THE EFFECT OF ADDITION OF ANTIBIOTICS ON THE KEEPING QUALITY OF BUCK SEMEN

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

Ram Krit Prasad

B. V. Sc. & A. H.
I. C. A. R. JUNIOR FELLOW
Post-Graduate Department of Gynaecology and Obstetrics
BIHAR VETERINARY COLLEGE
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Dated, the 25th May, 1976.

This is to certify that the work embodied in this Thesis entitled " THE EFFECT OF ADDITION OF ANTIBIOTICS ON THE KEEPING QUALITY OF BUCK SEMEN " is the bonafide work of Ram Krit Prasad 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.

Ram Krit Pd.

(RAM KRIT PRASAD).

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

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(R. K. PRASAD).

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DEDICATED

TO

MY PARENTS

C O N T E N T S

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INTRODUCTION

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Until recently the importance of goat was not much realised in India. But gradually goat, the poor man's cow is drawing the attention of husbandry-men.

According to the Eleventh All India Livestock Census released from the Directorate of Economics and Statistics, the goat population of India in 1972 stood at 68.024 millions and that of Bihar stood at 7.364 millions. The Agricultural Marketing Board (1967-68) estimated the production of goat meat in India to be 22 million kg on the basis of average carcass weight of 9.05 kg. The I.C.A.R. Pilot Project in Tamil Nadu (1967) and in Haryana (1969) revealed average goat meat yield to be 10.2 and 10.9 kg respectively (Singh, 1973).

But for economic and genetic reasons, application of artificial insemination in goat breeding is gaining ground. Reduction in the number of breeding bucks and replacement of numerous service stations by an Insemination Centre, saving of time, feeding cost of bucks and labour charges as well as better sexual health control are some of the most important points in favour of artificial insemination in goats.

For successful artificial insemination in cows the importance of good semen dilutors as well as additives like Penicillin and Streptomycin were proved by the works of

Branton and Prather (1954). But the results of Thacker and Almquist (1951) seemed to differ from those obtained by Branton and Prather (loc. cit.). Sahni and Roy (1969) reported better results in buck semen preservation with cow milk diluent.

In view of the conflicting results reported in literature, the present work was undertaken to examine the efficiency of egg yolk citrate and cow milk diluents in the preservation of buck semen and also to find out the suitable doses of Penicillin and Streptomycin as additives in different diluents. The object of this study was also to observe the keeping quality of buck semen specially in respect of pH, motility of sperms and the percentage of live sperms under different treatments at different hours of preservation.

Though the utility of various antibiotics as additives in different doses in bovine semen was studied by Sharma et al.(1962), no such work in case of buck semen could be traced in literature. Because the importance of such studies cannot be denied the present project was undertaken.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

The available relevant literature has been briefly reviewed.

Wagenaar (1946) found that the semen of fertile goats was yellow thick, had high motility and 1540 millions sperms per cm^3. Abnormal sperms varied from 6 to 30 per cent, whereas in infertile goat semen was thin watery, had slight motility, 52.5 millions sperms per cm^3 and 45 to 57 per cent abnormal sperms.

Wagner (1949) reported that Egg yolk (2 to 5%) Glucose phosphate diluent was superior to yolk free glucose-phosphate diluent for goat semen.

Dussardier and Szumowski (1952) reported that the highest motility average was seen in November and December, the average volume was noted to be 0.6 ml, concentration 3.60 millions per cubic mm, motility 75 per cent and pH 6.5.

Eaton and Simmons (1952) studied the semen character of Toggenburg and American bucks and found that the average volume was 0.65 ml, motility 1.5, concentration 2724 billions per cm^3 and abnormal sperms 8.46 per cent.

Shukla and Bhattacharya (1952) 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 millions per ml, sperm motility 3.8 to 5.0, abnormal sperm percentage to be 2.4 to

11.1 and colour to be creamy.

Mukherjee et al. (1953) found the average semen volume of seven goats to be 0.54 ± 0.11, initial motility 4.35 ± 0.11, sperm concentration 5368 ± 247.88 millions per ml and abnormal sperm percentage to be 3.35 ± 0.40.

Aehnelt and Brockmann (1955) studied semen samples from 4 male goats which were diluted in spermasol and yolk buffer prepared from eggs of New Hampshire, Sussex, White Leghorn and Brown Leghorn new. The diluted semen was stored at 6°C and examined daily. Spermatozoal motility and survival time were greatest when New Hampshire yolk buffer was used, followed by Sussex, White Leghorn and Brown Leghorn in decreasing order.

Sharma et al. (1957) concluded that in Betal buck semen volume averaged 1.3 cm^3 in summer and 0.8 cm^3 in winter; individual ejaculate varied from 0.2 to 1.2 cm^3, the amount increasing with body size. Sperm concentration averaged 5424.7 millions per cm^3 being lowest in spring and highest in winter.

Rathore and Mukherjee (1961) reported that when semen sample from Bikaneri ram was diluted (1:3) with egg yolk phosphate, fresh cow milk or egg yolk boric acid diluent and stored at 5°C for 72 hours, it was observed that dilution and storage significantly reduced head length and breadth of spermatozoa and the percentage of unstained spermatozoa. The percentage of fully and partially stained spermatozoa was significantly increased by storage.

Knoblauch (1962) reported that the average ejaculate volume of German imported buck was 1 ml, had creamy consistency, pH 6.6, forward motility 80 per cent and number of dead spermatozoa 20 per cent.

He further reported that semen diluted (1:5) in Sodium citrate with 10 or 20 per cent of egg yolk in which Penicillin had been added and stored for 1-7 days at +3°C showed 20 per cent forwarded motility.

Jelam and Nambiar (1965) reported that in 14 semen samples from 7 Jamnapari bucks, the average sperm motility was 72.3 ± 3.98 per cent and the percentage of unstained spermatozoa 72.0 ± 5.74 per cent. They also reported that when semen was diluted (1:20) in egg yolk citrate or goat milk and stored for 24 and 72 hours, the average motility was 54.4 ± 2.73 and 43.6 ± 2.69 respectively and the percentage of unstained spermatozoa was 61.27 ± 4.31 and 50.09 ± 3.26 respectively.

Kurian and Raja (1965) studied 47 semen samples from 6 Malabari bucks, collected at intervals of 7-12 days and found the average volume to be 0.4 - 1.2 ml, motility 60-90 per cent, pH 6.3 - 6.7, sperm concentration 2 - 3 millions per cmm, abnormal spermatozoa 6 - 12 per cent and live spermatozoa 85 - 95 per cent. They also reported that interval between collections did not affect semen character.

Misra and Sengupta (1965) reported that ejaculate volume, initial motility and percentage of live spermatozoa were significantly superior in case of Bikaneri ram than

Jamnapari buck as mentioned below :

Character	Ram	Buck
Volume (ml)	0.92 ± 0.05	0.65 ± 0.07
Initial motility	1.00 ± 0.42	2.30 ± 0.50
Sperm concentration ($x10^6$)	5313 ± 786	4080 ± 450
% of live sperms	87 ± 1.8	54 ± 4.9

Patel (1967) studied the characteristics of Jamnapari buck semen and found the average volume to be 0.815 ml, motility +5, concentration 1.5 millions per ml, abnormal sperms 5 per cent, pH 6.5 and live sperms 90.7 per cent.

Sahni and Roy (1967) studied the semen of Barbari bucks for volume, sperm motility, concentration and percentage of live spermatozoa and observed as follows :

Semen quality of the bucks used for insemination.

	Volume	Wave motion (0-5)	Sperm concentration ($x10^6$/ ml)	% of live sperm
Buck No. 4	0.71	4.7	1804	82.0
Buck No.831	0.54	4.9	2099	80.0

Fertility rate according to sperm treatment.

Sperm treatment.	Goat inseminated.	Settled	Percentage conception.
Neat	34	26	76.4
CUE	19	14	73.6
Cow milk	8	5	62.5

Saxena and Singh (1967) collected semen from 4 Bikaneri rams at weekly intervals for 6 weeks. The samples were diluted with skim milk yolk (SMY), egg yolk citrate (EYC), egg yolk glucose bicarbonate (EYGB) and Cornell University Extenders in the ratio of 1:20. After that 10 per cent, 25 per cent, 25 per cent and 20 per cent yolk was added to EMY, EYC, EYGB and CUE respectively. Diluted semen was examined at intervals of zero, 72, 120 and 168 hours (storage temperature 3 ± 1°C). Motility and live percentage remained highest in SMY followed by EYGB upto 168 hours of storage. But there was sharp fall in CUE and EYC for both characters upto 72 hours.

