged between 7.049 μ to 6.979 μ. Maximum decrease was noticed in EYCGGly followed by EYCGGlyCy. Statistical analysis of the data revealed no significant difference in the measurement of head length of spermatozoa due to diluents (Table 21).

Preserved Spermatozoa at 96 Hours:

The average of head length of individual bull and average head length ± S.E. and C.V. % of over all bulls are given in Table 22. The mean head length of spermatozoa varied from 6.969 to 6.806 μ. The data of head length were subjected to 'F' test and critical difference test shown in Tables 23 and 24, respectively. No significant difference was observed between EYCBGF vs WM, EYCBGF vs EYGB, EYCGGly vs EYCGGlyCy, EYCGGly vs WM, EYCGGlyCy vs WM and WM vs EYGB, but significant difference in head length between EYCBGF vs EYCGGly, EYCBGF vs EYCGGlyCy and highly significant difference between EYCGGly vs EYGB and EYCGGlyCy vs EYGB indicating thereby that the head length of EYCGGly and EYCGGlyCy was significantly lesser than EYCBGF and EYGB.

The over all mean value ± S.E. and C.V. % of head

length of spermatozoa at different hours of preservation are presented in Table 25. The mean head length varied from 7.097 to 6.882 μ. Statistical analysis of data showed significant difference at different hours of preservation (Table 26).

The critical difference test revealed no significant difference in the head length of spermatozoa between 0 hour, vs 24 hours, 0 hr vs 48 hrs, and 24 hrs vs 48 hrs. Significant difference in head length was noticed between 0 hr vs 72 hrs, 0 hr vs 96 hrs, 24 hrs vs 72 hrs, 24 hrs vs 96 hrs, 48 hrs vs 72 hrs, 48 hrs vs 96 hrs and 72 hrs vs 96 hrs. This indicated decrease in the head length during increase of storage period.

B. HEAD BREADTH

The effect of five diluents, viz., Egg-yolk-citrate-potassium-bicarbonate-glucose-fructose (EYCBGF), egg-yolk-citrate-glucose-glycine (EYCGGly), egg yolk-citrate-glucose-glycine-cysteine (EYCGGlyCy), whole milk (WM) and egg-yolk-glucose-bicarbonate (EYGB), on the head breadth of spermatozoa were studied. The head breadth of spermatozoa of fresh and preserved semen at 0, 24, 48, 72 and 96 hours were measured by ocular micrometer (1 ocular micrometer unit = 1.66 μ) and are presented diagrammatically in Fig. 3. The measurements of the head breadth of spermatozoa are presented in Tables 28 to 37. There was a gradual decrease in head breadth of spermatozoa with increase in storage hours. Decrease in head breadth was most marked in EYCGGlyCy and least marked in EYGB diluent.

Fresh and Preserved Semen at 0 Hour:

The mean values of head breadth of fresh and preserved spermatozoa at 0 hour, with their over all means ± S.E. and C.V. % are given in Table 28. The mean value of head breadth of fresh and preserved semen practically remained the same. Statistical analysis revealed no significant difference in head breadth of spermatozoa due to diluents (Table 29).

Preserved Semen at 24 Hours:

The mean values and their over all means ± S.E. with C.V. % of head breadth of spermatozoa of preserved semen at 24 hours are given in Table 30. The data of head breadth of 24 hours were subjected to 'F' test to see whether there is any significant difference in head breadth in different diluent. The result is shown in Table 31. From Table 31, it was concluded that there was no significant difference in head breadth in different diluent. Though there was slight difference in average head breadth in different diluents.

Preserved Semen at 48 Hours:

The mean values of head breadth of preserved spermatozoa at 48 hours and their over all means ± S.E. with C.V. % are presented in Table 32. There was slight variation in the head breadth of spermatozoa but the analysis of variance of the data revealed no significant difference in the head breadth of spermatozoa due to diluents (Table 33).

Preserved Semen at 72 Hours:

The mean values and their over all means ± S.E. and C.V. % of head breadth of spermatozoa of preserved semen at 72 hours are given in Table 34. The over all mean values in different diluents ranged from 4.763 - 4.730 μ. The data were treated statistically and the results are presented in Table 35. There was small variation due to diluents but the variation was not significant.

