STUDIES ON THE PRESERVATION OF BUCK SEMEN

BY

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THESIS

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DECLARATION

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

P.P. BALAKRISHNAN.

Mannuthy,
30-9-1979.

CERTIFICATE

Certified that the thesis entitled "STUDIES ON THE PRESERVATION OF BUCK SEMEN" is a record of research work done independently by Sri. P.P. Balakrishnan, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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

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C O N T E N T S

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DEDICATED

TO

THE MEMORY OF

MY BELOVED MOTHER

INTRODUCTION

INTRODUCTION

The common goat (Capra hircus) appears to have been domesticated from very ancient days. Goat has a definite place in the rural economy of our land and out of 3000 crores of rupees contributed by our livestock and poultry a sizable proportion is obtained from goats. Thus goat plays an important role in our national economy.

Goat Husbandry is an important livestock enterprise in Kerala. According to Livestock Census (1972) goat population in Kerala is 1.75 lakhs which stands second to cattle population. The one and only breed of livestock that Kerala possess is a breed of goat viz., 'Malabari goat'. Recently great emphasis is being made to improve this breed by cross breeding with exotic breeds like Sannen and Alpine.

The growing realisation of the genetic and economic possibilities of artificial insemination and the technical advances made in recent years have permitted a much greater utilisation of this method of breeding for the development and improvement of livestock industry. But artificial insemination in goats has not developed to the same stage of perfection as in the case of cattle even in advanced countries. The non availability of a suitable extender for the preservation of buck semen, has always been a limiting factor for the popularisation of artificial insemination in goats.

Though sufficient work has been done on the dilution and preservation of ram semen, similar studies on buck semen appears to be scanty. General survey of the literature shows that there has not been much reports on extensive attempts to study the preservation of buck semen. However, Hampel (1951), Blokhuis (1959) and Sahni and Roy (1969) have studied some of the standard semen diluents for preservation of buck semen.

Preliminary studies carried out in the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, indicated that buck semen can be successfully preserved with good motility up to 24 hours only (John, 1970). Considering the significant role the cross bred goats play in the rural economy of the State and the role of artificial insemination in the rapid improvement of the genetic potentialities, a detailed investigation on preservation of buck semen was undertaken with the ultimate object of evolving a suitable extender for the same.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The study of artificial insemination in goat was started as early as 1934 (Benedictovic, 1934). The diluents used were physiological salt solutions like Ringer's and Locks fluid, blood and serum. These diluents served mainly to increase the volume of semen rather than to preserve it for longer periods.

The real progress in semen preservation was made with the advent of egg yolk (Philips, 1939), egg yolk phosphate (Phillps and Lardy, 1940) and egg yolk citrate diluents (Salisbury et al., 1941). Willet and Salisbury (1942) and Mayer and Lasley (1945) reported the importance of yolk in protecting the sperms from cold shock. Blackshaw (1954), Mayer (1955) and Salisbury (1957) stated that this action of yolk was due to the presence of lecithin and lipoprotein in the yolk.

Roy (1957) pointed out that egg yolk coagulating enzyme present in the bulbourethral gland interfered in the keeping quality of buck semen. This enzyme, requiring the presence of calcium ions for its activity, was reported to cause denaturation of egg yolk resulting in the dilutor getting curdled and consequent loss of viability of spermatozoa. He further observed that addition of citrate, oxalate and phosphate inhibited this action. Iritani et al. (1964) reported

that a thermolabile egg yolk coagulating factor in buck semen, derived from the bulbourethral gland, was responsible for its poor keeping quality. They also found that this factor was eliminated when the sperm was washed and that addition of extract of cowper's gland maintained yolk coagulating activity. This coagulating factor did not affect egg white, milk or blood pigments. Jabnel (1954) observed that clumping of spermatozoa in the diluent was due to lack of carotinoid in egg and was marked in thin semen and increased by alkalinity. He also observed that keeping the diluent half an hour at 6°C would prevent agglutination.

Melrose and Stewart (1956) reported that there was no significant difference in fertility when 2.9 or 3.9 per cent citrate solution were used in the diluent. The level of yolk for optimum fertility and livability was reported to be 20 per cent (Swanson, 1949) and 20 to 30 per cent (Stewart et al., 1950; Achnelt, 1951; Holt, 1952 and Habibullin, 1952) in isotonic solution of sodium citrate. Almquist (1951) observed that 10 to 12.5 per cent of egg yolk in the diluent would not affect the fertility of bull sperm.

Rosenberger (1944) observed that buck semen diluted in Milovanovas glucose phosphate diluent at a ratio of 1:16 could be stored for longer periods at 10 to 12°C. He also observed

that out of 678 inseminations with this diluent, 612 goats became pregnant. Wanger (1949) reported that buck semen could be preserved upto 274 hours at 8°C in glucose phosphate diluents containing 2.5 per cent egg yolk. Schmidt et al. (1950) observed higher conception rate with semen diluted in glucose phosphate diluent at a ratio of 1:2 or 1:3 at 6 to 12°C storage. Using buck semen extended in glucose phosphate diluent and stored for 24 hours, Guha et al. (1951) obtained excellent fertility rate (60.9%). Hampel (1951) observed that buck semen diluted at 1:4 ratio with glucose phosphate diluent preserved better at 8°C than at 4°C.

Konger (1951) reported that there was significant reduction in motility and fertility when buck semen diluted in egg yolk citrate or egg yolk phosphate diluents was stored beyond 24 hours. Even the addition of hyaluronidase did not improve the motility and fertility. But the addition of sulphamine, homosulphamine and sodium citrate solution maintained the motility and fertility of semen up to 168 hours (Yoshika et al., 1951). Achnelt and Rosenwinkel (1953) compared various commercially prepared diluents with sodium citrate with or without egg yolk for extending buck semen and found that motility and conception rate was higher in sodium citrate diluent. Baudet et al. (1954), on a trial with various diluents, found that the fertilizing ability was lost as the storage time increased though motility was retained. Blokhuis (1957) observed

that 3.5 per cent sodium citrate with 5 per cent egg yolk was efficient in preserving the keeping quality of semen. Roy et al. (1959) obtained good viability of sperms when washed spermatozoa was preserved in egg yolk citrate, glycine egg yolk and egg yolk glycerol phosphate diluent. Blokhuis (1959) obtained a conception rate of 62.5 and 55.5 per cent respectively using egg yolk citrate and skim milk as extenders.

Knoblauch (1962) diluted buck semen in sodium citrate egg yolk dilutor at the rate of 1:5 and obtained good motility up to five days. He also observed a non-return rate of 86.6 per cent at 6 to 8 hours of storage. Jelam and Nambiar (1963) recorded 65.6 and 54.4 per cent motility at 24 and 72 hours of storage in egg yolk citrate diluent. Nasim et al. (1964) used sodium citrate egg yolk buffer containing antibiotics for preservation of buck semen at a ratio of 1:5 to 1:15 and obtained good forward motility up to 1 to 3 days storage at 38 to 40°F. They also reported good fertility rate when the diluted semen sample was used for insemination trials. Corteel (1967) compared three types of egg yolk citrate diluents containing antibiotics and found that the diluent containing phospholipids in association with yolk protein maintained good motility up to 12 to 16 days of storage but the fertility rate was low (26.9%). Patel (1967) carried out insemination trials with

buck semen diluted 1:4 to 1:20 with egg yolk citrate and obtained 98 per cent conception rate. John (1970) observed that semen could be stored without complete loss of motility till 120 hours in egg yolk citrate diluent. However, the motility dropped below 40 per cent beyond 48 hours of storage.

Gonan (1971) obtained good results with egg yolk citrate diluent containing 20 per cent egg yolk and 2.9 per cent sodium citrate stored at 2 to 3 hours at 18 to 20°C. Linzell (1971) found that buck semen could be diluted 1:4 to 1:20 with a glycerol egg yolk medium and stored for 1 to 2 days at 15°C with satisfactory motility. Fukhuhara (1973) observed that addition of 40 mg of chlorpromazine hydrochloride in egg yolk citrate buffer was beneficial for survival of buck spermatozoa. Hirore and Udatsu (1975) found that buck semen would retain 75 per cent motility up to three days at 25°C when diluted with 3 per cent sodium citrate and saturated with carbondioxide. The survival of sperm was found to be seven days. Schinder et al. (1975) used a diluent containing sodium citrate, glycine, fructose and egg yolk and noticed that spermatozoan motility would last for longer periods when the supernatant fluid of the diluent after 48 hours was used for preservation of semen. Koh and Ong (1977) observed that when buck semen was preserved in 2.9, 3.5 and 4 per cent sodium citrate egg yolk diluents,

sperm motility averaged 62.6, 46.7 and 24.3 per cent respectively. Wani et al. (1978) preserved buck semen at dilution rates of 1:1, 1:3, 1:6 and 1:10 of 20 per cent egg yolk citrate diluent and observed that 1:10 dilution rate was the best for sperm motility with 1:6 dilution rate ranking next at 5 to 7°C up to 24 to 48 hours.

Tyler and Tenabe (1952) claimed an improvement of survival rate of sperm by adding glycine to the diluent. Roy and Bishop (1954) found that buck semen when diluted in glycine egg yolk or egg yolk citrate maintained initial motility for a period of three days at 3°C. Roy et al. (1955) observed that motility of spermatozoa of bull, buffalo and ram was longer in egg yolk glycine than in yolk citrate diluent. In the case of washed buck spermatozoa also, egg yolk glycine diluent was more efficient than other diluents (Roy et al., 1959). Han (1957) found that addition of 0.5 per cent glycine to yolk citrate diluent apparently increased the motility of sperms without enhancing the conception rate. Ahmed (1955), Sha and Singh (1958) and Joshi and Singh (1968) obtained good motility of ram sperms in glycine containing diluents up to 144 hours of storage.

