Colour Additions in Different Dilutors and Their Influence 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

Devendra Pandey

B. V. Sc. & A. H.

R. A. U. JUNIOR FELLOW

Post-Graduate Department of Gynaecology and Obstetrics

BIHAR VETERINARY COLLEGE

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Dated, the 29th December, 1976

This is to certify that the work embodied in this Thesis entitled "COLOUR ADDITIONS IN DIFFERENT DILUTORS AND THEIR INFLUENCE ON THE KEEPING QUALITY OF BUCK SEMEN" is the bonafide work of Devendra Pandey 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 journal.

Devendra Pandey.

(DEVENDRA PANDEY)

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(D. PANDEY)

DEDICATED TO

MY

ELDEST GRAND FATHER

C O N T E N T S

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INTRODUCTION

INTRODUCTION

Goat keeping, though neglected during the last several years, is now getting an unprecedented boost up in our country. Scientific goat rearing and production now form the basis to its development.

Under the auspicious of the Indian Council of Agricultural Research various pilot projects on goat production have been launched in different parts of the country as for example in Tamil Nadu, Haryana and Assam for the improvement of goat farming in India. According to the 11th Livestock Census (1972) the goat population of India (excluding Arunachal Pradesh) was 68.024 millions and that of Bihar was 7.364 millions.

The estimated goat meat production in India was 22 millions kg on the basis of average carcas weight of 9.05 kg (Agri. Marketing Board of India 1967-68). In Tamil Nadu and Haryana the average goat meat production was estimated to be 10.2 kg and 10.9 kg respectively (Singh, 1973).

A well planned scientific breeding programme aims at improving the genetic potentialities per generation. Artificial insemination is the only tool available at present with which this genetic improvement can be brought about in a population, in the minimum time.

The colouring of semen of various breeds of bulls

has become a routine practice in various parts of the world (Rao, 1967). Such practice is very much desirable in large semen collection stations for breed identification. Where different breeds of the same species are used for semen collection. The National Association of Artificial Breeders used the following colour designation for identifying semen. Holstein, green; Ayrshire, purple; Brown Swiss, Brown; Jersey, Red and Guernsay, uncoloured egg yolk.

So far very little work is available on this aspect in India. So, a preliminary study has been undertaken with a view to find out the suitability of different colour additives for colouring buck semen and also to study, is any of these extra colour additives have got any deleterious or beneficial effect on the keeping quality of semen.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Neat semen.

Wagenaar (1946) observed that the semen of fertile bucks was yellow, thick, had high motility with 1540 millions sperms per cubic centimeter and 6-39% abnormal sperms whereas in the case of infertile bucks the semen was thin, watery, had slight motility and 52.5 millions sperms per cubic centimeter. The abnormal sperms varied from 45-57 per cent in such cases.

Eaton and Simmons (1952) studied the semen quality of 9 Taggenburg and 5 common American bucks over 400 observations and found the average volume to be 0.650 ml, motility 1.51, concentration 2.724 billions per cubic centimeter and abnormal sperms 8.46 per cent. Year to year variation in average were significant only in volume.

Dussardier and Szumowski (1952) observed that the highest motility average i.e. 75 per cent in buck semen was noted during November and December months. The average volume was found to be 0.6 ml, concentration 3,600 millions per cubic mm and pH 6.5.

Shukla and Bhattacharya (1952) studied the semen quality of 8 bucks for two years and reported the volume to be range from 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 abnormal sperm percentage to be 2.4 to 11.1 and colour to be creamy.

Mukherjee et al. (1953) conducted experiments on 7 bucks and noted the average volume of semen to be 0.54 ± 0.04, initial motility 4.35 ± 0.11, sperm concentration 5368 ± 247.88 millions per ml and abnormal sperms 3.35 ± 0.40 per cent.

Sharma et al. (1957) made observations on the semen samples collected from 3 Beetal bucks. They concluded that the average individual ejaculates varied from 0.2 to 1.2 ml, the amount increasing with body size. The average sperm concentration was found to be 5424.7 millions. Per cubic centimeter, being lowest inspring and highest in winter, motility was greatest in autumn and lowest in winter.

Knoblauch (1962) studied the semen samples from 19 white German imported bucks and found the average volume of ejaculate to be 1.0 ml, pH 6.6, forward motility 80 per cent, the number of dead spermatozoa 20 per cent and consistancy creamy.

Jalam and Nambiar (1965) evaluated semen samples of Jamnapari bucks. They found sperm motility average 72.3 ± 3.398 and unstained spermatozoa 72.7 ± 5.74. Further when they diluted the semen (1:20) in egg yolk citrate or goat milk, after 24 hours storage motility averaged 65.6 ± 3.63 and 59.3 ± 3.50 per cent respectively, and after 72 hours, 54.4 ± 2.73 and 43.6 ± 2.69 per cent respectively. After 72 hours storage the unstained spermatozoa average to be 61.27 ± 4.31 per cent and 50.09 ± 3.26 per cent respectively.

Kurian and Raja (1965) reported on semen samples collected from malabari bucks at the intervals of 7 to 12 days

and studied as follows : average volume 0.4 to 1.2 ml,motility 60-90 per cent, pH 6.3 to 6.7, sperm concentration 2-3 millions per cubic mm, abnormal spermatozoa 6-12 per cent and live 85-95 per cent. They also found that the interval between collections did not affect semen quality.

Misra and Sengupta (1965) studied the semen quality of Jamnapari bucks and found the average volume to be 0.65 ± 0.07, initial motility 2.3 ± 0.50, sperm concentration 4080 ± 450 millions per ml and percentage of live sperms 54 ± 4.9.

Patel (1967) observed the characteristics of Jamnapari buck semen and recorded 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 (1968) found that the mean motility of buck semen was 3.96 (80 per cent) and 3.63 (70 per cent) in Barbari and Jamnapari breeds respectively.

Tiwari et al. (1968) studied the quality of semen of Barbari and Sannen bucks and did not find any deterioration in semen production when the collections were taken one per day over a period of 21 days. Non-significant difference between breeds inseminal volume was recorded but highly significant difference in sperm concentration per millilitre and sperm number per ejaculate were observed between the two breeds ($P < 0.01$). The average volume was found to be 0.52 ± 0.06 and 0.997 ± 0.055, concentration 2035.62 ± 78.7 and 2393 ± 101.4 and live sperm 86.12 ± 0.886 and 84.65 ± 0.915 per cent respectively in Barbari and Sannen bucks. They further studied on the seminal attributes

of Jamnapari, Sannen x Jamnapari and Barbari bucks and found that if the period of collection was extended upto 40 days highly significant difference between breeds ($P < 0.01$) on volume and sperm number per ejaculate was observed. The average volume, concentration per ml, number of sperm per ejaculate, live sperm percentage and initial motility of Jamnapari, Sannen x Jamnapari and Barbari buck semen were found to be as follows.

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

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± 220.0	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

Mittal and Pandey (1972) reporting on the semen quality of Barbari and Jamnapari bucks stated that there was highly significant difference between bucks in relation to ejaculate, volume, sperm motility, concentration and percentage of viable spermatozoa after collection.

The average volume, sperm motility, sperm concentration, total sperm per ejaculate and live sperm percentage of Barbari and Jamnapari were as follows : -

Buck breed and number	Volume	Sperm motility	Sperm concentration per ml ($x10^6$)	Total serum per ejaculate ($x10^6$)	Live sperm percentage
	Overall average	Overall average	Overall average	Overall average	Overall average
Barbari I	0.433± 0.134	3.5± 0.172	2492 ± 101.3	1079 ± 138.0	69.9 ± 0.540
Barbari II	0.664± 0.249	3.75± 0.114	2256 ± 74.09	1498 ± 134.4	70.00± 0.655
Jamnapari I	0.88 ± 0.0933	3.88± 1.027	2788 ± 1531.6	2453 ± 142.8	72.53± 1.903
Jamnapari II	0.69 ± 0.840	3.96± 1.102	2674 ± 99.7	1845 ± 83.7	73.18± 2.021

Roy (1975) worked on the semen picture non-descript bucks and found the average 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 per cent and live percentage 87.81 ± 1.32.

D i l u e n t s

Wagner (1949) found that egg yolk (2.5 per cent) glucose phosphate was better diluent to yolk free glucose phosphate for buck semen.