Preserved Semen at 96 Hours:

The average of head breadth of individual bull and average head breadth ± S.E. of over all bulls and their C.V. % are given in Table 36. The over all mean values of head breadth in different diluents ranged from 4.703 to 4.677 μ showing slight variation due to diluents. The decrease in the head breadth was minimum in EYGB and maximum in EYCGGlyCy diluent. The data were subjected to analysis of variance and the results indicated that the variation in head breadth of preserved spermatozoa was statistically not significant (Table 37).

TABLE 1

Showing mean values of live spermatozoa (per cent) in fresh and preserved semen at zero hour

Bull number	Fresh	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	83.87	84.08	83.46	83.34	83.10	83.84
B	91.14	90.96	90.73	90.71	90.34	90.91
C	73.87	74.97	73.41	73.44	73.70	73.70
D	86.13	85.80	85.90	85.73	85.70	85.94
E	70.82	70.43	70.65	70.65	70.23	70.65
Over all average	81.79	81.58	81.44	81.37	81.09	81.63
S.E. ±	0.021	0.024	0.021	0.02	0.021	0.021
C.V. %	3.00	2.56	2.45	2.44	2.45	2.48

TABLE 2

Analysis of variance of live spermatozoa (per cent) of fresh and preserved semen at zero hour

Source of variation	df	SS	MS	F
Between dilutors	5	8.00	1.60	0.05 NS
Within dilutors	294	10096.80	34.34	
Total	299	10104.80		

NS denotes non-significant.

TABLE 3

Showing mean values of live spermatozoa (per cent) in preserved semen at 24 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	80.64	81.37	80.95	78.96	82.19
B	87.66	88.33	88.19	85.95	89.15
C	70.20	70.88	70.65	68.27	71.53
D	82.75	83.51	83.39	81.63	83.97
E	67.03	67.48	67.73	68.48	68.40
Over all average	78.14	78.83	78.71	76.47	79.61
S. E. ±	0.019	0.017	0.019	0.019	0.01
C. V. %	2.46	2.06	2.47	2.53	2.53

TABLE 4

Analysis of variance of live spermatozoa (per cent) of preserved semen at 24 hours

Source of variation	df	SS	MS	F
Between dilutors	4	132.78	33.19	1.05 NS
Within dilutors	245	7735.48	31.57	
Total	249	7868.26		

NS denotes non-significant

TABLE 5

Showing mean values of live spermatozoa (per cent) in preserved semen at 48 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	FYGB
A	76.82	78.76	78.73	73.63	79.67
B	84.81	85.78	85.92	80.79	86.64
C	66.90	68.10	68.20	62.57	69.27
D	80.01	80.84	81.03	76.13	81.74
E	64.40	64.55	65.32	61.48	66.02
Over all average	74.91	76.05	76.27	71.23	77.13
S. E. ±	0.019	0.019	0.019	0.016	0.018
C. V. %	2.49	2.54	2.64	2.30	2.42

TABLE 6

Analysis of variance of live spermatozoa (per cent) of preserved semen at 48 hours

Source of variation	df	SS	MS	F
Between dilutors	4	458.51	114.63	2.91 *
Within dilutors	245	7190.08	39.35	
Total	249	7648.59		

* denotes significant at 5 % level.

TABLE 7

Showing critical difference in live per cent of spermatozoa at 48 hours of preservation

Treatment	Mean difference	C. D. at 5 %	C. D. at 1 %
EYCBGF vs EYCGGly	0.76 NS	2.45	3.22
EYCBGF vs EYCGGlyCy	0.91 NS	2.45	3.22
EYCBGF vs WM	2.38 NS	2.45	3.22
EYCBGF vs EYGB	1.49 NS	2.45	3.22
EYCGGly vs EYCGGlyCy	0.15 NS	2.45	3.22
EYCGGly vs WM	3.14 *	2.45	3.22
EYCGGly vs EYGB	0.73 NS	2.45	3.22
EYCGGlyCy vs WM	3.29 **	2.45	3.22
EYCGGlyCy vs EYGB	0.58 NS	2.45	3.22
WM vs EYGB	3.87 **	2.45	3.22

NS denotes non-significant

* denotes significant at 5 % level

** denotes significant at 1 % level

TABLE 8

Showing mean values of live spermatozoa (per cent) in preserved semen at 72 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	73.12	74.80	73.70	69.07	76.00
B	80.56	81.83	80.76	75.42	83.24
C	63.60	63.50	61.92	58.35	65.60
D	76.93	77.24	75.42	71.53	78.75
E	60.80	61.22	59.90	56.78	62.80
Overall average	71.30	72.07	70.67	66.45	73.64
S.E. ±	0.016	0.018	0.018	0.011	0.018
C.V. %	2.34	2.57	2.60	2.25	2.42