Austin et al. (1968) reported the average volume of spanish goat ejaculate collected by electro ejaculation and artificial vagina method to be 1.93 and 0.84 ml, sperm concentration in millions per ml 1997 and 2436, motile sperms 75 and 80 per cent, percentage of live spermatozoa 87.1 and 79.5 and abnormal sperm percentage 6.3 and 7.2 respectively.

Joshi and Singh (1968) observed that skim milk yolk glucose and skim milk yolk glucose fructose diluents maintained significantly better sperm motility in ram semen than skim milk yolk, skim milk yolk glucose glycin, skim milk glucose bicarbonate and control diluent egg yolk citrate at 48, 96 and 144 hours of preservation. Skim milk yolk glucose and skim milk yolk glucose fructose gave significantly higher value for unstained spermatozoa percentage. No significant variations were noted in head length and head breadth

of spermatozoa in all the diluents upto 144 hours of storage.

Tiwari et al. (1968) reported that there did not occur any deterioration in semen production when bucks were placed on a collection schedule of one per day over a period of 21 days. The analysis of variance showed that the difference between breeds with regards to production of semen volume was not significant, but highly significant difference in sperm concentration per millilitre and sperm number per ejaculate was observed between the two breeds ($P < 0.01$) Barbari and Sannen. When the period of collection was extended to 40 days, the analysis of variance showed a highly significant difference ($P < 0.01$) in volume and sperm number per ejaculate between breeds. The difference between breeds with regard to sperm concentration per millilitre was only significant ($P < 0.05$). The average volume, concentration, and live sperm percentage in Barbari, Jamnapari, Sannen x Jamnapari buck semen was as follows:

Mean values (pooled data) of various semen attributes when collection were made in quick succession in three different breeds of bucks (Tiwari et al., 1968).

Breed	No.of collection	Volume	Sperm concentration per ml ($x10^6$)	No.of sperm per ejaculate ($x10^6$)	Live sperm percentage	Initial motility
Jamnapari	53	1.36± 0.05	3728.3± 192.4	3003.8± 220	85.3 ± 1.17	4.6 ± 0.09
Sannen x Jamnapari	54	1.37± 0.07	4995.5± 159.8	3494 ± 146	89.7 ± 0.5	5.0 ± 0
Barbari	52	0.94± 0.01	3650.2± 155.3	2028.5± 111.5	86.4 ± 0.8	4.2 ± 0.11

The average values of seminal attributes of Barbari and Jamnapari bucks were reported by Sahni and Roy (1969) to be 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	65.582

Dessouky et al. (1970) reported that semen from docked (two weeks after birth) ram was superior to undocked ram in respect of mass and individual motility, sperm count, methylene blue reduction time, pH value and percentage of abnormal sperm. But ejaculate volume and fructose content were better in semen of undocked rams. Egg yolk citrate gave the best result in terms of sperm motility followed by egg yolk citrate glycerol and egg yolk skim milk, egg yolk saline and egg yolk glycine extender.

Mittal and Pandey (1972) evaluated the semen quality of Barbari and Jamnapari bucks. Semen was collected once daily. They found that there was highly significant difference between bucks in relation to semen production and also regarding volume, sperm motility, sperm concentration and percentage of viable spermatozoa after collection.

Sahni and Tiwari (1973) reported on 442 native (Malpura Chokla and Jaisalmery) and crossbred (Rambouillet x native) ewes that were inseminated and said that ewes given

a single insemination with fresh semen diluted 1:3 with cow's milk, 40.1 per cent lambed and of ewes inseminated with fresh semen diluted with ewe's milk, 38.3 per cent lambed vs 17.3 and 22 per cent of ewes inseminated with semen diluted in the same way but stored at 8° - 10°C for 10 hours before use.

Roy (1975) studied with 50 semen samples from 5 non-descript bucks and reported the average mean volume to be 0.46 ± 0.01, colour - yellowish, concentration - 3658.5 ± 30.38 millions per ml, pH - 6.67 ± 0.28, motility - 78.60 ± 0.61, and live sperm percentage - 87.81 ± 1.32.

EFFECT OF ANTIBIOTICS AS A SEMEN ADDITIVE.

According to Almquist et al. (1946), Penicillin was not found to improve sperm motility, check bacterial growth, and above 750 oxford units levels, it was found to depress the ability of the sperm cells to live.

Knodt and Salisbury (1946) recommended the addition of Penicillin and Streptomycin as a means of preventing bacterial growth and of killing certain pathogenic organisms that may be present in the semen.

Almquist et al. (1948) reported that the addition of 250, 500 and 750 oxford units of Penicillin per ml of diluted bovine semen did not significantly reduce the ability of spermatozoa to maintain motility during storage period of 20 days. Levels of Penicillin ranging from 1000 to 2000 units per ml of diluted semen brought about a significant decrease

in spermatozoan livability during a 20 days storage period. When compared with untreated control samples, no significant decrease in maintenance of spermatozoan motility during a 6 days storage period occurred as the result of addition of 250, 500, 750 or 1000 units of Penicillin per ml of diluted semen, but higher levels of Penicillin were deleterious.

Almquist et al. (1948) reported that Streptomycin in varying doses were added to bull semen diluted (1:24) with egg yolk citrate. The semen samples were stored at 4.5°C and percentage of motile spermatozoa determined every two days for 20 days. Concentration upto 1000 units per ml had no significant effect on spermatozoan viability, whereas higher concentrations produced a significant decrease.

Effective control of bacterial growth in diluted bovine semen preserved for 20 days at 4.5°C with Penicillin 100 to 1000 I.U. plus Streptomycin 100 to 1000 micrograms per ml was reported by Almquist et al. (1949).

Significant increase in fertility level of bovine diluted semen with the addition of 1000 units of Penicillin and 1000 micrograms of Streptomycin per ml was also reported by Almquist and Prince (1950).

The use of a combination of 30-50 units of Penicillin and 0.02 to 0.04 mg Streptomycin per 1 cm^3 egg yolk citrate diluted bovine semen was effective against bacterial growth even 12 days after storage at +4°C. Larger doses of the antibiotics were found to be harmful to sperm motility and metabolism (Rozsa, 1950).

Improvement in fertility over the yolk citrate by addition of 1000 units per ml of Penicillin, 1000 micrograms per ml of Streptomycin was recorded by Foote and Bratton (1950).

Further addition of Penicillin, Streptomycin or a combination of these plus Polymyxin and Sulphanilamide to egg yolk citrate diluted bull semen increased the fertility level.

Best survival of bull sperm at temperatures from 0° - 29°C when treated with 200 units of Penicillin or Streptomycin in yolk citrate solution was reported by Murriel and Gonzaga (1951). With the former, the maximum period of survival was 4 days at 25 - 29°C and 5 days at temperatures below 14°C. Similar results were obtained with Streptomycin at the higher temperature but at temperatures from 0° to 5°C samples with Streptomycin survived for 7 days. In both cases however the longivity in untreated semen samples was almost equally good.

Myers and Almquist (1951) studied on samples of bovine semen and found that Aureomycin levels of 100-1000 micrograms were about equally as effective in controlling bacterial growth as 1000 units of Penicillin, 1000 micrograms of Streptomycin or a combination of 1000 units of Penicillin and 1000 micrograms of Streptomycin per ml of diluted semen. But Aureomycin at a concentration of 1000 micrograms per ml of diluted semen was highly toxic to spermatozoa than either Penicillin and/or Streptomycin at the above mentioned levels.

Hendrikse and Joling (1952) reported that Penicillin plus Streptomycin increased the fertility rate of bull semen for 1 to 4 days of storage.

Branton and Prather (1954) found that Streptomycin and Penicillin, either singly or in combination had a beneficial effect on livability and fertility of bovine spermatozoa due to their control of the bacterial flora of the semen. The beneficial effect may also be due to the effects of these agents on spermatozoan metabolism. Streptomycin and Penicillin each depressed the utilization of total reducing substances and fructose and the production of lactic acid by the spermatozoa.

Gokhale (1958) reported that the semen diluting medium with antibiotics (Penicillin 500 to 1000 I.U./ml and Streptomycin 500 to 1000 micrograms per ml) maintained a motility of 3 or above for some what longer period (18.2 hours).

