"INFLUENCE OF ADDITION OF CYSTEINE HYDROCHLORIDE AND EDTA ON PRESERVATION OF JAMUNAPARI BUCK SEMEN"

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)
PUSA (SAMASTIPUR), BIHAR

In partial fulfillment of the requirements
FOR THE DEGREE OF

Master of Veterinary Science

(Animal Reproduction, Gynaecology & Obstetrics)

By

Dr. SANTOSH KUMAR

Registration No.- M/VOG/76/2007-08

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY & OBSTETRICS
BIHAR VETERINARY COLLEGE
PATNA - (BIHAR)

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BIHAR VETERINARY COLLEGE
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2011

Dedicated

To

Respected Guru

And

My Parent

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BIHAR VETERINARY COLLEGE, PATNA- 800 014
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Assistant professor(sr. Grade)
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CERTIFICATE-I

This is to certify that the thesis entitled ***"Influence of addition of cysteine hydrochloride and EDTA on preservation of Jamunapari buck semen"*** submitted in partial fulfillment of the requirements for the award of degree of Master of Veterinary Science **(Animal Reproduction, Gynaecology & Obstetrics)** of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa, Samastipur, is the record of bonafide research work carried out by **Dr. Santosh kumar, Registration No.: M/VOG/76/2007-08** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

This is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

25/6/2012

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Major Advisor

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DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

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CERTIFICATE-II

We, the undersigned members of the advisory committee of **Dr. Santosh kumar, Registration No.: M/VOG/76/2007-08,** a candidate for the degree of **Master of Veterinary Science** with major in **Animal Reproduction, Gynaecology & Obstetrics**, have gone through the manuscript of the thesis and agree with the thesis entitled"***Influence of addition of cysteine hydrochloride and EDTA on preservation of Jamunapari buck semen.***"** It may be submitted by **Dr. Santosh Kumar** in partial fulfillment of the requirements for the degree.

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CERTIFICATE-III

This is to certify that the thesis entitled ***"Influence of addition of cysteine hydrochloride and EDTA on preservation of Jamunapari buck semen"*** submitted by **Dr. Santosh kumar, Registration No.: M/VOG/76/2007-08** in partial fulfillment of the requirements for the Degree of Master of Veterinary Science **(Animal Reproduction, Gynaecology & Obstetrics)** of the **Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa, Samastipur,** was examined and approved on ..23..07.../2011.

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B.V.C., Patna ***(Santosh Kumar)***

Dated: 25.06.2018

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Chapter-I

INTRODUCTION

INTRODUCTION

Goat is very useful animal for mankind. It is also called poor man's cow but it has been accused to be enemy of crops. Its milk contains very small fat globules, easily digestable and useful for newborn baby and old age people. It is easy source of milk and meat. It can be housed in very small space and feed consumption is lower than that of cow & buffalo. India is a religious country where religious barrier is main constraint. Beef is prohibited for Hindu and pork is prohibited for Muslim community. This barrier is removed by goat meat (chevon) accepted very gladly by the all religions and harmony is maintained in the society. In Indian market, the cost of chevon is the highest among all meats. Over all goat as a source of revenue in terms of milk, meat, hide and fur production for our national economy is very much accountable.

According to 17th animal Census report of India in 2003 total population of goat was 12,436 crore about 25% of total livestock population of India. According to report of total world goat population, India has 17% of world goat population. The position of India in goat population is 2nd in world [FAO, 2003]. India having about 23 well defined goat breeds which is about 20% of the total goat population. The majorities (about 75%) of

the Indian goats are considered as mixed and non-descript in different state.

Jumunapari (also known as Jamanpari) is biggest and most majestic breed of goats in India, mostly found in Jamuna, and Chambal Valley. The breed has been extensively utilized to upgrade indigenous breeds of goat for milk and meat purpose in India.

Cross breeding has been accepted as an efficient way for genetic improvement of indigenous goat all over India. In India, goats are kept in large number by landless and poor farmers. An artificial insemination holds promise in the grading up programme. However, its application in goat breeding is yet to be carried out extensively but the application of artificial insemination technique in field condition for goat has not got momentum till now. Though artificial inseminations have been adopted with a limited success in some organized goat farms.