TABLE 9

Analysis of variance of live spermatozoa (per cent) in preserved semen at 72 hours

Source of variation	df	SS	MS	F
Between dilutors	4	566.59	141.65	5.32 **
Within dilutors	245	6523.86	26.63	
Total	249	7090.45		

** denotes significant at 1 % level.

TABLE 10

Showing critical difference in live per cent of spermatozoa at 72 hours of preservation

Treatment	Mean difference	C.D. at 5 %	C.D. at 1 %
EYCBGF vs EYCGGly	0.49 NS	2.02	2.65
EYCBGF vs EYCGGlyCY	0.40 NS	2.02	2.65
EYCBGF vs WM	3.01 **	2.02	2.65
EYCBGF vs EYGB	1.50 NS	2.02	2.65
EYCGGly vs EYCGGlyCy	0.89 NS	2.02	2.65
EYCGGly vs WM	3.50 **	2.02	2.65
EYCGGlyCy vs EYGB	1.01 NS	2.02	2.65
EYCGGlyCy vs WM	2.61 *	2.02	2.65
EYCGGlyCy vs EYGB	1.90 NS	2.02	2.65
WM vs EYGB	4.51 **	2.02	2.65

NS denotes non-significant

* denotes significant at 5 % level

** denotes significant at 1 % level

TABLE 11

Showing mean values of live spermatozoa per cent in preserved semen at 96 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	68.92	67.32	66.72	61.48	71.32
B	76.27	74.62	74.67	69.23	78.59
C	59.90	58.60	57.28	52.52	62.00
D	73.21	71.83	71.21	66.80	75.01
E	57.07	55.73	55.00	51.40	59.72
Average	67.30	65.82	64.73	60.42	69.57
S.E. ±	0.015	0.015	0.014	0.013	0.015
C.V. %	2.46	2.44	2.39	2.38	2.21

TABLE 12

Analysis of variance of live spermatozoa per cent of preserved semen at 96 hours

Source of variation	df	SS	MS	F
Between dilutors	4	826.78	206.70	9.01 **
Within dilutors	245	5621.81	22.95	
Total	249	6448.59		

** denotes significant at 1 % level

TABLE 13

Showing critical difference in live percentage of spermatozoa at 96 hours of preservation

Treatments	Mean difference	C.D. at 5 %	C.D. at 1 %
EYCBGF vs EYCGGly	0.90 NS	1.86	2.45
EYCBGF vs EYCGGlyCy	1.55 NS	1.86	2.45
EYCBGF vs WM	4.11 **	1.86	2.45
EYCBGF vs EYGB	1.40 NS	1.86	2.45
EYCGGly vs EYCGGlyCY	0.65 NS	1.86	2.45
EYCGGly vs WM	3.21 **	1.86	2.45
EYCGGly vs EYGB	2.30 *	1.86	2.45
EYCGGlyCy vs WM	2.56 **	1.86	2.45
EYCGGlyCy vs EYGB	2.95 **	1.86	2.45
WM vs EYGB	5.51 **	1.86	2.45

NS denotes non-significant

* denotes significant at 5 % level

** denotes significant at 1 % level

TABLE 14

Showing mean values of head length (in microns) of fresh and preserved spermatozoa at zero hour

Bull number	Fresh	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	6.840	6.856	6.824	6.857	6.857	6.857
B	7.403	7.420	7.402	7.403	7.403	7.403
C	6.990	6.974	6.990	6.991	6.989	6.990
D	6.890	6.890	6.890	6.875	6.889	6.873
E	7.353	7.336	7.385	7.371	7.353	7.369
Average	7.095	7.095	7.098	7.099	7.098	7.098
S. E. ±	0.038	0.038	0.039	0.039	0.038	0.039
C. V. %	3.80	3.80	3.80	3.80	3.80	3.80

TABLE 15

Analysis of variance of head length of fresh and preserved spermatozoa at zero hour

Source of variation	df	SS	MS	F
Between dilutors	5	0.05	0.01	0.14 NS
Within dilutors	294	22.12	0.07	
Total	299	22.17		