Roy et al. (1957) studied the neat semen quality of bucks with different diluents. They found that egg yolk citrate was better than glycerophosphate egg yolk and glycine egg yolk.

Tiwari et al. (1968) studied the effect of dilution on buck semen (1:1 to 1:10) in respect of the percentage of motile sperms at two storage intervals (6 hours and 36 hours). They observed that the percentage of decrease in motility from the storage period of 6 to 36 hours was maximum in lowest (1:1) and minimum in highest (1:10) dilution. A decline of 38-39 per cent was recorded in the dilution rate of 1:3 and 1:5.

Colour additions to diluted semen.

Phillips (1945) gave a practical method of colouring semen for identification purpose. He used 5 per cent solution of Methylene blue, Janus green, thionin, alizarin sulphonate, nite blue sulfate, neutral red and sudan III for the experiment. The conclusion was th t when nite sulfate, neutral red and sudan III were added to the egg yolk buffer semen, no appreciable detrimental effect was observed on the motility for several days. Neutral red and sudan III were especially remarkable in this respect. Methylene blue, janus green, thionin and alizarin sulphonate were not satisfactory.

Almquist (1946) studied the effect of certain coaltar dyes used for semen identification on the livality and fertility of bull spermatozoa. He conducted experiment with red, green, brown and purple colour preparations in laboratory and field conditions. One drop of any dye solution for each 10 ml of

dilutor was able to maintain the motility of sperms as good as in the case of uncoloured controls. In field study, when the same dye was used at a lower concentration, no significant difference infertility was observed between coloured and uncoloured samples.

Ostrowski and Ruwisch (1959) conducted in vitro experiments with three North American and two German stains for staining diluted bull semen and also deep frozen semen. The stains used were strawberry red, emerald green, purple grape and two German stains, red and pink. The conclusion was that the colours had no adverse influence on diluted semen stored at 5°C, or on deep frozen semen (-80°C) after 14 days of storage.

Clamohoy and Foote (1963) studied the effect of dyes on the survival of bull spermatozoa. Strawberry shade red emerald shade green, purple shade grape, brown, lemon yellow, and blue certified food colours were added to yolk citrate, glycerol and skimmilk glycerol extenders at the rate of 0.6 ml per 100 ml of extender. The observed no effect on sperm survival during a period of 30 days when stored at -79°C. Further observation was that the same concentration of all food colours except brown had no harmful effect on bull sperm when stored for 12 days at 5°C in CUE, 50 per cent yolk citrate and skimmilk extenders. The brown food colour was found to be spermicidal ($P<0.01$).

Rao (1967) tested anrang keshar, chocolate brown food colour and neutral red for colouring of bull semen. He

reported that the difference in livability of semen samples coloured with these dyes was not significant.

Tiwari and Sahni (1967) compared the indigenous dyes (yellow and orange) with neutral red and sudan III as colour additives for the identification of buffalo, sheep and goat semen. It was interesting to record that the indigenous dyes were as good as the other two dyes. None of the dyes had depressed the viability of spermatozoa upto 54 hours.

Sahni and Roy (1971) studied the influence of certain colour additives viz. neutral red, sudan III, orange and yellow on keeping quality and identification of sheep and goat semen. They observed that these dyes did not produce any adverse effect on the keeping quality of semen samples, stored upto 54 hours at 5-7°C.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was conducted on the semen samples obtained from five local non-descript bucks. The animals were between 3 and 4 years of age and were maintained under the same environmental and managemental conditions at the Department of Gynaecology and Obstetrics, Bihar Veterinary College, Patna. The bucks were kept on grazing and a concentrate mixture at the rate of 500 gms per buck per day. The concentrate feed consisted of -

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

Methods of semen collection.

Collections were taken by the Artificial vagina method. The artificial vagina temperature was maintained at 45° to 50°C. Collections were taken at weekly interval. 10 collections per buck and a total of 50 samples were examined. Collections were generally taken in the forenoon and immediately after collection the semen was taken to the laboratory for examination.

Evaluation of neat semen.

Following seminal attributes were studied :

(1) Volume.

(2) Colour.

(3) Mass activity.

(4) Hydrogen-ion concentration (pH).

(5) Concentration of sperms.

(6) Live sperm percentage.

The volume of the ejaculate was recorded directly from the graduated collection tube.

Colour was observed by naked eye.

Mass activity of neat semen (initial motility) was examined under low power objective of the microscope without using cover slips. The mass activity was graded under the following five categories (Tomar, 1970) : -

(1) <u>+5 Motility</u> - The observer finds only very quick wave motions in the field. It is very difficult to trace when the eddies are formed or disintegrated. There is only wave after wave in the field. Swirls are created with mass movement of spermatozoa.

(2) <u>+4 Motility</u> - The swirls and eddies are comparatively not so rapid as in +5 grade. The swirls can be observed to move towards the extermities.

(3) <u>+3 Motility</u> - The swirls are slow and are scattered in the field. Individual movements of spermatozoa are discernable. Grossly over 40 to 60% of spermatozoa are progressively motile. Rest of the spermatozoa are weak in motion.

(4) <u>+2 Motility</u> - Swirls are absent. Individual

movements of spermatozoa are more evident in the field. Grossly the observer finds not more than 40% of spermatozoa in progressive motion. Other sperms are weak in motility. These may have oscillating, undulating or circular movements. The nonprogressive movement may also be characterised as "throbing movement".

(5) +1 Motility - No wave motions are observed in the film, and only about 20% of spermatozoa may have progressive movement. Rest of the spermatozoa show throbing movement.

(6) Zero Motility - The spermatozoa are not motile.

Hydrogen-ion concentration (pH).

The determination of seminal pH was done by narrow range Nitrazine papers (B.D.H.).

Concentration of sperms.

The semen was throughly mixed by rotating the tube between the palms. The thoroughly mixed semen was sucked upto 0.5 mark of the R.B.C. diluting pipette. Then 3 per cent sodium chloride solution was sucked upto 101 mark. The pipette was shaken gently to have thorough mixing of the semen in the bulb. The semen was thus diluted 200 times. First 2 to 3 drops of the fluid (the fluid contained in the stem of the pipette) were discarded.

The counting chamber was used first under 10x and then under 40x objective. A cover slip was put on the chamber.

1 to 2 drop of the diluted semen was put on the side of the cover slip. The semen (diluted 200 times) spread throughout the counting chamber.

Under the 40x, objective of the microscope the number of sperms present in 5 big squares of the counting chamber, one each on the four corners and one in the centre, were counted to get the concentration of sperms in the semen.

The calculation was done as mentioned below:

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

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

Therefore n x 5 was the number of sperms present in 0.1 cmm of diluted semen.

Therefore nx5x10 was the number of sperms present in 1 cmm of diluted semen.

Therefore nx5x10x200 was the number of sperms present in 1 cmm of original neat semen.

Therefore nx5x10x200x1000 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 nx10,000,000.

Live sperm percentage.

The percentage of live sperms was calculated by using the compound eosin - nigrosin stain according to the

method suggested by Swanson et al. (1951).

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

The solution was warmed in water bath for 30 minutes and filtered after cooling through Whatman's filter paper No.42.

Preparation of slides.

One drop of neat semen was mixed with 2-3 drops of the compound stain in a clear warm watch glass. Thorough mixing was ensured by blowing through a pipette and then the mixture was allowed to stand for one minute. Thin smears were drawn on two clear, grease free slides and dried quickly in air. The slides were marked with grease pencil indicating the semen sample number, group number, sub-group number and hour of preservation. The smear was examined under oil immersion lens.

Live spermatozoa did not take any stain and appeared colourless. The dead spermatozoa took the eosin stain. The sperms partially stained anteriorly or posteriorly were considered dead. The nigrosin stain presented a very homogenous violet background. A total of 100 sperms were counted at random in a slide under different fields of the microscope and the percentage of live sperms was calculated.

Preparation of dilutors.

The following two dilutors were used in the experiment.

(i) Egg yolk citrate.

(ii) Whole cow milk.

Egg yolk citrate diluent.