Sahni and Roy (1969) obtained an average motility of 70.5, 26.8 and 6.8 per cent at 6, 54 and 126 hours in glycine egg yolk diluents. Similarly, John (1970) also reported an

averageinitial motility of 70.00,per cent with 64.04, 62.62, 37.91 and 12.70 per cent at 24, 48, 72 and 96 hours at a dilution of 1:10. The motility dropped below 40 per cent at 72 hours of storage. On the contrary, Adler and Rasbech (1956) and Storm (1956) did not observe any improvement in motility by the addition of glycine to the diluent.

Milk was used for a long time in experiments with sperm (Donne, 1837; Koelliker, 1905 and Hoffman, 1905). However, it was Michajilov (1950) who successfully used milk for diluting bull semen. The successful use of heated milk as a semen diluent was later reported by Jacquet (1951), Thacker and Almquist (1951), Jacquet and Cassou (1952), Sanfile (1952), Weiss (1952), Schmidt and Kroll (1953), Almquist (1954), Tomar and Desai (1961), Kale (1963), Gupta et al. (1974) and Greeshmohan (1976).

Vandemark (1951) obtained better fertility rate with chemically treated unheated pasteurised milk than yolk citrate. Thacker and Almquist (1953) used boiled homogenised milk and pasteurised skim milk and observed that sperm survival was equal to that of yolk citrate diluent. Sacke et al. (1956) studied the effect of time and temperature on viability of bull sperm and found that good motility was obtained when skim milk was heated at 87 to 97°C for 10 minutes. Adler and Rasbech (1956) and Kerruish (1956) observed significant

difference in conception rate between diluted semen in heated skim milk and yolk citrate. Salisbury (1957) reported that skim milk proved as good as yolk citrate in terms of conception rate.

Dauzier (1956) reported that goat semen stored in skim milk for periods below eight hours gave a conception rate of 64 per cent eventhough good motility was maintained satisfactorily for longer periods. Dauzier and Dumesnil (1958) obtained good motility with satisfactory conception rate (62%) when milk was used for preservation of buck semen. Hill et al. (1958) using reconstituted skim milk as ram semen diluent obtained satisfactory motility for a period of 15 days at 1:10 dilution. Melrose et al. (1958) reported that use of nine per cent spray dried reconstituted skim milk powder in combination with streptomycin had a higher conception rate.

Blokhuis (1959) carried out fertility trials with buck semen diluted in skim milk and obtained a conception rate of 55.5 per cent. Fisher and Kandera (1960) did not observe any significant difference in conception rate when powdered skim milk and whole milk were used along with yolk streptomycin. Ahamed (1963) found that heated skim milk was inferior to heated homogenised whole milk as diluent. Jelam and Nambiar (1963) reported an average motility of 59.3 and

43.6 respectively after 24 and 72 hours when goat milk was used as semen diluent at a dilution of 1:20. Ron and Aamdal (1963) reported that the motility of goat spermatozoa diluted with dried milk was 47 per cent and 13 per cent after 48 hours and 160 hours of storage respectively.

Joshi and Singh (1968) studied the efficiency of five diluents, viz., skim milk yolk, skim milk yolk glucose, skim milk yolk glucose fructose, skim milk yolk glucose glycine, skim milk yolk glucose bicarbonate and found that skim milk yolk glucose and skim milk yolk glucose fructose diluents maintained significantly better motility than the other diluents when stored at 48, 96 and 144 hours at 3 ± 1°C with ram semen. Pavlovic and Vardin (1968) obtained favourable results with skim milk diluent for ram semen. The conception rate was highest when semen was diluted 11 times with reconstituted unheated powdered skim milk.

Tewari et al. (1968) observed that the sperm motility was 16.09, 27.6, 36.4 and 50.4 per cent in the dilution rate of 1:1, 1:2, 1:5 and 1:10 respectively, when stored at 5 to 7°C for six hours in cow's milk. The corresponding figures at 30 hours of storage were 6.9, 16.8, 22.4 and 35.0 per cent respectively. Sahni and Roy (1969) found that milk and milk containing diluents were significantly superior to diluents containing yolk for preservation of buck semen.

Sahni and Tewari (1973) carried out fertility trials with semen diluted in cows milk and ewes milk and found that the lambing rate with freshly diluted semen and semen stored for 10 hours at 8 to 10°C was 40 per cent and 20 per cent respectively. Petruzzi and Tarantini (1974) reported that semen diluted in powdered or homogenised pasteurised cows milk with or without egg yolk gave better motility after storage for 96 hours at 5°C. John and Raja (1975) obtained an average motility of 70.6 and 70.7 per cent initially, 64.0 and 60.5 per cent after 48 hours, 33.0 and 27.5 per cent after 96 hours of storage with cows and goats milk respectively at dilution of 1:10 at 4 to 6°C.

Sahni and Roy (1969) reported that the average motility of buck semen diluted with Cornell University Extender (CUE) was 75.5, 38.0 and 7.3 per cent at 6, 54 and 126 hours respectively. Koh and Ong (1977) also observed similar results with CUE.

Ron and Aamdal (1963) using Illini variable temperature (IVT) diluent for preservation of buck semen, obtained motility of 70 and 53 per cent at 48 and 168 hours of storage respectively. Aamdal et al. (1965) found a change in the colour, consistency and lack of motility in goat semen using IVT diluent containing 10 per cent egg yolk, stored for 10 hours at 15 to 20°C. They also noted that when semen was

stored at 5°C these changes occurred somewhat later and was attributed to the formation of lysolecithin.

Pillai (1971), on a study on the preservation of buck semen at room temperature in coconut milk extender (CME) observed that buck semen could be preserved in CME up to 24 hours at a dilution of 1:100, 1:150 and 1:200. But it was found that the livability of sperm was superior in 1:200 dilutions than the other two dilutions. Sahni and Roy (1972) reported that egg yolk citrate, cows milk and CUE were satisfactory for preserving buck semen at room temperature (30-35°C) for a storage period of 2 to 3 hours.

Lopatko (1971) observed no significant difference in sperm survival rate between Tris diluent and glucose yolk citrate diluent. Founger (1976) used Tris glycerol egg yolk diluent for freezing buck semen and obtained recovery rate of 60 to 80 per cent.

Gonazatez Stagnaro (1976) found that semen from Nubian buck diluted in Laciphos or reconstituted skim milk showed good forward motility. Masuda et al. (1972) reported that five per cent solution of acetone soluble egg yolk lipids preserved 88.3 per cent of prefreezing motility as compared to 77.3 per cent by 10 per cent egg yolk in buck semen.

Hartel (1967) found that percentage of forward motility of spermatozoa was greater and lasted considerably longer in bucks fed 11 g nitrate in gelatin capsule. Elwishy et al. (1971) noticed highly significant monthly and seasonal variation on motility of diluted semen stored at 4 to 5°C. Sahni and Roy (1972) used certain colour additives like Neutral red, Sudan III orange and yellow for identification of buck semen and did not find any adverse effect on keeping quality at 5 to 7°C. Fukhuhara (1973) observed that the presence of pyruvates, acetates, lactate, glucose, fructose and mannose in the diluent maintained motility. Citrate, fumerate, maleate, succinate and L-betaglutarate slightly increased oxygen uptake but did not maintain motility. Galactose, xylose, ribose, maltose, lactose and sucrose had no effect on oxygen uptake or motility. Montigny (1976) observed good motility in whole buck semen stored at 4°C for one day.

MATERIAL AND METHODS

MATERIAL AND METHODS

Semen samples collected from eight cross bred bucks (6 Sannen x Malabari and 2 Alpine x Malabari) in the age group of 1½ to 2 years belonging to the 'All India Co-ordinated Research Project on Goats for Milk Production' attached to the College of Veterinary and Animal Sciences, Mannuthy, formed the material for the study. These bucks were maintained on standard feeding and managemental conditions.

Semen samples were collected by means of artificial vagina as described by Perry (1969) observing strict aseptic precautions. A total of 64 ejaculates were utilised for the study. All the semen samples were subjected to routine evaluation tests immediately after collection and only those samples with good initial motility were used for the trial. The composition of the various extenders used for the study are as follows:

(1) Egg yolk citrate diluent (EYC)

Egg yolk	25 ml
Sodium citrate (Analar)	1.96 g
Distilled water to	100 ml

(2) Egg yolk glycine diluent (EYG)

Glycine	2 g
Egg yolk	30 ml
Distilled water to	100 ml

(3) Egg yolk glucose citrate diluent (EYGC)

Glucose	5 g
Egg yolk	10 ml
Sodium citrate dihydrate	1.96 g
Distilled water to	100 ml

(4) Egg yolk glucose sodium bicarbonate diluent (EYGB)

Glucose	3 g
Sodium bicarbonate	0.15 g
Egg yolk	30 ml
Distilled water to	100 ml

(5) Egg yolk glucose sodium bicarbonate milk diluent (EYGBM)

Glucose	2 g
Sodium bicarbonate	20 mg
Egg yolk	20 ml
Goat milk	40 ml
Distilled water to	100 ml

(6) Illini variable temperature diluent (IVT)

Glucose	3 g
Sodium citrate	2 g
Sulphanilamide	300 mg
Sodium bicarbonate	210 mg
Potassium chloride	40 mg
Egg yolk	10 ml
Distilled water to	100 ml

(Not saturated with carbondioxide)

(7) Coconut milk extender (CME)

Coconut water	17 ml
Egg yolk	7 ml
Sodium citrate dihydrate	2.2 g
Sulphanilamide	0.3 g
Dihydrostreptomycin	0.135 g
Crystalline penicillin	0.060 g
Polymyxin B sulphate	0.010 g
Mycostatin (10 mg in 50 ml)	1 ml
Catalase	15,000 unit
Distilled water to	100 ml

(Sodium hydroxide 10% solution few drops to adjust pH to 7.4)

The coconut milk extender was prepared according to the technique described by Norman (1964).