NS denotes non-significant

TABLE 16

Showing mean values of head length (in microns) of preserved spermatozoa at 24 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	6.841	6.824	6.842	6.857	6.856
B	7.403	7.386	7.386	7.386	7.403
C	6.958	6.957	6.973	6.956	6.990
D	6.873	6.873	6.840	6.874	6.874
E	7.336	7.369	7.353	7.353	7.352
Average	7.082	7.082	7.079	7.085	7.095
S. E. ±	0.041	0.040	0.041	0.038	0.040
C. V. %	4.10	3.53	4.10	3.81	3.94

TABLE 17

Analysis of variance of head length of preserved spermatozoa at 24 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.02	0.005	0.061 NS
Within dilutors	245	20.14	0.082	
Total	249	20.16		

NS denotes non-significant

TABLE 16

Showing mean values of head length (in microns) of preserved spermatozoa at 24 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	6.841	6.824	6.842	6.857	6.856
B	7.403	7.386	7.386	7.386	7.403
C	6.958	6.957	6.973	6.956	6.990
D	6.873	6.873	6.840	6.874	6.874
E	7.336	7.369	7.353	7.353	7.352
Average	7.082	7.082	7.079	7.085	7.095
S. E. ±	0.041	0.040	0.041	0.038	0.040
C. V. %	4.10	3.53	4.10	3.81	3.94

TABLE 17

Analysis of variance of head length of preserved spermatozoa at 24 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.02	0.005	0.061 NS
Within dilutors	245	20.14	0.082	
Total	249	20.16		

NS denotes non-significant

TABLE 18

Showing mean values of head length (in microns) of preserved spermatozoa at 48 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	6.825	6.823	6.825	6.842	6.858
B	7.386	7.369	7.352	7.353	7.385
C	6.922	6.922	6.922	6.938	6.954
D	6.858	6.840	6.824	6.858	6.874
E	7.319	7.319	7.335	7.320	7.352
Average	7.062	7.055	7.052	7.062	7.085
S.E. ±	0.036	0.037	0.037	0.035	0.035
C.V. %	3.68	3.69	3.69	3.40	3.53

TABLE 19

Analysis of variance of head length of preserved spermatozoa at 48 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.02	0.005	0.075 NS
Within dilutors	245	16.33	0.067	
Total	249	16.35		

NS denotes non-significant

TABLE 20

Showing mean values of head length (in microns) of preserved spermatozoa at 72 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	6.808	6.708	6.725	6.741	6.825
B	7.286	7.269	7.269	7.286	7.353
C	6.890	6.873	6.873	6.907	6.940
D	6.825	6.792	6.776	6.841	6.841
E	7.286	7.253	7.269	7.285	7.302
Average	7.019	6.979	6.982	7.012	7.049
S.E. ±	0.035	0.038	0.037	0.036	0.035
C.V. %	3.42	3.72	3.72	3.57	3.55

TABLE 21

Analysis of variance of head length of preserved spermatozoa at 72 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.15	0.036	0.53 NS
Within dilutors	245	16.57	0.068	
Total	249	16.72		

NS denotes non-significant

TABLE 22

Showing mean values of head length (in microns) of preserved spermatozoa at 96 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	6.757	6.656	6.657	6.774	6.791
B	7.237	7.123	7.104	7.204	7.285
C	6.757	6.640	6.641	6.708	6.807
D	6.724	6.389	6.572	6.707	6.759
E	7.153	7.055	7.054	7.103	7.205
Average	6.926	6.813	6.806	6.899	6.969
S.E. ±	0.037	0.040	0.028	0.035	0.051
C.V. %	3.75	4.11	2.94	3.62	5.16

TABLE 23

Analysis of variance of head length of preserved spermatozoa at 96 hours

Source of variation	df	SS	MS	F
Between dilutors	4	1.09	0.27	3.57 *
Within dilutors	245	18.68	0.08	
Total	249	19.77		

* denotes significant at 5 % level

TABLE 24

Showing critical difference within diluents at 96 hours of preservation

Treatments	$\bar{X}$ difference	C. D. at 5 %	C. D. at 1 %
EYCBGF vs EYCGGly	0.113 *	0.110	0.144
EYCBGF vs EYCGGlyCy	0.120 *	0.110	0.144
EYCBGF vs WM	0.027 NS	0.110	0.144
EYCBGF vs EYGB	0.043 NS	0.110	0.144
EYCGGly vs EYGGGlyCy	0.007 NS	0.110	0.144
EYCGGly vs WM	0.086 NS	0.110	0.144
EYCGGly vs EYGB	0.176 **	0.110	0.144
EYCGGlyCy vs WM	0.093 NS	0.110	0.144
EYCGGlyCy vs EYGB	0.163 **	0.110	0.144
WM vs EYGB	0.070 NS	0.110	0.144