The buffer solution was prepared by dissolving 2.90 gms of sodium citrate dihydrate (B.D.H.) in 100 ml of glass distilled water. The buffer solution was sterilised by autoclaving for 30 minutes at 15 lbs pressure and then kept in a refrigerator for further use.

Only infertile hen's eggs were used for collection of yolk. The eggs were wiped with a swab of alcohol. The egg shell was then broken. Albumin was discarded and the yolk was placed on a sterilized filter paper. The yolk membrane was broken and the yolk was collected in a measuring cylinder. One part of egg yolk and one part of buffer solution were mixed thwroughly and thus the diluent was prepared.

Whole milk diluent.

Pure cow milk was obtained from a local milkman in the evening hours. It was heated in a water bath at 92°C-95°C for 10 minutes. Over and under heating was avoided. The heated milk was then cooled down to room temperature and kept over night in a refrigerator. Next morning it was filtered through a thin swab of cotton to remove the fat which had collected at the surface. It was thus ready for use.

Preparation of dye.

Three different dyes selected for the experiment

were as under :

i) Neutral red indicator (Renal of Hungary, $C_{16}H_{16}N_4CHI$ - M. wet - 288.78).

ii) Orange G (B.D.H.).

iii) Sudan III (B.D.H.).

100 mg of Neutral red was dissolved in 100 ml of glass distilled water to make the solution 0.1 per cent.

To make 0.1 per cent solution of orange G, 100 mg of orange G was dissolved in 100 ml of glass distilled water.

Sudan III is not soluble in water. Therefore firstly 100 mg of sudan III was dissolved in 33 ml of Absolute alcohol, and then 67 ml of glass distilled water was added to make it 0.1 per cent solution. All the three dyes were kept in a refrigerator for further use.

Dilution of semen.

Semen dilution in the case of both the dilutors (egg yolk citrate and whole cow milk) were done in the ratio of 1:10. The dilutors were also brought to room temperature (30°-32°C) before use. The test tubes were gently rotated between the palms for uniform distribution of sperms in the dilutors.

The diluted semen samples were divided into the following groups:

Group A. Egg yolk citrate group.

In this group, four subgroups were made -

(i) In this subgroup no colour additives were used.

(ii) In this subgroup 2 microdrops of neutral red (0.1% solution) were added.

(iii) To this subgroup 2 microdrops of orange G (0.1% solution) were added.

(iv) To this sub-group of diluted semen 2 microdrops of sudan III (0.1% solution) were added.

Group B. Whole Cow milk extender.

In this group also 4 subgroups were made -

(i) This subgroup no colour additives were added in diluted semen sample.

(ii) In this subgroup of diluted semen sample 2 microdrops of neutral red (0.1% strength) were added.

(iii) To this subgroup 2 microdrops of orange G (0.1% strength) were added.

(iv) To this sub-group 2 microdrops of sudan III (0.1% strength) were added.

Immediately after dilution and additions of colour additives the semen sample number, it group and subgroup number were written on the test tubes.

Evaluation of diluted semen was done at [illegible]o, 24, 48 and 72 hours of preservation for Hydrogen-ion concentration, motility of sperms and percentage of live sperms of both the extenders in all subgroups.

To find out the hydrogen-ion concentration and live sperm percentage the methods which have described in neat semen

were adopted.

Motility of sperms.

For grading the individual motility of sperms the movements of individual sperms were taken into consideration. The semen was diluted 100 times for this purpose. A droplet of diluted semen was taken on a clean glass slide, gently warmed upto 32°C, covered with a cover slip and examined under high power (40x) of the microscope. Sperms with progressive motility only were considered. Motility of all the groups and subgroups were examined at o, 24, 48 and 72 hours of preservation.

The motility grading after dilution was done according to the table of Herman and Swanson (1941) as given below: -

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.

Grade	Percentage of live sperm	Movement
2.5 to 3.0	50 to 60% motile sperms	Wave formation with slight whorls which moves slowly across the field.
3.0 to 3.5	60 to 70% motile sperms.	Rapid vigorous movement, waves and whorls or eddies form and change with rapidity.
3.5 to 4.0	70 to 80% motile sperms.	Rapid vigorous movement, waves and whorls or eddies form and change with rapidity.
4.0 to 4.5	80 to 90% motile sperms.	Movement and churning of the swrirls 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.

Preservation of semen.

The tube was wrapped in cotton wool and put in another bigger test tube labelled with sample number group and subgroup number. The test tubes were kept in a beaker half filled with water at 25 to 30°C and kept in a refrigerator for the gradual cooling. After 2 to 3 hours when the water attainded 4°C temperature it was removed and tubes were kept in the empty beaker. On the beaker the hours of preservation were marked.

After 24, 48 and 72 hours respectively the corresponding beaker was taken out. The semen tubes were thawed one by one by keeping them between the palms till it attainded the body temperature. The tubes were placed in water bath at 30°C and the samples were again examined.

Statistical analysis.

Statistical analysis of data were done as per Snedecor and Cochran (1967).

R E S U L T S

R E S U L T S

NEAT SEMEN

Colour :

The colour of the semen obtained from non-descript local bucks from different collections was found to be yellowish (Table - I).

Volume :

The average volume of ejaculate was recorded to be 0.47 ± 0.06 ml (Table - I).

Hydrogen-ion concentration :

The mean pH of neat semen, on the basis of study of 50 samples from different ejaculates was found to be 6.68 ± 0.53 (Table - I).

Sperm concentration per ml :

In the present study the mean concentration of sperms per ml turned out to be 3963 ± 83.62 millions on the basis of 50 samples of semen from different ejaculates (Table-I).

Mass activity :

Based on 50 semen samples obtained from different ejaculates the mass activity of sperms in neat semen was found to be 81.23 ± 1.02 per cent (Table - I).

TABLE - I.

Mean and S.E. of different attributes of neat semen of local non-descript bucks.

No. of observations	Colour	Volume in ml	Concentration of sperms millions/ml	pH	Mass activity percentage of sperms	Live percentage of sperms
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
50	Yellowish	0.47±0.06	3963 ± 85.62	6.68±0.53	81.23 ± 1.02	86.32 ± 2.76

Live sperm percentage :

In the present study the mean live percentage of sperms in buck semen was found to be 86.32 ± 2.76 (Table-I).

VARIOUS ATTRIBUTES OF SEMEN IN EGG YOLK CITRATE AND WHOLE COW MILK DILUENTS WITH DIFFERENT COLOUR ADDITIVES

Hydrogen-ion concentration.

pH in EYC extender :

The mean pH of semen diluted in egg yolk citrate extender and with no dye additive at 0, 24, 48 and 72 hours of preservation was found to be 6.65 ± 0.18, 6.52 ± 0.15, 6.29 ± 0.32, and 6.12 ± 0.11 respectively (Table - II).

With neutral red dye additive in egg yolk citrate extender the mean pH values obtained were 6.65 ± 0.29, 6.51 ± 0.19, 6.28 ± 0.26 and 6.10 ± 0.21 under 0, 24, 48 and 72 hours of preservation respectively (Table - II).

In case of addition of orange G in egg yolk citrate extender at 0, 24, 48 and 72 hours of preservation, the mean pH values were recorded to be 6.62 ± 0.38, 6.47 ± 0.18, 6.28 ± 0.17 and 6.08 ± 0.23 respectively (Table - II).

With the addition of sudan III dye in egg yolk citrate the mean pH at 0, 24, 48 and 72 hours of preservation was recorded to be 6.60 ± 0.16, 6.46 ± 0.22, 6.27 ± 0.11 and 6.01 ± 0.26 respectively (Table - II).

The mean pH values of diluted semen samples in egg

TABLE - II.

Mean pH values with S.E. of buck semen in Egg Yolk Citrate (E.Y.C.) diluent with different dyes at different hours of preservation.