(8) Kiew or Varohm diluent-1 (Kiew-I)

Glucose	6 g
Disodium ethylene diamine tetra acetate (EDTA)	370 mg
Sodium bicarbonate	120 mg
Sodium citrate dihydrate	375 mg
Glass distilled water to	100 ml

(9) Kiew or Varohm diluent-2

Potassium chloride	40 mg
Sodium bicarbonate	210 mg
Glucose	600 mg
Sodium citrate dihydrate	2 g
Glass distilled water to	100 ml

Kiew or Varohm diluent-1 and 2 were mixed at a 2:1 ratio and the resulting mixture (Kiew-II) was used for dilution of semen.

(10) Glucose glycine EDTA sodium bicarbonate citrate diluent (GGEBC)

Glucose	4.5 g
Glycine	350 mg
Disodium ethylene diamine tetra acetate (EDTA)	250 mg
Sodium bicarbonate	150 mg
Sodium citrate	800 mg
Distilled water to	100 ml

(11) Tris diluent

Fructose	1.25 g
Citric acid	1.70 g
Tris (hydroxymethyl) amino-methane	3.25 g
Glycerol	8 ml
Distilled water to	92 ml

(12) Egg yolk glucose glycine citrate diluent (EYGGC)

Glucose (5%)	30 parts
Glycine (4%)	25 parts
Sodium citrate (2.9%)	25 parts
Egg yolk	20 parts

(13) Egg yolk fructose glycine citrate diluent (EYFGC)

Fructose (5%)	30 parts
Glycine (4%)	25 parts
Sodium citrate dihydrate (2.9%)	25 parts
Egg yolk	20 parts

(14) Whole cows milk (CM)

Whole cows milk was heated to 93 to 95°C for 10 to 15 minutes in a water bath. The milk was then cooled and left overnight in a refrigerator. This was further filtered

just before dilution through sterile cotton so that the filterate was free of masses of milk fat and was homogenised. The extender was brought to room temperature before mixing with semen.

(15) Whole goats milk (GM)

Goat milk was also processed for use as extender in the same line as that of cows milk.

(16) Skim milk diluent (SM)

Skim milk was heated to 93 to 95°C for 10 to 15 minutes in a water bath. It was then cooled and left overnight in a refrigerator. The extender was brought to room temperature before mixing with semen.

(17) Cornell University Extender (CUE)

Egg yolk	20 parts
Stock solution	80 parts

Composition of stock solution

Sodium citrate dihydrate	1.45 g
Sodium bicarbonate	210 mg
Potassium chloride	400 mg
Glucose	300 mg
Glycine	937 mg

Sulphanilamide	300 mg
Distilled water to	100 ml

(18) Skim milk citrate fructose glycine diluent (SMCFG)

Skim milk	50 parts
Stock solution	50 parts

Composition of stock solution

Sodium citrate (2.9%)	40 parts
Fructose (5%)	35 parts
Glycine (4%)	25 parts

(19) Milk citrate fructose glycine diluent (MCFG)

Milk	50 parts
Stock solution	50 parts

Composition of stock solution

Sodium citrate (2.9%)	40 parts
Fructose (5%)	35 parts
Glycine (4%)	25 parts

All the extenders except coconut milk extender were fortified with 1000 unit of crystalline penicillin and 1000 micro grams of dihydrostreptomycin per ml of diluent.

Dilution and preservation

The semen samples were diluted in various extenders at a rate of 1:10 by split sample technique and the percentage of motile sperms assessed. The diluted samples were filled in 5 ml sterile glass ampoules, labelled and stored in the refrigerator at 5°C. Motility was assessed at 12 hour interval up to 120 hours or until total cessation of motility.

Rating of motility was done objectively under microscope giving an arbitrary score of 0 to 100 depending on the percentage of progressive motile sperms. Based on the results of preliminary observations 12 extenders viz., EYC, EYG, Kiew-I, Kiew-II, EYFGC, EYGC, EYGB, EYGBM, IVT, GGEBC, EYGGC, SM were eliminated from further studies due to poor preservability.

Preservation at room temperature in coconut milk extender (CME)

Thirty two semen samples were diluted with CME at the ratio of 1:10 and 1:200 using split sample technique. The diluted semen was filled in 2 ml sterile plastic vials, sealed airtight and stored in a dark place at room temperature (22.6 to 36.4°C). The motility of semen was recorded at 12 hours interval up to 96 hours or until total cessation of motility.

The pH of the solution was adjusted to 7.4 by adding few drops of sodium hydroxide (10%) solution. The extender prepared was stored at 5°C in the refrigerator and brought to room temperature just before use.

The data collected were subjected to statistical analysis as per Snedecor and Cochran (1967).

Limited number of semen samples extended in Tris, SMCFG, MCFG diluents were used for inseminations immediately after collection and dilution to assess the fertility in terms of conception rate.

RESULTS

RESULTS

With the ultimate object of evolving a suitable extender for buck semen, 64 semen samples were extended in six diluents viz., Tris diluent, skim milk citrate fructose glycine diluent (SMCFG), milk citrate fructose glycine diluent (MCFG), whole cows milk (CM), whole goats milk (GM), Cornell University Extender (CUE) and 32 semen samples in coconut milk extender (CME). Semen samples collected from eight cross bred bucks were diluted at a fixed rate of 1:10 with these different extenders. In CME semen samples were extended at dilution rate of 1:10 and 1:200. These semen samples were preserved at 5°C and the samples extended in CME were preserved at room temperature 22.6 to 36.4°C. Each semen sample was then subjected to assessment of motility at fixed intervals of 12 hours. The data pertaining to the preservation of various samples are presented in Tables 1 to 8 and in Figures 1 to 2.

Preservation at 5°C

The data on the preservation of buck semen in six extenders at 5°C are presented in Tables 1 to 4. It was observed from the Table 1 that initial motility of semen diluted in Tris, SMCFG, MCFG, CM, GM and CUE was 87.11, 84.92, 82.89, 80.78, 80.47 and 86.56 per cent respectively. Statistical analysis of data revealed significant difference between the

various diluents (Table 2). However, the variations in motility between Tris, SMCFG and CUE, between SMCFG and MCFG, between MCFG and CM and between CM and GM were not significant.

At 12 hours of storage the percentage of progressive motile sperms was 82.11, 77.73, 77.34, 71.48, 70.94 and 76.88 respectively for Tris, SMCFG, MCFG, CM, GM and CUE diluents (Table 1). The percentage of reduction of motility of sperms at 12 hours of preservation was 5.00, 7.19, 5.55, 9.30, 9.53 and 9.68 respectively in Tris, SMCFG, MCFG, CM, GM and CUE diluents (Table 3). On analysis, it was revealed that the differences in motility between SMCFG, MCFG and CUE and between GM and CM diluents were not significant (Table 2).

The motility of sperms at 24 hours storage in Tris, SMCFG, MCFG, CM, GM and CUE was 75.39, 70.94, 69.53, 62.42, 60.39 and 65.47 per cent respectively (Table 1). The sperm motility was significantly different between diluents on comparison at 24 hours of storage (Table 2). However, the variation in motility between SMCFG and MCFG and between CM and GM was not statistically significant. The percentage of reduction in motility during storage from 12 to 24 hours period was 6.72, 6.79, 7.81, 9.06, 10.55 and 11.41 respectively in Tris, SMCFG, MCFG, CM, GM and CUE diluents (Table 3).

During 36 hours of storage, the percentage of progressive motility was observed to be 70.47, 63.28, 63.98, 55.39,

53.05 and 54.69 in Tris, SMCFG, MCFG, CM, GM and CUE respectively (Table 1). The reduction of sperm motility from 24 to 36 hours of storage was 4.92, 7.66, 5.55, 7.03, 7.34 and 10.78 per cent in the respective diluents (Table 3). No significant difference in sperm motility was observed between SMCFG and MCFG and also between CM, GM and CUE (Table 2).

When the semen was stored for 48 hours, the percentage of progressive motility in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively was 62.97, 58.52, 55.39, 49.77, 46.41 and 43.36 (Table 1) and the percentage of decline in motility was 7.50, 4.76, 8.59, 5.62, 6.64 and 11.33 respectively in the diluents (Table 3). Statistical analysis revealed significant differences between all the diluents (Table 2).

The percentage of progressive motility of sperms at 60 hours of preservation in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively was 56.25, 51.33, 49.61, 38.75, 39.06 and 35.08 (Table 1). From the table it could be seen that the differences in motility between the diluents were significantly different except between SMCFG and MCFG and between CM and GM (Table 2). The percentage of decrease in sperm motility during 48 to 60 hours of storage was 6.72, 7.19, 5.78, 11.02, 7.35 and 8.28 respectively (Table 3).

At 72 hours of storage, the percentage of motile sperms was 49.90, 41.41, 41.33, 33.20, 29.30 and 25.55 respectively

in these diluents (Table 1). The percentage of motility between the diluents was significantly different except between SMCFG and MCFG (Table 2). The decrease in sperm motility during this period of storage was respectively 6.35, 9.92, 8.28, 5.55, 9.76 and 9.53 per cent (Table 3).

Semen preserved at 84 hours in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively retained 42.73, 32.42, 35.70, 25.39, 20.47 and 16.25 per cent progressive motility (Table 1) and the reduction of motility during 72 to 84 hours of storage was 7.17, 8.99, 5.63, 7.81, 8.83 and 9.30 per cent respectively (Table 3). Analysis of data showed that differences between the various diluents were statistically significant.

The percentage of motility at 96 hours in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively were 37.42, 23.83, 28.67, 17.27, 12.11 and 9.92 (Table 1) and the reduction in motility of sperms during 84 to 96 hours of storage period was observed to be 5.31, 8.59, 7.03, 8.12, 8.36 and 6.33 per cent respectively (Table 3). The differences in the percentage of progressive motility between all diluents were significant except between GM and CUE which was not significantly different (Table 2).