The productive efficiency of superior quality bucks could be most economically exploited if semen is stored with minimum deterioration of its fertilizing ability. To maintain the fertilizing ability of semen, suitable extenders and additives should be used.

Several techniques have been utilized to improve the motility as well as fertility of spermatozoa by incorporating various additives i.e. chelating agents,

sulphahydryl compound in fresh chilled and frozen semen of goats. Many investigators reported that the efficacy and suitability of certain additives i.e. EDTA and cysteine HCl in Improving quality of semen in terms of either morphology, preservability, freezability or fertility in cattle, ram and buffalo semen (Shannon and Curson, 1983; Saxena and Tripathi, 1984; Singh et al. 1989; Dhami and Sahni, 1994 and Kumar et al. 1996 a , 1996 b). EDTA is a powerful chelating agent which form stable compounds with heavy metals (Abdou and El Guindi, 1977). It is also postulated that EDTA protects against amino acid oxidase released by dead sperm and probably reduces cold shock also (Salisbury et al. 1978). The beneficial effect of cysteine HCl has been reported due to its restrictive effect on aerobic metabolism and stimulation of anaerobic metabolism (Sengupta et al. 1969).

The available literatures indicate a lack of information about the role of EDTA and cysteine HCl as additives in preservation of Jamunapari buck Semen.

Therefore, present study was undertaken with the following objectives.-

(i) Evaluation of Jamunapari buck Semen under agro climatic condition of Patna.

(ii) To observe the effect of cysteine hydrochlorides on preservability of Jamunapari buck Semen based on their seminal characteristics.

(iii) To observe the effect of EDTA on preservability of Jamunapari buck semen based on their seminal characteristics.

*****

Chapter-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Semen Characteristics :

Mukherjee et al. (1952) studied the semen quality of male goat and found the sperm concentration 5368.0±247.88 millions/ml and abnormal percentage of spermatozoa 3.35±0.40.

Shukla and Bhattacharya (1952) reported to be the average volume of buck semen 0.46 to 0.78 ml ,sperm concentration 1726 to 3240 millions/ml, mass motility 3.8 to 5.0, and abnormal sperm percentage 2.4 to 11.1.

Sharma et al. (1957) observed that in Betal buck average semen volume was 1.3 ml in winter; it was increasing with body size. Average Sperm concentration 5424.7 millions per ml.

Jelam and Nambiar (1965) found that in 14 semen samples of Jamunapari bucks, the average sperm motility was 72.3±3.98 percent and the percentage of unstained spermatozoa 72.0±5.74 percent. They also reported that when semen was diluted (1:20) in egg yolk citrate or goat milk and stored for 24 and 72 hrs. the average motility was 54±2.73 and 43.6±2.69 respectively and the

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 percent, pH 6.3-6.7, sperm concentration 2-3 millions per cm3, abnormal spermatozoa 6-12 percent and live spermatozoa 85-95 percent. They also reported that interval between collections did not affect semen characters.

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

Character	Ram	Buck
Volume (ml)	0.92±0.05	0.65±0.07
Initial Motility	81 ±0.42	82±0.50
Sperm concentration($x10^6$)	5313±786	4080±450
% of live sperm	87±1.8	54±4.9

Patel (1967) studied the characteristics of Jamunapari buck semen and found the average volume to be 0.815 ml, mass motility +5, concentration 1.5 millions/cmm, abnormal sperms 5 percent and live sperms 90.7%.

Tiwari et. al. (1968) in an assessment on the quality of semen of Barbari and saanen bucks recorded the sperm

millions/cmm, abnormal sperms 5 percent and live sperms 90.7%.

Tiwari et. al. (1968) in an assessment on the quality of semen of Barbari and saanen bucks recorded the sperm concentration as 2313.95±169.75 and 4652.14±205.58 millions/ml. and the percentage of live spermatozoa as 90.3 ±1.63 and 88.59 ±1.40 respectively.

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 percent, percentage of live spermatozoa 87.1 and 79.5 and abnormal sperm percentage 6.3 and 7.2 respectively

Sahni and Roy (1969) in a study on the quality of semen of Barbari and Jamunapari bucks recorded the average sperm concentration to be 1910 and 2241 millions /ml and the percentage of live spermatozoa to be 69.49 and 63.58., respectively.

