

**STUDIES ON
BUCK SEMEN WITH REFERENCE
TO THE USE OF EGG YOLK CITRATE
AND COW MILK EXTENDERS**

Thesis

Submitted to the Faculty of Veterinary Science,
and Animal Husbandry

RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR,

*in partial fulfilment of the requirements
for the degree of*

MASTER OF SCIENCE (VETERINARY)

IN

GYNAECOLOGY AND OBSTETRICS

By

Rajendra Prasad Roy

B. V. Sc. & A. H. (R. A. U.)

JUNIOR RESEARCH FELLOW (R. A. U.)

Post-Graduate Department of Gynaecology and Obstetrics

**BIHAR VETERINARY COLLEGE
PATNA.**

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Dated, the 18th November, 1975.

This is to certify that the work embodied
in this Thesis entitled "STUDIES ON BUCK SEMEN
WITH REFERENCE TO THE USE OF EGG YOLK CITRATE
AND COW MILK EXTENDERS" is the bonafide work of
Gajendra Prasad Roy and was carried out under my
guidance and supervision.

B.K. Singh

(B.K. SINGH).

C E R T I F I C A T E

Certified that the research work
incorporated in this Thesis has not
been published in part or in full
in any other journal.

G. P. Roy.
(GAJENDRA PRASAD ROY).

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

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(G. P. ROY).

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I N T R O D U C T I O N

Goat has rightly been regarded as poor man's cow. But in India, though it is valued as giver of milk for children, the sick and the old it has been accused of being destructive to young forests. Due to heavy pressure on land on account of tremendous increase in human and livestock population, goats have been deprived of proper grazing facilities. They are therefore forced to take up whatever forms of food comes their way. Economic conditions in India are such that call for more stress on goat breeding. In general, goats serve man kind with meat, milk as well as hides and this they have been doing from pastoral days by remaining constant companion of mankind.

According to All India Livestock Census (1966) the goat population in the country was about 65 million. According to Livestock Census of Bihar (1972) the total number of goats in this province was 4442056. In 1967-68 the Agriculture Marketing Board estimated the production of 224 million Kg of goat meat on the basis of average carcass weight of 9.05 Kg. The pilot project conducted by I.C.A.R. in Tamil Nadu (1967) and in Haryana (1969) revealed average goat meat yield to be 10.2 and 10.9 Kg. respectively (Singh, 1973).

Singh and Moore (1968) estimated the average milk production in Indian goats to be about 58 Kg as against milk

of cow and buffalo which is about 173 Kg and 491 Kg respectively.

In cow's milk iron is scarce but in goat milk iron is 8 to 10 times more than that of cow's milk. As against acidic nature of cow's milk the goat's milk is found to be alkaline in effect (Lall, 1969).

Goat meat contains high quality and readily digestible protein and supplies all the essential amino acids necessary for body building and is also an excellent source of supply of important minerals and trace elements etc. It is rich in Vit. A and is also a good source of Vit. B (Thiamin B₁, Riboflavin B₂ and Niacin). As a quality meat producer also it is expected that goat should prove superior to pig and lamb because of low fat content in the carcass (Singh and Sanger, 1970). The production of goat skin in India, a major item of export, was estimated to be nearly 274.19 lacs each year (Lall, 1962). The contribution of goat as an earner of foreign exchange for the country amounts to Rs. 142.20 lacs per year in the form of hides and skin alone which are primarily exported to U.S.A. and U.S.S.R. (Singh and Sanger, 1970). Randhawa (1969) estimated that 70 lac pounds of goat hair costing approximately Rs. 72 lacs is obtained from Indian goats every year.

To have more return from the goat, better quality of goat need be developed. But the number of better bucks are very few in proportion to the high demand. In view of this Artificial Insemination in goats seems to be the best way for development. For successful preservation of buck

semen a good extender is needed. Work on this aspect has been very scarce. So the present study was undertaken to observe the characters of buck semen and to find out a suitable dilutor for its preservation.

*

REVIEW OF LITERATURE

The importance of Artificial Insemination in goat has gained momentum with the realization of importance of goat in the national economy. But literature on study of back semen especially in India is very scarce.

The use of egg yolk containing diluents has opened up new avenues for the wide expansion of the artificial breeding by the technique of Artificial Insemination.

Review of literature on back semen has been grouped under three heads as follows :-

(1) REVIEW OF LITERATURE

- (i) Egg yolk containing diluents
- (ii) Milk diluent

1.1.1. Egg yolk :

Sharma and Srinivasan (1952) collected back semen regularly throughout the year from two adult bucks and recorded average ejaculate volume to be 0.6 ml, average pH to be 6.5, average number of sperm per ejaculate to be 2000 million, concentration average to be 3.50 million/cm³, average sperm motility to be 75% and average abnormal sperm percentage to be 2.75 with no significant seasonal variation.

Sexual quality of 12 male goats of 1 to 12 years of age was studied by Sharma et al. (1952) and they reported the average sperm volume to be 0.54 ± 0.11 having initial

REVIEW OF LITERATURE

The importance of Artificial Insemination in goat has gained momentum with the realisation of importance of goat in the national economy. But literature on study of buck semen specially in India is very scarce.

The use of egg yolk containing diluents has opened up new avenues for the wide expansion of the artificial breeding by the technique of Artificial Insemination.

Review of literature on buck semen has been grouped under three heads as follows : -

- (i) Neat semen
- (ii) Egg yolk containing diluents
- (iii) Milk diluent

Neat semen :

Dussardier and Szumowski (1952) collected buck semen regularly throughout the year from two adult bucks and recorded average ejaculate volume to be 0.6 ml, average pH to be 6.5, average number of sperms per ejaculate to be 2200 million, concentration average to be 3.60 million/cmm, average sperm motility to be 75% and average abnormal sperm percentage to be 2.75 with no significant seasonal variation.

Semen quality of 14 male goats of 1 to 1½ years of age was studied by Mukherjee et al. (1952) and they reported the average semen volume to be 0.54 ± 0.11 having initial

motility of 4.35 ± 0.11 . They further observed the sperm concentration and percentage of abnormal sperms to be 5368 ± 247.88 million/ml and 3.35 ± 0.40 respectively.

Shukla and Bhattacharya (1952) studied the semen quality of goats for two years and reported the average volume of buck semen to be 0.46 to 0.78 ml, pH 6.2 to 6.5, sperm concentration 1726 to 3240 million per ml, sperm motility 3.8 to 5.0 and abnormal sperm percentage to be 2.4 to 11.1 whereas they observed the colour of neat semen to be creamy.

Knoblauch (1962) studied White German improved buck semen and found the average ejaculate volume to be 1 ml, colour ivory, consistency creamy, pH 6.6, motility 80% and dead spermatozoa 20%.

Kurian and Raja (1965) studied 47 Malabari buck semen samples, collected at intervals of 7 - 12 days from 6 bucks and found the average volume to be 0.4 - 1.2 ml, motility 60 - 90%, pH 6.3 - 6.7, sperm concentration 2 - 3 million per cmm, abnormal spermatozoa 6 - 12% and live spermatozoa 85 - 95%. No significant effect of interval between collections on semen characteristics could be found by them.

The normal values for semen collected from Spanish goats, by electro ejaculation and artificial vagina methods as recorded by Austin et al. (1968) showed average volume of ejaculate to be 1.93 and 0.84 ml, sperm concentration in million per ml 1997 and 2436, motile spermatozoa 75 and 80%, percentage of live spermatozoa 87.1 and 79.5 and abnormal sperm

percentage 6.3 and 7.2% respectively.

The average, volume, initial motility, sperm concentration and percentage of live sperms in the neat semen of Barbari and Jamnapari bucks as reported by Sahni and Roy (1969) were as follows :

Buck	Volume (ml)	Initial motility (0 - 5)	Sperm concentration (x 10 ⁸ /ml)	Live sperm (%)
Barbari	0.66	3.694	19.10	69.49
Jamnapari	0.94	3.634	22.412	63.582

Egg yolk containing diluents :

Use of hen's egg yolk as diluting medium for semen by Phillips and Lardy (1939) opened up a new way in the expansion of artificial insemination programme.

Wagner (1949) reported that though egg yolk added to glucose phosphate diluent in 1:1 ratio reduced goat sperm viability, the addition of small amount of egg yolk (25%) increased the viability. Aandal et al. (1955) studied the conception rate of ewes inseminated with Dala and Merino ram semen diluted in egg yolk citrate and stored for 24 hours at 4°C and obtained better result than when inseminated with semen diluted in egg yolk glycine.

Aehnelt et al. (1955) studied semen samples from 46 goats. The semen samples were diluted in spermasol and

yolk buffer added from eggs of New Hampshire, Sussex, White Leghorn and Brown Leghorn new. The diluted semen samples were stored at 6°C and examined daily. Spermatozoal motility and survival time were greatest when New Hampshire's egg yolk with buffer was used, followed by Sussex, White Leghorn and Brown Leghorn in decreasing order.

Blockhuis (1957) reported that goats when inseminated with semen diluted in a 3% sodium citrate buffer, containing 5% egg yolk, showed much better results than when inseminated with semen diluted in a similar buffer containing 20% egg yolk.

Roy et al. (1959) studied the effect of various diluents on goat semen and found egg yolk citrate to be the best among all. According to Lunca et al. (1961) the survival of ram spermatozoa was the best in egg yolk citrate diluent.

Bejalski and Kastyak (1961) made comparative studies on the influence of different diluents on the survival of ram spermatozoa. According to them semen samples diluted with E.Y.C. + Fructose, E.Y.C. + Glucose, E.Y.C. + Glycocol or E.Y.C. + Glycocol + Glycerol and stored at 2°C showed sperm survival to be the best in E.Y.C. + Glycocol and E.Y.C. + Fructose, although differences between the diluents were small.

Knoblauch (1962) experimented on Malabari buck semen diluted in the ratio of 1:5 in sodium citrate with 10% or 20% egg yolk to which penicillin had been added and stored

for 1 - 7 days at 3°C and found 20% motility on the 5th day of storage.

Jelam et al. (1965) reported that when 14 semen samples were collected from 7 Jamnapari bucks and diluted in the ratio of 1:20 in egg yolk citrate and stored for 24 hours and 72 hours the average motility was found to be 54.4 ± 2.73 and 43.6 ± 2.69 and averaged percentage of unstained sperms 61.27 ± 4.31 and 50.09 ± 3.26 respectively.