NS denotes non-significant

* denotes significant at 5 % level

** denotes significant at 1 % level

TABLE 25

Showing mean values of head length (in microns) of spermatozoa at different hours of preservation

Hours of preservation	Head length	S. E. ±	C. V. %
0 hr	7.097	0.039	3.80
24 hrs	7.085	0.018	3.95
48 hrs	7.063	0.016	3.54
72 hrs	7.008	0.036	3.57
96 hrs	6.882	0.017	4.07

TABLE 26

Analysis of variance of head length of spermatozoa at different hours of preservation

Source of variation	df	SS	MS	F
Between hours	4	7.90	1.98	28.29 **
Within hours	1295	95.20	0.07	
Total	1299	103.10		

** denotes significant at 1 % level

TABLE 27

Showing critical difference in head length at different hours of preservation

Hours	$\bar{X}$ difference	C. D. at 5 %	C. D. at 1 %
0 vs 24	0.012 NS	0.043	0.057
0 vs 48	0.034 NS	0.043	0.057
0 vs 72	0.089 **	0.043	0.057
0 vs 96	0.215 **	0.043	0.057
24 vs 48	0.022 NS	0.045	0.059
24 vs 72	0.077 **	0.045	0.059
24 vs 96	0.203 **	0.045	0.059
48 vs 72	0.055 *	0.045	0.059
48 vs 96	0.181 **	0.045	0.059
72 vs 96	0.126 **	0.045	0.059

NS denotes non-significant

* denotes significant at 5 % level

** denotes significant at 1 % level

TABLE 28

Showing mean value for head breadth (in microns) of fresh and preserved spermatozoa at zero hour

Bull number	Fresh	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	4.929	4.946	4.929	4.863	4.963	4.980
B	4.963	4.963	4.946	4.980	4.963	4.963
C	4.980	4.980	4.963	4.980	4.963	4.980
D	4.912	4.912	4.929	4.895	4.929	4.929
E	4.946	4.963	4.946	4.929	4.963	4.946
Average	4.946	4.953	4.943	4.929	4.956	4.960
S. E. ±	0.003	0.002	0.010	0.040	0.040	0.007
C. V. %	1.21	0.20	1.42	2.02	2.11	1.01

TABLE 29

Analysis of variance of head breadth of fresh and preserved spermatozoa of zero hour

Source of variation	df	SS	MS	F
Between dilutors	5	0.16	0.032	1.45 NS
Within dilutors	294	6.39	0.022	
Total	299	6.55		

NS denotes non-significant

TABLE 30

Showing mean values for head breadth (in microns) of preserved spermatozoa at 24 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	4.929	4.912	4.846	4.895	4.963
B	4.878	4.929	4.913	4.913	4.929
C	4.878	4.878	4.896	4.861	4.895
D	4.929	4.846	4.861	4.844	4.878
E	4.963	4.929	4.912	4.929	4.929
Average	4.915	4.899	4.886	4.888	4.919
S. E. ±	0.01	0.01	0.01	0.01	0.01
C. V. %	1.63	2.24	2.25	1.84	1.63

TABLE 31

Analysis of variance of head breadth of preserved spermatozoa at 24 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.02	0.005	0.5 NS
Within dilutors	245	2.47	0.01	
Total	249	2.49		

NS denotes non-significant

TABLE 32

Showing mean values for head breadth (in microns) of preserved spermatozoa at 48 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	4.812	4.812	4.779	4.878	4.895
B	4.794	4.828	4.844	4.778	4.810
C	4.810	4.794	4.730	4.812	4.827
D	4.762	4.827	4.778	4.747	4.796
E	4.827	4.844	4.829	4.828	4.847
Average	4.801	4.821	4.792	4.809	4.835
S. E. ±	0.01	0.01	0.02	0.02	0.017
C. V. %	1.66	1.45	2.71	2.69	2.69

TABLE 33

Analysis of variance of head breadth of preserved spermatozoa at 48 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.05	0.012	1.00 NS
Within dilutors	245	3.15	0.012	
Total	249	3.20		