Sharma and Arora, (1970). Studied the semen characteristics of Beetal bucks and observed the values for volume as 0.517±0.006 ml, sperm concentration 5166.27±176.55 millions per ml, live sperm percentage 88.70±1.30 and abnormal sperm percentage 8.58±1.47.

Highly significant difference between buck of Barbari and Jamnapari breeds in relation to semen production in terms of volume, sperm motility, sperm concentration and percentage of viable spermatozoa after collection was reported by Mittal and Pandey (1972

Igboeli (1974) found an average ejaculate volumes 0.67±0.03 and 1.34±0.05 ml in the native Zambian and Boer bucks, respectively, as the semen was collected twice weekly for six weeks. Ejaculate volume did not differ between first and second week of collection.

Koh (1975) recorded the average ejaculate volume to be 0.85±0.04 ml, sperm concentration 3974.8±130.8 millions per ml, motility 85.0±0.76 percent and percentage of abnormalities to be 3.11±0.28 for the semen of cross bred goats (Kambing x Jamunapari) aged 2-4 years.

Roy (1975) studied with 50 semen samples from 5 non-descript bucks and reported an 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.

Vinha (1975) reported that the sperm concentration was highest in summer (1752380 spermatozoa/mm3) and lowest in Autumn (1348636/mm3) ; in Anglo Nubian goat.

Kang and Chung (1976) observed that sperm concentration 9.86 x 108 and 16.02 x 108 /ml ($P<0.05$) of Korean native goats in Nov. – Dec. and July – Sept., respectively.

Patil and Raja, (1978) studied the semen characteristics of Malabari bucks and observed the ejaculate volume to be average 0.50 ml, sperm concentration to be 3534 millions/ml, percentage of live spermatozoa to be 61.38% and percentage of abnormal spermatozoa to be 4.34% respectively

Saxena and Tripathi, (1979) reported as the average volume 0.37 ml, sperm concentration 4795.0 x 106/ml, 77.65 percent live sperm and 6.84 percent abnormal sperm in Jamunapari bucks.

Mohan et al. (1980) mean values of sperm concentration per ml and total sperm count per ejaculate in Pashmina bucks were $35.21\pm1.18 \times 10^{8}$ and $21.50\pm0.92 \times 10^{8}$, respectively.

Prasad et al. (1980) studied the normal values for different semen characters of Saanen, Barbari and Jamunapari x Barbari cross bred goats and found the average ejaculate volume as 0.70 ± 0.022, 0.72 ± 0.032 and 0.69 ± 0.024 ml, sperm concentration 2820.90 ± 7.427, 2117.65 ± 32.445 and 2375.47 ± 7.095 millions/ml and live

sperm percentage 91.93±0.988, 92.53±0.292 and 91.07±0.544 respectively.

Saxena and Tripathi (1980) reported an avege mass motility of 3.78±0.07 (0-5 scale), sperm concentration 4795.0±292.97 x 106 /ml., percentage of live sperm 77.65 and percent abnormal sperm 6.84 in Jamnapari buck.

Sinha et al. (1981) observed in Jamunapari bucks, the average volume was 0.345±0.045 ml, mass motility was 4.020±0.145 and sperm concentration was 3600.146±318.145 millions/ml.

Sinha et al. (1981) found that season and age had a significant effect on sperm concentration (x 106/ml) of Jamunapari bucks, the range varied from 2986.95±333.33 in rainy season to 3837.08±435.99 in winter.

Mann (1981) reported that in African dwarf goats ejaculate volume to be average 0.77±0.26 ml, sperm concentration to be 3.22±1.22 x 109 /ml and percentage of morphologically abnormal spermatozoa to be 13.45±8.77 respectively.

Dabas et al. (1982) reported that average acrosomal abnormalities in Jamunapari bucks were 5.23 percent.

Mittal (1982) reported that in Barbari bucks ejaculate volume averaged 0.69 ml, sperm concentration averaged 2472 millions /ml and percentage of live spermatozoa was 72.9 percent.

Singh et al. (1982) in a study on the seminal quality of Jamunapari bucks recorded the mean ejaculate volume to be 0.86±0.09, live sperm percentage to be 80.90±2.32 and sperm concentration to be 2293±72.8 millions/ml.

Traldi (1983) reported the average sperm motility 90.6±6.14 percent at the onset of maturity and 90.5±5.16 percent at 12 months of age for Moxoto bucks.