During 108 hours of preservation, the percentage of progressive motility was noted to be 28.52, 19.14, 20.23, 10.70, 7.42 and 5.70 respectively (Table 1). Motility

differed significantly between Tris, SMCFG, MCFG, CM, GM and CUE diluents at 108 hours of storage except between SMCFG and MCFG and between GM and CUE diluents (Table 2). The reduction of sperm motility during this period was 8.90, 4.69, 8.44, 6.57, 4.69 and 4.22 per cent for the respective diluents.

The motility of sperms at 120 hours was 19.30, 13.91, 12.42, 6.48, 3.20 and 2.81 per cent respectively in the various diluents (Table 1). The reduction in motility of sperms during 108 to 120 hours storage period was observed to be 9.22, 5.23, 7.81, 4.22, 4.22 and 2.89 per cent respectively in these diluents (Table 3). On statistical analysis, no significant difference in motility was observed between SMCFG and MCFG and between CM and CUE diluents (Table 2).

Analysis of the data also revealed that motility of sperms in the different extenders varied significantly ($P < 0.01$) at different periods of time (Table 4). Motility was maintained up to a level of 50 per cent during 60 hours in Tris, SMCFG, MCFG diluents. In CUE, motility dropped to 43.36 per cent at 48 hours of storage. From the Table 1 it could be seen that motility of sperms in three extenders viz., Tris, SMCFG, MCFG was significantly higher than in CM, GM and CUE (Fig. 1).

Preservation at room temperature in CME

The data on the trials conducted with CME in 1:10 and

1:200 dilution are presented in Tables 5 to 7 and in Figure 2. It may be observed from the Table 5 that the initial motility in 1:10 and 1:200 dilutions were 67.18 and 83.28 respectively and at 12 hours of preservation the values were 24.21 and 66.41 respectively.

The percentage of motility at 24 hours of preservation in 1:10 and 1:200 dilution were 3.43 and 52.34 respectively and at 36 hours the values were only 1.40 and 39.98 per cent. When semen was preserved for 48, 60, 72, 84 and 96 hours in 1:200 dilution the percentage of progressive motile sperms were 29.27, 19.38, 12.81, 7.50 and 3.28 respectively whereas in 1:10 dilution no motility was observed beyond 36 hours (Table 5).

The percentage of reduction of motility at 12, 24 and 36 hours were 42.97, 20.78 and 2.03 respectively in 1:10 dilution whereas in 1:200 dilution the reduction was 16.87, 14.07, 12.96, 10.16, 9.84, 6.57, 5.31 and 4.22 at 12, 24, 36, 48, 72, 84 and 96 hours of storage. Statistical analysis revealed significant difference in motility between 1:10 and 1:200 dilution at all periods of storage (Tables 6 and 7). Better preservability was observed at 1:200 dilution in CME than at 1:10 (Fig. 2).

The percentage of conception of insemination was

53.2 per cent (33 out of 62) in MCFG, 52 per cent (13 out of 25) in SMCFG and 44 per cent (11 out of 25) in Tris diluents (Table 8).

Table 1. Percentage of motility in different extenders during varying periods of storage at 5°C.

Extenders	Hours of storage										
	0	12	24	36	48	60	72	84	96	108	120
Tris diluent	87.11	82.11	75.39	70.47	62.97	56.25	49.90	42.73	37.42	28.52	19.30
SMCFG	84.92	77.73	70.94	63.38	58.52	51.33	41.41	32.42	23.83	19.14	13.91
MCFG	82.89	77.34	69.53	63.98	55.39	49.61	41.33	35.70	28.67	20.23	12.42
CM	80.78	71.48	62.42	55.39	49.77	38.75	33.20	25.39	17.27	10.70	6.48
GM	80.47	70.94	60.39	53.05	46.41	39.06	29.30	20.47	12.11	7.42	3.20
CUE	86.56	76.88	65.47	54.69	43.36	35.08	25.55	16.25	9.92	5.70	2.81

Mean of 64 observations.

Table 2. Comparison of motility in different extenders during varying periods of storage at 5°C.

Extenders	Hours of storage										
	0	12	24	36	48	60	72	84	96	108	120
Tris	A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1
SMCFG	B_1	B_2	B_2	B_2	B_2	B_2	B_2	B_2	B_2	B_2	B_2
MCFG	B_2	B_3	C_2	B_3	C_3	B_2	B_2	C_3	C_3	B_2	B_2
CM	C_2	C_4	D_3	C_4	D_4	C_3	C_3	D_4	D_4	C_3	C_3
GM	C_3	C_4	D_3	C_4	E_5	C_3	D_4	E_5	E_5	D_4	D_4
CUE	D_1	B_3	E_4	C_4	F_6	D_4	E_5	F_6	E_5	D_4	D_4

Note: Diluents with entries having neither letter nor numeral as common are significantly different ($P \leq 0.01$).

Table 3. Rate of decline of sperm motility (%) in different extenders during varying periods of storage at 5°C.

Extenders	Hours of storage									
	0-12	12-24	24-36	36-48	48-60	60-72	72-84	84-96	96-108	108-120
Tris	5.00	6.72	4.92	7.50	6.72	6.35	7.17	5.31	8.90	9.22
SMCFG	7.19	6.79	7.66	4.76	7.19	9.92	8.99	8.59	4.69	5.23
MCFG	5.55	7.81	5.55	8.59	5.78	8.28	5.63	7.03	8.44	7.81
CM	9.30	9.06	7.03	5.62	11.02	5.55	7.81	8.12	6.57	4.22
GM	9.53	10.55	7.34	6.64	7.35	9.76	8.83	8.36	4.69	4.22
CUE	9.68	11.41	10.78	11.33	8.28	9.53	9.30	6.33	4.22	2.89

Table 4. Analysis of variance. Percentage of motile sperms at 5°C.

Source	df	SS	MSS	F
Between bucks	7	8822.579	1260.368	47.421**
Between diluents	5	172236.535	34447.307	1296.083**
Between time of storage	10	2407376.337	240737.634	9057.778**
Error	4201	111655.509	26.578	
Total	4223	2700090.960		

** Highly significant (P < 0.01).

Table 5. Percentage of motility in CME at room temperature during varying periods of storage.

Extender	Hours of storage								
	0	12	24	36	48	60	72	84	96
CME 1:10 dilution	67.18	24.21	3.43	1.40	-	-	-	-	-
CME 1:200 dilution	83.28	66.41	52.34	39.38	29.22	19.38	12.81	7.50	3.28

Mean of 32 observations.

Table 6. Analysis of variance. Percentage of motile sperms at room temperature preservation (1:10 dilution).

Source	df	SS	MSS	F
Between bucks	7	168.750	24.107	0.966
Between time of storage	3	89551.562	29850.520	1197.229**
Error	117	2917.188	24.933	
Total	127	92637.500		

** Highly significant ($P < 0.01$).

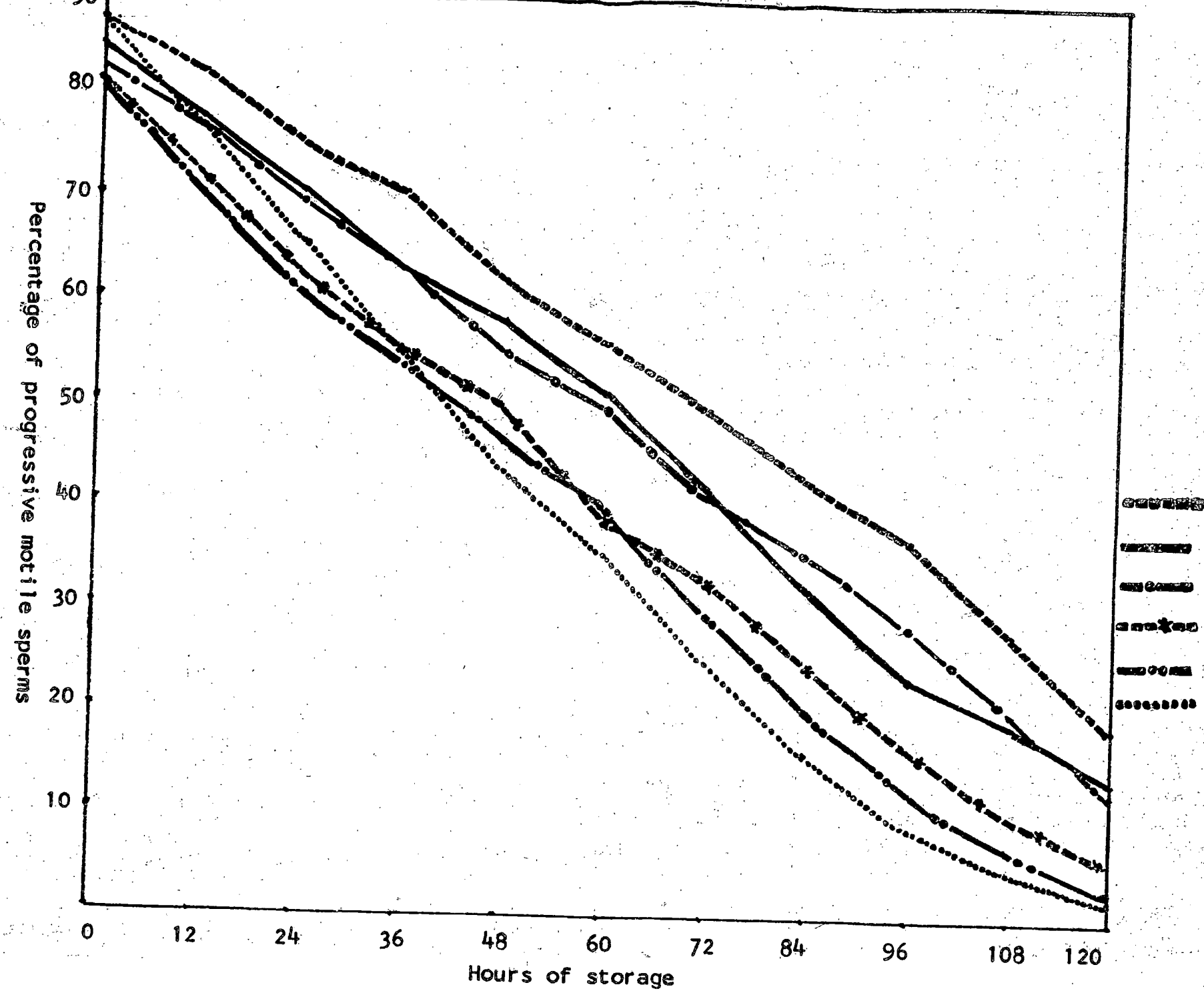
Table 7. Analysis of variance. Percentage of motile sperms at room temperature preservation (1:200 dilution).