Saxena and Singh (1967) collected semen from 4 Bikaneri rams at weekly interval for 6 weeks. The samples were diluted with skim milk yolk (S.M.Y.), egg yolk citrate (E.Y.C.), egg yolk glucose bicarbonate (E.Y.G.B.) and Cornell University Extenders in the ratio of 1:20. After that 10%, 25%, 25% and 20% yolk was added to S.M.Y., E.Y.C., E.Y.G.B. and C.U.E. respectively. Diluted semen was examined at intervals of 0, 72, 120 and 168 hours (storage temperature $3 \pm 1^\circ\text{C}$). Motility and live percentage remained highest in S. M. Y. followed by E.Y.G.B. upto 168 hours of storage. But there was sharp fall in C.U.E. and E.Y.C. for both characters upto 72 hours.

Joshi and Singh (1968) found that the survival of ram spermatozoa was higher in egg yolk citrate fructose glycine diluent than in diluents lacking in glycine.

Sahni and Roy (1969) reported the percentage of motile sperms in buck semen after 6, 54 and 126 hours of preservation at 5°C in E.Y.C. diluent to be 66.3, 24.5 and 6.1 respectively.

Milk diluent :

Milk as a medium to extend semen for artificial insemination has been reported from time to time by many workers (Thacker and Almquist, 1951; Sobek, 1951; Almquist et al., 1954; Jaskowski, 1956; Tomar and Desai, 1961 and Kale, 1963).

Dauzier et al. (1954) studied the effect of various diluents on ram semen. They reported that the spermatozoal motility was preserved for longest period in a diluent containing 3 part 3% citrate and 1 part egg yolk. Motility was not preserved so long in milk diluent. But spermatozoal motility was better in skim milk heated for 10 minutes than in milk treated in other ways. Motility was the poorest in saline diluent.

IsTvan (1956) inseminated 535 ewes with ram semen diluted in boiled cow milk and reported that 75.5% ewes lambed after one insemination.

According to Malikov (1957) there were 175 control ewes inseminated with 0.05 ml undiluted semen, 116 and 146 ewes inseminated respectively with 0.1 and 0.05 ml semen diluted in egg yolk citrate and 47 and 64 ewes respectively with 0.1 and 0.05 ml semen diluted in boiled cow's milk. The average conception rate was 73, 81, 66, 66, and 53% respectively.

Ganceve (1961) diluted ram semen 10 and 20 times in heated cow's skim milk and inseminated 272 and 136 ewes

respectively. The lambing percentage was noted to be 82.21 and 83.33 respectively.

Efendieva (1963) studied the effect of dilution rate of ram semen in various diluents on conception rate and found optimum results with semen diluted in the ratio of 1:1 in boiled cow milk. The conception rate was reduced when the dilution rate was increased.

Varadin (1964) reported that motility and survival of ram spermatozoa in diluent made from powdered skim milk were inferior than that in spermasol milch egg yolk dilutor.

Furedean et al. (1967) reported that the ram spermatozoal motility and survival were greater when upto 1% glucose and 5% yolk were added to semen diluted with skim milk heated to 90 - 95°C for 10 - 15 minutes than when 1% sugar was added.

Sahni and Roy (1967) found 71.6% conception in Barbari goat inseminated either with neat buck semen or with buck semen diluted in heated cow milk in the ratio of 1:1. The results with neat or diluted semen did not differ. The percentage of motile buck spermatozoa diluted with cow milk and preserved for 6, 54 and 126 hours at 5°C were reported to be 73, 39.8 and 12.2 respectively by Sahni and Roy (1969). They also found that milk and milk containing diluents were significantly superior to yolk containing diluents except the Cornell University Extender.

Sahni and Tiwari (1973) made comparative studies

on cow's milk diluent and ewe's milk diluent. According to them, when ewes were inseminated with fresh semen diluted in the ratio of 1:3 in cow's milk, 40.1% lambing occurred and when ewes were inseminated with semen diluted in ewe's milk, the lambing percentage was 38.3.

Amir et al. (1973) studied the influence of dilutions on ram semen. They conducted the experiment over 3 seasons with 450 ewes and showed that semen diluted in skim milk in the ratio of 1:10 brought about the same conception rate as that obtained with undiluted semen. They also reported that 1:1 dilution did not lower the conception rate.

*

EXPERIMENTAL

Five local non-descript bucks between 2 and 5 years of age were utilized for the present experiment. These animals were maintained at the Department of Gynecology, Bihar Veterinary College, Patna. During experimental period, the bucks were kept on grazing along with concentrate mixture consisting of -

Grownup rice	-	1 part
Wheat bran	-	1 part
Crushed maize	-	2 parts.

For experimental : MATERIALS AND METHODS The mixture was given at the rate of 500 gms per buck per day.

Method of semen collection :

Semen was collected by Artificial vagina method. Collections were taken always in the morning hours and one collection per week from every buck was taken. At the time of collection the artificial vagina was provided with an inflating bag covered with rubber cone and semen collecting tube. During the process of collection the temperature of artificial vagina was maintained between 40° and 55°C.

Its effect on semen :

Analyses of different attributes of semen were

MATERIALS AND METHODS

Five local nondescript bucks between 2 and 3 years of age were utilized for the present experiment. These animals were maintained at the Department of Gynaecology, Bihar Veterinary College, Patna. During experimental period, the bucks were kept on grazing along with concentrate mixture consisting of -

Groundnut cake	-	1 part
Wheat bran	-	1 part
Crushed maize	-	2 parts.

The experimental animals received concentrate mixture at the rate of 500 gms per buck per day.

Method of semen collection :

Semen was collected by Artificial vagina method. Collections were taken always in the morning hours and one collection per week from every buck was taken. At the time of collection the Artificial vagina was provided with an insulating bag covering rubber cone and semen collecting tube. During the process of collection the temperature of Artificial vagina was maintained between 40° and 55°C.

Evaluation of semen :

Evaluation of different attributes of neat semen

like colour, volume, pH, mass motility, concentration of sperms live percentage and percentage of abnormal sperms was done. The evaluation was done immediately after collection. Then the neat semen was diluted in the proportion of 1:10 in both egg yolk citrate and cow milk extenders separately. Each neat semen sample was divided into two parts. One part for diluting with egg yolk citrate and the other with whole cow milk extenders. Thus 50 samples, each in egg yolk citrate and milk extenders were studied.

Concentration of sperms :

The semen sample: was thoroughly mixed in a watch glass to make even distribution of sperms. This semen sample was sucked upto 0.5 mark of the R.B.C. diluting pipette (containing the red head). After that 3% sodium chloride solution was sucked upto 101 mark. The two ends of the pipette were closed by means of fingers and it was shaken gently just to ensure the thorough mixing of the semen in the bulb. In this way the semen was diluted 200 time.

Now the counting chamber was focused first under 10 x and then under 40 x objectives. A cover slip was put on the counting chamber. 2 to 3 drops of fluid (the fluid contained in the stem of the pipette) were discarded. Then a drop of the fluid was kept at the side of the cover slip. The fluid spread throughout the counting chamber which was filled with the diluted semen.

Now under the 40 x objective, the number of sperms present in 5 big squares of the counting chamber was counted. The 5 big squares selected for counting were 4 at the corners and one in the centre in order to get the random concentration of sperms. The calculation was done as below:

The volume of counting chamber was 1 mm x 1 mm x 0.1 mm.

Let the number of sperms present in 5 big squares be n .

Therefore $n \times 5$ was the number of sperms present in 0.1 mm of diluted semen.

Therefore $n \times 5 \times 10$ was the number of sperms present in 1 mm of diluted semen.

Therefore $n \times 5 \times 10 \times 200$ was the number of sperms present in 1 cm of original neat semen.

Therefore $n \times 5 \times 10 \times 200 \times 1000$ was the number of sperms present in 1 ml of semen samples.

That is the number of sperms in 1 ml of neat semen was $n \times 10000000$.

Initial motility of spermatozoa in neat semen :

A drop of neat semen was spread uniformly over a clean and dry glass slide maintained at 37°C. Initial motility was graded under low (10x) power on the basis of the mass movement of the spermatozoa and the sperm was graded grossly

into 5 categories.

- (i) + 5 motility : - There was only wave after wave.
- (ii) + 4 motility : - The swirls and eddies were not so rapid as in 5 grade. The swirls were observed to move towards extremities.
- (iii) + 3 motility : - The swirls were slow and scattered in the field.
- (iv) + 2 motility : - Swirls were absent. But individual movements of spermatozoa were more evident in the field.
- (v) + 1 motility : - No wave motions were observed in the field. Only 20% sperms had progressive movement.

Live sperm percentage :

To find out live sperm percentage, the eosin and nigrosin method of staining was used.

Preparation of stain :

The compound stain was prepared by dissolving 1 gm of Eosin (B.D.H.), 5 gms of Nigrosin (Water soluble, B.D.H.) and 3 gms of Sodium citrate dihydrate (B.D.H.) in 100 ml of glass distilled water.

This solution was warmed in water bath for 30 minutes and filtered after cooling with Whatman's Filter Paper No. 41 and 42.

Preparation of slide :

One drop of semen was placed on a clean and dry watch glass and a large drop of the compound stain was added to it and thoroughly mixed by blowing air gently through a pipette and allowed to stand for one and half minutes. Thin smear was drawn on clean, grease free slides and dried quickly in air. The smear was examined under Oil Immersion Lens (100x). Live spermatozoa were colourless. The dead spermatozoa took pink colour due to Eosin. Sperms under Oil Immersion Lens were counted at random under different fields of microscope and the percentage of live sperms was determined.

Abnormal sperm percentage :

Abnormal sperms were detected with the help of Methylene blue and Eosin stains. Methylene blue stain was prepared by dissolving 1 gm of Methylene blue in 100 ml of glass distilled water. Eosin stain was prepared by dissolving 2 gm of Eosin (B.D.H.) in 100 ml of glass distilled water. Smear of semen was prepared and dried in air. Then it was flooded with 1% Methylene blue and left for 20 minutes and washed gently in running tap water. Again the smear was flooded with 2% Eosin solution and left for 5 minutes. Then it was washed gently under running tap water. The wet slide was dried up by means of a blotting paper and examined under Oil Immersion Lens to find out the abnormalities of sperms. The percentage of abnormal sperms was calculated at different hours of preservation.

Evaluation after dilution :

The diluted semen samples were kept in refrigerator at 5°C and examined at zero, 24, 48 and 72 hours of preservation. Motility of sperms, live and abnormal percentage and pH were determined.