Bhatia (1960) reported the use of 500 I.U./ml Penicillin and 0.01 mg/ml Streptomycin for the storage of bull semen at National Dairy Research Institute, Bangalore.

Perry (1960) reported that a combination of Streptomycin at 500 to 1000 micrograms per ml and Penicillin at 500 to 1000 units per ml of diluted semen was satisfactory in maintaining sperm viability during several days of storage.

Marinov (1962) studied on 260 ejaculates from 6 bulls and found beneficial effects of Penicillin and Streptomycin on sperm survival in diluted semen at 0° - 3°C, except

when concentrations added were high.

Singh (1965) suggested the routine use of Penicillin in the dose of 1000 I.U./ml of diluted semen, Streptomycin in the dose of 1000 micrograms per ml of diluted semen and Sulphanilamide at the rate of 0.3 gm per 200 ml in semen diluents for preservation of bull and buffalo semen.

Penicillin in doses of 12,500 - 500,000 I.U./100 ml of diluted semen were reported to reduce sperm motility by Golubeva (1970). The same worker further reported that a dose of 50,000 I.U. Streptomycin reduced motility and resistance, a dose of 100,000 I.U. was almost lethal and doses of 250,000 or 500,000 I.U. were lethal for spermatozoa.

Tomar (1970) reported that Penicillin and Streptomycin together were effective in controlling most of the bacterial growth. Penicillin G sodium was commonly used, but Procaine penicillin was also not harmful to spermatozoa. It was sound procedure to use 1,000 units of Penicillin and 1000 micrograms of Streptomycin per ml of diluted semen.

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MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was undertaken on 5 local non-descript bucks maintained at the Department of Gynaecology, Bihar Veterinary College, Patna under similar environmental and managemental conditions. The experimental animals were kept on grazing and in addition to this half kilogram of concentrate mixture was supplied at the rate of per animal per day. The concentrate mixture consisted of the following ingredients : -

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

Common salt and mineral mixture was also supplied.

METHOD OF SEMEN COLLECTION.

One collection per buck per week was taken by Artificial vagina method. The temperature of Artificial vagina was maintained at about 45°C. The bucks were allowed one or two false mounts before taking collection (Branton *et al.*, 1952).

Immediately after collection the semen samples were taken in the laboratory and kept at 32°C in water bath. Evaluation of neat semen was done and then the samples were diluted and preserved for further examination.

EVALUATION OF SEMEN.

The quantity and colour was directly noted from the semen collection tube and the pH of neat semen was also estimated.

Initial motility of spermatozoa in neat semen :

After collection, each ejaculate was immediately examined for initial motility. The motility examination under low power microscope, was made with one droplet of semen on the slide without cover slip. From these observations the initial motility ratings were made into 5 categories (Tomar, 1970).

(i) +5 motility - There was only wave after wave.

(ii) +4 motility - The swirls and eddies were not 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 movement of spermatozoa were more evident in the field.

(v) +1 motility - No wave motion were observed in the field. Only 20 per cent sperms had progressive motility. Rest of the spermatozoa show throbbing movement.

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 times.

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 = 0.1 cmm.

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

Therefore n x 5 was the number of sperms present

in 0.1 cmm of diluted semen.

Therefore n x 5 x 10 was the number of sperms present in 1 cmm of diluted semen.

Therefore n x 5 x 10 x 200 was the number of sperms present in 1 cmm of original neat semen.

Therefore n x 5 x 10 x 200 x 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 x 10000000.

The neat semen was then diluted in the ratio of 1:10 in both egg yolk citrate and cow milk extenders separately and the following groups were made :

Group A. Egg yolk citrate extender group :

In this group, four subgroups were made -

(i) In this subgroup no additives were used.

(ii) To this subgroup of diluted semen sample Benzyle Penicillin (Alembic) 500 I.U./ml plus Streptomycin sulphate (Ambistrin-S, Squibb) 500 micrograms per ml were added.

(iii) To this subgroup Benzyle Penicillin (Alembic) 1000 I.U./ml plus Streptomycin sulphate (Ambistrin-S, Squibb) 1000 micrograms per ml were added.

(iv) To this subgroup of diluted semen sample Benzyle Penicillin (Alembic) 1500 I.U./ml plus Streptomycin sulphate (Ambistrin-S,Squibb) 1500 micrograms per ml were added.

Group B. Cow milk extender group :

In this group also four subgroups were made -

(i) In this subgroup of diluted semen sample no additives were added.

(ii) To this subgroup Benzyle Penicillin (Alembic) 500 I.U./ml plus Streptomycin sulphate (Ambistrin-S, Squibb) 500 micrograms per ml were added.

(iii) In this subgroup Benzyle Penicillin (Alembic) 1000 I.U./ml plus Streptomycin sulphate (Ambistrin-S, Squibb) 1000 micrograms per ml were added.

(iv) To this subgroup Benzyle Penicillin (Alembic) 1500 I.U./ml plus Streptomycin sulphate (Ambistrin-S, Squibb) 1500 micrograms per ml were added.

All the semen samples were examined at zero, 24, 48, and 72 hours of preservation. The semen samples were evaluated for the following attributes at different hours of preservation and in different extenders : -

(i) Hydrogen ion concentration.

(ii) Motility of sperms.

(iii) Percentage of live sperms.

PREPARATION OF EGG YOLK CITRATE DILUTOR.

Egg yolk citrate extender was prepared according to the formula of Salisbury et al. (1941). The buffer solution was prepared after dissolving 2.90 gms of Sodium Citrate dihydrate (B.D.H./Analar) in 100 ml of glass distilled water.

Buffer solution prepared for use as semen extender was sterilized by autoclaving for half an hour at 15 lb pressure and kept in a refrigerator for longer use.

Only infertile fresh hen's eggs were used for the preparation of dilutor. Egg shell was washed thoroughly in tap water and then wiped with rectified spirit. The egg shell was then broken, the shell membrane removed and the white of the egg (Albumin) was discarded.

The egg yolk was placed on a sterilized filter paper (What's man No. 41 and 42). The membrane over the yolk was removed carefully by a forcep and it was collected in a measuring cylinder. One part of egg yolk and two parts of buffer solution as mentioned above were mixed thoroughly. And thus the diluent was prepared.

PREPARATION OF COW MILK EXTENDER.

Fresh cow milk was procured and heated in a water bath at 92° - 95°C for 10 minutes in a sterile flask avoiding over heating and under heating. Heated milk was allowed to cool down to room temperature and then kept over night in the refrigerator. Next morning the milk was filtered through a thin layer of cotton and it was ready for use.

DILUTION OF SEMEN.

The dilutors were prepared as described earlier and kept in 10 ml test tubes in water bath at 32°C. The test tubes containing the dilutors were properly labelled. Only

freshly prepared dilutors were used. Care was taken that the temperature of dilutor and semen remained at 30° - 32°C at the time of dilution. The dilution was done in the ratio of 1:10 in both the egg yolk citrate and cow milk extenders. The test tubes were gently rotated between the palms for uniform distribution of sperms in the dilutors.

PRESERVATION OF SEMEN.

Diluted semen was put in clean and sterilized small test tube. This test tube was wrapped with cotton wool and put in another bigger test tube. The test tube was labelled for date of collection, name of additives and name of dilutor. The test tube containing the diluted semen sample was 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 when the water of the beaker attained the temperature of 4°C, it was removed and the tubes was kept in the same empty beaker for preservation at 4° ± 1°C (Muller, 1962). The diluted semen sample was examined at zero hour and further at 24, 48 and 72 hours of preservation.

SPERM MOTILITY.

The movement of individual sperm was taken into consideration while grading the motility. The semen was diluted in the ratio of 1:100 with the dilutors used. A drop of diluted semen was taken on a thin, clean and sterilized glass slide and gently warmed to body temperature. Then it was

covered with coverslip and examined under high power magnification. Motility of all the diluted semen samples were examined at zero, 24, 48 and 72 hours of preservation.

The grading of sperm motility after dilution was done by 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 sperm	Movement.
0	No motility	No movement.
0.5 to 1.0	10 to 20% motile sperms.	Movement weak and oscilatory. 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.	Wave formation with slight whorls which moves slowly across the field.
2.5 to 3.0	50 to 60% motile sperms.	Wave formation with slight whorls which moves slowly across the field.