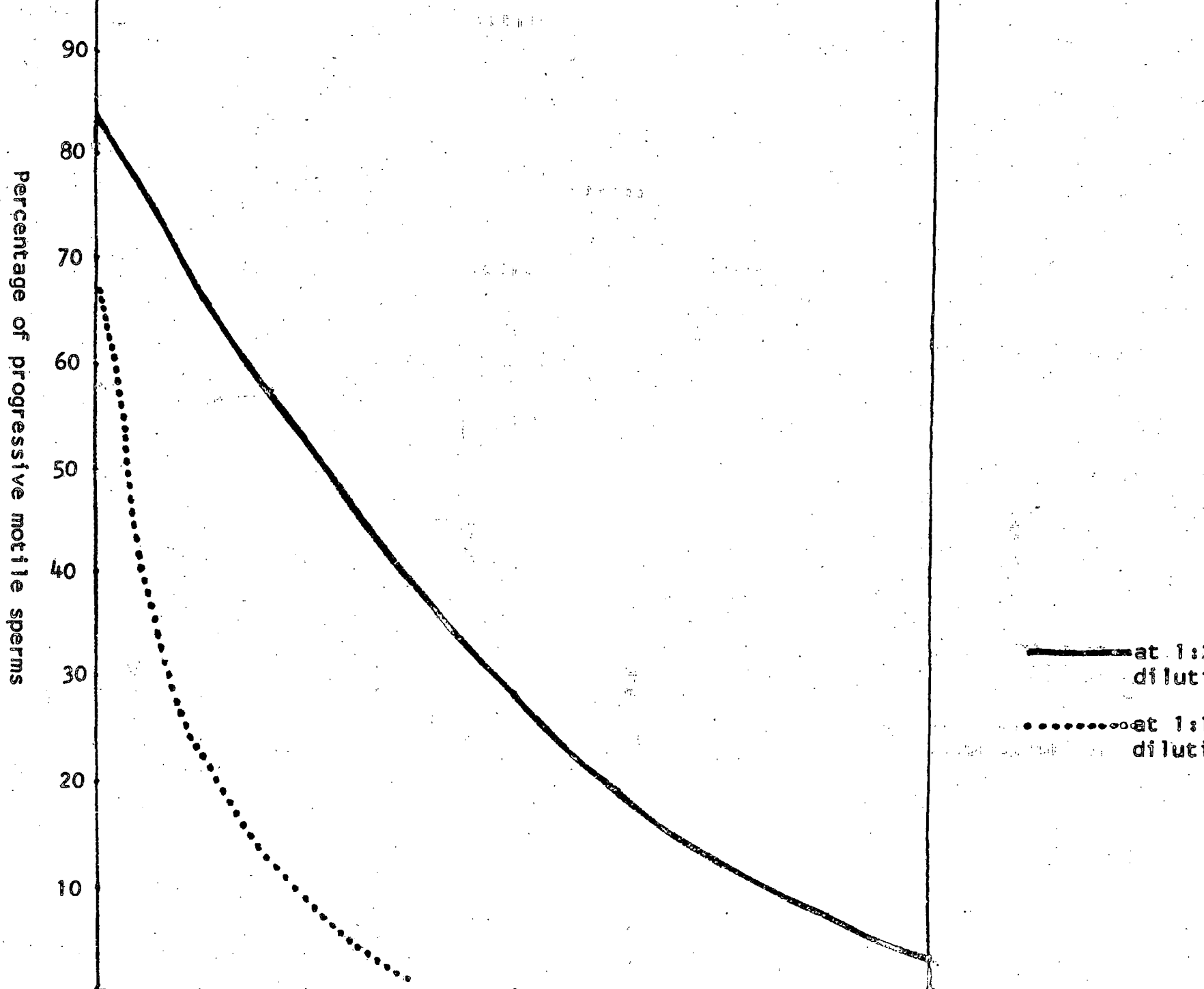
Source	df	SS	MSS	F
Between bucks	7	260.330	37.190	0.969
Between time of storage	8	197418.750	24677.343	643.007**
Error	272	10438.889	38.378	
Total	287	208117.969		

** Highly significant ($P < 0.01$).

Table 8. Conception rate with different diluents.

Name of diluents	Number of goats inseminated	Number conceived	Percentage of conception
Milk citrate fructose glycine	62	33	53.2
Skim milk citrate fructose glycine	25	13	52.0
Tris	25	11	44.0
Total	112	57	50.8


Percentage of progressive motile sperms
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
108
120
Hours of storage


90
80
70
60
50
40
30
20
10
Percentage of progressive motile sperms
at 1:
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DISCUSSION

DISCUSSION

The present study was undertaken with the object of evolving a suitable extender for preservation of buck semen. Ejaculates from eight healthy cross bred bucks were extended in 19 different diluents by split sample technique. Based on preliminary observations, 12 extenders were eliminated from further studies due to poor preservability. The data on the preservation of buck semen at 5°C in six extenders viz., Tris diluent, skim milk citrate fructose glycine diluent (SMCFG), milk citrate fructose glycine diluent (MCFG), whole cows milk (CM), whole goats milk (GM) and Cornell University Extender (CUE) and also at room temperature preservation in coconut milk extender (CME) were collected and statistically analysed.

The data presented in Table 1 showed that initial motility of buck semen diluted in Tris, SMCFG, MCFG, CM, GM and CUE was 87.11, 84.92, 82.89, 80.75 and 86.56 per cent respectively. Analysis of the data revealed significant difference in initial motility between various diluents. The data indicated that buck semen showed good initial motility in all the diluents studied. Dauzier (1956) and Sahni and Roy (1969) reported good initial motility for buck semen in skim milk extenders. On the contrary, Ahamed (1963) observed that skim milk was inferior to whole milk for dilution of buck semen.

Sahni and Roy (1969) and John and Raja (1975) reported initial motility of 73.1 and 70.6 per cent respectively in cows milk. On the other hand, Tewari et al. (1968) observed only 50.4 per cent initial motility in cows milk. Jelam and Nambiar (1963), Sahni and Roy (1969) and John and Raja (1975) obtained an initial motility of 72.3, 74.3 and 73.7 per cent respectively with goats milk which is comparable to the present findings. The initial motility of 86.56 per cent in CUE is also comparable to that reported by Sahni and Roy (1969) and Koh and Ong (1977).

The percentage of sperm motility at 12 hours of storage was 82.11, 77.73, 77.34, 71.48, 70.94 and 76.88 respectively with Tris, SMCFG, MCFG, CM, GM and CUE diluents. Perusal of available literature did not reveal any data on preservation of buck semen at 12 hours of storage.

The percentage of motility was observed to be 75.39, 70.94, 69.53, 62.42, 60.39 and 65.47 in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively at 24 hours of storage. Comparable results at 24 hours of storage with cows milk and goats milk were reported by Jelam and Nambiar (1963) and John and Raja (1975).

When the samples were stored for 36 hours in Tris, SMCFG, MCFG, CM, GM and CUE diluents, the percentage of progressive motility was 70.47, 63.28, 63.98, 55.39, 53.05 and

54.69 respectively. This is not in agreement with the findings of Tewari et al. (1968) who reported only 35 per cent motility in cows milk at 30 hours of storage.

The percentage of motile sperms at 48 hours was 62.97, 58.52, 55.39, 49.77, 46.41 and 43.36 respectively in Tris, SMCFG, MCFG, CM, GM and CUE diluents. Jelam and Nambiar (1963) and John and Raja (1975), however, observed better motility at the same period of storage in goats milk and cows milk. This may be attributed to the difference in breed of the bucks studied.

Semen preserved for 60 hours in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively retained 56.25, 51.33, 49.61, 38.75, 39.06 and 35.08 per cent of progressive motility. The data revealed that only three diluents viz., Tris, SMCFG, MCFG were suitable for storage up to 60 hours whereas the other three diluents showed motility rate below 40 per cent. This is comparable to the findings of Sahni and Roy (1969) who also observed a poor motility below 40 per cent in CM, GM and CUE diluents at 54 hours of storage.

During 72 hours of storage the sperm motility was 49.90, 41.41, 41.33, 33.20, 29.30 and 25.25 per cent respectively in Tris, SMCFG, MCFG, CM, GM and CUE diluents. The motility rate in cows milk and goats milk was inferior to the

earlier reports (Jelam and Nambiar, 1963 and John and Raja, 1975).

The present investigation revealed that beyond 72 hours of preservation, motility above 40 per cent was observed only in Tris diluent. The other diluents showed motility below 40 per cent. This is in agreement with the findings of Sahni and Roy (1969) and John and Raja (1975) who also observed a rapid decline in motility beyond 72 hours of storage. However, Jelam and Nambiar (1963) reported a higher motility at 72 hours of storage in goats milk.

Buck semen preserved in CME showed initial motility of 67.18 per cent in 1:10 dilution and 83.28 per cent in 1:200 dilution. Pillai (1971) also observed similar results. At 12 hours of storage the motility in 1:10 dilution was observed to be 24.21 per cent and in 1:200 dilution, 66.41 per cent. At 24 hours of storage, the motility in 1:10 dilution rate was further reduced to a negligible percentage of 3.43 per cent while in 1:200 dilution the samples maintained 52.34 per cent motility. John (1970) did not observe any motility at 24 hours in 1:10 dilution. The present findings is therefore in accordance with that of John (1970) and Pillai (1971) who had reported similar trend in the maintenance of motility at 1:200 dilution rate.