Preparation of Egg Yolk Citrate Extender :

The buffer solution was prepared by dissolving 2.90 gms of sodium citrate dihydrate (B.D.H.) in 100 ml of glass distilled water. This solution was sterilized by autoclaving for half an hour on 15 lb pressure and it was kept in refrigerator for longer use.

Addition of Egg Yolk to the buffer solution :

Only infertile fresh hen's eggs were used in the experiment. The eggs were cleaned by washing in water and then with a swab of alcohol. The egg shell was then broken. The white of the egg (Albumin) was discarded and the yolk was placed on a sterilized filter paper (What's man No.41 and 42). The yolk membrane was removed and yolk was collected in a measuring cylinder. One part of egg yolk and one part of buffer solution were mixed thoroughly. And thus the diluent was prepared.

Preparation of whole cow milk extender :

Pure cow milk was taken in a sterile glass container

from a particular cow.

This milk was poured into a sterile flask and heated from 22° to 95°C in a water bath for a period of 10 minutes. After this the milk was poured in a sterile test tube from the flask and then the test tube was kept in a refrigerator at 5°C and left over-night. This milk sample was taken out next morning and filtered through a cotton-swab in another sterile test tube. As a result of refrigeration much of the fat that had collected at the surface did not pass through the cotton filter, and the filtrate contained only very small fat globules. Thus the diluent was ready for use.

The dilutors were prepared, as described earlier and kept in 5 ml test tubes. The test tubes containing the dilutors were properly labelled. These test tubes were kept in a water bath at 32°C for 2 minutes. Care was taken to see that the temperature of the dilutor and that of the semen remained at 30° - 32°C at the time of dilution. The test tubes were gently rotated between the palms to ensure uniform distribution of sperms in the dilutor.

Preservation of semen :

The diluted semen was put in a clean and sterilized test tube. This tube was wrapped in cotton and put in another bigger test tube which was labelled for date of collection and the dilutor used. The same process was followed both for BYC

and cow milk extender groups. The test tubes containing the diluted semen samples were placed in a beaker having 250 ml of water at 25° - 30°C and kept in a refrigerator for gradual cooling. After 2 - 3 hours, the water contained in the beaker which had attained the temperature of 4°C was discarded and the test tubes were kept in the empty beaker. The diluted semen samples were examined at zero hour and further at intervals of 24, 48 and 72 hours of preservation.

Motility of sperm in diluted semen :

The movement of individual sperm was taken into consideration while grading the motility of sperm in diluted semen. The semen was diluted in the ratio of 1:100 with the dilutors used. A drop of diluted semen was placed on a thin, clean and sterilized glass slide and warmed to body temperature. Then it was covered with a cover slip and examined under high power (45x) magnification. Motility of sperms in both groups of diluted semen was examined at 0 hour, 24 hours, 48 hours and 72 hours of preservation.

The grading of motility was done according to the method advocated by Herman and Swanson (1941) and the grades were given on the basis of the table of Herman and Swanson (1941) which is given here under :

Grade	{Percentage of live sperms}	Movement
0	No motility.	No movement.

Grade	Percentage of live sperms	Movement
0.5 to 1.0	10 to 20% motile sperms.	Movement weak and oscillatory, no waves.
1.0 to 1.5	20 to 30% motile sperms.	Movement mainly vigorous but no waves and eddies.
1.5 to 2.0	30 to 40% motile sperms.	Movement mainly vigorous but no waves and eddies.
2.0 to 2.5	40 to 50% motile sperms.	Waves formation with slight whorls which moves slowly across the field.
2.5 to 3.0	50 to 60% motile sperms.	Waves formation with slight whorls which moves slowly across the field.
3.0 to 3.5	60 to 70% motile sperms with swirling motion.	Rapid and vigorous movement, waves and whorls or eddies form and change with great rapidity.
3.5 to 4.0	70 to 80% motile sperms.	Rapid and vigorous movement, waves and whorls or eddies form and change with great rapidity.
4.0 to 4.5	80 to 90% motile sperms.	Movement and churning of the swirls and eddies are extremely rapid and can be compared with a tide in the sea.
4.5 to 5.0	90 to 100% motile sperms.	Movement and churning of the swirls and eddies are extremely rapid and can be compared with a tide in the sea.

R E S U L T S

Different activities of soil spores

Galaxy :

Seven samples of five non-descript local bushes from different collections were investigated under yellow colonies which varied from light yellow to deep yellow shades.

Table :

The mean value was found to be 0.46 ± 0.01 ml by analyzing R E S U L T S and was incorporated in Table - 1. This average value is furnished in Table - 1.

Table - 1

The overall mean spore concentration was calculated as 1698.5 ± 36.56 million per ml of spores in 50 samples collected from local bushes used in this experiment. This average value is given in Table - 1.

Spore concentration (ml) :

The average pH was found to be 6.67 ± 0.03 and this figure is incorporated in Table - 1.

Spore concentration of spores :

The mean initial spore concentration was calculated

to be 78.60 ± 0.61 in 50 samples of local non-descript buck semen. This value is shown in Table - I.

Live sperm percentage :

The mean percentage of live sperms was estimated to be 87.81 ± 1.32 from smears stained with eosin nigrosin compound stain by counting 300 spermatozoa per smear in 50 samples of buck semen. This value is furnished in Table - I.

Abnormal sperm percentage :

The mean percentage of abnormal spermatozoa was recorded to be 5.00 ± 0.12 . This result is furnished in Table - I.

TABLE - I.

Mean values of different attributes of neat caprine semen along with S.E.

No. of obser- vations	Colour	Volume (ml.) (Mean \pm S.E.)	Sperm concen- tration (million per ml) (Mean \pm S.E.)	pH (Mean \pm S.E.)	Motility % (Mean \pm S.E.)	Live sperm % (Mean \pm S.E.)	Abnormal sperm % (Mean \pm S.E.)
50	Yellow	0.46 \pm 0.01	3658.5 \pm 30.38	6.67 \pm 0.28	78.60 \pm 0.61	87.81 \pm 1.32	5.00 \pm 0.12

Different attributes of diluted semen :

Hydrogen ion concentration (pH) :

The average pH of diluted semen was observed to be 6.67 ± 0.16 , 6.50 ± 0.11 , 6.34 ± 0.28 and 6.17 ± 0.07 in E.Y.C. diluent and 6.66 ± 1.00 , 5.92 ± 0.78 , 5.79 ± 0.65 and 5.67 ± 0.69 in cow milk diluent at 0, 24, 48 and 72 hours of preservation respectively (Table - II).

Statistical analysis revealed non-significant difference between diluents as well as between hours of preservation (Table - III).

TABLE - II.

Mean values of pH of buck semen along with S.E. under different hours of preservation in E.Y.C. and Milk diluents.

Hours of preservation	Number of observations	pH	
		E.Y.C. Mean \pm S.E.	Milk. Mean \pm S.E.
0 hour	50	6.67 ± 0.16	6.66 ± 1.00
24 hours	50	6.50 ± 0.11	5.92 ± 0.78
48 hours	50	6.34 ± 0.28	5.79 ± 0.65
72 hours	50	6.17 ± 0.07	5.67 ± 0.69

Fig.

Showing mean seminal pH in different diluents at different hours of preservation.

1 big division = 0.5 pH.

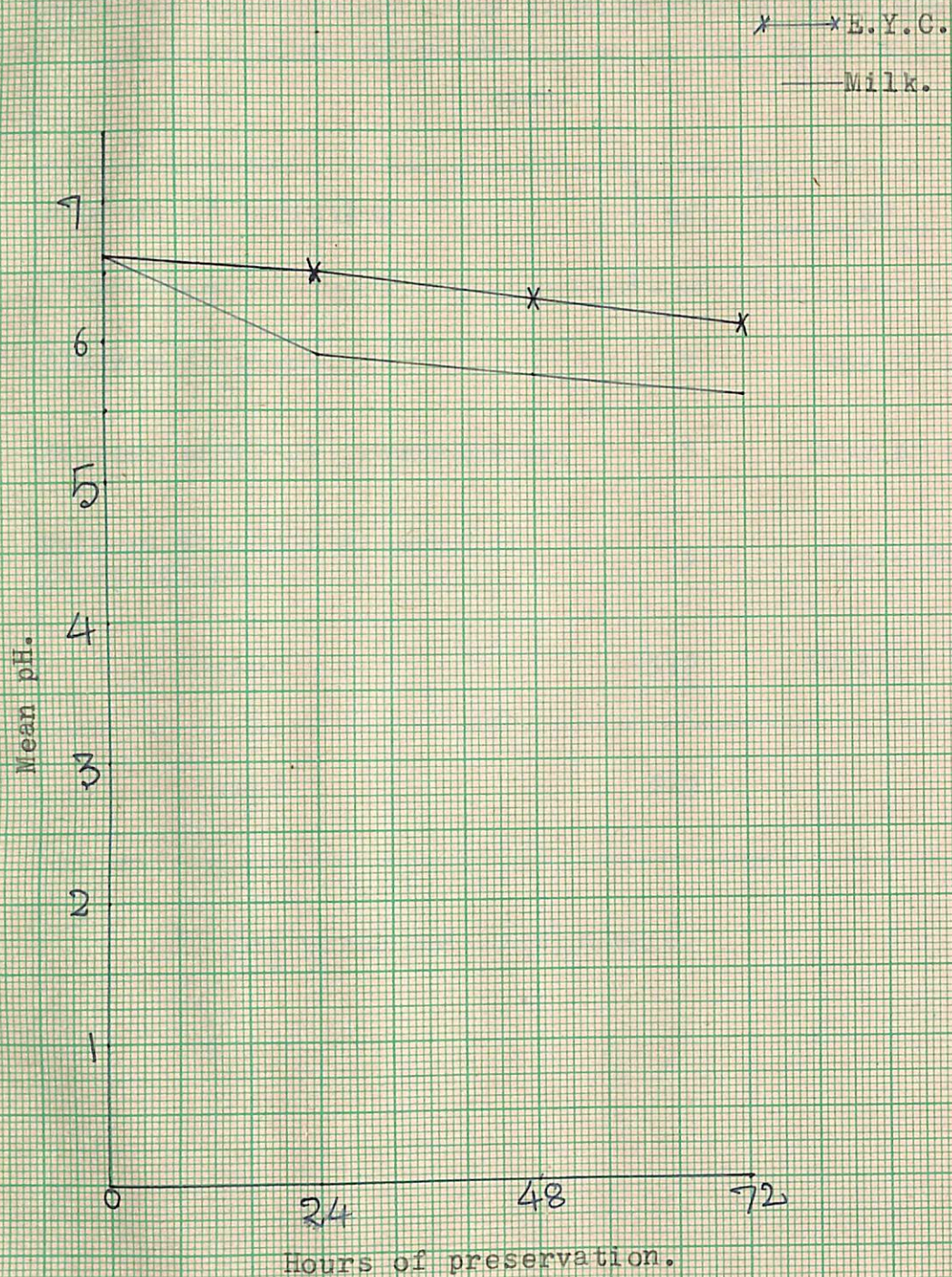


TABLE - III.