Grade	Percentage of live sperm	Movement.
3.0 to 3.5	60 to 70% motile sperms with swirling motion.	Rapid vigorous movement, waves and whorls or eddies form and change with great rapidity.
3.5 to 4.0	70 to 80 motile sperms.	Rapid vigrous 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 a 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 a sea.

PERCENTAGE OF LIVE SPERMATOZOA.

The percentage of live spermatozoa was estimated with the help of Eosin and Nigrosin stain (Swanson et al.,1951).

The compound stain was prepared by dissolving 1 gm of Eosin (B.D.H. water soluble), 5 gms Nigrosin (B.D.H. water soluble) and 3 gms Sodium citrate dihydrate (B.D.H./Analar) in 100 ml of glass distilled water. The solution was warmed in a water bath for 30 minutes and filtered after cooling.

Before use, the stain was kept in a beaker of warm water at 37°C for some time. One drop of semen sample was placed on a clean, watch glass and a large drop of compound stain was added to it. The semen and the stain were thoroughly mixed by gentle blowing, through a pipette and allowed to stand for one minute. Thin smear was drawn on a clean, grease free slide and dried quickly in air. The smear was examined under oil immersion lens (100x). Live spermatozoa were colourless. The dead spermatozoa took pink colour of Eosin. The spermatozoa taking partial stain anteriorly or posteriorly were counted as dead. The Nigrosin stain presented homogenous violet background. A total of 300 spermatozoa under Oil Immersion Lens were counted at random under different microscopic fields and the percentage of live sperms was determined.

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*

R E S U L T S

R E S U L T S

Neat semen.

Colour :

The colour of semen samples obtained from five non-descript local bucks from different collections was found to vary from light yellow to yellow shades. Therefore they were put under yellowish colour.

Volume :

The volume of semen ejaculate from five bucks was obtained to be 0.48 ± 0.02 ml on the basis of fifty semen samples collected from them (Table - I).

Concentration of sperm per ml :

Based on fifty semen samples collected under the experiment, the mean concentration of sperms per ml was found to be 3832.4 ± 48.69 millions (Table - I).

Motility percentage of sperms :

The mean initial mortility percentage of sperms from fifty semen samples obtained from bucks was calculated to be 79.78 ± 0.73 (Table - I).

Hydrogen ion concentration (pH) :

The mean pH of fifty semen samples from bucks was computed to be 6.71 ± 0.32 (Table - I).

Live sperm percentage :

Study of fifty semen samples collected from five bucks under this experiment, the mean value of live sperm percentage was found to be 88.63 ± 2.19 (Table - I).

Different attributes of diluted semen under different doses of Penicillin and Streptomycin.

Hydrogen ion concentration (pH) :

The mean pH of diluted semen was observed to be 6.58 ± 0.21, 6.50 ± 0.10, 6.41 ± 0.19, 6.21 ± 0.43 and 6.60 ± 0.29, 6.51 ± 0.73, 6.30 ± 0.39, 6.20 ± 0.38 in egg yolk citrate and cow milk diluents at zero, 24, 48, and 72 hours of preservation at 0 - 0 dosing of Penicillin and Streptomycin respectively (Table - II).

The pH values of diluted semen in egg yolk citrate and cow milk diluents with different doses of Penicillin and Streptomycin under different hours of preservation have been tabulated under Table - II.

Analysis of variance run could not reveal any significant difference between diluents, between hours of preservation, between doses and also among interactions as regards the pH of semen (Table - III).

Percentage of sperm motility :

The mean motility percentage of sperms in the egg yolk citrate diluent at 0 - 0 dosing of Penicillin and

TABLE - 1.

Showing mean and S.E. of the different attributes of neat semen of non-descript buck.

Number of observations.	Colour	Volume (ml)	Concentration of sperm (million/ml)	pH	Initial motility %	Live sperm %
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
50	Yellowish	0.48 ± 0.02	3832.4±48.69	6.71 ± 0.32	79.78±0.73	88.63±2.19

TABLE - II.

Mean values of pH of buck semen along with S.E. under different hours of preservation with different doses of Penicillin and Streptomycin in EYC and cow milk diluents.

Hours of preservation.	No. of observations	pH							
		EYC				Cow milk			
		0 I.U. penicillin + 0 Microgram Streptomycin per ml	500 I.U. Penicillin + 500 Micrograms Streptomycin per ml	1000 I.U. Penicillin +1000 Micrograms Streptomycin per ml	1500 I.U. Penicillin +1500 Micrograms Streptomycin per ml	0 I.U. Penicillin + 0 Microgram Streptomycin per ml	500 I.U. Penicillin + 500 Micrograms Streptomycin per ml	1000 I.U. Penicillin +1000 Micrograms Streptomycin per ml	1500 I.U. Penicillin +1500 Micrograms Streptomycin per ml
		Mean ± S.E.	Mean ± S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean ± S.E.
0	50	6.58±0.21	6.58±0.31	6.60±0.23	6.61±0.18	6.60±0.29	6.61±0.63	6.63±0.78	6.61±0.53
24	50	6.50±0.10	6.51±0.13	6.52±0.19	6.52±0.21	6.51±0.73	6.51±0.62	6.40±0.37	6.30±0.47
48	50	6.41±0.19	6.40±0.21	6.40±0.23	6.40±0.17	6.30±0.39	6.30±0.51	6.27±0.73	6.20±0.42
72	50	6.21±0.43	6.21±0.33	6.21±0.10	6.20±0.27	6.20±0.38	6.15±0.67	6.10±0.79	6.00±0.49

TABLE - III.

Analysis of variance showing effect of diluents, hours of preservation as well as different doses of Penicillin and Streptomycin on the pH of buck semen.

Sources of variation	df	pH M. S.
Between diluents.	1	0.63 NS
Between hours of preservation.	3	0.87 NS
Between doses.	3	1.10 NS
Between diluents x hours of preservation.	3	0.32 NS
Between diluents x doses.	3	0.68 NS
Between hours of preservation x doses.	9	0.18 NS
Between diluents x hours of preservation x doses.	9	0.23 NS
Error.	768	1.02
Total.	799	

NS denotes non-significant.

Streptomycin under zero, 24, 48, and 72 hours of preservation were found to be 74.77 ± 1.56, 57.67 ± 1.18, 34.32 ± 1.13 and 22.86 ± 0.97 respectively (Table - IV).

In case of cow milk diluent under 0 - 0 dosing with Penicillin and Streptomycin, the average motility percentage of sperms came out to be 64.44 ± 1.78, 41.32 ± 0.97, 20.83 ± 0.91, 10.12 ± 0.57 at zero, 24, 48, and 72 hours of preservation respectively (Table - IV).

The mean motility of sperms with different doses of Penicillin and Streptomycin under different hours of preservation in egg yolk citrate and cow milk diluents have been incorporated in Table - IV.

Analysis of variance run to find out the effect of diluents, hours of preservation and doses revealed highly significant differences, although the interactions did not turn out to be significant (Table - V).

Live sperm percentage :

The mean live sperm percentage of buck semen in egg yolk citrate and cow milk diluents at zero, 24, 48, and 72 hours of preservation under 0 - 0 dosing with Penicillin and Streptomycin was estimated to be 86.67 ± 2.18, 78.12 ± 1.82, 60.48 ± 1.92, 40.13 ± 0.63 (Egg Yolk Citrate) and 72.93 ± 0.69, 60.17 ± 0.91, 39.48 ± 0.45, 31.28 ± 0.19 (cow milk) respectively (Table - VI).