Sinha and Singh, (1982) reported the average volume, mass motility, sperm concentration, live sperm percentage and abormal sperm percentage to be 0.446±0.011ml,4.439±0.065, 2440.15±40.085 millions / ml, 84.45±0.414 and 7.87±0.214 respectively in Black Bengal bucks. The corresponding values in Saanen were 0.72±0.016 ml, 4.507±0.048, 2780.30±3568 millions/ml, 85.21±0.402 and 6.196±0.273 respectively.

Baviskar (1985) reported average sperm concentration to be 3145.45± 25.11 millions/ml.; average live sperm count to be 84.22±0.26 percent and average percentage of total sperm abnormality to be 7.85±0.34 in Osmanabadi bucks.

Pandey et al. (1985) reported an average semen volume 0.96 ml. and 0.79 ml. and an average mass activity to be 89.66 and 78.85 percent, average live sperms were 82.23 and 71.44 percent and sperm concentration 4503.256±114.75 and 2280.05±116.3 millions/ml., in Saanen and Barbari bucks respectively.

Singh et al. (1985) conducted comparative studies on seminal quality of pure and cross bred bucks. They recorded the average values for sperm concentration, live sperm percentage and total abnormal sperm percentage to be 2619.58±213.024, 2910.33±283.893 and 2851.67±211.708 millions/ml; 91.07±1.103, 90.33±0.608 and 86.37±1.0 and 2.12±0.155, 2.0±0.178 and 2.11±0.11 respectively in Black Bengal, Jamunapari and Cross bred (Jamunapari x Black Bengal) bucks.

Memon et al. (1986) reported regarding collection of semen of bucks with artificial vagina and electro ejaculator & reported average percentage of normal acrosome as 96.0±1.0 and 92.0±3.0 respectively. Thereby indicating that average percentage of spermatozoa with normal acrosome was greater for samples collected by artificial vagina than by electro ejaculator (i.e. Bailey ejaculator).

Sinha (1986) reported the average values of seminal attributes, live sperm percentage, head abnormality

percentage, tail abnormality percentage and acrosomal abnormality percentage in Black Bengal bucks 83.15±0.64, 0.03±0.03, 2.78±0.14, 0.88±0.11 & in Jamnapari 83.52± 0.48 0.06±0.04, 2.85±0.12, 1.23±0.09 respectively & in Barbari 83.15 ± 0.56, 0.02 ± 0.02, 2.67 ± 0.16 and 1.21 ± 0.11 respectively.

Bakshi et al. (1987) reported that the sperm concentration of Angora and 1/8 exotic Angora × 7/8 local Angora goat as 2597.98±86.38 and 2810.45±96.77×106/ml., percent live spermatozoa as 86.69±1.4 and 86.68±1.10 respectively.

Mittal (1987) recorded the mean values for sperm concentration (x108) to be 28.12, 26.71, 26.21, 26.32 and 27.12 live sperm percentage 80.0, 80.8, 78.2, 82.3 and 79.1 and abnormal sperm percentage 6.21, 5.72, 6.83, 7.47 and 9.23 during spring, summer, monsoon, autumns and winter seasons respectively in indigenous and crossbred goats.

Patnaik et al. (1991) reported the average semen volume, average sperm concentration, percentage of live sperm and total sperm abnormalities as 0.44±0.24 ml.; 2309.17±95.89 millions per ml.; 83.83±1.02 and 5.42±0.31 percent respectively in Ganjan bucks.

Knoblauch (1992) reported an average ejaculate volume of German imported buck to be 1 ml., with creamy

colour and thick consistency, pH 6.6, forward progressive motility 80 percent and number of dead spermatozoa 20 percent.

Sinha (1992) made a comparative study on various seminal attributes of Bettal, Black Bengal and Cross bred bucks and found the mean sperm concentration 2788.194±27.826, 2550±18.066 and 2604.722±17.703 millions/ml, live sperm percentage 89.55±0.646, 85.61±0.549 and 87.65±0.403 and percentage of head and tail abnormality 0.083±0.031, 2.69±0.144, 0.111±0.035, 2.11±0.118 and 0.125±0.025, 2.82±0.091 respectively.