Analysis of variance table showing effect of diluents as well as hours of preservation on the pH of buck semen.

Sources of variation	df.	pH
		M. S.
Between diluents.	1	2952.91 NS
Between hours of preservation.	3	2832.67 NS
Between diluents x hours of preservation.	3	135.6 NS
Error	392	3063.52
Total	399	

NS indicates non-significant.

Motility :

The average motility of sperm in diluted semen was recorded to be 78.60 ± 1.23 , 60.00 ± 1.12 , 41.00 ± 1.00 and $24.40 \pm 0.62\%$ in E.Y.C. diluent and 62.80 ± 1.08 , 44.75 ± 0.88 , 25.80 ± 0.63 and $12.60 \pm 0.12\%$ in cow milk diluent at 0, 24, 48 and 72 hours of preservation respectively (Table-IV).

Highly significant differences in motility between different diluents as well as between different hours of preservation were recorded (Table - V).

TABLE - IV.

Mean sperm motility % of buck semen along with S.E. under different hours of preservation in E.Y.C. and cow milk diluents.

Hours of preservation	Number of observations	Motility percentage	
		E.Y.C. Mean \pm S.E.	Milk Mean \pm S.E.
0 hour	50	78.60 ± 1.23	62.80 ± 1.08
24 hours	50	60.00 ± 1.12	44.75 ± 0.88
48 hours	50	41.00 ± 1.00	25.80 ± 0.63
72 hours	50	24.40 ± 0.62	12.60 ± 0.12

Fig.

Overall mean motility percentage at different hours of preservation.

1 big division = 4% motility

X — X E.Y.C.

— Milk.

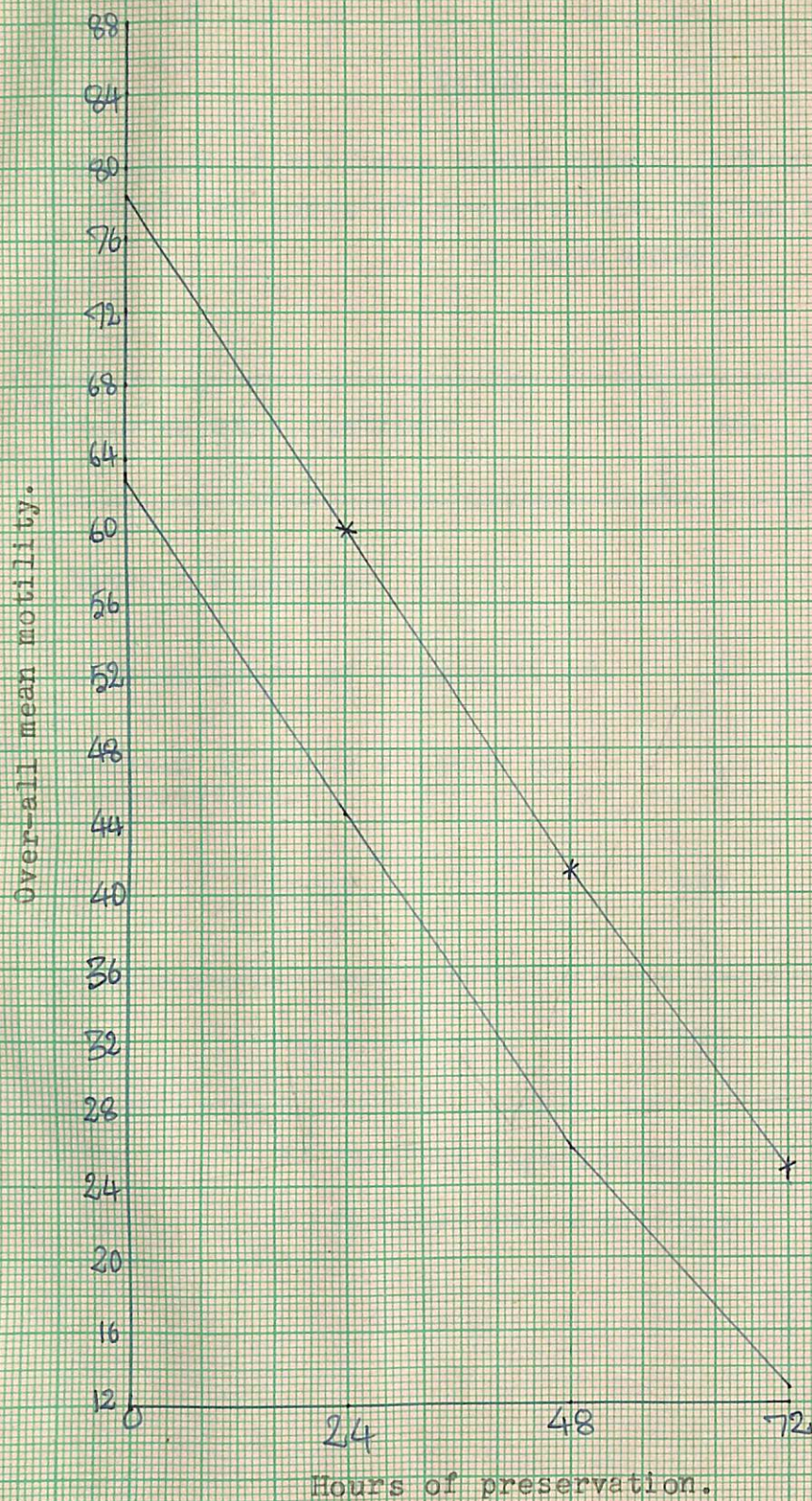


TABLE - V.

Analysis of variance table showing effect of diluents as well as hours of preservation on the sperm motility in buck semen.

Sources of variation	df.	Motility percentage M.S.
Between diluents.	1	1786.39**
Between hours of preservation.	3	1820.12**
Between diluents x hours of preservation.	3	244.79 NS
Error	392	369.63
Total	399	

** denotes significant at 1% level.

NS denotes non-significant.

Live sperm percentage :

The mean percentage of live sperms was recorded to be 84.22 ± 1.82 , 76.60 ± 1.08 , 60.44 ± 0.92 and 43.00 ± 0.45 in E.Y.C. diluent and 70.88 ± 0.93 , 58.86 ± 0.63 , 41.66 ± 0.28 and 25.98 ± 0.13 in cow milk diluent at 0, 24, 48 and 72 hours of preservation respectively (Table - VI).

Different hours of preservation as well as different diluents had highly significant influence on the live sperm percentage of non-descript buck semen (Table - VII).

TABLE - VI.

Mean values of live sperm percentage in buck semen along with S.E. under different hours of preservation in E.Y.C. and cow milk diluents.

Hours of preservation	Number of observations	Live sperm percentage	
		E.Y.C. Mean \pm S.E.	Milk Mean \pm S.E.
0 hour	50	84.22 ± 1.82	70.88 ± 0.93
24 hours	50	76.60 ± 1.08	58.86 ± 0.63
48 hours	50	60.44 ± 0.92	41.66 ± 0.28
72 hours	50	43.00 ± 0.45	25.98 ± 0.13

Fig.

Showing mean percentage of live spermatozoa in different diluents at different hours of preservation.

1 big division = 4% live sperms

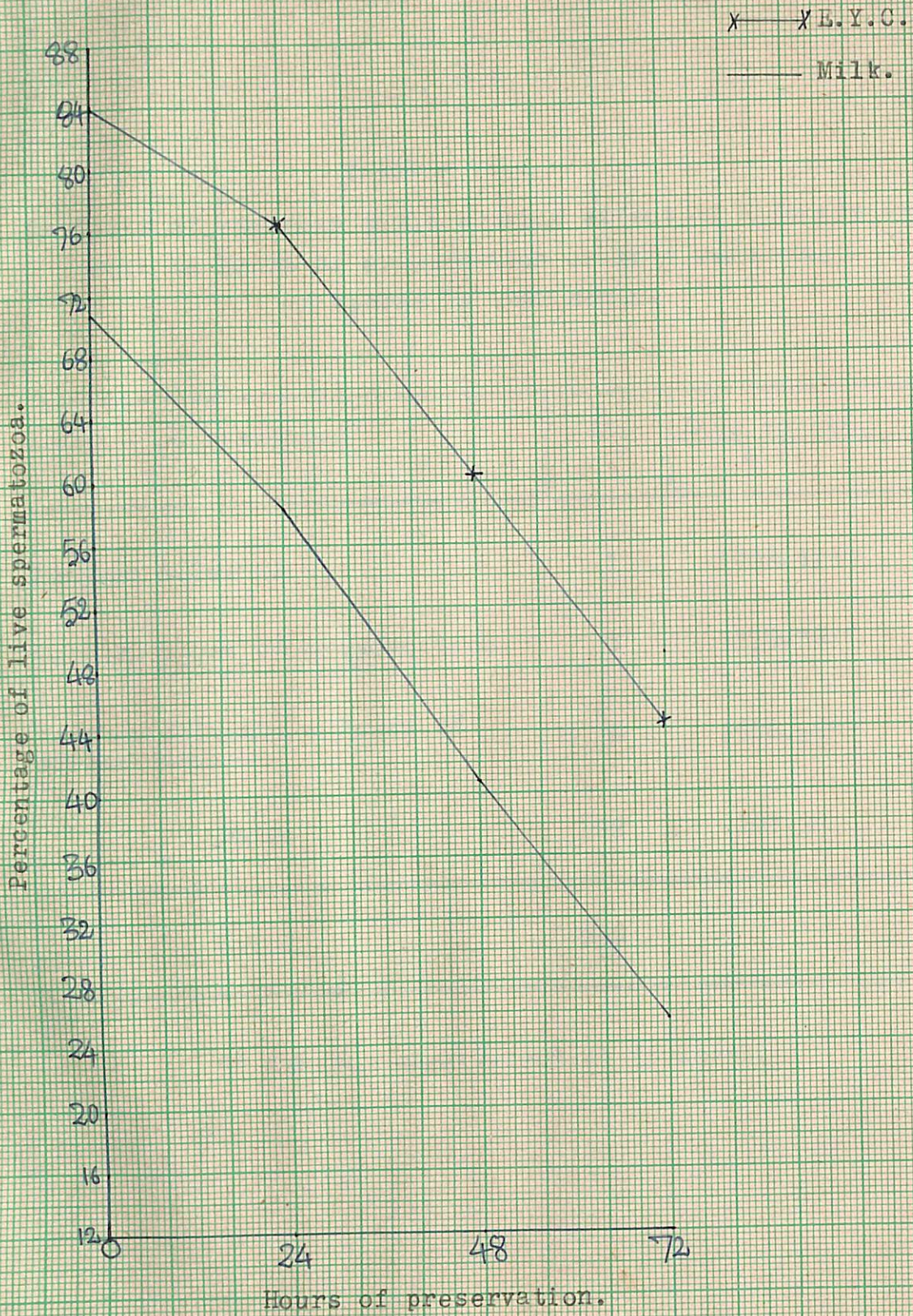


TABLE - VII.