The present study also revealed that preservation at

room temperature in CME at 1:200 dilution is preferable to other dilution rates. Optimum dilution rate is dependent on the total number of live spermatozoa required for successful artificial insemination. This was estimated to be 15 million in cows (Norman et al., 1960). Similar data are not available for buck semen.

From the foregoing paragraphs it could be observed that buck semen maintained satisfactory motility in Tris, SMCFG, and MCFG diluents up to 60 hours at 1:10 dilution and only up to 24 hours in CME at 1:200 dilution.

Norman et al. (1958) suggested that Tris (hydroxymethyl) aminomethane has considerable promise as reactivator which could be directly added to the diluent medium. Bomstein and Steberal (1959) reported that organic buffers particularly amine buffers such as Tris have been successfully used for preservation of semen. Nahas (1961) observed that Tris had a good buffering capacity and was relatively non toxic to living cells. It is known to penetrate many types of cells rather readily in the undissociated form but it is not known whether it readily penetrates the sperm cell thereby acting as an intracellular buffer. Tris buffer also appeared to be less toxic in the critical temperature (Davis et al., 1963). Another practical advantage of Tris diluent was reported to be a clear visualization of spermatozoa during microscopic

examination which facilitated easier and more accurate evaluation of semen quality unlike in the case of milk diluents (Chaube and Sengupta, 1972). The beneficial effects of Tris diluent presently observed might be due to the above factors.

The present study also revealed that buck semen could be preserved up to 60 hours with good motility in SMCFG, MCFG diluents. The two diluents in addition to milk, contain glycine and fructose in good proportion. Beneficial effects of milk as a semen diluent have been reported by Thacker and Almquist (1951), Jacquet and Cassou (1952), Sanfile (1952), Weiss (1952), Almquist (1954), Tomar and Desai (1961), Kale (1963), Gupta et al. (1974) and Greeshmohan (1976).

The beneficial effects of glycine to prolong the life span of spermatozoa have been reported by Roy and Bishop (1954), Gabriel (1955), Roy et al. (1955), Sakala (1957), Stower and Budhussain (1957) in bull semen; Sha and Singh (1956), Joshi and Singh (1968) in ram semen; Roy et al. (1959), Sahni and Roy (1969) and John (1970) in buck semen. Beneficial effect of glycine might be due to the synthesis of creatine and adenosine which may be important for the regulation of glycolysis and also as a nutrient (Roy, 1957).

Addition of fructose and other sugars in the dilutor has been shown to improve the viability of bovine spermatozoa

(Kampschmidt et al., 1951). Fructose, the only glycosible sugar present in the semen (Mann, 1948), was reported to improve the sperm survival and motility when added to the diluent (Dimitro Poulos, 1954 and Perez, 1954). The present observation of maintenance of better motility in diluents containing milk, glycine and fructose could be attributed to the above factors.

Low motility rates were obtained from second day onwards in diluents containing egg yolk. Roy (1957) opined that the enzyme present in the secretions of bulbourethral glands interferes with the preservation of buck semen by coagulating the egg yolk. The poor keeping quality of buck semen in diluents containing egg yolk observed in the present study could be attributed to this factor.

It may, therefore, be concluded that buck semen can be stored at 5°C with good results up to 60 hours in Tris, SMCFG, MCFG extenders and up to 36 hours in CM, GM and CUE diluents at dilution rate of 1:10. Satisfactory motility could be maintained only up to 24 hours in CME at dilution rate of 1:200.

In a limited trial to assess the fertility of the three diluents viz., Tris, SMCFG, MCFG, semen samples extended in these diluents were used for insemination in a small

herd of goats and conception rates ranging from 44 to 53.2 per cent were observed on first insemination. Blokhuis (1959), Roy et al. (1959), Ron and Aamdal (1963), Sahni and Roy (1967) and Wani et al. (1978) have also reported conception rates ranging from 48 to 60 per cent when diluted samples were used within 24 hours. However, Knoblauch (1962) obtained only 33.3 per cent conception rate with 24 hours old semen.

In conclusion, it may also be stated that, although further elaborate trials are required to assess the fertility of the three extenders viz., Tris, SMCFG, MCFG, preliminary studies reveal that these extenders have normal fertility as reported earlier by many workers.

SUMMARY

SUMMARY

In the present investigation an attempt has been made to evolve a suitable extender for buck semen.

Semen collected from eight cross bred bucks (6 Sannen x Malabari and 2 Alpine x Malabari) belonging to the "All India Co-ordinated Research Project on Goats for Milk Production" formed the material for the study. A total of 64 ejaculates were collected and were extended in the following diluents: Egg yolk citrate diluent (EYC), Egg yolk glycine diluent (EYG), Egg yolk glucose glycine citrate diluent (EYGGC), Egg yolk glucose citrate diluent (EYGC), Egg yolk fructose glycine citrate diluent (EYFGC), Egg yolk glucose sodium bicarbonate diluent (EYGB), Egg yolk glucose sodium bicarbonate milk diluent (EYGBM), Illini variable temperature diluent (IVT), Coconut milk extender (CME), Kiew-I diluent, Kiew-II diluent, Glucose glycine EDTA sodium bicarbonate citrate diluent (GGEBC), Cornell University Extender (CUE), Tris diluent, Whole cows milk (CM), Whole goats milk (GM), Skim milk diluent (SM), Skim milk citrate fructose glycine diluent (SMCFG), Milk citrate fructose glycine diluent (MCFG).

During the course of the experiment, twelve diluents were eliminated from further studies due to poor preservability. The remaining diluents viz., Tris, SMCFG, MCFG, CM, GM

and CUE were used for further studies. The data on preservation of buck semen in CME at room temperature were also collected. The results obtained are summarised below.

Preservation at 5°C

The initial motility was observed to be 87.11, 84.92, 82.89, 80.78, 80.47 and 86.56 per cent respectively in Tris, SMCFG, MCFG, CM, GM and CUE. Statistical analysis revealed significant difference between the various diluents except between Tris, SMCFG and CUE, between SMCFG and MCFG, between MCFG and CM and between CM and GM.

At 12 hours the motility decreased to 82.11, 77.73, 77.34, 71.48, 70.94 and 76.88 per cent respectively for the various diluents. Statistical analysis revealed that the difference in motility between SMCFG, MCFG and CUE and between GM and CM diluents was not significant.

The motility of sperms at 24 hours of storage in Tris, SMCFG, MCFG, CM, GM and CUE diluents was 75.39, 70.94, 69.53, 62.42, 60.39 and 65.47 per cent respectively. Significant difference was observed between the various diluent comparisons except between SMCFG and MCFG and between CM and GM.

Percentage of motile sperms at 36 hours of storage

was 70.47, 63.28, 63.98, 55.39, 53.05 and 54.69 in the respective diluents. No significant difference in sperm motility was observed between SMCFG and MCFG and also between CM, GM and CUE diluents.

During 48 hours of storage, the percentage of progressive motility was observed to be 62.97, 58.52, 55.39, 49.77, 46.41 and 43.36 respectively in Tris, SMCFG, MCFG, CM, GM and CUE diluents. Statistical analysis revealed that all the diluents were significantly different from each other.

The percentage of progressive motility of sperms at 60 hours of preservation in Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively was 56.25, 51.33, 49.61, 38.75, 39.06 and 35.08. On statistical analysis, significant difference was observed between all the diluents except between SMCFG and MCFG and between CM and GM.

The percentage of motility at 72 hours of storage was 49.90, 41.41, 41.33, 33.20, 29.30 and 25.55 respectively in Tris, SMCFG, MCFG, CM, GM and CUE diluents. Statistical analysis showed significant difference between all the diluent comparisons except between SMCFG and MCFG.

The motility percentage declined to 42.73, 32.42, 35.70, 25.39, 20.47 and 16.25 in Tris, SMCFG, MCFG, CM, GM

and CUE diluents after 84 hours of storage. Analysis showed that the differences between all the diluent comparisons were statistically significant.

At 96 hours of storage, Tris, SMCFG, MCFG, CM, GM and CUE diluents respectively retained 37.42, 23.83, 28.67, 17.27, 12.11 and 9.92 per cent of progressive motility. The differences in the motility between the diluents were significant except between CM and GM.

At 108 hours of storage in Tris, SMCFG, MCFG, CM, GM and CUE diluents, the percentage of motility was 28.52, 19.14, 20.23, 10.70, 7.42 and 5.70 respectively. Analysis showed no significant difference between SMCFG, MCFG and between GM and CUE diluents.

Motility at 120 hours of storage in Tris, SMCFG, MCFG, CM, GM and CUE diluents were 19.30, 13.91, 12.42, 6.42, 3.20 and 2.81 per cent respectively. Analysis revealed significant differences between the various diluents except between SMCFG and MCFG and between CM and CUE diluents.

Preservation at room temperature in CME

Semen preserved in coconut milk extender (CME) at 1:10 and 1:200 dilution showed that semen could be successfully

preserved up to 24 hours at 1:200 dilution whereas at 1:10 dilution the motility dropped 3.43 per cent at 24 hours of storage. At 48 hours of preservation, there was a decline in motility up to 29.22 per cent.

The percentage of conception with Tris, SMCFG and MCFG was 44.0, 52.0 and 53.2 respectively.

In general, it could be stated that Tris, SMCFG and MCFG were superior to other diluents in the preservation of buck semen at 5°C. Coconut milk extender can also be successfully used for preservation of buck semen at room temperature up to 24 hours at a dilution of 1:200.

REFERENCES

*Aamdal, J., Lyngset, O. and Fossum, K. (1965). Toxic effect of lysolecithin on sperm. Anim. Breed. Abstr. 34 (2) : 1330.