Singh (1992) conducted an exhaustive study on various seminal characteristics of Beetal, Black Bengal & Cross bred bucks and recorded the mean volume to be 1.27±0.039, 0.47±0.011 and 0.54±0.009 ml, mass activity 4.63±0.05, 4.25±0.043 and 4.36±0.073, sperm concentration 2793.33±11.715, 2546.30±20.842 and 2589.17±10589 millions/ml, live sperm percentage 89.72 0.533, 84.59 0.510 and 86.04 0.343 head abnormality percentage 0.75±0.092, 0.85±0.113 and 0.96±0.07 and tail abnormality percentage 3.04±0.127, 4.72±0.131 and 4.13±0.084 respectively in Beetal, Black Bengal and Cross bred bucks.

Das and Raj Konwar, (1993) reported to be average ejaculate volume was 0.79±0.02 ml, sperm concentration 4401.05±112.07 millions/ml, percentage of viable spermatozoa 90.25 and percentage of sperm abnormalities 5.89±0.06 in Beetal bucks

Puranik et al. (1993) reported average ejaculate volume as 0.56±0.3 ml. and 0.58±0.2 ml; percentage of motile spermatozoa 70.1±2.0 and 75.0±1.9; sperm concentration 3079±24.5 and 2763±86.3 (x106) per ml. for Osmanabadi and Crossbred bucks, respectively.

Singh and Raja (1995) reported to be percentage of live spermatozoa in Sirohi buck semen 84.80±0.05.

Ahmed et al. (1997) reported an average ejaculated volume, live percentage, mass activity and average spermatozoa concentration 0.77 ml.; 84.5, 3.19 (0-5 scale); 2.77 billions/cmm. of Saanen buck, in the autumn season.

2.2. Effect of EYC

Aehmelt and Brockmann (1955) studied semen samples from 4 male goats which were diluted in sperm sol and yolk buffer prepared from eggs of New Hamshire, Sussex, White Leghorn and Brown Leghorn. The diluted semen was stored at 6°C and examined daily. Spermatozoal motility and survival time were greater

when New Hampshire Yolk buffer was used, followed by Sussex, White Leghorn and Brown Leghorn in decreasing order.

Rathore and Mukherjee (1961) reported that when semen sample from Bikaneri ram was diluted 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 was significantly increased by storage. They further reported that semen diluted in sodium citrate with 10 or, 20 percent of egg yolk in which penicillin had been added and stored for 1 - 7 days at +30C showed 20 percent forward motility.

Saxena and Singh (1967) collected semen from 4 Bikaneri rams at weekly intervals for 6 weeks. 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 percent, 25 percent, 25 percent and 20 percent yolk was added to SMY, EYC, EYGB and CUE respectively. Diluted semen was examined at intervals of zero, 72, 120 and 168 hours (storage temperature 3±10C). 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 up to 72 hours.

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 diluents 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 variation was noted in head length and head breadth of spermatozoa in all diluents upto 144 hours of storage.

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.

Sahni and Tiwari (1973) reported on 442 native (Malpura chokla and Jaisalmery) and crossbred (rambouillet x native) ewes those were inseminated and said that ewes given a single insemination with fresh semen diluted 1:3 with cow's milk, 40.1 percent 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.

Deka (1984) diluted semen samples in four extenders namely Tris egg yolk citrate fructose glycerol, SMEYFG, EYCFG and REGY and reported that the percentage of motile sperm varied between extenders significantly.

2.3 Semen Additives:

Popov (1968) observed that bull sperm survival is related to the amount of non polar groups and to pH. The index of sperm survival with addition of EDTA increased to 160.9 whereas in glcose yolk citrate (control) it was 96.8.

Sengupta et al. (1969) studied the preservation of buffalo spermatozoa in diluents supplemented with certain additives. At 96 hours of storage Cysteine HCl and EDTA continued to exhibit highly significant beneficial effect as compared to control. Cysteine was also found to exert highly significant ($P<0.01$) beneficial effect on 4 to 120 hours of storage in a number of yolk based diluents and the improvement being greater with progress of time.

Abdou and El Guindi, (1977) conducted a study on rate of anaerobic fructolysis of bull semen in citrate glycine plus 5 or 10 mM disodium EDTA and citrate glycine alone. They observed that both the levels maintained higher sperm motility during all storage periods (upto 360 mts at 300C) as compared with control medium. The overall inhibition effects after 360 mts. for lower and higher level of EDTA were 22.3 and 27.2 respectively. Motility percentage after 360 mts.in control diluent and diluent containing 5 and 10 mM EDTA were 60, 80 and 80 respectively.