Hours of preservation	Number of observations	No dye additive	2 microdrops of 0.1% neutral red dye in 1 ml of diluted semen	2 microdrops of 0.1% orange G dye in 1 ml of diluted semen	2 microdrops of 0.1% sudan III dye in 1 ml of diluted semen.
		Mean±S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	6.65±0.18	6.65 ± 0.29	6.62 ± 0.38	6.60 ± 0.16
24	50	6.52±0.15	6.51 ± 0.19	6.47 ± 0.18	6.46 ± 0.22
48	50	6.29±0.32	6.28 ± 0.26	6.28 ± 0.17	6.27 ± 0.11
72	50	6.12±0.11	6.10 ± 0.21	6.08 ± 0.23	6.01 ± 0.26

yolk citrate with different colour additives at different hours of preservation have been tabulated in Table - II.

pH in whole cow milk extender :

At 0, 24, 48 and 72 hours of preservation with no addition of dyes in cow milk extender the seminal pH was found to be 6.59 ± 0.58, 5.89 ± 0.69, 5.76 ± 0.71 and 5.63 ± 0.61 respectively (Table - III).

At 0, 24, 48 and 72 hours of preservation in the case of the sub-group containing neutral red additive in the milk diluent the seminal pH was recorded to be 6.53 ± 0.69, 5.88 ± 0.73, 5.75 ± 0.59 and 5.61 ± 0.68 respectively (Table - III). But in the case of addition of orange G and at the same hours of preservation i.e. from 0 to 72 hours the seminal pH was recorded to be 6.50 ± 0.78, 5.80 ± 0.63, 5.80 ± 0.73 and 5.58 ± 0.57 respectively (Table - III).

With the addition of Sudan III dye in semen diluted in milk diluent and preserved at 0, 24, 48 and 72 hours the seminal pH was recorded to be 6.52 ± 0.61, 5.80 ± 0.67, 5.72 ± 0.78 and 5.52 ± 0.62 respectively.

The pH values of diluted semen in cow milk extender with different colour additives under different hours of preservation have been incorporated in Table - III.

The analysis of variance could not reveal any significant difference in seminal pH between hours of preservation, between diluents or between additive dyes and in other interactions (Table - IV).

TABLE - III.

Mean seminal pH values with S.E. at different hours of preservation with different colour additives in whole cow milk extender.

Hours of preservation	Number of observations	No dye additive	2 microdrops of 0.1% neutral red dye in 1 ml of diluted semen	2 microdrops of 0.1% orange G dye in 1 ml of diluted semen	2 microdrops of 0.1% sudan III dye in 1 ml of diluted semen
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	6.59 ± 0.58	6.53 ± 0.69	6.50 ± 0.78	6.52 ± 0.61
24	50	5.89 ± 0.69	5.88 ± 0.73	5.80 ± 0.63	5.80 ± 0.67
48	50	5.76 ± 0.71	5.75 ± 0.59	5.80 ± 0.73	5.72 ± 0.78
72	50	5.63 ± 0.61	5.61 ± 0.68	5.58 ± 0.57	5.52 ± 0.62

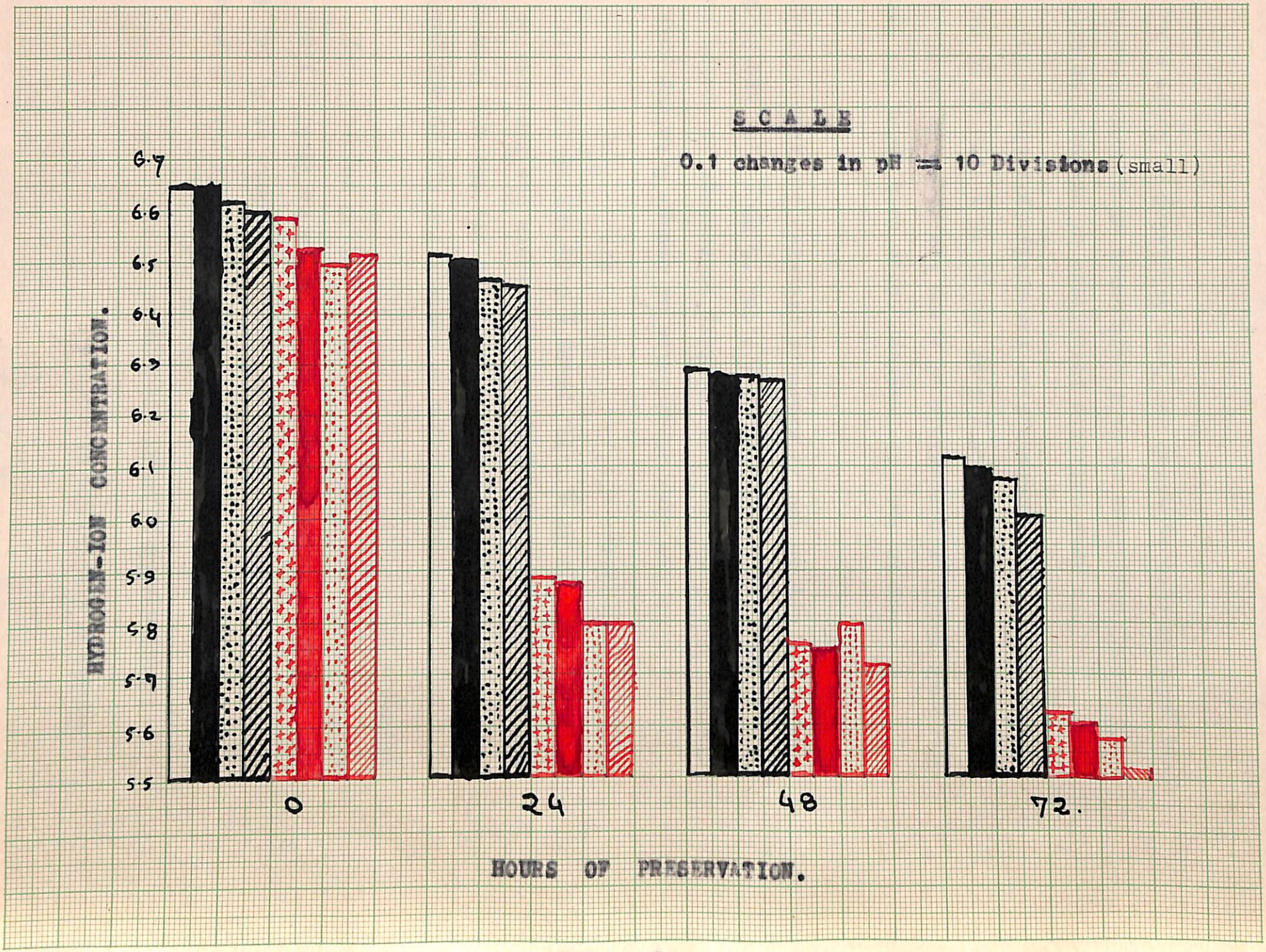
SCALE
0.1 changes in pH = 10 Divisions (small)
HYDROGEN-ION CONCENTRATION.
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5
0
24
48
72.
HOURS OF PRESERVATION.

FIG. - I.

Motility percentage of sperms.

In egg yolk citrate extender :

In the no treatment subgroup in egg yolk citrate extender the mean motility percentage of sperms at 0, 24, 48 and 72 hours of preservation was noted to be 74.87 ± 1.88, 58.59 ± 1.81, 34.18 ± 1.67 and 23.98 ± 1.02 respectively (Table - V).

With the addition of neutral red dye in egg yolk citrate extender and preservation at 0, 24, 48, and 72 hours the mean motility percentage in buck semen was recorded to be 74.12 ± 1.15, 58.72 ± 1.78, 36.25 ± 0.89 and 26.12 ± 2.12 respectively (Table - V).

In the case of addition of orange G in egg yolk citrate extender, the mean motility percentage of sperms turned out to be 73.82 ± 1.67, 58.53 ± 1.66, 35.83 ± 1.01 and 26.02± 1.73 at 0, 24, 48 and 72 hours of preservation respectively (Table - V).

But in the EYC subgroup added with sudan III dye the mean motility percentage of sperm was found to be 70.13± 2.19, 52.35 ± 1.91, 33.64 ± 0.92 and 23.76 ± 1.32 at 0, 24, 48 and 72 hours of preservation respectively (Table - V).

The mean motility percentage of sperms with different colour additives at different hours of preservation in egg yolk citrate extender has been tabulated in Table - V.

TABLE - V.

Mean motility percentage of sperms along with S.E. in buck semen at different hours of preservation with different colour additives in egg yolk citrate (E.Y.C.) extender.