NS denotes non-significant

TABLE 34

Showing mean values of head breadth (in microns) of preserved spermatozoa at 72 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	4.746	4.746	4.730	4.746	4.763
B	4.730	4.746	4.714	4.730	4.747
C	4.715	4.714	4.714	4.698	4.746
D	4.761	4.761	4.745	4.745	4.795
E	4.762	4.762	4.746	4.730	4.762
Average	4.743	4.746	4.730	4.730	4.763
S. E. ±	0.01	0.01	0.014	0.014	0.014
C. V. %	2.32	1.89	2.11	2.11	2.10

TABLE 35

Analysis of variance of head breadth of preserved spermatozoa at 72 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.04	0.008	0.73 NS
Within dilutors	245	2.66	0.011	
Total	249	2.70		

NS denotes non-significant

TABLE 36

Showing mean values for head breadth (in microns) of preserved spermatozoa at 96 hours

Bull number	EYCBGF	EYCGGly	EYCGGlyCy	WM	EYGB
A	4.664	4.663	4.648	4.681	4.680
B	4.649	4.682	4.649	4.665	4.697
C	4.681	4.647	4.664	4.649	4.648
D	4.729	4.730	4.714	4.730	4.746
E	4.730	4.730	4.712	4.713	4.746
Average	4.691	4.690	4.677	4.688	4.703
S. E. ±	0.014	0.014	0.014	0.013	0.016
C. V. %	2.13	2.13	2.31	2.03	2.43

TABLE 37

Analysis of variance of head breadth of preserved spermatozoa at 96 hours

Source of variation	df	SS	MS	F
Between dilutors	4	0.02	0.005	0.45 NS
Within dilutors	245	2.75	0.011	
Total	249	2.77		

NS denotes non-significant

DISCUSSION

DISCUSSION

Live percentage of spermatozoa:

The present study was undertaken to find out the percentage of live spermatozoa in different diluents at 0, 24, 48, 72 and 96 hours of preservation. The dilutors studied were egg yolk-citrate-potassium-bicarbonate-glucose-fructose (EYCBGF), egg yolk-citrate-glucose-glycine (EYCGGly), egg yolk-citrate-glucose-glycine-cysteine (EYCGGlyCy), whole milk (WM) and egg yolk-glucose-bicarbonate (EYGB).

The live percentage of spermatozoa in different diluents at 0 hour of preservation remained practically the same. The differences in live percentage of spermatozoa at 24 hours of preservation was also recorded to be non-significant (Table 4). At 48 hours of storage, significant increase in live percentage of spermatozoa was recorded only in EYGB in comparison to WM. At 72 hours of preservation every dilutor was significantly superior than WM and no significant difference in live percentage of spermatozoa was recorded between EYCBGF, EYCGGly, EYCGGlyCy and EYGB, indicating almost equal capacity to preserve the spermatozoa for a period upto 72 hours. The WM was noticed to be significantly inferior than all other dilutors and significant increase in the live percentage of spermatozoa was observed in EYGB in comparison to all other dilutors except EYCBGF at 96 hours of preservation. Statistically there was no difference in EYCBGF vs EYCGGly,