Analysis of variance table showing effect of diluents as well as hours of preservation on live percentage of sperms in buck semen.

Sources of variation	df.	Live sperm% M.S.
Between diluents.	1	1987.32**
Between hours of preservation.	3	1879.32**
Between diluents x hours of preservation.	3	2508.56**
Error	392	201.91
Total	399	

** denotes significant at 1% level.

Abnormal sperm percentage.

The mean percentages of abnormalities of sperms were 5.48 ± 0.20 , 6.02 ± 0.19 , 6.44 ± 0.09 and 6.88 ± 0.36 in E.Y.C. diluent and 5.80 ± 0.24 , 6.40 ± 0.18 , 6.76 ± 1.19 and 7.16 ± 0.23 in cow milk diluent at 0, 24, 48 and 72 hours of preservation respectively (Table - VIII).

Analysis of variance showed that the different diluents and hours of preservation had non-significant effect on this seminal attribute (Table - IX).

TABLE - VIII.

Mean values of abnormal sperm percentage of buck semen along with S.E. under different hours of preservation in E.Y.C. and cow milk diluents.

Hours of preservation	Number of observations	Abnormal sperm percentage	
		E.Y.C. Mean \pm S.E.	Milk Mean \pm S.E.
0 hour	50	5.48 ± 0.20	5.80 ± 0.24
24 hours	50	6.02 ± 0.19	6.40 ± 0.18
48 hours	50	6.44 ± 0.09	6.76 ± 0.19
72 hours	50	6.88 ± 0.36	7.16 ± 0.23

Fig.

Showing over-all percentage of abnormal sperms at different hours of preservation.

1 big division = 0.5% abnormal sperm.

— E.Y.C.

— Milk.

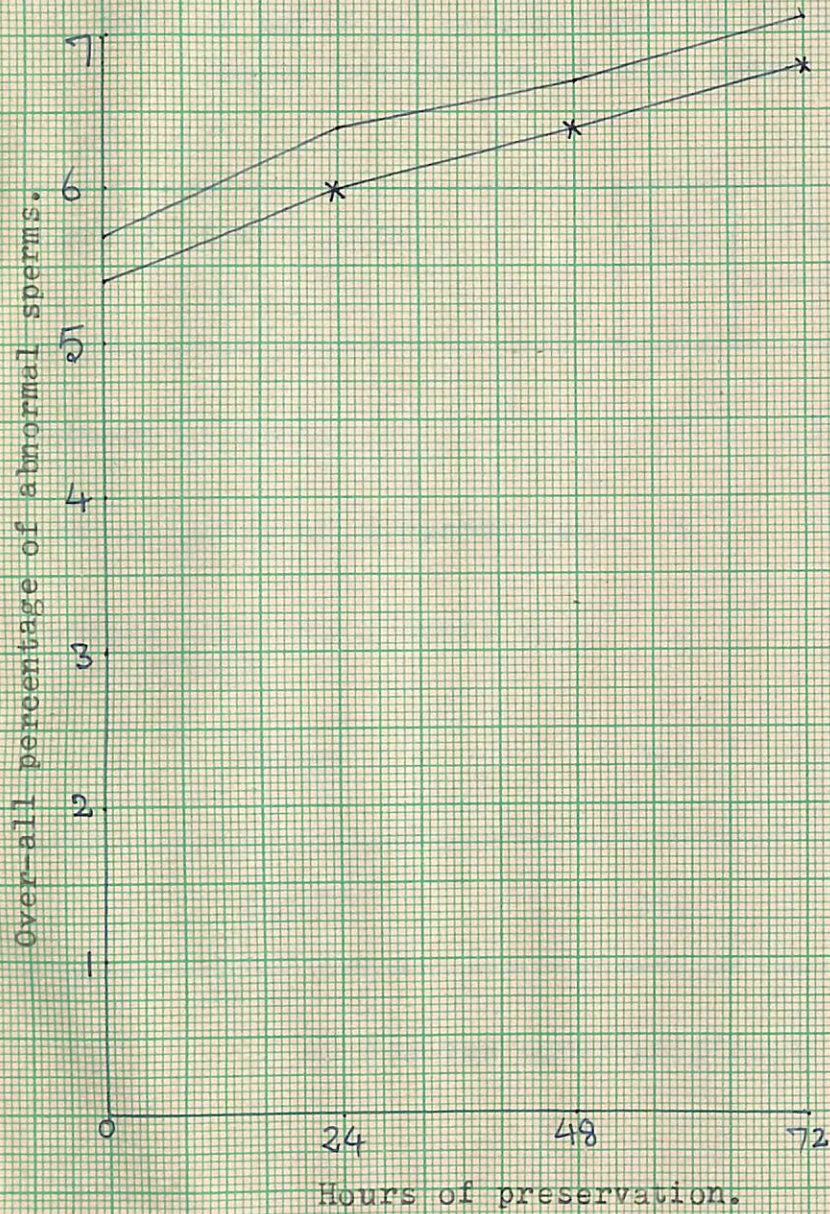


TABLE - IX.

Analysis of variance table showing effect of diluents as well as hours of preservation on the abnormal sperm percentage of buck semen.

Sources of variation	df.	Abnormal sperm percentage M. S.
Between diluents.	1	322.63 NS
Between hours of preservation.	3	174.86 NS
Between diluents x hours of preservation.	3	20.63 NS
Error	392	198.97
Total	399	

NS denotes non-significant.

TABLE - X.

Mean values of different attributes of caprine semen along with E.V.C. under different hours of preservation in E.V.C. and cow milk diluents.

Hours of preservation	No. of observations	Live sperm %		pH		% of sperm motility		Abnormal sperm	
		E.V.C. Mean \pm S.E.	Milk Mean \pm S.E.	E.V.C. Mean \pm S.E.	Milk Mean \pm S.E.	E.V.C. Mean \pm S.E.	Milk Mean \pm S.E.	E.V.C. Mean \pm S.E.	Milk Mean \pm S.E.
0 hour	50	84.22 \pm 1.82	70.88 \pm 0.93	6.67 \pm 0.16	6.66 \pm 1.00	78.60 \pm 1.23	62.80 \pm 1.08	5.48 \pm 0.20	5.80 \pm 0.24
24 hours	50	76.60 \pm 1.08	58.86 \pm 0.63	6.50 \pm 0.11	5.92 \pm 0.78	60.00 \pm 1.12	44.75 \pm 0.88	6.02 \pm 0.19	6.40 \pm 0.18
48 hours	50	60.44 \pm 0.92	41.66 \pm 0.28	6.34 \pm 0.28	5.79 \pm 0.65	41.00 \pm 1.00	25.80 \pm 0.63	6.44 \pm 0.09	6.76 \pm 0.19
72 hours	50	43.00 \pm 0.45	25.98 \pm 0.13	6.17 \pm 0.07	5.67 \pm 0.69	24.40 \pm 0.62	12.60 \pm 0.124	6.88 \pm 0.36	7.16 \pm 0.23

TABLE - XI.

Analysis of variance table showing effect of diluents as well as hours of preservation on different attributes of caprine semen.

Sources of variation	df.	Live sperm % N.S.	pH N.S.	Motility % N. S.	Abnormal sperm % N. S.
Between diluents.	1	1987.32**	2952.91 NS	1786.39**	322.63 NS
Between hours of preservation.	3	1879.32**	2832.67 NS	1820.12**	174.86 NS
Between diluents x hours of preservation.	3	2508.56**	135.6 NS	244.79 NS	20.63 NS
Error	392	201.91	3063.52	369.63	198.97
Total	399				

** denotes significant at 1% level.

NS denotes non-significant.

DISCUSSION

RESULTS

Volume

The average volume obtained from 30 samples of milk taken collected from 5 nondescript cows was found to be 0.45 ± 0.01 ml. Bhattacharya (1952) reported the average volume of milk taken from cows to be 0.34 ± 0.01 ml whereas Bhattacharya (1952) obtained high value for the volume of milk taken from cows 0.45 ± 0.01 ml. DISCUSSION seemed to agree with the results reported by Bhattacharya (1952) but varied from the findings of Bhattacharya (1952) and Bhattacharya (1952). The difference in the values might be attributed to the difference of goats used in the present study, variation of seasons and feeding schedules also might be the factors for difference in results. This result did not also seem to agree with the observations of Bhattacharya and Roy (1952), who recorded the average volume of milk taken from goats in Jammu and Kashmir to be 0.55 and 0.44 ml respectively. The present findings also did not tally with those obtained by Anwar and Hage (1955), who reported the average volume of milk taken from goats to be 0.4 to 1.2 ml. Bhattacharya and Bhattacharya (1952) recorded the average volume to be 0.5 ml and this result to be a bit on the higher side than the present observation, whereas the findings of Bhattacharya (1952) and Bhattacharya (1952) are similar to the present findings.