Hours of preservation	Number of observations	No dye additive	2 microdrops of 0.1% neutral red dye in 1 ml of diluted semen	2 microdrops of 0.1% orange G dye in 1 ml of diluted semen	2 microdrops of 0.1% sudan III dye in 1 ml of diluted semen
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	74.87 ± 1.88	74.12 ± 1.15	73.82 ± 1.67	70.13 ± 2.19
24	50	58.59 ± 1.81	58.72 ± 1.78	58.53 ± 1.66	52.35 ± 1.91
48	50	34.18 ± 1.67	36.25 ± 0.89	35.83 ± 1.01	33.64 ± 0.92
72	50	23.98 ± 1.02	26.12 ± 2.12	26.02 ± 1.73	25.76 ± 1.32

In whole cow milk extender :

In no dye addition subgroup, at 0, 24, 48 and 72 hours of preservation in the cow milk extender the mean motility percentage of sperms was found to be 64.21 ± 2.18, 40.98 ± 1.12, 27.81 ± 0.96 and 15.77 ± 0.76 respectively (Table - VI).

In the case of neutral red dye subgroup, at 0, 24, 48 and 72 hours of preservation the mean motility percentage of sperms turned out to be 66.31 ± 1.39, 43.95 ± 0.91, 29.47± 0.98 and 16.82 ± 0.21 respectively (Table - VI).

With the addition of orange G dye in the milk diluter group the mean motility percentage of sperms was recorded to be 66.16 ± 1.88, 42.86 ± 1.91, 29.01 ± 1.23 and 15.97 ± 1.00 respectively (Table - VI).

With the addition of sudan III dye in milk dilutor group the mean sperm motility percentage at 0, 24, 48 and 72 hours of preservation was found to be 63.89 ± 1.73, 39.17 ± 1.63, 25.89 ± 1.07 and 13.76 ± 0.57 respectively (Table - VI).

The mean motility percentage of sperms at different hours of preservation with different dyes in cow milk extender has been tabulated in Table - VI.

Statistical analysis revealed highly significant differences in mean motility percentage of sperms between hours of preservation as well as between extenders. All other treatments and interactions turned out to be non-significant (Table-VII).

TABLE - VI.

Mean motility percentage along with S.E. of sperms in buck semen with different colour additives at different hours of preservation in whole cow milk extender.

Hours of preservation	Number of observations	No dye additive Mean±S.E.	2 microdrops of 0.1% neutral red dye in 1 ml of diluted semen Mean ± S.E.	2 microdrops of 0.1% orange G dye in 1 ml of diluted semen Mean ± S.E.	2 microdrops of 0.1% sudan III dye in 1 ml of diluted semen. Mean ± S.E.
0	50	64.21±2.18	66.31 ± 1.39	66.16 ± 1.88	63.89 ± 1.73
24	50	40.98 ±1.12	43.95 ± 0.91	42.86 ± 1.91	39.17 ± 1.63
48	50	27.81 ±0.96	29.47 ± 0.98	29.01 ± 1.23	25.89 ± 1.07
72	50	15.77 ±0.76	16.82 ± 0.21	15.97 ± 1.00	13.76 ± 0.57

TABLE - VII.

Analysis of variance showing the effects of different hours of preservation, different extenders, different additives and their interactions on the motility percentage of sperms in buck semen.

Sources of variation	df	M. S.
Between extenders.	1	713.32**
Between hours of preservation.	3	589.34**
Between dyes.	3	125.67 NS
Between extender x hours of preservation.	3	79.67 NS
Between extender x dyes.	3	68.35 NS
Between hours of preservation x dyes.	9	79.46 NS
Between extenders x hours of preservation x dyes.	9	37.81 NS
Error	768	93.81
Total:	799	

** denotes significance at 1% level.
NS denotes non-significance.

S C A L E

1% change in Motility = 1 Division(small)

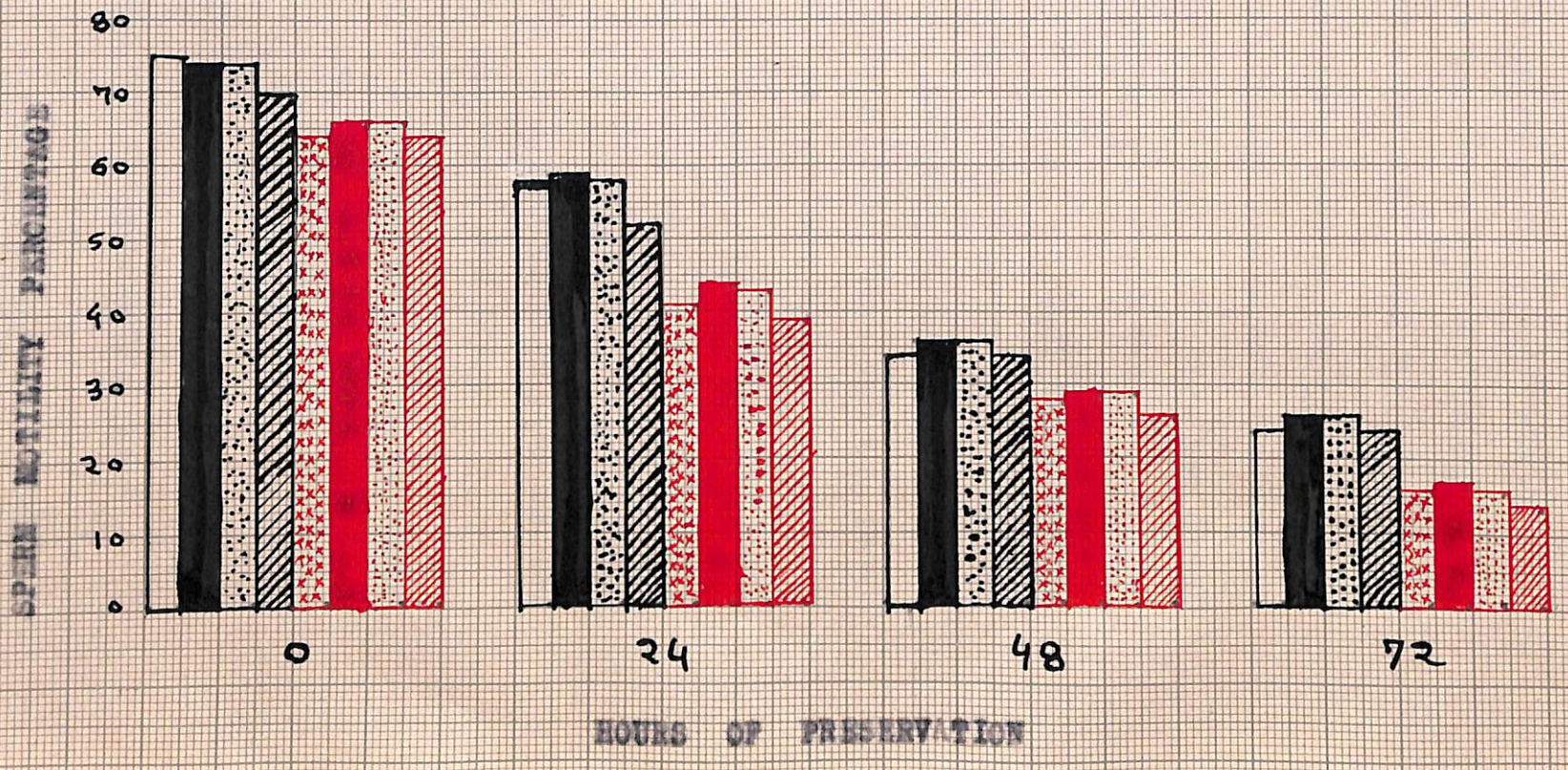

FIG. - II

Showing sperm motility percentage at different hours of preservation.

EYC group :

(i) No addition of dyes.

(ii) 2 microdrops of Neutral red (0.1% solution) per ml of diluted semen.

(iii) 2 microdrops of Orange G (0.1% solution) per ml of diluted semen.

(iv) 2 microdrops of Sudan III (0.1% solution) per ml of diluted semen.

Whole Cow milk group :

(i) No addition of dyes.

(ii) 2 microdrops of Neutral red (0.1% solution) per ml of diluted semen.

(iii) 2 microdrops of Orange G (0.1% solution) per ml of diluted semen.

(iv) 2 microdrops of Sudan III (0.1% solution) per ml of diluted semen.