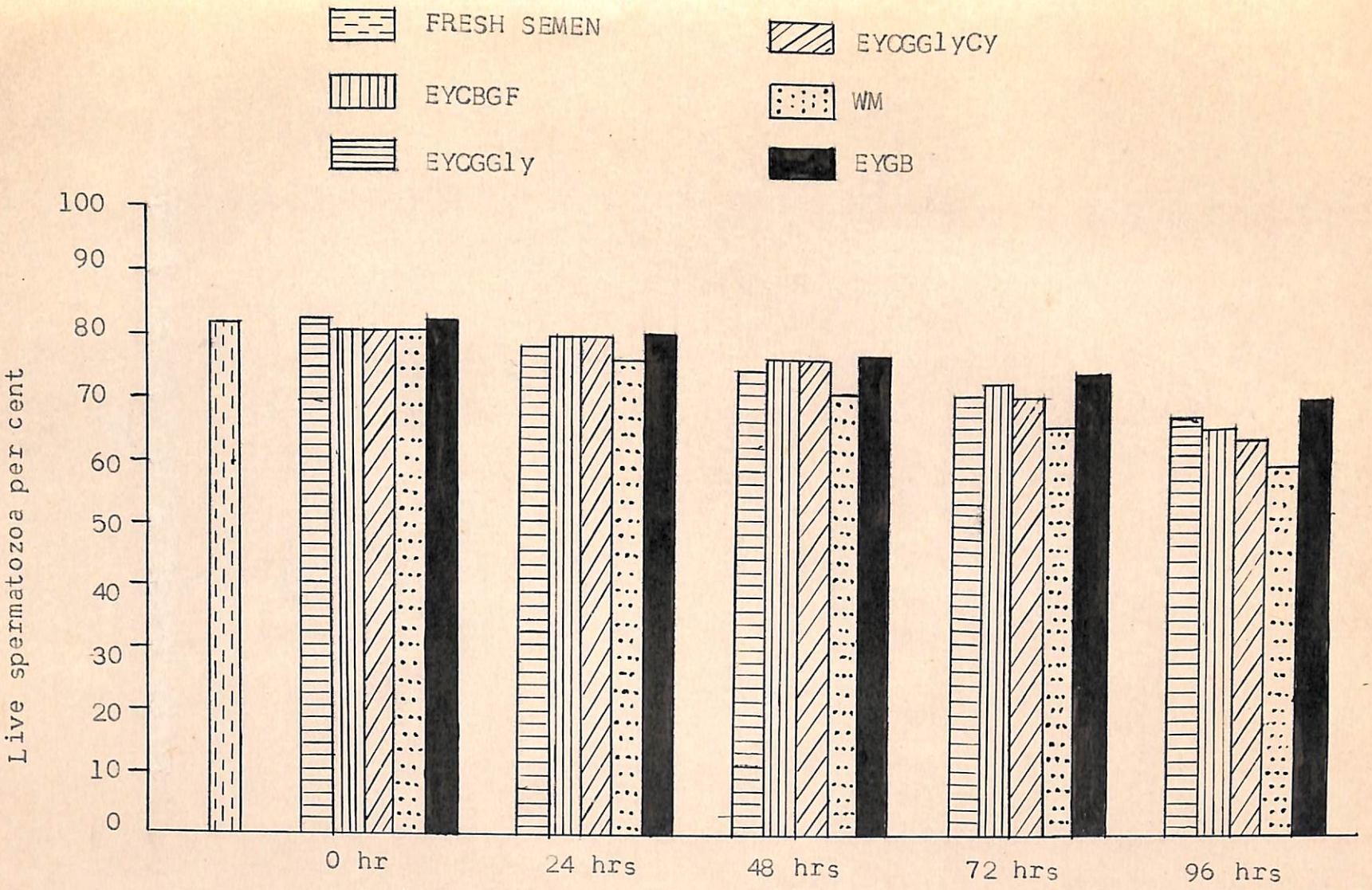

Figure - 1. Live spermatozoa per cent in different diluents at different hours of preservation.

EYCBGF vs EYCGGlyCy, EYCGF vs EYGB and EYCGGly vs EYCGGlyCy. The mean values of live spermatozoa per cent at 96 hours of storage showed least decrease in EYGB followed in order by EYCBGF, EYCGGly, EYCGGlyCy and WM.

The superiority of EYGB to preserve buffalo spermatozoa for a longer duration of time are in agreement with the findings of other workers, who after making comparative study with other commonly employed semen diluents for the preservation of buffalo spermatozoa at refrigerator temperature, concluded egg yolk-glucose-bicarbonate to be the most suitable (Srivastava and Prabhu, 1956; Gokhale, 1958; Sharma and Mahajan, 1961b; Nayudu, 1961; Mahajan and Sharma, 1963; Sharma and Sengar, 1965 and Kumar, 1966). However, Tomar and Desai (1961b) had obtained better results with EYCBGF than EYGB at very long period of storage but both the diluents persued nearly the same path of efficacy for about 4 days of storage period. EYGB was also reported to be better than egg yolk-glucose-glycine (Singh et al., 1970) and egg yolk-citrate-glucose-glycine (Kumar, 1966).

Kale (1963) and Joshi et al. (1967) noticed + 3 motility in cows milk for 3 days with buffalo semen, however, autoclaved milk was stated to be much inferior than EYGB (Srivastava and Prabhu, 1956). Mahajan and Sharma (1963) reported that EYGB was significantly superior to diluents having milk as a major constituent. In the present finding WM was significantly inferior than other diluents at 96 hours

of preservation. Although the milk dilutor did not prove as effective as other dilutors to maintain the live spermatozoa per cent but its mean value of live spermatozoa per cent was found to be more than 60 %. This finding does not come in way in the popularity and uses of this dilutor.