Vidyachand et al. (1979) reported the preservation of buffalo semen at variable room temperature in some modified diluents. semen from 6 Murrah buffalo bulls was diluted in the following diluents : (1) coconut milk extender (CME); (2) CME+cysteine hydrochloride; (3) CME+ glucose; (4) CME + glucose + cysteine hydrochloride; (5) modified Cornell University Extender (CUE) + cysteine hydrochloride; (6) modified IVT diluent + cysteine hydrochloride. After 48 h of storage at room tem., sperm motility was significantly higher for diluent 2 than for all other diluents (56.9 Vs 53.0-55.5%), but after 96 and 144 h there were no significant differences.

Shannon and Curson, (1983) studied the effects of Cysteine HCl and EDTA on in vitro survival at 370C and fertility of bovine semen. They used two diluents

containing either 5 or 20% egg yolk. Some of the diluents were saturated with N2 so that O2 concentration was reduced 40%. Cysteine was observed to depress sperm survival at 370C in diluents at 100% air saturation, but enhanced survival when air saturation was reduced to 40%. EDTA enhanced sperm survival at 370C and the concentration of EDTA required to achieve the maximum increase in survival was dependent on egg yolk concentration.

Saxena and Tripathi (1984) conducted a study on the preservation of ram semen at 3-50C in Russian dilutor with supplementation of EDTA and Cysteine HCl. They reported some beneficial effect of EDTA and Cysteine HCl with Russian dilutor on survivability of spermatozoa.

Nadroo et al. (1988) investigate the effect of addition of glycine, cysteine, glutathione and ethylene diamine tetra-acetic acid (EDTA) individually or in combination of egg yolk citrate extender on preservation of semen of Jersey at 4±10C. The sperm motility and livability decline gradually in all the dilutors during storage. The variations in the abnormalities of the spermatozoa during storage in all the dilutors were negligible. The results revealed slight beneficial effect of addition of glycine and cysteine over others to EYC extender.

Singh et al. (1989) observed that the percentage of sperm motility and live count for buffalo semen was significantly ($P<0.01$) higher in citric acid whey fortified with 0.1% and 0.2% Cysteine HCl upto 96 hours of storage at refrigerant temperature.

Puranik et al. (1994) conducted studies on preservation of Osmanabadi and Cross bred buck semen at refrigerator temperature and reported that the tris dilutor was better than laciphos giving 52% motility at 72 hours of storage in both the breeds. Addition of EDTA in tris dilutor gave 60% motile sperm for both breeds at 72 hours of preservation.

Dhami and Sahni, (1994) reported that inclusion of 0.1% Cysteine HCl or EDTA in three diluents viz Tris, Citrate and Lactose had significant beneficial effect towards improving quality/freezability and fertility or frozen/liquid semen of bovines.

Kumar et al. (1996) reported better results with EDTA and Cysteine HCl for preservation of bovine semen in milk diluents.

Kumar et al. (1996) reported that incorporation of EDTA of Cysteine HCl for preservation of Friesian and Murrah semen in tris dilutors had shown better results. It was also reported that Cysteine HCl was superior to EDTA for both the species.

Singh et al. (2002) reported that post preservation seminal attributes were better in TEYFC extender incorporated with cysteine HCl as compared to other extenders, indicating that TEYFC is an ideal semen diluent as compared to EYC and that inclusion of 0.01% cysteine HCl in both extenders showed beneficial effect towards improving the keeping quality of liquid semen of Beetal bucks.

Funahashi and sano (2005) reported that a semen extender with cystein improved the viability, chromatin structure and membrane integrity of boar sperm cells during liquid preservation.