DISCUSSION

NEAT SEMEN

Volume :

The average volume obtained from 50 samples of buck semen collected from 5 nondescript bucks was found to be 0.46 ± 0.01 ml. Mukherjee et al. (1952) reported the average volume of neat semen of buck per ejaculate to be 0.54 ± 0.11 ml whereas Shukla and Bhattacharya (1952) obtained high value for the volume of buck semen ranging from 0.46 to 0.78 ml. The present findings seemed to agree with those reported by Mukherjee et al. (loc. cit.) but varied from the findings of Shukla and Bhattacharya (loc. cit.). The differences in the volume might be attributed to breed difference of goats used in the present study, variation of seasons and feeding schedules also might be the factors for differences in results. This result did not also seem to agree with the observations of Sahni and Roy (1969), who recorded the average volume of buck semen in Barbari and Jamnapari bucks to be 0.66 and 0.94 ml respectively. The present findings also did not tally with those obtained by Kurian and Raja (1965), who recorded the average volume of Malabari buck semen to be 0.4 to 1.2 ml. Dussardier and Szumowski (1952) recorded the average ejaculate volume to be 0.6 ml and this seemed to be a bit on the higher side than the present observation, whereas the findings of Knoblauch (1962) 1 ml volume and Austin (1968)

1.93 ml volume (electro ejaculate), 0.84 ml (A.V.method) seemed to vary widely from the present findings. This discrepancy in the average volume of buck semen might be due to different breeds of goats used by different workers, method of collection and feeding and managerial practices etc.

Colour :

The present study showed the colour of neat buck semen to be yellowish in general though light yellow and deep yellow shades were not uncommon. Creamy colour of neat buck semen was recorded by Shukla and Bhattacharya (loc.cit.).

According to them the thin creamy samples on the average showed higher pH than thick ones. But no difference in values of initial motility of spermatozoa was recorded between thick and thin creamy samples. Knoblauch (loc. cit.) found buck semen to be of ivory colour with creamy consistency. Investigation under report found the buck semen colour to be yellow. Difference in breeds and feeds provided might be responsible for this.

Hydrogen ion concentration (pH) :

Present case showed the average pH of neat semen to be 6.67 ± 0.28 (Table - I), whereas Kurian and Raja (loc. cit.) obtained the pH of Malabari buck semen to be 6.3 to 6.7. Knoblauch (loc. cit.) recorded the average pH of White German buck semen to be 6.6, whereas Dussardier and Szumowski (1952) obtained average pH of buck semen to be 6.5. Shukla and

Bhattacharya (1952) recorded the pH of buck semen ranging from 6.2 to 6.5. The present values obtained seemed to be a bit on the higher side. This might be due to different breeds, seasons as well as fluctuations in feeding and managerial practices.

Sperm concentration :

The present investigation recorded the average sperm concentration of neat buck semen to be 3658.5 ± 30.38 million per ml (Table - I). This value seemed to be very close to that obtained by Dussardier and Szumawski (loc.cit.), who recorded an average sperm concentration in buck semen to be 3600 million per ml. But the finding differed from the values obtained by Sahni and Roy (1969), who reported it to be 1910 and 2241 million per ml in Barbari and Jannapari respectively. Shukla and Bhattacharya (loc. cit.) recorded the sperm concentration in buck semen to be ranging from 1726 to 3240 million per ml in different months of the years which was much lower than that obtained in the present study. Mukherjee (loc. cit.) obtained the sperm concentration of buck semen to be 5368 ± 247.88 million per ml which was much higher than the present finding. This difference might be explained on the ground of breed variability, environmental variables etc.

Initial motility of sperm :

The spermatozoal motility of buck semen in the present investigation was recorded to be 78.60 ± 0.61 per

cent (Table - 1). The value obtained in the present investigation seemed to tally with those reported by Dussardier and Szumowski (loc. cit.), who obtained 75% motility. Shukla and Bhattacharya (loc. cit.) reported the initial spermatozoal motility of buck semen to range from 3.8 to 5.0 in different months of year. Their values seemed to be a bit higher than that obtained in the present case. The present values were not within the range obtained by Sahni and Roy (loc. cit.) who reported 3.69 and 3.634 motility in the five point scale in Barbari and Jamnapari bucks respectively. Fluctuations in feeding and managerial practices and breed difference of bucks for study might be responsible for this wide variation in the findings.

Live sperm percentage :

In nondescript bucks the mean live sperm percentage in neat semen was recorded to be 87.81 ± 1.32 as against 69.49 and 63.63 reported by Sahni and Roy (loc. cit.) in Barbari and Jamnapari bucks respectively. The values obtained by Kurian and Raja (loc. cit.) seemed to be in close agreement with the present finding, because this value was 93% in Malabari buck semen. This difference in the values obtained might be due to different factors like variations in the breeds of goat, agroclimatic variables, method of semen collection and feeding of the bucks etc.

Abnormal sperm percentage :

The value of abnormal sperm percentage in the seminal fluid of nondescript bucks was obtained to be 5.00 ± 0.12 (Table - I) in this study. But Austin et al. (loc.cit.) found the abnormal sperm percentage in Spanish goat by electro ejaculation and by Artificial Vagina methods to be 6.3 and 7.3 respectively. Kurian and Raja (loc. cit.) obtained the abnormal sperm percentage in Malabari buck semen to be 6 to 12. Shukla and Bhattacharya (loc. cit.) obtained the values of abnormal sperm percentage ranging from 2.4 to 11.1 during different months of year. Mukherjee et al. (1952) also reported the percentage of abnormal sperms to be 3.35 ± 0.40 . The difference in the percentage of abnormal sperms reported by various workers might be attributed to breed difference, difference in the method of semen collection and seasonal variations.

EFFECT OF E.Y.C. AND MILK DILUENTS ON DIFFERENT CHARACTERISTICS OF BUCK SEMEN:

Hydrogen ion concentration (pH) :

The pH of buck semen at 0 hour of preservation in E.Y.C. diluent was found to be 6.67 ± 0.16 . At 24 hours the pH came down to 6.50 ± 0.11 and at 48 hours the seminal pH was 6.34 ± 0.28 as against 6.17 ± 0.07 at 72 hours of preservation (Table - II). This was expected with the increasing

hours of preservation as the pH came down gradually due to increase in acidity. Though the pH of neat semen revealed its acidic nature, the increasing acidity was marked at 72 hours of preservation.

In case of milk diluent the pH of diluted buck semen showed sharp fall ranging from 6.6 ± 1.00 at 0 hour to 5.67 ± 0.69 at 72 hours. The fall was particularly marked at 0 to 24 hours of preservation. The pH of semen was 6.6 at 0 hour which declined to 5.92 ± 0.78 at 24 hours. There after the fall in pH was rather uniform and steady (Table II).

Analysis of variance of pH at different hours of preservation in different diluents revealed no significant difference between hours of preservation and between diluents (Table III). The increased acidity of stored diluted buck semen with increasing hours of preservation might be due to formation of lactic acid during storage and thus taking the semen on the acidic side.

Motility percentage of sperms :

The motility percentage of sperms in E.Y.C. diluent was recorded to be 78.60 ± 1.23 , 60.00 ± 1.12 at 0 hour and 24 hours respectively and 41.00 ± 1.00 at 48 hours and 24.40 ± 0.62 at 72 hours (Table IV). Perusal of motility percentage as depicted in Table IV revealed sharp fall in the motility percentage with increasing hours of preservation. The order of decline in motility percentage of sperms between

different hours of preservation seemed to be of the same order.

In the milk diluent the motility percentage of sperms at 0, 24, 48 and 72 hours was recorded to be 62.80 ± 1.08 , 44.75 ± 0.88 , 25.80 ± 0.63 and 12.60 ± 0.124 respectively (Table IV). In the case of milk diluent sharp decline in motility percentage with increasing hours of preservation was noted. The motility percentage showed a sharp decline from the very beginning in contrast to the findings with E.Y.C. diluent in which the decline was more steady. This might be due to non-addition of buffer in the milk diluent.

The difference in mean motility percentage in different diluents was found to be highly significant at different hours of preservation. The diluents were also found to influence this attribute of buck semen highly significantly (Table V). The present findings seemed to differ with the observation of Jelam et al. (1965) who reported the average motility percentage of buck semen to be 54.4 and 43.6 after storage for 24 and 72 hours respectively.

This difference might be due to difference in breed, method of collection of semen and feeding and managerial variations and dilution rate of semen with different diluents.

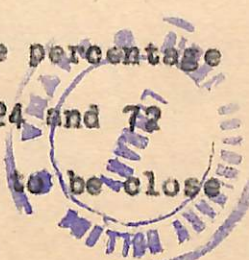
Live sperm percentage :

The live percentage of spermatozoa in buck semen with E.Y.C. diluent was found to be 84.22 ± 1.82 , 76.60 ± 1.08 ,

60.44 \pm 0.92 and 43.00 \pm 0.45 at 0, 24, 48 and 72 hours of preservation respectively whereas in case of milk diluent the values at 0, 24, 48 and 72 hours of preservation were found to be 70.88 \pm 0.93, 28.86 \pm 0.63, 41.66 \pm 0.28 and 25.98 \pm 0.13 respectively (Table - VI). The study of Table VI revealed that the minimum live sperm percentage suitable for artificial insemination (60%) was recorded upto 48 hours of preservation in E.Y.C. diluent, but this value was not maintained even at 24 hours with the use of milk diluent. Lasley and Bogert (1943) reported that semen samples with less than 50% live spermatozoa might be of questionable fertility. Table VI showed sharp fall in live sperm percentage between 24 to 48 hours and 48 to 72 hours of preservation. Though there was decline in the value between 0 to 24 hours of preservation, it was not remarkable. It further revealed that in case of milk diluent the fall in live sperm percentage between 0 to 24 hours was higher in comparison to that in E.Y.C. diluent. At other different hours of preservation the decline in live sperm percentage was almost of the same order with increasing hours of preservation in E.Y.C. diluent.

Diluents were found to influence the trait highly significantly. The hours of preservation also had a significant influence in the fluctuation of live sperm percentage (Table VII).

Jelam et al. (1965) reported the average percentage of unstained buck sperms to be 61.27 and 60.09 at 24 and 72 hours respectively in E.Y.C. diluent, which seemed to be close



to the value obtained by present study.

Abnormal sperm percentage :

The abnormal sperm percentage in E.Y.C. diluent was found to be 5.48 ± 0.20 , 6.02 ± 0.19 , 6.44 ± 0.09 and 6.88 ± 0.36 at 0, 24, 48 and 72 hours of preservation respectively (Table VIII). In case of milk diluent the abnormal sperm percentages were 5.80 ± 0.24 , 6.40 ± 0.18 , 6.76 ± 0.19 and 7.16 ± 0.23 at 0, 24, 48 and 72 hours of preservation respectively (Table VIII).