The satisfactory results obtained from EYGB and EYCBGF may be related to the presence of glucose and fructose in the diluting medium. The metabolism of spermatozoa in bull is predominately of a glycolytic charactor. According to Mann (1954), mammalian spermatozoa possesses only a negligible reserve of intra-cellular glycogen and depend therefore, under anaerobic conditions, on an extra-cellular source of energy. Bull spermatozoa glycolyse glucose, and fructose and the presence of these sugars is beneficial to sperm motility. The deleterious effect of salt diluents on spermatozoa is also partly overcome by the inclusion of certain organic substances. It has been shown that the addition of small amounts of glucose to a yolk buffer diluent is conducive for the bovine spermatozoa (Salisbury and VanDemark, 1945). Kampschmidt et al. (1951) studied the effects of increased glucose level and lowered electrolyte content, and observed that the replacement of buffer salts, citrate or phosphate with a metabolisable sugar led to an improvement in semen storage until the concentration of salt was so low that the buffering capacity was affected. Kok (1952) also observed superior results by adding fructose to the egg yolk-citrate

dilutor. Presence of potassium ion also helped the preservation of buffalo bull spermatozoa (Mann, 1954, Tomar and Desai, 1961b).

It has been found by many workers including Mann, 1954 and Flipse, 1956 that glycine is also metabolised by bull spermatozoa. Roy and Bishop (1954) had suggested that the beneficial effect of glycine may be due to its low electrolyte content, and the role played by it in the synthesis of creatine and adenosin, which may be important for the regulation of glycolysis. But presence of electrolyte in the egg yolk-glycine medium has an adverse effect on the liveability of the spermatozoa (Tomar and Desai, 1961c). This may be the reason for the glycine containing dilutors that they failed to maintain a very good percentage of live spermatozoa in the present experiment. Thus, in the light of the present study, it may be mentioned that optimum concentration of glucose in the dilutors is a necessity for the maintenance of live spermatozoa in buffalo bull semen.

D I S C U S S I O N

Morphology of spermatozoa:

The concensus of opinion is that the fertilizing capacity of spermatozoa is dependent to a great extent on the dimensional variations of the head (Savage et al., 1927; Lagerlof, 1934; Krajnc, 1964).

The head is the most vital part of the spermatozoa, which is made up chiefly of nucleus covered anteriorly by the acrosome. The nucleus is composed of deoxyribonucleic acid (DNA). The acrosome is composed of an inner and outer layer; it arises from the golgi apparatus of the spermatid as it differentiates into a sperm (Hafez, 1969). Acrosome has vital role in fertilization and its deformities will make the bull sterile (Marshall, 1968; Hafez, 1969). Blom (1945) and Hancock (1952) reported that loss of acrosomal cap is the first stage in degeneration of the spermatozoa.

In the present study the observations recorded on the dimensions of head in relation to preservation at different hours of storage in different diluents have been furnished in Tables 14 to 37. It is evident that the dimensions of head showed a steady decrease with the increase in storage hours. On statistical analysis the decrease in head length was found to be significant. This observation is incorboration with the findings of Mukherjee (1961), who recorded significant

decrease in head measurement of spermatozoa after 72 hours of preservation and Krishnnaya (1966), who observed significant drop in head dimensions between fresh and preserved spermatozoa at 24, 48 and 72 hours of preservation. This change in head measurements occurred due to its dilution and is inconsistant with the observations of Cimpeanu (1968).

There was, however, no significant difference in the measurement of head length in different diluents upto 72 hours of preservation but the mean values of measurements in different diluents indicated a trend of marked decrease in EYCGGly and EYCGGlyCy diluents. After 96 hours of preservation, a statistically significant decrease was recorded in EYCGGly and EYCGGlyCy diluents in comparison to EYGB and EYCBGF. Least decrease was recorded in EYGB followed by EYCBGF, WM, EYCGGly and EYCGGlyCy (vide Table 22). The differences in the mean values of head breadth of spermatozoa preserved in different diluents upto 96 hours were not significant, but the mean values established a trend which showed maximum decrease in EYCGGlyCy and EYCGGly dilutor. The order for the mean values of head breadth in the five semen diluents at 96 hours of storage were EYGB, EYCBGF, WM, EYCGGly and EYCGGlyCy. This observation is substantiated with the observations of Mukherjee and Dott (1960), Mukherjee (1961), Pandey et al. (1961) and Rathore and Mukherjee (1963). These workers recorded the significant decrease at 72 hours of preservation, however, in the present study, the decrease could be evident