Live sperm percentage.

In egg yolk citrate extender :

In no dye addition subgroup, at 0, 24, 48 and 72 hours of preservation in egg yolk citrate extender, the mean live sperm percentage was found to be 86.19 ± 2.36, 79.02 ± 2.13, 61.17 ± 1.68 and 40.32 ± 0.99 respectively (Table-VIII).

Under the treatment subgroup with neutral red dye in egg yolk citrate extender, the mean live sperm percentages was recorded to be 86.29 ± 1.97, 80.06 ± 1.87, 63.72 ± 2.10 and 45.76 ± 1.12 at 0, 24, 48 and 72 hours of preservation respectively (Table - VIII).

But in the case of egg yolk citrate extender subgroup treated with orange G dye, the mean live sperm percentage at 0, 24, 48 and 72 hours of preservation was found to be 85.88 ± 2.14, 80.01 ± 1.96, 62.40 ± 1.98 and 44.87 ± 1.24 respectively (Table - VIII).

With sudan III dye in egg yolk citrate extender group the mean live sperm percentage at 0, 24, 48 and 72 hours of preservation turned out to be 85.68 ± 1.92, 79.63 ± 1.73, 60.87 ± 1.76 and 40.66 ± 0.49 respectively (Table - VIII).

The mean live sperm percentage in the case of different colour additives and at different hours of preservation in egg yolk citrate extender has been incorporated in Table - VIII.

TABLE - VIII.

Mean live sperm percentage along with S.E. in buck semen in egg yolk citrate (E.Y.C.) extender at different hours of preservation with addition of different dyes.

Hours of preservation	Number of observations	No dye additive	2 microdrops of 0.1% neutral red dye in 1 ml of diluted semen	2 microdrops of 0.1% orange G dye in 1 ml of diluted semen	2 microdrops of 0.1% sudan III dye in 1 ml of diluted semen
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	86.19±2.36	86.29 ± 1.97	85.88 ± 2.14	85.68 ± 1.92
24	50	79.02±2.13	80.06 ± 1.87	80.01 ± 1.96	79.63 ± 1.73
48	50	61.17±1.68	63.72 ± 2.10	62.40 ± 1.98	60.87 ± 1.76
72	50	40.32 ±0.99	45.76 ± 1.12	44.87 ± 1.24	40.66 ± 0.49

In whole **cow milk extender** :

In cow milk extender with no dye addition at 0, 24, 48 and 72 hours of preservation, the mean live sperm percentage was obtained to be 71.86 ± 0.89, 60.92 ± 0.77, 40.14 ± 0.63 and 30.39 ± 0.36 respectively (Table - IX).

With neutral red dye addition in milk extender, the mean live sperm percentage of buck semen at 0, 24, 48 and 72 hours of preservation was recorded to be 71.80 ± 0.83, 63.67± 1.19, 45.72 ± 0.78 and 36.82 ± 0.38 respectively (Table - IX).

In the case of orange G dye addition subgroup, the mean live sperm percentage at 0, 24, 48 and 72 hours of preservation was found to be 71.63 ± 1.14, 62.83 ± 1.41, 45.16 ± 0.69 and 36.08 ± 0.26 respectively (Table - IX).

In the case of subgroup containing sudan III dye, at 0, 24, 48 and 72 hours of preservation the mean live sperm percentage was observed to be 71.15 ± 0.87, 60.86 ± 0.93, 40.04 ± 0.58 and 30.43 ± 0.32 respectively (Table - IX).

The mean live sperm percentage with different colour additives and at different hours of preservation in cow milk extender has been incorporated in Table - IX.

The analysis of variance revealed highly significant differences in the mean live sperm percentage between extenders and between hours of preservation. All other treatments and interactions showed non-significant differences (Table - X).

TABLE - IX.

Mean live sperm percentage along with S.E. of buck semen in whole cow milk extender at different hours of preservation with different colour additives.

Hours of preservation	Number of observations	No dye additive	2 microdrops of 0.1% neutral red dye in 1 ml of diluted semen	2 microdrops of 0.1% orange G dye in 1 ml of diluted semen	2 microdrops of 0.1% sudan III dye in 1 ml of diluted semen
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0	50	71.86±0.89	71.80 ± 0.83	71.63 ± 1.14	71.15 ± 0.87
24	50	60.92±0.77	63.67 ± 1.19	62.83 ± 1.41	60.86 ± 0.93
48	50	40.14±0.63	45.72 ± 0.78	45.16 ± 0.69	40.04 ± 0.58
72	50	30.29±0.36	36.82 ± 0.38	36.08 ± 0.26	30.43 ± 0.32

TABLE - X.

Analysis of variance showing the effects of extenders, addition of different dyes and different hours of preservation and their interactions on the live sperm percentage in buck semen.

Sources of variation	df	M. S.
Between extenders.	1	1632.17**
Between hours of preservation.	3	1011.23**
Between dyes.	3	183.27 NS
Between extenders x hours of preservation.	3	111.36 NS
Between extenders x dyes.	3	78.32 NS
Between hours of preservation x dyes.	9	69.91 NS
Between extenders x hours of preservation x dyes.	9	112.76 NS
Error.	768	306.77
Total:	799	

** denotes significance at 1% level.
NS denotes non-significance.

FIG. - III

Showing live sperm percentage at different hours of preservation.

EYC group :

(i) No addition of dyes.

(ii) 2 microdrops of Neutral red (0.1 % solution) per ml of diluted semen.

(iii) 2 microdrops of Orange G (0.1% solution) per ml of diluted semen.

(iv) 2 microdrops of Sudan III (0.1% solution) per ml of diluted semen.

Whole Cow milk group :

(i) No addition of dyes.

(ii) 2 microdrops of Neutral red (0.1% solution) per ml of diluted semen.

(iii) 2 microdrops of Orange G (0.1% solution) per ml of diluted semen.

(iv) 2 microdrops of Sudan III (0.1% solution) per ml of diluted semen.

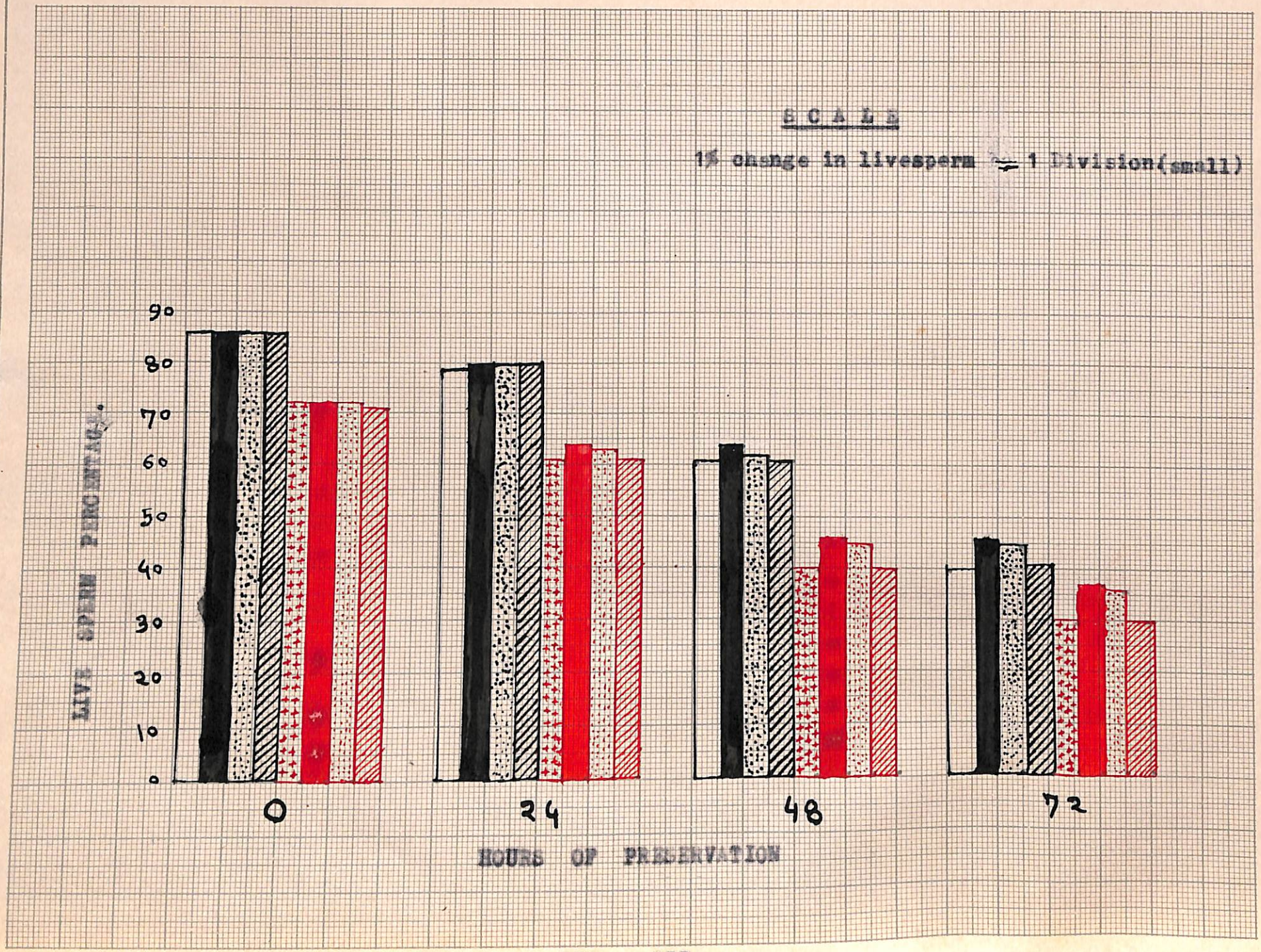
SCALE
1% change in livesperm = 1 Division(small)
LIVE SPERM PERCENTAGE
90
80
70
60
50
40
30
20
10
0
0
24
48
72
HOURS OF PRESERVATION