*Achnelt, E. (1951). Comparative experimental testing of the diluents, egg yolk spermasol, egg yolk citrate. Anim. Breed. Abstr. 21 (1) : 201.

*Achnelt, E. and Rosenwinkel, S. (1953). Dilution and storage of goat semen. Anim. Breed. Abstr. 22 (2) : 607.

*Adler, H.C. and Rasbech, N.O. (1956). Glycine semen diluent with special reference to the storage of bull semen. Anim. Breed. Abstr. 26 (3) : 1325.

*Adler, H.C. and Rasbech, N.O. (1956). Investigation on skim milk and cream as semen diluents. Anim. Breed. Abstr. 26 (3) : 1324.

Ahamed, S.I. (1955). Effect of glycine on storage of ram semen. J. Agri. Sci. 46 : 164-167.

*Ahamed, M. (1963). Dilution studies. Effect of seminal plasma level and milk diluents. Anim. Breed. Abstr. 33 (3) : 2335.

Almquist, J.O. (1951). The fertility of bovine semen in dilutor containing varying amount of egg yolk. J. Dairy Sci. 34 : 763-766.

Almquist, J.O. (1954). Dilutors for bovine semen. V. A comparison of heated milk and egg yolk citrate as dilutors for semen from bulls of high and low fertility. J. Dairy Sci. 37 : 1308-1315.

*Baudet, C., Dauzier, L., Decard, R. and Wintenberger, S. (1954). Notes on artificial insemination applied to sheep and goat. Anim. Breed. Abstr. 23 (1) : 236.

*Benedictovic, S. (1934). An experiment on artificial insemination in goats. Anim. Breed. Abstr. 2 (2) : 112.

Blackshaw, A.W. (1954). The prevention of temperature shock of bull and ram semen. Aust. J. Biol. Sci. 7 : 573-582.

*Blokhuis, J. (1957). Artificial insemination in goat. Vet. Bull. 28 (6) : 1946.

*Blokhuis, J. (1959). The artificial insemination of goat in the province of Utrecht. Anim. Breed. Abstr. 27 (3) : 1408.

Bomstein, R.A. and Steberal, E.A. (1959). Preservation of washed bovine spermatozoa in synthetic medium at room temperature. Expt. Cell. Research. 18 : 217.

Chaube, L.K. and Sengupta, B.P. (1972). An efficacious extender for invitro conservation of buffalo sperm viability at 4°C: A note. Indian J. Anim. Sci. 42 (3): 165-166.

*Corteel, J.M. (1967). Survival of goat spermatozoa in whole or fractioned egg yolk associated with a sodium citrate buffer solution and antibiotics. Anim. Breed. Abstr. 36 (3) : 2752.

*Dauzier, L. (1956). Some results obtained from the artificial insemination of ewes and goats in France. Anim. Breed. Abstr. 24 (4) : 1734.

*Dauzier, L. and DuMesnil, F. (1958). The artificial insemination of sheep and goat. Anim. Breed. Abstr. 27 (2) : 864.

Davis, I.S., Bratton, R.W. and Foote, R.H. (1963). Livability of bovine spermatozoa at 5°C in tris buffered and citrate buffered yolk glycerol extender. J. Dairy Sci. 46 : 57-60.

*Dimitro poulos, E. (1954). Comparative values of several diluents for improving the storage period and improving fertility of bull semen. Anim. Breed. Abstr. 22 (4):1464.

*Donne, A. (1837). Nou velles experiences surless animal cules spermatiques et sur quelques une des causes de lasterilite chez la Femme. Paris: Les Libraires de Medecine. Cited: Jackson, K.F. and Mann, T.(1960)Biochemical appraisal of milk diluent for semen. J. Agri. Sci. 54 : 166-169.

Elwishy, A.B., Elsawf, S.A., Elmikkani, F. and Omer, A.A. (1971). Motility and seasonal variation in sexual activity of male Demascus goats. Indian J. Anim. Sci. 41 (7) : 562-569.

*Fisher, J. and Kandera, L. (1960). Notes on the dilution of bull semen with powdered milk. Anim. Breed. Abstr. 28 (4) : 1940.

*Founger, J.A. (1976). Uterine insemination with frozen semen in goats. Anim. Breed. Abstr. 45 (3) : 1421.

Fukhuhara, R. (1973). Effect of various substrates on respiration, glycolysis and motility of goat spermatozoa. Jap. J. Zootech. Sci. 44 (5) : 271-274.

Fukhuhara, R. (1973). Effect of chlor promazine hydrochloride on the viability and metabolism of goat and bull spermatozoa. Jap. J. Zootech. Sci. 44 (6) : 338-342.

*Gabriel, R.W. (1955). The effect of fructose and glycine on certain characteristics of bovine spermatozoa. M.S. Thesis. University of Minesota.
Cit. Salisbury, G.W. (1957).

*Gonan, B. (1971). A study of artificial insemination in Angora does inseminated with diluted and stored buck semen. Anim. Breed. Abstr. 39 (4) : 4827.

*Gonazatez Stagnaro, C. (1976). Artificial insemination with frozen semen in goat. Anim. Breed. Abstr. 45 (2): 828.

Greeshmohan, G.C., George, and Razden, M.N. (1976). A note on partialy defatted cow milk as a dilutor for buffalo bull semen preservation. Indian J. Anim. Sci. 46 (10): 551-553.

Guha, S., Kohli, M.L. and Bhattacharya, D. (1951). Artificial insemination in sheep and goats at Izatnagar. Indian J. Vet. Sci. 21 (2) : 171-176.

Gupta, A.K., Singh, B.P. and Nomer, N.S. (1974). In vitro preservation of bull semen in boiled milk. Indian Vet. J. 51 (6) : 401-403.

*Habibullin, H.H. (1952). Fertilisation and viability of progeny in dairy cattle breeding. Anim. Breed. Abstr. 21 (1) : 221.

Hampel, J. (1951). Cited Blokhuis, J. (1962). Artificial insemination in the goat. The Semen of Animals and Artificial Insemination by J.P. Maule. Commonwealth Agricultural Bureaux, Farnham Royal Bucks, England. 1st Ed. pp. 257-258.

*Han, J. (1957). Glycine as an additive to bull semen. Anim. Breed. Abstr. 28 (2) : 664.

*Hartel, M. (1967). The influence of feeding nitrate on the semen of bucks. Anim. Breed. Abstr. 36 (4) : 3783.

*Hill, J.R., Hurst, V. and Godley, W.C. (1958). A comparison of reconstituted skim milk and egg yolk sodium citrate as extender for ram semen. Vet. Bull. 28 (7) : 2326.

*Hirore, K. and Udatsu, S. (1975). Survival of goat spermatozoa stored at 25°C. Anim. Breed. Abstr. 44 (10): 4871.

*Hoffman, L. (1905). Ost. Mschr. Tierheilk. Tierzucht. 29 : 1. Cited Jacobsen, K.F. and Mann, T. (1960). Biochemical appraisal of a milk filuent for semen. J. Agri. Sci. 54 : 166-169.

Holt, A.F. (1952). Variation in dilutor composition and its effect on the fertility of bovine semen. Vet. Rec. 64 : 818.

Iritani, A., Nishikawa, Y. and Nagasawa, S. (1964). Studies on the egg yolk coagulating enzyme in goat semen. Jap. J. Anim. Reprod. 10 : 44-51.

*Jabnel, J. (1954). Agglutination of spermatozoa in yolk citrate semen diluent. Vet. Bull. 26 (3) : 1026.

*Jacquet, J. (1951). Some biological media for the preservation of bull semen. Anim. Breed. Abstr. 20 (4): 1633.

*Jacquet, J. and Cassou, R. (1952). Influence de la dilution du sperme dans un nouveau milieu a base de lait ecreme sur la fe condite des taureaux. Anim. Breed. Abstr. 21 (1) : 226.

Jelam, L.V. and Nambiar, K.G. (1963). Effect of diluent on the viability and biometrics of buck spermatozoa. Kerala Vet. 4 (1) : 1-5.

John, K.J. (1970). A study on the preservation of buck semen. Dissertation submitted to the Kerala Veterinary College and Research Institute, Mannuthy for the Senior Officers' Refresher Course.

John, K.J. and Raja, C.K.S.V. (1975). A study on the preservation of buck semen. Kerala J. vet. Sci. 4 (1): 90-93.

Joshi, J.D. and Singh, G. (1968). Studies on ram semen diluents. Indian J. Vet. Sci. 38 (4) : 575.

Joshi, J.D. and Singh, G. (1968). Effect of skim milk series of diluents on the livability and morphology of ram spermatozoa. Indian J. Vet. Sci. 38 (4) : 583.

Kale, S.N. (1963). Preservation of buffalo semen in milk and some milk containing dilutors. Indian Vet. J. 40 (7) : 425-430.

Kampschmidt, R.F., Mayer, D.T., Herman, H.A. and Dickerson, G.E. (1951). Viability of bulls spermatozoa as influenced by electrolyte concentration, buffer efficiency and added glucose. J. Dairy Sci. 34 : 45-51.

*Kerruish, B.M. (1956). A field trial comparison of milk and egg yolk citrate as semen dilutors. Anim. Breed. Abstr. 24 (4) : 1648.

*Knoblauch, H. (1962). Investigation on goat ejaculates. Anim. Breed. Abstr. 31 (2) : 1309.

*Koh, S.H. and Ong, C.H. (1977). Comparison of storage, motility of chilled goat semen extended in different diluents. Anim. Breed. Abstr. 45 (1) : 282.

Koelliker, A. (1905). Z. Wiss. Zool. 7 : 201. Cited Jacobsen, K.F. and Mann, T. (1960). Biochemical appraisal of a milk diluent for semen. J. Agri. Sci. 54 : 166-169.

*Konger, M. (1951). Storage, dilution and use of ram semen in artificial breeding of sheep. Anim. Breed. Abstr. 21 (2) : 798.

Linzell, J.L. (1971). Goats. The UFAW Hand Book on the Care and Management of Farm Animals by C.W. Hume. Churchill Livingstone, London. 1st Ed. pp. 94.