The fluctuation in abnormal sperm percentage with increasing hours of preservation was found to be rather slight and non-significant. The diluents also were found to have no significant effect on this attribute of the buck semen (Table IX).

Keeping in view the facts discussed above it could be concluded that milk diluent was in no way superior to E.Y.C. diluent for preservation of buck semen. This is contrary to the report of Sahni and Roy (loc. cit.) who found a better keeping quality of buck semen in milk diluent than in the case of E.Y.C. extender.

*

S U M M A R Y

1. Live sperm percentage, abnormal sperm percentage, pH and motility after different hours of preservation from 0 to 72 hours at 24 hourly intervals in L.Y.C. and boiled whale oil with diluents were studied for one complete academic year on the semen obtained from 5 non-descript bucks of approximately 2 to 3 years of age.

2. The attributes for heat semen were also studied. The colour of heat semen was observed to be yellowish and the peak volume (ml), sperm concentration (million/ml), pH, motility percentage, live sperm percentage and abnormal sperm percentage of heat semen of non-descript bucks were found to be 0.45 ± 0.01 , 3638.5 ± 30.38 , 6.87 ± 0.22 , 78.60 ± 0.41 , 87.81 ± 1.32 , 5.00 ± 0.12 respectively.

3. Highly significant difference between L.Y.C. and boiled diluents as regards live sperm percentage and motility was reported. Hours of preservation were also found to affect these two traits highly significantly. The mean pH and abnormal sperm percentage did not show any significant difference between diluents as well as between different hours of preservation.

4. The mean live sperm percentage at 0, 24, 48 and 72 hours of preservation in L.Y.C. were found to be 64.22 ± 1.52 , 76.6 ± 1.08 , 68.44 ± 0.32 , 43.00 ± 0.43 respectively as against the values for the same hours of preservation in boiled diluent

S U M M A R Y

1. Live sperm percentage, abnormal sperm percentage, pH and motility after different hours of preservation from 0 to 72 hours at 24 hourly interval in E.Y.C. and boiled whole cow milk diluents were studied for one complete academic year on the semen obtained from 5 nondescript bucks of approximately 2 to 3 years of age.
2. The attributes for neat semen were also studied. The colour of neat semen was observed to be yellowish and the mean volume (ml), sperm concentration (million/ml), pH, motility percentage, live sperm percentage and abnormal sperm percentage of neat semen of nondescript bucks were found to be 0.46 ± 0.01 , 3658.5 ± 30.38 , 6.67 ± 0.28 , 78.60 ± 0.61 , 87.81 ± 1.32 , 5.00 ± 0.12 respectively.
3. Highly significant difference between E.Y.C. and milk diluents as regards live sperm percentage and motility were recorded. Hours of preservation were also found to affect these two traits highly significantly. The mean pH and abnormal sperm percentage did not show any significant difference between diluents as well as between different hours of preservation.
4. The mean live sperm percentage at 0, 24, 48 and 72 hours of preservation in E.Y.C. were found to be 84.22 ± 1.82 , 76.6 ± 1.08 , 60.44 ± 0.92 , 43.00 ± 0.45 respectively as against the values for the same hours of preservation in milk diluent

came to 70.88 ± 0.93 , 58.6 ± 0.63 , 41.66 ± 0.28 , 25.98 ± 0.13 respectively.

5. The seminal pH in E.Y.C. diluent at 0, 24, 48 and 72 hours was recorded to be 6.67 ± 0.16 , 6.50 ± 0.11 , 6.34 ± 0.90 , 6.17 ± 0.07 respectively as against 6.66 ± 1.00 , 5.92 ± 0.78 , 5.79 ± 0.65 , 5.67 ± 0.69 in milk diluent for the same hours of preservation.

6. The motility percentage of sperm in E.Y.C. diluent at 0, 24, 48 and 72 hours of preservation was found to be 78.60 ± 1.23 , 60.00 ± 1.12 , 41.00 ± 1.00 , 24.40 ± 0.62 respectively in E.Y.C. diluent whereas for milk diluent the corresponding values were 62.80 ± 1.08 , 44.75 ± 0.88 , 25.80 ± 0.63 , 12.60 ± 0.12 respectively.

7. In E.Y.C. diluent the abnormal sperm percentage at 0, 24, 48 and 72 hours of preservation was observed to be 5.48 ± 0.20 , 6.02 ± 0.19 , 6.44 ± 0.09 , 6.88 ± 0.36 respectively whereas the corresponding values in milk diluent turned out to be 5.80 ± 0.24 , 6.40 ± 0.18 , 6.76 ± 0.19 , 7.16 ± 0.23 respectively.

The observations of present study indicated E.Y.C. to be a better diluent for buck semen in comparison to boiled cow milk.

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Fig. 1 : Showing mass motility
of sperms.



Fig. 2 : Showing live sperms.



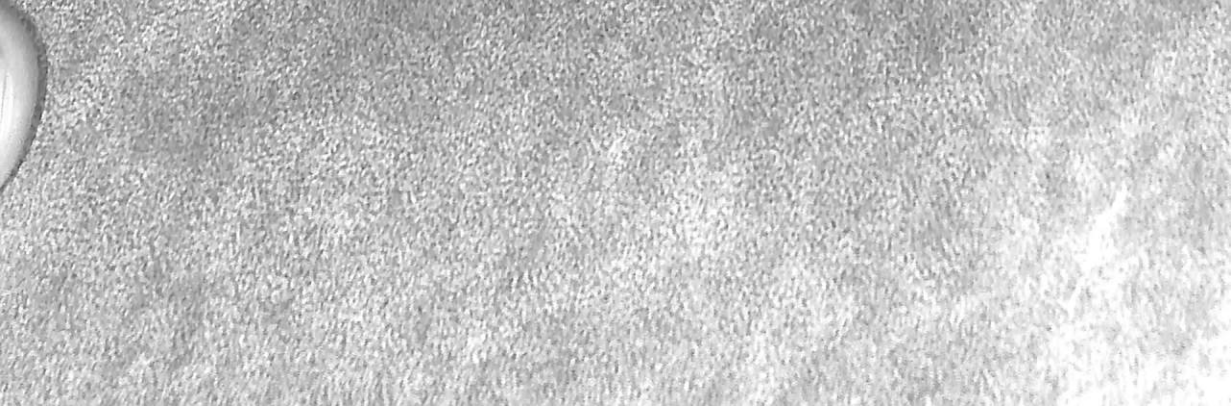
Fig. 1 : Showing mass motility
of sperms.



Fig. 2 : Showing live sperms.



Fig. 3 : Showing abnormal sperms
with coiled tail.



BIBLIOGRAPHY

- Amiel, J. and Wagner, J. (1939). Investigation of sheep. *Physiology and nutrition*. Ford, Vol. 2, 2: 1939. (Cited from *Ann. Breed. Abst.*, 21: 1939).
- Amiel, J. and Wagner, J. (1939). Sheep nutrition using egg yolk from 1939-40. *Ann. Breed. Abst.*, 21: 1939. (Cited from *Ann. Breed. Abst.*, 21: 1939).
- Amiel, J., Wagner, J., Lohr, J., Lohr, J. and Pennington-
Pinto, G. (1939). The effect of the ratio of
dilution of the yolk on differential dilution on
the concentration of the yolk. *Ann. Breed. Abst.*,
21: 1939. (Cited from *Ann. Breed. Abst.*, 21: 1939).
- Baker, J. V., Lohr, J. V., Lohr, J. V. and Lohr, J. V. (1939).
Germinal values for sheep collected from human
and by two methods. *Ann. Breed. Abst.*, 21: 1939-72. (Cited from *Ann. Breed. Abst.*, 21: 1939).
- Baker, J. V. and Lohr, J. V. (1939). Comparative studies on
the effect of certain dilutions on the survival of
the ovum. *Physiology and nutrition*. Ford, Vol. 2, 2: 1939-72. (Cited from *Ann. Breed. Abst.*, 21: 1939).
- Baker, J. V. (1939). The practical use of artificial insemination with goats. *Physiology and nutrition*. Ford, Vol. 2, 2: 1939-72. (Cited from *Ann. Breed. Abst.*, 21: 1939).
- Baker, J. V., Lohr, J. V. and Lohr, J. V. (1939). Preservation of the ovum after dilution and maturation of its fertilizing ability. *Physiology and nutrition*. Ford, Vol. 2, 2: 1939-72. (Cited from *Ann. Breed. Abst.*, 21: 1939).

B I B L I O G R A P H Y

- Aandal, J. and Hogset, I. (1955). Insemination of Sheep. Preliminary communication. Nord. Vet. Med. 7: 309-14. (Cited from Anim. Breed. Abst., 23: 395).
- Aehnelt, E. and Brockmann, P. (1955). Semen dilution using egg yolk from different breed of fowl. Suppl. Fort Pft. Zuchthyg. Hanstier besan, 5(6): 69-72. (Cited from Anim. Breed. Abst., 23: 396).
- Amir, D., Schindler, H., Ryal, E., Lehrer, A.R. and Kempenich-Pinto, O. (1973). The effect of the ratio of dilution of ram semen in different diluents on the conception rate. Annales de Biologie Animale, Biochimie, Biophysique, 13(1): 1-5. (Cited from Anim. Breed. Abst., 41: 457).
- Austin, J.W., Leidy, R.B., Krise, G.M. and Hupp, E.W. (1968). Normal values for semen collected from Spanish Goat by two methods. J. appl. Physiology, 24: 369-72. (Cited from Anim. Breed. Abst., 36: 623).
- Bejalski, S. and Kastyak, L. (1961). Comparative studies on the effect of certain diluents on the survival of ram semen. Plodnosci, nieplodonose Zwierzat domowych. II. Zeszprobl. Postep. Naukroln. Polsk. Akad. Nauk. No. 31: 163-168. (Cited from Anim. Breed. Abst., 33: 188).
- Blokhuis, J. (1957). The practical use of artificial Insemination with goats. Tijdschr. Diergeneesk., 81: 570-81. (Cited from Anim. Breed. Abst., 25: 403).
- Dauzier, L., Thibault, C. and Wintenberger, S. (1954). Preservation of ram semen after dilution and maintenance of its fertilising ability. Ann. Endocr. (Paris), 15: 341-50. (Cited from Anim. Breed. Abst., 23: 292).