FIG. - III.

D I S C U S S I O N

NEAT SEMEN

Ejaculate volume.

On the basis of 50 observations taken from 5 non-descript bucks, the average ejaculate volume was recorded to be 0.47 ± 0.06 ml. The semen was collected at intervals of one week. Shukla and Bhattacharya (1952) found the ejaculate volume of buck to range from 0.46 to 0.78 ml whereas Mukherjee *et al*. (1953) reported the mean ejaculate volume of buck to be 0.54 ± 0.04. It seemed that the ejaculate volume obtained by Shukla and Bhattacharya (loc. cit.) was rather high. The value obtained in the present study seemed to agree with the values reported by other workers in the field. In Barbari and Jamnapari bucks the average ejaculate volume was found out by Sahni and Roy (1969) to be 0.65 and 0.89 ml respectively. This was a higher value in comparison to that reported in the present study. The small body size of the local nondescript bucks used in the present study might be the cause that the disagreement in the findings have arisen. Kurian and Raja (1965) working with Malabari bucks found the ejaculate volume to vary from 0.4 to 1.2 ml. Among the other Indian workers in the field, Mittal and Pandey (1972) reported the seminal volume per ejaculate of Barbari bucks to vary from 0.433 ± 0.134 to 0.664 ± 0.249 as against that from Jamnapari bucks which varied from 0.69 to 0.88 ml. Tiwari *et al*. (1968) reported the mean volume per

ejaculate in case of Sannen, Jamnapari, Barbari and Sannen x Jamnapari crosses to be 0.997 ± 0.055, 1.36 ± 0.05, 0.94 ± 0.01 and 1.37 ± 0.07 ml respectively. In Jamnapari bucks, Patel (1967), and Mishra and Sengupta (1965) recorded the mean seminal volume per ejaculate to be 0.815 and 0.65 ml respectively. Sharma et al. (1957) reported the ejaculate volume in Betal bucks to vary from 0.2 to 1.2 cm^3. Roy (1975) observed 0.46 ± 0.01 ml to be the average ejaculate volume in the case of nondescript bucks in Bihar.

Dussardier and Szumowski (1952) recorded the average volume of buck semen per ejaculate to be 0.6 ml whereas Knoblauch (1962) found the average ejaculate volume to be 1.0 ml in White German improved bucks. Working with Toggerburg goats, Eaton and Simmons (1952) showed that the average volume of seminal ejaculate was 0.65.

The present finding seemed to be very close to that reported by Roy (loc. cit.) and Mukherjee et al. (loc. cit.). But wide variation from those reported by Sahni and Roy, Kurian and Raja, Dussardier and Szumowski, Mittal and Pandey, Knoblauch, Austin et al., Eaton and Simmons, Misra and Sengupta, Patel and Tiwari et al. (loc. cit.) were recorded. The wide differences in the findings of the different workers might be due to the variables like season, feeding and managemental practices, and breed differences.

Colour.

Though the present study revealed the colour of the buck semen to be yellowish, this did not conform with the

findings of Shukla and Bhattacharya (loc. cit.) who reported creamy colour of bucks semen. Ivory colour of buck semen with creamy consistency was observed by Knoblauch (loc. cit.). In the case of Sannen goat in Zeeland, Wagenaar (loc. cit.) reported yellow thick semen with high motility in fertile goats whereas watery, thin in infertile ones. In Jamnapari buck semen, Patel (loc. cit.) recorded creamy to slight yellow colour. According to Roy (loc. cit) the colour of buck semen was yellowish, though light and deep yellow colours were also seen.

Hydrogen-ion concentration.

In the present study the average pH of neat semen of local nondescript bucks was recorded to be 6.68 ± 0.53 as against 6.67 ± 0.28 reported by Roy (loc. cit.). The range of pH of neat semen was reported to be 6.3 to 6.7 in case of Malabari bucks by Kurian and Raja (loc. cit.). According to Patel (loc. cit.) seminal pH range in Jamnapari bucks was 6.2 to 6.8 and the average pH was 6.5. Shukla and Bhattacharya (loc. cit.) showed the pH of buck semen to be 6.2 to 6.5. In the case of exotic bucks, Dussardier and Szumowski (loc. cit.) observed the mean seminal pH to be 6.5 as against the finding of Knoblauch (loc. cit.) which was 6.6 in White German improved bucks. The variation in pH reading of buck semen, as has been recorded by different workers in the field might be due to different factors like differences in feeding, managemental practices, sequence of ejaculate, breed difference and influence of season etc.

Mass activity.

The average mass activity in freshly collected semen of nondescript bucks was recorded to be 81.23 ± 1.02% (Table - I). Dussardier and Szumowski (loc.cit.) reported 75% motility in buck semen. The present finding seems to confirm with the findings of above workers. But the value obtained by Roy (loc. cit.) was 78.60 ± 0.61% in local nondescript bucks. But their results were much lower than that in the present case. Whereas Tiwari et al. (loc. cit.) reported the mass activity in the cases of Jamnapari, Sannen x Jamnapari and Barbari bucks to be 4.6 ± 0.09, 5.0 ± 0.0 and 4.2 ± 0.11 respectively. Patel (loc. cit.) reported +5 motility in Jamnapari buck semen whereas Sahni and Roy (1967) found the initial motility in Barbari bucks to be 4.7 to 4.9. In the case of Jamnapari bucks the initial motility as reported by Misra and Sengupta (loc. cit.) was 2.3 ± 0.50. This seemed to be very low. Kurian and Raja (loc. cit.)found 60 to 90% motility in the semen of Malabari bucks. Mittal and Pandey (loc. cit.) reported 3.5 ± 0.172 to 3.75 ± 0.114 and 3.88 ± 1.027 to 3.96 ± 1.102 spermatozoa motility in Barbari and Jamnapari bucks respectively.

The present finding seemed to tally with those reported by Roy (loc. cit.) and Dussardier and Szumowski (loc. cit.) but it differed from the findings of Shukla and Bhattacharya, Sahni and Roy, Mittal and Pandey and Tiwari et al. (loc. cit.). Differences in breed, feeding standards, managemental practices, season and place might be attributed

to be the causes of variation in the results obtained by different workers.

Sperm concentration.