Table - VI incorporates the values of live sperm percentage of buck semen under different diluents with

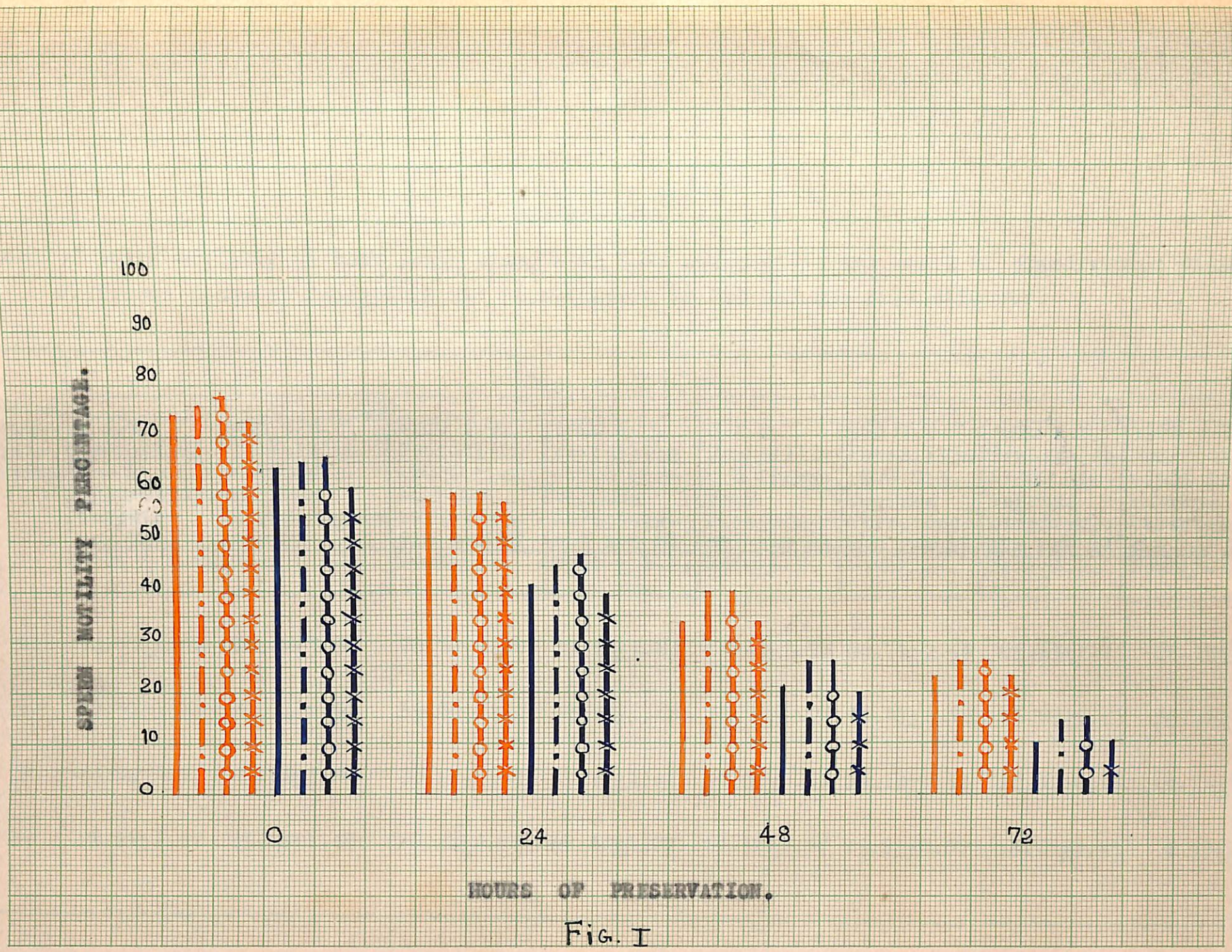
SPERM MOTILITY PERCENTAGE.
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
HOURS OF PRESERVATION.

Fig. I

TABLE - IV.

Mean values of sperm motility in buck semen along with S.E. under different hours of preservation with different doses of Penicillin and Streptomycin in EYC and cow milk diluents.

Hours of preservation.	No. of observations	Motility %							
		EYC				Cow milk			
		0 I.U. Penicillin + 0 Microgram Streptomycin per ml	500 I.U. Penicillin + 500 Micrograms Streptomycin per ml	1000 I.U. Penicillin +1000 Micrograms Streptomycin per ml	1500 I.U. Penicillin +1500 Micrograms Streptomycin per ml	0 I.U. Penicillin + 0 Microgram Streptomycin per ml	500 I.U. Penicillin +500 Micrograms Streptomycin per ml	1000 I.U. Penicillin +1000 Micrograms Streptomycin per ml	1500 I.U. Penicillin +1500 Micrograms Streptomycin per ml
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	74.77±1.56	76.20±1.37	76.78±1.23	73.18±1.63	64.44±1.78	64.78±2.43	65.69±1.11	60.12±2.18
24	50	57.67±1.18	59.05±1.97	59.68±1.72	57.32±1.68	41.32±0.97	45.47±1.19	46.95±0.91	39.61±1.02
48	50	34.32±1.13	39.82±1.63	40.24±1.01	33.73±1.91	20.83±0.91	25.78±0.65	26.32±0.76	19.72±1.03
72	50	22.86±0.97	25.89±1.48	26.48±0.87	23.12±1.02	10.12±0.57	13.89±0.87	14.77±0.91	9.78±0.46

TABLE - V.

Analysis of variance showing effect of diluents, different doses of Penicillin and Streptomycin and different hours of preservation on the sperm motility in buck semen.

Sources of variation	df	Motility % M. S.
Between diluents.	1	967.52**
Between hours of preservation.	3	673.67**
Between doses.	3	583.69**
Between diluents x hours of preservation.	3	68.32 NS
Between diluents x doses.	3	59.73 NS
Between hours of preservation x doses.	9	108.37 NS
Between diluents x hours of preservation x doses.	9	26.38 NS
Error.	768	169.63
Total.	799	

** denotes significant at 1% level.

NS denotes non-significant.

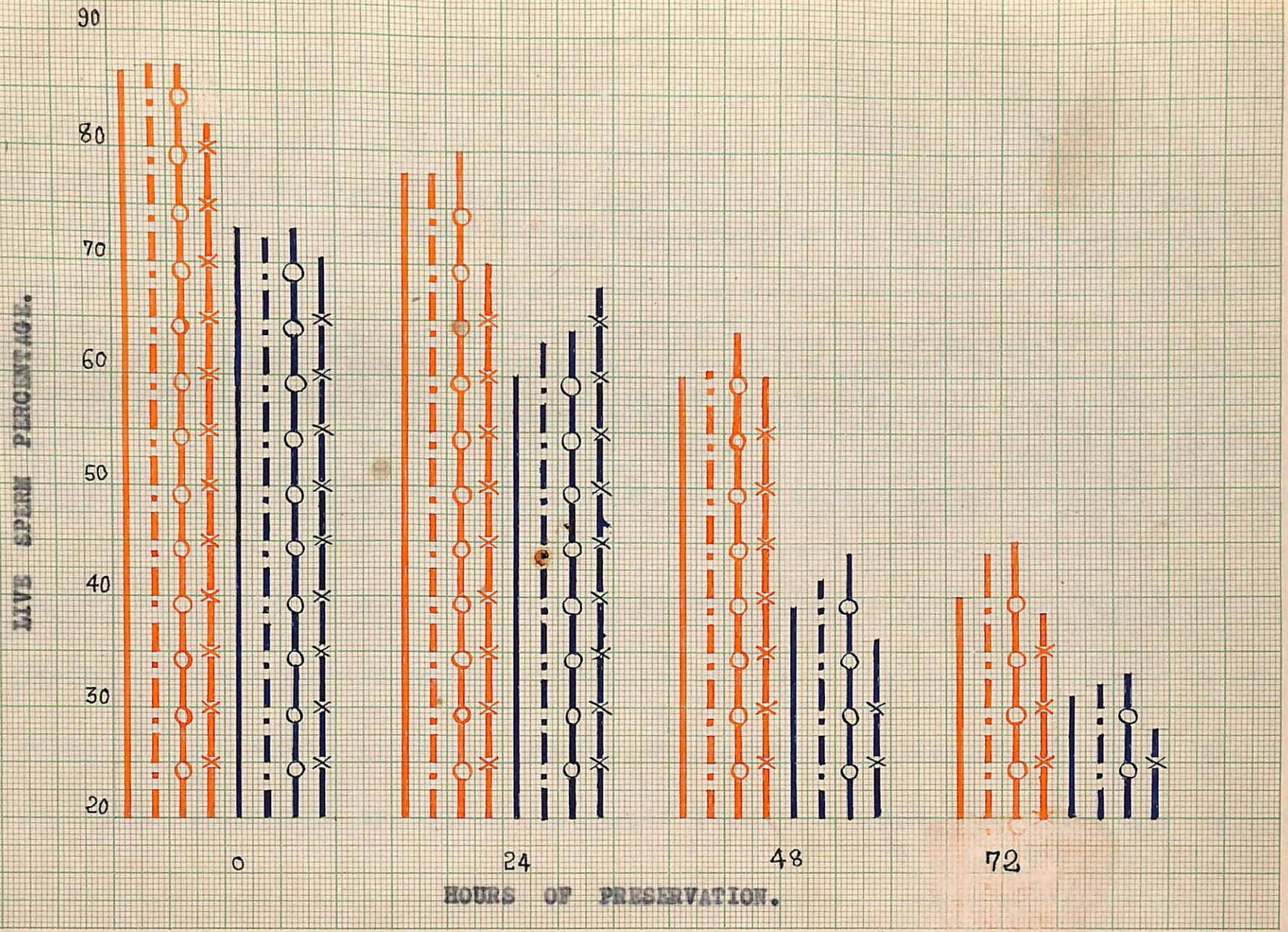
90
80
70
60
50
40
30
20
LIVE SPERM PERCENTAGE.
0
24
48
72
HOURS OF PRESERVATION.