*Lopatko, M.I. (1971). Tris phosphate diluent for ram semen. Anim. Breed. Abstr. 42 (8) : 3211.

Mann, T. (1948). Fructose content and fructolysis in semen. Practical application in the evaluation of semen quality. J. Agri. Sci. 38 : 323-331.

Masuda, H., Nishikawa, M. and Nagasawa, S. (1972). Studies on the substance in egg yolk which affect the metabolism of spermatozoa V. Substance protecting against cold shock and freezing injury. Jap. J. Zootech. Sci. 43 (8) : 417-423.

*Mayer, D.T. (1955). The chemistry and certain aspect of the metabolic activities of mamalian spermatozoa. Cited Physiology of Reproduction and Artificial Insemination of Cattle by Salisbury, G.W. and Van Demark, N.L. (1961). W.H. Freemen and Company, Sanfrancisco and London. 1st Ed. pp. 410-435.

Mayer, D.T. and Lasely, J.F. (1945). The factor in egg yolk affecting the resistence, storage potentialities and fertilising capacity of mamalian spermatozoa. J. Anim. Sci. 4 : 261-269.

Melrose, D.R. and Stewart, D.L. (1956). The effects of fertility of changes in the composition of the normal egg yolk semen diluent. British Vet. J. 112 : 536-540.

Melrose, D.R., Stewart, D.L. and Bruce, W. (1958). Comparative fertility studies of bovine semen diluents containing powdered skim milk, fresh milk, skim milk, glycine and egg yolk. Vet. Rec. 70 : 433-438.

Michajilov, N.M. (1950). Sperm dilution in the milk. J. Amer. vet. med. Ass. 117 : 337.

*Montigny, G. De. (1976). Artificial insemination of goats in France. Anim. Breed. Abstr. 44 (1) : 547.

Nahas, G.G. Ed. (1961). Invitro and invivo effect of amine buffers. Ann. N. Y. Acd. Sci. 93 : 333. Cited Davis, I.S., Bratton, R.W. and Foot, R.M. (1963).

*Nasim, M., Amjad Ali, M., Wahiduzzaman, M. and Shamsuddin, M. (1964). A study on fertility of semen on Black Bengal buck with new technique of insemination for goats. Anim. Breed. Abstr. 34 (2) : 1335.

Norman, C. (1964). Further studies on the preservation of mammalian sperm at variable temperatures. Proc. Vth Int. Congr. on Ani. Reprod. and Art. Insem. 4 : 269-274.

Norman, C., Johnson, C.E., Porterfield, I.D. and Dunbar Jr. R.S. (1958). Effect of pH on the life span and metabolism of bovine semen kept at room temperature. J. Dairy Sci. 41 : 1803.

Norman, C., Johnson, C.E., Porterfield, I.D., Golderburg, E. and Dunbar, Jr. R.S. (1960). Physiology and Biochemistry of germ cells V. Fertilising capacity of bovine sperm maintained at room temperature in coconut milk extender. Anat. Rec. 137 : 244.

*Pavlovic, A. and Vardin, M. (1968). The best diluent for ram semen. Anim. Breed. Abstr. 36 (4) : 3776.

Patel, J.K. (1967). Artificial insemination in goats. Indian Vet. J. 44 (6) : 509-511.

*Perez Y Perez, F. (1953). The effect of differences in pH between semen and diluents on the viability of ass spermatozoa. Anim. Breed. Abstr. 23 (1) : 124.

Perry, E.J. (1969). The Artificial Insemination of Farm Animals. Oxford and IBH Publishing Co., Delhi, Calcutta, Bombay. 4th Ed. pp. 219.

*Petruzzi, V. and Tarantini, S. (1974). Survival of ram spermatozoa at 5°C in milk extender. Anim. Breed. Abstr. 44 (3) : 1220.

*Phillips, P.H. (1939). Preservation of bull semen. Anim. Breed. Abstr. 8 (1) : 130.

Phillips, P.H. and Lardy, H.A. (1940). A yolk buffer pabulam for the preservation of bull semen. J. Dairy Sci. 23 : 399-404.

Pillai, V.B. (1971). A study on the efficiency of C.M.E. as a diluent for the preservation of buck semen at room temperature. Dissertation submitted to the Kerala Veterinary College and Research Institute, Mannuthy for the Senior Officers' Refresher Course.

Report of the National Commission on Agriculture (1976). Ministry of Agriculture and Irrigation, Government of India, New Delhi. pp. 211.

*Ron, I. and Aamdal, J. (1963). Experiments with insemination of goat. Anim. Breed. Abstr. 32 (4) : 3122.

*Rosenberger, G. (1944). The practise of artificial insemination in goats. Anim. Breed. Abstr. 12 (4) : 204.

Roy, A. (1957). Egg yolk coagulating enzyme in the semen and cowpers gland of the goat. Nature 179 : 318.

Roy, A. and Bishop, M.W.H. (1954). Effect of glycine on survival of bull spermatozoa invitro. Nature 174 : 746-747.

Roy, A., Srivastava, R.K. and Pandey, M.D. (1955). Preservation of buffalo semen in glycine buffer. Curr. Sci. 24 : 246.

Roy, A., Gupta, D.N. and Pandey, M.D. (1959). Studies on the reproductive physiology of goat I. Preservation of spermatozoal viability invitro. Indian J. Vet. Sci. 29 (2) : 49-54.

Sacke, R.G., Almquist, J.O. and Flipse, R.J. (1956). Dilutors for bovine semen VII. Effect of time and temperature of heating skim milk upon the livability of bovine spermatozoa. J. Dairy Sci. 39 : 90-96.

Sahni, K.L. and Roy, A. (1967). A study on the sexual activity of the Barbari goat (Capra hircus L.) and conception rate through artificial insemination. Indian J. Vet. Sci. 37 (4) : 269-270.

Sahni, K.L. and Roy, A. (1969). Influence of season on semen quality of rams and effects of dilutors and dilutions on invitro preservation. Indian J. Anim. Sci. 39 (1) : 1-13.

Sahni, K.L. and Roy, A. (1972). The influence of colour additives on the keeping quality and identification of sheep and goat semen. Indian J. Anim. Sci. 42 (3): 214.

Sahni, K.L. and Roy, A. (1972). A note on the effect of two storage temperature on the keeping quality of sheep and goat semen in different diluents. Indian J. Anim. Sci. 42 (8) : 580-583.

Sahni, K.L. and Tewari, S.B. (1973). Artificial insemination of sheep with diluted and stored semen in milk diluent. Indian Vet. J. 50 (6) : 536-539.

*Sakala, J. (1957). Improvements in the preservation and distribution of ram semen. Anim. Breed. Abstr. 26 (2): 873.

*Salisbury, G.W. (1957). Recent development with bull semen diluents. Anim. Breed. Abstr. 25 (2) : 111-123.

Salisbury, G.W., Fuller, H.K. and Willet, E.L. (1941). Preservation of bovine spermatozoa in yolk citrate diluent and field result from its use. J. Dairy Sci. 24: 905-910.

*Sanfile, V. (1952). Milk as a semen diluent. Anim. Breed. Abstr. 22 (4) : 1485.

Schinder, H., Amir, D., Laor, M. and Barnea, R. (1975). Insemination trial with goat under field condition. Dairy Goat J. 53 (1) : 18-19.

*Schmidt, K. and Kroll, H. (1953). Conservation of bull semen. I. Experiments invitro with various additions. Anim. Breed. Abstr. 21 (4) : 1731.

*Tyler, A. and Tenabe, T.Y. (1952). Motile life of spermatozoa in glycine and yolk citrate diluent at high and low temperature. Anim. Breed. Abstr. 22 (2) : 572.

Vandemark, N.L. (1951). Skim milk as a partial replacement for yolk citrate in a bovine semen extender. Cited Salisbury, G.W. (1957).

*Wanger, H. (1949). Experiences and experiments in the insemination of goat. Anim. Breed. Abstr. 19 (3) : 1286.

Wani, G.M., Sinha, N.K. and Sahni, K.L. (1978). Investigation on dilution preservation and storage of buck semen. Cited Sinha, N.K. Artificial insemination in goats. Paper presented at Summer Institute on Goat Production, Mathura.

*Weiss, K. (1952). Semen dilution with milk. Anim. Breed. Abstr. 21 (3) : 1275.

*Willet, E.L. and Salisbury, G.W. (1942). The effect of various dilutors, cooling rate, temperature of storage and some other factors on the livability of spermatozoa in stored sample of bull semen. Anim. Breed. Abstr. 11 (1): 231.

*Yoshika, Z., Indo, Y. and Torizuka, T. (1951). Experiments on the storage of semen and the insemination of the stored semen in sheep and goat. Anim. Breed. Abstr. 20 (1) : 245.

*Original not consulted.

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ABSTRACT OF A THESIS

Submitted in partial fulfilment
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Department of Animal Reproduction

COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

1979

ABSTRACT

With the ultimate object of evolving a suitable extender for buck semen, 64 ejaculates from eight healthy cross bred bucks (6 Sannen x Malabari and 2 Alpine x Malabari) selected at random from "All India Co-ordinated Research Project on Goats for Milk Production", Mannuthy, were used for the preservation studies. Six diluents viz., Tris, Skim milk citrate fructose glycine (SMCFG), Milk citrate fructose glycine (MCFG), Whole cows milk (CM), Whole goats milk (GM) and Cornell University Extender (CUE) were stored at 5°C and at room temperature in Coconut milk extender (CME). Buck semen could be stored up to 60 hours in Tris, SMCFG and MCFG with good motility at 5°C. In CME, semen could be stored only up to 24 hours in 1:200 dilution. Egg yolk containing diluents proved to be least suitable for preservation of buck semen at 5°C. The percentage of conception rate was 44.0, 52.0 and 53.3 with Tris, SMCFG and MCFG diluents respectively.

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