The present investigation showed the mean sperm concentration in neat semen of nondescript local bucks to be 3963 ± 83.62 millions per ml (Table - I). The value for this trait as recorded by Roy (loc. cit.) was 3658.5 ± 30.38 millions per ml which appears to be nearer to the findings of the present study. In the case of Barbari and Jamnapari bucks, the values obtained by Sahni and Roy (loc. cit.) was 1908 and 2241 millions per ml respectively. Shukla and Bhattacharya (loc. cit.) reported the sperm concentration in buck semen to be 1726 to 3240 millions per ml as against 5368 ± 247.88 millions per ml as recorded by Mukherjee (loc. cit.). Tiwari et al.(loc. cit.) obtained the average sperm concentration in neat buck semen from Jamnapari, Sannen x Jamnapari and Barbari to be 3728.3 ± 192.4, 4995.5 ± 159.8 and 3650.2 ± 155.3 millions per ml respectively. In case of Betal bucks,Sharma et al.(1957) found the sperm concentration to be 5424.7 millions per ml. Misra and Bengupta (loc. cit.) reported the sperm concentration in Jamnapari bucks to be 4080 ± 450 millions per ml. 2.724 billions per cm^3 was the value reported by Eaton and Simmons (loc. cit.). Wagenaar (1946) found the concentration of sperm in fertile Sannen bucks to be 1540 millions per cm^3. Mittal and Pandey (1972) working on Jamnapari and Barbari bucks found the sperm concentration to be 2674 ± 99.7 to 2788±153.6

and 2256.6 ± 74.09 to 2492 ± 101.3 millions per ml respectively. The average sperm concentration was registered to be 3600 millions per ml by Dussardier and Szumowski (loc. cit.).

The present finding seemed to tally with those of Roy, and Dussardier and Szumowski (loc. cit.), although wide variations from the values obtained by Shukla and Bhattacharya, Mukherjee et al., Kurian and Raja and Misra and Sengupta (loc. cit.) have been recorded. This might be due to differences in breed, feeding and managemental practices, seasonal influence, frequency of semen collection or due to methodological differences.

Live sperm percentage.

In the case of neat semen the average live sperm percentage was recorded to be 86.32 ± 2.78 (Table - I) as against 87.81 ± 1.32 as reported by Roy (loc. cit.) in case of local non-descript bucks. Mittal and Pandey (loc. cit.) reported this value to be 70% in Barbari bucks. In the case of Jamnapari bucks the live sperm percentage as reported by Misra and Sengupta (loc. cit.) was 54 ± 4.9 against 72-73% recorded by Mittal and Pandey (loc. cit.) and 90.7% reported by Patel (loc. cit.) in the same breed. In the case of White German improved bucks, Knoblauch (1962) found 80% live sperms. In Malabari bucks mean live sperm percentage was recorded to be as high as 85 to 95% by Kurian and Raja (loc. cit.). The great divergence in the results reported by different workers might be due to variable factors like breed, methods of semen

collection, feeding and management practices and agroclimatic conditions etc. The present study seems to agree with the high value of live sperm percentage in neat semen (90.7%) as obtained by Patel (loc. cit.) in Jamnapari bucks.

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

Hydrogen-ion concentration of semen.

In egg yolk citrate :

It appeared that with increasing hours of preservation in EYC irrespective of addition of dyes there was a fall in seminal pH (Table - II), but it was not found to be statistically significant. In the case of no dye addition subgroup, the fall in pH was from 6.65 to 6.12, a difference of 0.53 from 0 to 72 hours. In the case of neutral red subgroup also the pH fell down from 6.65 to 6.10 during 0 to 72 hours of preservation. In the case of orange G addition subgroup the seminal pH fell from 6.62 to 6.08 during 0 to 72 hours of preservation, whereas in the case of Sudan III treated subgroup the fall in pH during 0 to 72 hours was 0.59 (Table - II).

The analysis of variance revealed no statistically significant difference between extenders, between hours of preservation and between dye additives. All the interactions also turned out to be non-significant (Table - IV).

In whole cow milk extender :

In the case of milk extender the pH in general was

found to be lesser in every treatment subgroup than those in egg yolk citrate extender. But this difference was also found to be non-significant. Besides, the order of fall in seminal pH in milk extender was higher than in the case of egg yolk citrate diluent. But this difference in the two dilutor groups was also found to be non-significant (Table - IV). As is expected, with increasing hours of preservation the pH became more acidic. This might be due to formation and accumulation of lactic acid (Table - III and IV) consequent upon metabolic activities of sperms.

Motility percentage of sperms.

In egg yolk citrate extender :

Sharpest fall in the motility percentage in egg yolk citrate group was recorded during 24 to 48 hours of preservation in all the treatment subgroups and this was from 18.71 to 22.70%. The lowest fall in motility percentage was observed during 48 to 72 hours of preservation and it was from 9.81 to 10.13%. The fall in motility percentage during 0 to 24 hours was recorded to be in between the above two and it was from 17.28 to 15.29% (Table - V).

In whole cow milk extender :

In the case of milk extender, the fall in motility percentage was very sharp from 0 to 24 hours of preservation and it was 22.36 to 24.72%, irrespective of addition of dyes. The fall in motility percentage between 24 to 48 and 48 to 72

hours of preservation was almost of similar order and it was 13.17 to 14.52% during 24-48 hours and 12.04 to 13.03% during 48-72 hours of preservation (Table - VI).

The analysis of variance revealed highly significant difference between extenders and between hours of preservation. But addition of dyes did not seem to have any significant influence on the motility percentage of sperms (Table - VII). Although treatment with neutral red and orange G showed higher values for motility at 0, 24, 48 and 72 hours of preservation in comparison to Sudan III subgroups, the difference was statistically non-significant and it was concluded that addition of dyes had no influence on sperm motility (Table VI and VII).

Live sperm percentage.

In egg yolk citrate extender :

Live sperm percentage showed sharpest fall with increasing hours of preservation. The fall was maximum in case of no treatment subgroup from 0 to 72 hours. The sharpest fall to be recorded was during 48 to 72 hours. In case of neutral red subgroup also the sharpest fall was recorded during 48 to 72 hours. Orange G dye treated subgroup also showed the sharpest fall almost equally during 24-48 and 48-72 hours of preservation. In the case of Sudan III subgroup also the sharpest fall was observed in between 48 to 72 hours.

Among the dyes, neutral red proved to be the best and Sudan III the worst, but no statistically significant

difference could be found due to the effect of the dyes (Table - VIII).

In whole cow milk extender :

In no additive subgroup the fall in live sperm percentage was maximum during 24 to 48 hours. Similar was the case with neutral red, orange G and Sudan III treated subgroups (Table - IX).

The present observations are similar to the observations of Sahni and Roy (1971) who found that colour additives (neutral red, Sudan III and orange) could be used only for the identification of stored samples of goat semen. Colour additives did not produce any adverse effect on the keeping quality of goat semen upto 72 hours as noted in this experiment.

Phillips (1945), Almquist (1946), Tiwari and Sahni (1967) and Rao (1967) observed that dyes could be safely used for colouring diluted semen samples for identification purpose. In the present study it had been seen that dyes did not improve appreciably the keeping quality of goat semen but they also did not produce any detrimental effect. Hence, neutral red, orange G and Sudan III could be safely used for the colouring of goat semen for identification purposes.

It was also observed in the present study that whole cow milk was far inferior to EYC as a diluent and could not be recommended for use in the case of goats.

S U M M A R Y

S U M M A R Y

Experiments on the effect of addition of 3 dyes - neutral red, orange G and sudan III on different seminal attributes like pH, motility percentage and live sperm percentage of nondescript local buck were conducted.

Different ejaculate samples obtained from 5 nondescript local bucks were examined at 0, 24, 48 and 72 hours of preservation.

For evaluation of neat semen, volume, colour, mass activity, pH sperm concentration and live sperm percentage were examined.

Effect of different dilutors and hours of preservation on seminal pH of bucks tested out to be non-significant. Treatment of semen with different dyes also revealed non-significant effect on pH in EYC as well as in whole cow milk diluted buck semen.

Highly significant effects of dilutors and hours of preservation were observed only in motility percentage and live percentage of sperms but addition of different dyes did not seem to have any significant influence on the diluted semen.

The addition of different dyes did not show any adverse effect on pH, motility percentage and live percentage of sperms, hence they are safe for use with buck semen for identification.

It was also noted that egg yolk citrate was a better diluent for buck semen than boiled whole cow milk.

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