FIG II

TABLE - VI.

Mean values of live sperm percentage of buck semen along with S.E. under different hours of preservation with different doses of Penicillin and Streptomycin in EYC and cow milk diluents.

Hours of preservation	No. of observations	Live sperm %							
		EYC				Cow milk			
		0 I.U. Penicillin + 0 Microgram Streptomycin per ml	500 I.U. Penicillin +500 Micrograms Streptomycin per ml	1000 I.U. Penicillin +1000 Micrograms Steptomycin per ml	1500 I.U. Penicillin +1500 Micrograms Streptomycin per ml	0 I.U. PPenicillin + 0 Microgram Streptomycin per ml	500 I.U. Penicillin +500 Micrograms Streptomycin per ml	1000 I.U. Penicillin +1000 Micrograms Streptomycin per ml	1500 I.U. Penicillin +1500 Micrograms Streptomycin per ml
		Mean±S.E.	Mean±S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	86.67±2.18	86.82±1.94	87.38±1.08	82.46±0.92	72.93±0.69	71.98±0.89	73.27±0.58	70.63±1.72
24	50	78.12±1.82	77.93±0.92	79.82±1.10	70.31±2.17	60.17±0.91	62.93±1.19	63.89±1.67	67.76±1.23
48	50	60.48±1.92	60.56±1.17	63.82±0.63	59.81±1.37	39.48±0.45	41.69±0.88	43.79±0.21	36.12±0.36
72	50	40.13±0.63	43.78±0.72	45.18±1.32	38.68±0.97	31.28±0.19	32.08±0.24	33.12±0.31	28.43±0.67

different dosing and hours of preservation.

Statistical analysis seemed to reveal highly significant differences between diluents, between hours of preservation and between doses of Penicillin and Streptomycin respectively, although no significant interactions could be obtained (Table - VII).

TABLE - VII.

Analysis of variance showing effect of diluents, different doses of Penicillin and Streptomycin and hours of preservation on live percentage of sperms in buck semen.

Sources of variation	df	Live sperm % M. S.
Between diluents.	1	1367.12**
Between hours of preservation.	3	976.73**
Between doses.	3	683.52**
Between diluents x hours of preservation.	3	182.13 NS
Between diluents x doses.	3	63.19 NS
Between hours of preservation x doses.	9	58.32 NS
Between diluents x hours of preservation x doses.	9	103.67 NS
Error.	768	203.69
Total.	799	

** denotes significant at 1% level.

NS denotes non-significant.

***
*

DISCUSSION

D I S C U S S I O N

NEAT SEMEN.

Volume of ejaculate :

The average volume of ejaculate of 5 non-descript bucks was found to be 0.48 ± 0.02 on the basis of 50 samples collected at intervals of one collection per week (Table-I). The finding of Mukherjee et al. (1953) showed that the average ejaculate volume of buck was 0.54 ± 0.11 ml as against the high values for this trait reported by Shukla and Bhattacharya (1952), who reported the ejaculate volume of buck to be ranging from 0.44 to 0.78 ml. The results of the present study seemed to agree with the lowest value recorded by Shukla and Bhattacharya (loc. cit.). But as a whole the values obtained in the present study were much closer to those reported by Mukherjee et al. (loc. cit.). Sahni and Roy (1969) working with Barbari and Jamnapari bucks reported the average volume of semen to be 0.66 and 0.94 ml respectively. In Malabari bucks, Kurian and Raja (1965) showed the average volume of semen to range from 0.4 to 1.2 ml whereas Dussardier and Szumowski (1952) reported the average ejaculate volume of buck semen to be 0.6 ml and this seemed to be a bit on the higher side than the present observation. In 1962 Knoblauch found the average volume of buck semen to be 1.00 ml. Austin et al. (1968) found the ejaculate volume to vary widely on the basis of difference in methods of collection. He reported the

volume to be 1.93 ml by electroejaculation as against 0.84 ml by artificial vagina method.

Mittal and Pandey (1972) found the volume of ejaculate from Barbari bucks to vary from 0.433 ± 0.134 to 0.664 ± 0.249 as against 0.88 ± 0.093 to 0.69 ± 0.84 from Jamnapari bucks. Tiwari et al. (1968) worked with Sannen, Jamnapari, Barbari and Sannen x Jamnapari cross and recorded the ejaculate volumes to be 0.997 ± 0.055, 1.36 ± 0.05, 0.94 ± 0.01, 1.37 ± 0.07 ml respectively. Patel (1967) recorded the volume of Jamnapari buck ejaculate to be 0.815 ml whereas Misra and Sengupta (1965) recorded the average volume of semen ejaculate to be 0.65 ± 0.07 in the same breed. Sharma et al. (1957) found the average ejaculate volume of Betal bucks to be 1.3 ml in summer and 0.8 ml in winter whereas individual ejaculate volume was found to vary widely from 0.2 to 1.2 ml. Eaton and Simmons (1952) working with nine Toggenburg bucks reported the average volume of seminal ejaculate to be 0.65 ml. They also reported highly significant seasonal differences as regards the volume of semen. Roy (1975) recorded the average volume of semen from non-descript bucks to be 0.46 ± 0.01 ml.

The present finding, though seemed to agree with those of Mukherjee et al. (loc. cit.), Roy (loc. cit.) and the lowest volume recorded by Shukla and Bhattacharya (loc. cit.), it was found to widely disagree with those reported by Sahni and Roy, Kurian and Raja, Dussardier and Szumowski, Mittal and Pandey, Knoblauch and Austin (loc. cit.).

The widely varying differences among the findings of different workers on the ejaculate volume might be due to variations of season, feeding, breed differences, as well as the environmental variables.

Colour :

In the present study it was noted that the colour of buck semen was yellowish, although it ranged between various shades from light yellow to yellow (Table - I). This observation was not in agreement with that reported by Shukla and Bhattacharya (loc. cit.) who found the buck semen to be cream coloured. They also reported that thin creamy samples were on the average showing higher pH than the thick ones, although no significant variations in initial motility of spermatozoa between thin and thick creamy samples could be recorded. Knoblouch (loc. cit.) reported ivory colour of buck semen with creamy consistency. The variations in colour reported by different workers in this field might be attributed to breed differences, feeding variations or seasonal and climatic influences. Human error also should be an important factor in colour recordings. Wagenaar (1946), working with Sannen goats in Zeeland, found that in fertile goats the colour of semen was yellow thick with high motility whereas in infertile goats it was watery thin with slight motility. Patel (loc. cit.) reported the colour of Jamnapari buck semen to range from creamy to slight yellow.

Roy (loc. cit.) reported the colour of buck semen to be generally yellowish though light and deep yellow colours

are not uncommon.

Hydrogen ion concentration (pH) :

The average pH of neat semen obtained from 5 local non-descript bucks, in the present case turned out to be 6.71 ± 0.32 (Table - I). Roy (loc. cit.) reported the average pH of neat semen from non-descript bucks to be 6.67± 0.28. In Malabari bucks, Kurian and Raja (loc. cit.) reported the seminal pH to range from 6.3 to 6.7. In Jamnapari breed, Patel (loc. cit.) reported the average pH to be 6.5 though it ranged from 6.2 to 6.8. Dussardier and Szumowski (1952) recorded the average pH of buck semen to be 6.5 as against the findings of Knoblauch (loc. cit.), who recorded the pH of semen in white German bucks to be 6.6. Shukla and Bhattacharya (loc. cit.) found the pH of buck semen to range from 6.2 to 6.5. The difference in pH values as recorded by different workers might be due to variations in feeding, managemental practices and differences in breed, ejaculate volumes, weight of bucks or due to seasonal influence etc.