- Dussardier, M. and Szumowski, P. (1952). The semen of the male goat. *Rec. Med. Vet.*, 128: 628-35. (Cited from *Anim. Breed. Abst.*, 23: 134).
- Efendieva, T.P. (1963). The effect of the rate of dilution of ram semen with various diluents on conception. *Referat 2. Zivotrvet.* 1964 (10) No. 10. 58:126. (Cited from *Anim. Breed. Abst.*, 32: 503).
- Furedean, T., Barbulescu, I. and Popovici, P. (1967). Improving the diluents for ram semen. *Lucr. Stient, Inst. Cerc. Zootch.*, 25: 459-70. (Cited from *Anim. Breed. Abst.*, 36: 989-90).
- Ganceve, T. (1961). The artificial insemination of ewes with semen diluted 10 and 20 times. *Izv. cent, naucvoizsded. Inst. Biol. Pat, Razmnozav. Selskostop. Zivotn (Sofija)*, 1: 139-41). (Cited from *Anim. Breed. Abst.*, 32: 343).
- Gupta, A.K., Singh, B.P. and Tomar, N.S. (1974). In vitro preservation of bull semen in boiled milk. *Indian Vet. J.*, 51: 401-03.
- Herman, H.A. and Swanson, E.W. (1941). Variations in dairy bull semen with respect to its use in artificial insemination. *Agric. Exp. Sta. Misso. Res. Bull.* No. 326. (Cited by Kumar, B., M.Sc.(Vet.) Thesis 1973, Rajendra Agricultural University, Bihar).
- Ištvan, S. (1956). The effectiveness of using milk as a diluent for ram semen. *Ovcevodstvo*, 4: 33-37. (Cited from *Anim. Breed. Abst.*, 25: 60).
- Jelam, L.V. and Nambiar, K.G. (1965). Effect of diluents on the viability and biometrics of buck spermatozoa. *Kerala Vet.*, 4: 1-5.

- Joshi, J.D. and Singh, G. (1968). Effect of egg yolk citrate series diluents on the livability and morphology of ram spermatozoa. *Indian J.Vet.Sci.*, 38:575-82.
- Knoblauch, H. (1962). Investigation on goat ejaculates. *Zuchthyg. Fort Pflstor. Besam. Haustier.*, 6: 9-22. (Cited from *Anim.Breed.Abst.*, 31: 227).
- Kurian, N.J. and Raja, C.P.S.V. (1965). Studies on the semen characteristics of Malabari bucks. *Kerala Vet.*, 4: 31-33.
- Lall, H.K. (1962). Handbook of Animal Husbandry. 1st edn., p. 93. Indian Council of Agricultural Research, New Delhi.
- _____ (1969). Pasupalan Vigyan. 1st edn., p. 79 (First Bhag), Bhartiya Krishi Anusandhan Parishad, New Delhi.
- Lasley, J.P. and Bogart, R. (1943). Agr.Expt. Sta. Misso. Res. Bull., 376. (Cited by Tomar, N.S., M.V.Sc. (A.H.). Artificial Insemination and Reproduction of Cattle and Buffaloes, 1970, First edn., p. 79. Saroj Prakashan, 646 Katra, Allahabad-2).
- Livestock Census of Bihar (1972). Unpublished.
- Lunca, N. et al. (1961). The survival of ram spermatozoa in various diluents. *Lucr. Sti. Inst. Agron. "N. Bălcescu" (Bucuresti)*, 5: 45-53. (Cited from *Anim. Breed. Abst.*, 31: 494).
- Malikov, D.I. (1957). Insemination of ewes with a reduced quantity of semen. *Ovcerodstvo.*, 3(a): 21-22. (Cited from *Anim. Breed. Abst.*, 31: 494).

- Mukherjee, D.P., Roy, A. and Bhattacharya, P. (1952). The effect of feeding thyroprotein on semen quality and semen physiological conditions of goats. Indian J. Vet. Sci., 23: 1-7.
- Phillips, P.H. and Lardy, H.A. (1939). A yolk buffer pabulum for the preservation of bull semen. J.Dairy Sci., 25: 399-404.
- Randhawa, M.S. (1969). Bharat Me Krishi Aur Pasupalan. 2nd edn., p. 330. Indian Council of Agricultural Research, New Delhi.
- Roy, A., Gupta, D.N. and Pande, M.D. (1959). Studies on the reproductive physiology of goat. 1. Preservation of spermatozoal viability in vitro. Indian J. Vet.Sci., 29: 49-54.
- Sahni, K.L. and Roy, A. (1967). A study on the sexual activity of Barbari goat and conception rate through A.I. Indian J.Vet.Sci., 37: 269-78).
- _____ (1969). Influence of season on semen quality of rams and effects of dilutors and dilutions on in vitro preservation. Indian J. Anim. Sci., 39: 1-14.
- _____ and Tiwari, S.B. (1973). Artificial Insemination of sheep with diluted stored semen in milk diluents. Indian Vet.J., 5: 536-39.
- Saxena, V.B. and Singh, G. (1967). Preservation of Ram spermatozoa. 1. affects on motility, livability and morphology of ram spermatozoa in four semen diluents. J.Anim.Morph.Physiol., 14: 114-20.

- Shukla, D.D. and Bhattacharya, P. (1952). Seasonal variation in reaction time and semen quality of goats. Indian J. Vet. Sci. & Anim. Husb., 22: 179-90.
- Singh, S.N. and Sanger, O.P.S. (1970). Final technical report of the P.L. 480, Research project No.A.7-A.H.-80. On the investigation of milk and meat potentialities of Indian goat. Department of Animal Husbandry and Dairy, R.B.S.College. Bich. Puri. Agr., India.
- Singh, C.M. (1973). Souvenir, 20th Indian Veterinary Conference. Bangalore, Oct. 15 to Oct. 18th, 1973, p.12.
- Singh, H. and Moore, E.N. (1968). Livestock and poultry production. 2nd edn., p. 331. Prentice-Hall of India Private Limited, New Delhi.

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**"INFLUENCE OF SEMINAL IONIC
CONCENTRATION ON PRESERVABILITY OF
LARGE WHITE YORKSHIRE AND TAMWORTH X
DESI (T & D) BOARS SEMEN"**



ABSTRACT

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ABSTRACT

A study was conducted on 72 semen ejaculates of 4 large white Yorkshire and 4 Tamworth x Desi (T & D) boars maintained under the Adhoc Research Project at B.V.C., Patna. Semen collection was done by gloved hand technique over oestrus sows at 5 days interval.

The mean value of different semen characteristics were strained volume 160.34 ± 1.08 and 132.73 ± 3.89 ml, Gel volume 41.47 ± 0.18 and 34.70 ± 0.19 ml, Total volume 220.38 ± 0.80 and 185.27 ± 0.79 ml, individual sperm motility 84.13 ± 0.41 and 82.22 ± 0.58 percent, Live sperm percentage 83.22 ± 0.48 and 80.19 ± 0.67 , sperm concentration 275.48 ± 0.35 and 268.70 ± 0.31 million per ml, progressive motility 72.27 ± 0.91 and 70.55 ± 0.83 and pH 7.60 ± 0.03 and 7.40 ± 0.04 in large white Yorkshire and T & D boars respectively.

The stained volume, Gel volume, Total volume, individual sperm motility, live sperm, sperm concentration and pH differed significantly between breeds. There was no significant difference in progressive motility of spermatozoa between breeds.

The mean incidence of head abnormality, midpiece abnormality, tail abnormality, proximal cytoplasmic droplet and distal cytoplasmic droplet in large white Yorkshire and T & D boars were 2.42 ± 0.03 and 2.53 ± 0.02 , 0.41 ± 0.03 and

0.55 \pm 0.02, 2.01 \pm 0.02 and 2.31 \pm 0.01, 1.20 \pm 0.002 and 1.30 \pm 0.02 and 0.58 \pm 0.09 and 1.19 \pm 0.008 percent respectively.

Significant difference was observed in Tail abnormality of spermatozoa between breeds. But non-significant difference was observed for Head abnormality, midpiece defect, proximal cytoplasmic droplet defect and distal cytoplasmic droplet defect.

The mean incidence of damaged apical ridge, missing apical ridge and loose acrosomal cap in large white Yorkshire and T & D boars were 0.62 \pm 0.03 and 0.63 \pm 0.03, 0.00 and 0.00 and 1.41 \pm 0.06 and 1.42 \pm 0.06 percent respectively.

No significant difference was observed in acrosomal morphology of spermatozoa between breeds.

The mean concentration of calcium, magnesium, inorganic phosphorus, zinc, copper, cobalt and manganese estimated in the present study were 4.20 \pm 0.25 and 4.09 \pm 0.28, 8.89 \pm 0.418 and 8.85 \pm 0.410, 1.79 \pm 0.10 and 1.75 \pm 0.04, 17.10 \pm 0.11 and 16.98 \pm 0.15, 0.156 \pm 0.001 and 0.154 \pm 0.001, 0.049 \pm 0.005 and 0.044 \pm 0.006 and 0.020 \pm 0.003 and 0.018 \pm 0.001 mg/100ml in large white Yorkshire and T & D boars respectively.

No significant difference was observed in seminal minerals concentration between breeds.

Semen samples having 70% or more progressive motility were finally diluted with Kiev extender based on concentration

of spermatozoa in the semen. Then, those samples were preserved in B.O.D. incubator at 18 – 20°C for 24, 48 and 72 hours. Those preserved semen samples were brought at room temperature and then were utilized to study the different characteristics of spermatozoa for assessing preservability of the bar semen.

The sperm motility and live sperm percentage in large white Yorkshire boars were non-significantly higher than that in T & D boars semen. The tail abnormality in large white Yorkshire boar semen were significantly lower in comparison of T & D boars semen, whereas the incidence of head abnormality were non significantly lower in large white Yorkshire boar semen in comparison of T & D boar semen. Further, it was observed that a significant decrease in percentage of sperm motility and live sperm and significant increase in incidence of head and tail abnormality were recorded with increase in duration of preservation in both breeds.

The mean percentage of damaged apical ride, missing apical ridge and loose acrosomal cap in large white Yorkshire boars were non-significantly lower than T & D boars semen.

A significant increase in percentage of damaged apical ridge, missing apical ridge and loose acrosomal cap were recorded with increase in preservation time in semen of both breeds.

The mean seminal minerals concentration in large white Yorkshire boars were non-significantly higher than T & D boars semen. Non-significant decrease in minerals concentration was recorded with increase in hours of preservation in semen samples of both breeds.