Initial motility :

The present study revealed that the initial motility of sperms of non-descript buck semen was 79.78 ± 0.73 per cent (Table - I). Dussardier and Szumowski (loc. cit.) obtained 75 per cent motility in the case of buck neat semen. The initial spermatozoal mortility of buck semen was found to range from 3.8 to 5.0 in different months of the year by Shukla and Bhattacharya (1952). Sahni and Roy (loc. cit.) reported 3.694

and 3.634 to be the initial motility of sperms in case of Barbari and Jamnapari bucks respectively. According to Tiwari et al. (loc. cit.), the initial motility of sperms in Jamnapari, Sannen x Jamnapari and Barbari bucks was 4.6 ± 0.09, 5.0 ± 0 and 4.2 ± 0.11 respectively. Patel (loc.cit.) reported +5 motility of sperms in case of Jamnapari bucks whereas Sahni and Roy (1967) reported 4.7 to 4.9 motility in case of Barbari bucks. Misra and Sengupta (loc. cit.) reported the initial motility of sperms in case of Jamnapari bucks to be 2.3 ± 0.50. Eaton and Simmons (loc. cit.) reported the motility of sperms in case of Toggenburg bucks to be 1.51. Kurian and Raja (loc. cit.) reported 60 to 90 per cent motility in Malabari buck neat semen on the basis of a study of 49 semen samples. Mittal and Pandey (loc. cit.) found 3.5 ± 0.172 to 3.75 ± 0.114 and 3.88 ± 0.027 to 3.88 ± 1.027 sperm motility in Barbari and Jamnapari bucks respectively. Roy (loc. cit.) reported initial spermatozoal motility in the case of bucks to be 78.60 ± 0.61 per cent.

The present value obtained was very close to that reported by Roy (loc. cit.) and Dussardier and Szumowski (loc. cit.) but the values reported by Shukla and Bhattacharya, Sahni and Roy, Mittal and Pandey and Tiwari et al. (loc. cit.) were found to differ from the findings of the present study. The differences in results might be due to feeding, managemental and breed differences. Different seasons and place also might have influenced the results.

Sperm concentration :

The sperm concentration in neat semen obtained from non-descript bucks under the present investigation turned out to be 3832.4 ± 48.69 millions per ml (Table-I). Roy (loc. cit.) obtained the value for this trait also in non-descript bucks to be 3658.5 ± 30.38 millions per ml. Sahni and Roy (1969) working with Barbari and Jamnapari buck semen reported the value to be 1910 and 2241 millions per ml respectively. Shukla and Bhattacharya (loc. cit.) working in different months of the year found the sperm concentration in buck semen to range from 1726 to 3240 millions per ml, whereas Mukherjee et al. (loc. cit.) recorded this value to be 5368 ± 247.88 millions per ml.

Tiwari et al. (loc. cit.) reported the average sperm concentration per ml ($x\ 10^6$) of neat semen to be 3728.3 ± 192.4, 4995.5 ± 159.8 and 3650.2 ± 155.3 in respect of Jamnapari, Sannen x Jamnapari cross and Barbari bucks respectively. Sahni and Roy (1967) reported the sperm concentration per ml to be 1804 and 2099 millions respectively in case of two Barbari bucks. Whereas Misra and Sengupta (loc. cit.) recorded the sperm concentration per ml ($x\ 10^6$) to be 4080 ± 450. Sharma et al. (loc. cit.) reported the average sperm concentration in Betal bucks to be 5424.7 millions per ml. The above finding was much higher than that recorded in the present study. Eaton and Simmons (loc. cit.) found the average number of sperms in buck semen to be 2.724 billions per cm^3. They also reported highly significant

influence of seasons and breeds on this character. Wagenaar (1946) reported that in the case of fertile Sannen bucks the lowest count of sperm was 1540 millions per cm^3 whereas in case of infertile bucks the highest sperm concentration was recorded to be 52.5 millions per cm^3.

Kurian and Raja (loc. cit.) obtained sperm concentration in Malabari goats to be 2 to 3 millions per cmm. Mittal and Pandey (1972) recorded sperm concentration to be 2674 ± 98.7 to 2788 ± 153.6 and 2256.6 ± 74.09 to 2492 ± 101.3 millions per ml in Jamnapari and Barbari bucks respectively. Dussardier and Szumowski (loc. cit.) recorded an average sperm concentration in buck semen to be 3600 millions per ml.

The values obtained in the present investigation seemed to tally with the findings of Roy (loc. cit.) and Dussardier and Szumowski (loc. cit.), but it differed widely from those reported by Sahni and Roy, Shukla and Bhattacharya, Mukherjee et al., Kurian and Raja and Misra and Sengupta (loc. cit.).

Breed differences, variations in feeding and environmental variables might be the reasons that can be adduced to explain this differences in results.

Live sperm percentage :

The mean live sperm percentage in neat semen of non-descript bucks was found to be 88.63 ± 2.19 (Table - I) as against 87.81 ± 1.32 obtained by Roy (loc. cit.). Sahni

and Roy (1969) obtained the live sperm percentage in Barbari and Jamnapari bucks to be 69.49 and 63.63 respectively.There was close agreement between the present findings and the values obtained by Kurian and Raja (loc. cit.) in the Malabari bucks (93%). Mishra and Sengupta (loc. cit.) recorded live sperm percentage in Jamnapari bucks to be 54 ± 4.9 as against 72-73 per cent obtained by Mittal and Pandey (loc. cit.) and 90.7 per cent obtained by Patel (loc. cit.) in Jamnapari bucks. In Barbari bucks, Mittal and Pandey (loc. cit.) noticed the live sperm percentage to be nearly 70 per cent as against 80-82 per cent recorded in Barbari bucks by Sahni and Roy (1967). Knoblauch (1962) obtained 80 per cent value of this attribute in German imported buck semen. The wide variations in results obtained by different workers in this field might be due to agroclimatic variables, differences in breeds, methods of semen collection and feeding differences etc.

EFFECT OF ADDITION OF ANTIBIOTICS ON THE KEEPING QUALITY OF BUCK SEMEN :

Hydrogen ion concentration (pH).

Egg yolk citrate diluent group :

It is evident from Table - II that with no addition of antibiotics, the mean pH of semen in EYC diluent was 6.58 ± 0.21, 6.50 ± 0.10, 6.41 ± 0.19, 6.21 ± 0.43 at zero, 24, 48 and 72 hours of preservation respectively. From the above, it was quite clear that with increasing hours of

preservation, the semen became more and more acidic as expected. But analysis of variance revealed no significant effect of hours of preservation on the pH of buck semen (Table-III).

With addition of 500 I.U. Penicillin and 500 micrograms Streptomycin per ml of diluted semen, the pH value was noted to be 6.58 ± 0.31, 6.51 ± 0.13, 6.40 ± 0.21 and 6.21 ± 0.33 at zero, 24, 48 and 72 hours of preservation respectively (Table - II).

With the addition of 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted semen, the pH value was 6.60 ± 0.23, 6.52 ± 0.19, 6.40 ± 0.23 and 6.21 ± 0.10 at zero, 24, 48 and 72 hours of preservation in EYC respectively (Table - II). With the addition of 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen, the pH at zero, 24, 48 and 72 hours of preservation was recorded to be 6.61 ± 0.18, 6.52 ± 0.21, 6.40 ± 0.17 and 6.20 ± 0.27 respectively (Table - II). It seemed that variations in pH due to increasing hours of preservation was non-significant. No significant difference in pH values with addition of different doses of antibiotics could also be recorded (Table - III).

Cow milk diluent group :

Mean pH of semen in milk diluent with no addition of antibiotics, at zero, 24, 48 and 72 hours of preservation was noted to be 6.60 ± 0.29, 6.51 ± 0.73, 6.30 ± 0.39 and

6.20 ± 0.38 respectively (Table - II).

With addition of 500 I.U. Penicillin and 500 Micrograms Streptomycin per ml, the pH value of semen after zero, 24, 48 and 72 hours of preservation was recorded to be 6.61 ± 0.63, 6.51 ± 0.62, 6.30 ± 0.51 and 6.15 ± 0.67 respectively (Table - II). With 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml, the pH of buck semen was found to be 6.63 ± 0.78, 6.40 ± 0.37, 6.27 ± 0.73 and 6.10± 0.79 after zero, 24, 48 and 72 hours of preservation in cow milk diluent respectively (Table - II).

With 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml, the seminal pH at zero, 24, 48 and 72 hours of preservation was recorded to be 6.61 ± 0.53, 6.30 ± 0.47, 6.20 ± 0.42 and 6.00 ± 0.49 respectively (Table - II).

No sharp fall of pH upto 72 hours of preservation could be recorded. This seems to be at variance with the findings of Roy (loc. cit.) who noted a sharp fall in pH of diluted buck semen in milk diluent. The above mentioned worker also found particularly marked sharp fall from 0-24 hours of preservation nothing like this could recorded in the present study. The fall in pH could be said to be rather uniform and steady (Table - II). The highest pH value with milk diluent was obtained at zero hour under the doses 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml (6.63 ± 0.78) whereas the lowest seminal pH was found to be 6.00 ± 0.49 at 72 hours of preservation in milk diluent under the dose of 1500 I.U. Penicillin and 1500 micrograms Streptomycin

per ml (Table - II). The increased acidity of buck semen from zero to 72 hours of preservation might be due to the lactic acid formation during storage of semen.

The addition of different doses of antibiotics did not have significant effect on the pH of semen samples at different hours of preservation (Table - III). The interaction between diluents and hours of preservation, between diluents x hours x doses turned non-significant in the case of seminal pH.

Motility percentage of sperms.

Motility percentage at different hours of preservation and with different dosing of antibiotics in Egg yolk citrate and cow milk diluent were recorded in Table - IV.

Egg yolk citrate diluent group :

Table - IV revealed that at zero hour of preservation maximum motility percentage was shown in the group treated with 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted semen in EYC diluent (76.78±1.23), whereas the minimum motility percentage was recorded in the dose group of 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml (73.18 ± 1.63).

At 24 hours of preservation nearly 17 per cent fall in percentage of motility was recorded in all the treatment groups. But the maximum motility percentage was found in the group added with 1000 I.U. Penicillin and 1000

micrograms Streptomycin per ml of diluted semen (59.68 ± 1.72). Here again the lowest motility percentage was found in the group treated with 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen (57.32±1.68).

At 48 and 72 hours of preservation also, treatment with 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted semen gave the highest motility percentage that is 40.29 ± 1.01 and 26.48 ± 0.87 respectively.

The lowest sperm motility at 48 hours of preservation was recorded in the group treated with 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen (33.73 ± 1.91). But at 72 hours of preservation the minimum motility percentage was found in the no treatment group (22.86 ± 0.97) Table - IV.

Thus it was evident that in the EYC diluent, the maximum motility percentage was recorded uniformly with 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted semen at all stages of preservation. The minimum motility percentage at zero, 24, and 48 hours of preservation in EYC was noted in the group treated with 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen. But at 72 hours of preservation the lowest motility percentage was found in the no treatment group.

The analysis of variance (Table - V) showed that hours of preservation, dosing and diluents had highly significant effect on the motility percentage.

Cow milk diluent group :

In this diluent also, like that in EYC, the treatment with 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted semen gave the maximum motility percentage at all the stages of preservation i.e. 65.69 ± 1.11, 46.95 ± 0.91, 26.32 ± 0.76 and 14.77 ± 0.91 at zero, 24, 48 and 72 hours of preservation respectively (Table - IV).

The lowest motility percentage in milk diluent was found in the group with addition of 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen. At all the stages of preservation, this dosing showed the lowest mean motility percentage (at zero, 24, 48 and 72 hours of preservation the values were 60.12 ± 2.18, 39.61 ± 1.02, 19.72 ± 1.03 and 9.78 ± 0.46 respectively) (Table - IV).

The sharp difference in the motility percentage, between EYC and cow milk diluent groups is evident from Table - IV. At all the stages of preservation and in all the treatment groups, the cow milk diluent exhibited significantly lower value than the EYC diluent.

Statistical analysis (Table - V) also revealed highly significant difference between diluents. Thus from what has been mentioned above it seems sthat EYC diluent has a sharp edge over that of milk diluent so far as motility percentage of sperms is concerned. As was expected with increasing hours of preservation fall in motility percentage was recorded in both diluent groups and the order of difference also seems to be of similar nature in both these diluents.

Live sperm percentage.

Egg yolk citrate diluent group :

At all the stages of preservation ranging from 0 to 72 hours, the live sperm percentage was found to be highest in the 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml group. The values were 87.38 ± 1.08, 79.82 ± 1.10, 63.82 ± 0.63 and 45.18 ± 1.32 at zero, 24,48 and 72 hours respectively (Table - VI).

The lowest live sperm percentage was obtained at all the stages of preservation in the treatment group of 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen. At zero, 24, 48 and 72 hours of preservation with 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml dosing the live sperm percentage was recorded to be 82.46 ± 0.92, 70.31 ± 2.17, 59.81 ± 1.37 and 38.68 ± 0.97 respectively (Table - VI).

Sharp fall in live sperm percentage with increasing hours of preservation was recorded. The fall in live sperm percentage between 0 to 24 hours was 8 to 12 per cent but from 24 to 48 hours the fall was sharper still being 11-18 per cent, whereas from 48 to 72 hours the fall in live sperm percentage was still sharper ranging from 17 to 21 per cent.

Cow milk diluent group :

Like that in EYC dilutor, the treatment with 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of

diluted semen showed the highest value of live sperm percentage at all the stages of preservation except at 24 hours. The values were 73.27 ± 0.58, 43.79 ± 0.21 and 33.12 ± 0.31 at zero, 48 and 72 hours of preservation respectively (Table-VI).

At 24 hours of preservation, with 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen, the maximum live sperm percentage was 67.76 ± 1.23 as against the minimum live sperm percentage 62.93 ± 1.19 which was noted in no treatment group (Table - VI).

At all the stages of preservation except at 24 hours, the minimum live sperm percentage was recorded with the addition of 1500 I.U. Penicillin and 1500 micrograms Streptomycin per ml of diluted semen (Table - VI).

The fall in live sperm percentage in milk diluent with different doses of antibiotics was not sharp at any particular hour of preservation. But at different hours of preservation, the fall was marked and almost of similar order as that recorded in EYC.

Highly significant difference between dilutors, between hours of preservation and between doses in respect of live sperm percentage was recorded. Although none of the interactions turned out to be significant (Table - VII).

From what has been stated above, it was found that diluents, hours of preservation, and doses did not affect seminal pH of bucks, although motility percentage and live

sperm percentage were highly significantly affected. Milk diluent constantly gave poor results than EYC.

As regards, dosing, it was found that 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted semen yielded the best results in case of motility and live sperm percentage. Therefore, the present study seems to indicate EYC to be a better dilutor and 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml to be the best suited dose for addition to diluted semen. No literature could be found regarding the addition of antibiotics in buck semen. So the present findings could not be compared with any previous work.

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S U M M A R Y

S U M M A R Y

Studies were made on neat semen as well as diluted semen samples obtained from 5 local non-descript bucks. The effects of different doses of antibiotics and different hours of preservation were also observed.

In the case of neat semen, mean ejaculate volume was 0.48 ± 0.02 ml, pH 6.71 ± 0.32, initial motility percentage 79.78 ± 0.73, concentration of sperm per ml 3832.4 ± 48.69 millions and live sperm percentage 88.63 ± 2.19. The colour of ejaculate varied from light yellow to yellow shades. Egg yolk citrate and cow milk were used as diluents for the preservation of buck semen.

Addition of different doses of antibiotics (Penicillin and Streptomycin) did not have significant effect on the pH of diluted semen samples at any stage of preservation in both the EYC and cow milk diluents.

The motility and live sperm percentages were recorded with the addition of different doses of antibiotics at all the stages of preservation in both diluents.

Highly significant effects of diluents, hours of preservation as well as different doses of antibiotics on motility and live sperm percentages were recorded.

In comparison to the EYC extender, significantly lower motility and live sperm percentages at all the stages

of preservation ranging from zero to 72 hours were recorded by the use of milk diluent. Thus the present study seems to point to the superiority of EYC over the milk diluent.

Addition of 1000 I.U. Penicillin and 1000 micrograms Streptomycin per ml of diluted buck semen seemed to be the best of all dosings.

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