Raval et al. (2006) studied the effect of extender additives on sperm and acrosome abnormalities during cryopreservation of triplebred (HF X JX Kankrej) bull's semen. They used extender-additives, viz. cysteine Hcl (0.1%) and EDTA (0.1%) in Tris fructose yolk glycerol diluent using split sample technique (1:10 dilution) on sperm and acrosome abnormalities duirng cryopreservation. The mean percentages of total sperm abnormalities at initial, pre freeze and post-thaw stages were 7.44±0.16, 9.07±0.17 and 13.57±0.20 ($P<0.01$), respectively. The overall mean percentages of sperms with head, midpiece and tail abnormalities in fresh semen were 2.33±0.06, 1.37±0.06 and 3.78±0.12, respectively. The corresponding values at pre-freeze and

post-thaw stages were 2.94±0.10, 2.11±0.08, 4.10±0.14 and 4.35±0.13, 2.78±0.09, 6.45±0.18, respectively. The mean percentages of sperms with damaged acrosome at initial, pre-freeze and post-thaw stages were 7.67±0.19, 10.13±0.24 and 16.30±0.28 ($P<0.01$), respectively. The values of swollen, ruffled, denuded and detached acrosome in fresh semen were 2.25±0.06, 2.03±0.08, 2.00±0.09 and 2.55±0.10%, respectively. The corresponding pre-freeze values were 2.73±0.09, 2.43±0.09, 2.22±0.09 and 3.17±0.12%, and post-thaw values 3.69±0.12, 3.79±0.13, 3.63±0.012 and 5.32±0.16%, respectively. In general, the values of all traits were significantly ($P<0.01$) lower both at pre-freeze and post-thaw stage in the presence of EDTA and cysteine Hcl as compared to that on control. Tris diluent indicating beneficial effect of both the additives towards sperm protective against cryo-damage.

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Chapter-III

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Experimental animals:

The present study was conducted on the semen samples collected from six Jamunapari bucks belonging to the age group of 1.5-2.5 years and maintained under identical feeding and managemental conditions at I.C.A.R Eastern Zone, Patna.

2. Collection of semen:

Semen samples were collected twice a week by artificial vagina technique. Bucks were subjected to 2 to 3 false mounts before taking collection. Ejaculates were collected in graduated test tube of 5 ml capacity and the collected semen was brought to the laboratory for evaluation. Total 24 collections from each of 6 bucks (i.e. 144 semen ejaculates) were utilized for the study.

3. Evaluation of semen:

The seminal ejaculates were evaluated for the following attributes:

3.1. Macroscopic evaluations: volume, Colour & consistency of ejaculated semen were observed and recorded.

Fig. I: Photographs showing collection of semen and preparation of EYC.

The volume was recorded directly from the graduated collection tube. The Colour and consistency of ejaculates were observed by naked eye in adequate light.

3.2. Microscopic Evaluation: for Mass activity, Progressive motility, Live sperm percentage, Sperm concentration, and percentage of abnormal spermatozoa.

3.2.1. Mass Activity:

Mass activity of semen was assessed following the procedure of Hancock (1951). A clean and sterilized glass slide was taken. A drop of semen was poured over the glass slide and it was examined under low power objective of the microscope (10x) without using cover slip. The mass activity was graded from 0 to +5 based on intensity of swirls and eddies as per Tomar, 1976. Semen samples showing mass activity of grade +4 (80%) and above were included for further processing i.e. dilution & preservation.

3.2.2. Progressive motility:

The individual sperm motility percentage was calculated by following standard method.

Haemocytometer method was used to assess the progressive motility of spermatozoa. RBC pipette was utilized for dilution. Semen buffer solution or, 2% sodium citrate dehydrate solution was used for dilution of semen. Total non-motile spermatozoa where counted in the

chamber. Then the same charge hoemocytometer was subjected to heat for killing of spermatozoa. Now total spermatozoa in the chamber were counted. The difference marked out between the total and non-motile sperms counts gave an estimate of motile spermatozoa. The percentage of motile spermatozoa was calculated as follows-

$$\% \text{ motile spermatozoa} = \frac{\text{Total no. of spermatozoa–Non motile spermatozoa}}{\text{Total no. of spermatozoa}} \times 100$$

3.2.3. Live sperm percentage:

The Eosin-nigrosin staining technique (Hancock, 1951) was employed to determine the percentage of live spermatozoa.

The compound stain of Eosin-nigrosin was prepared by dissolving 5 gm of Eosin, 30 gm of nigrosin in 300 ml. distill water. The solution was warmed in water bath for 30 minutes and was filtered after cooling with the help of Whatman's filter paper no.40.

On a clean grease free glass slide, a drop of neat semen was taken; to it four drops of nigrosin-eosin were added. A thin smear was drawn on slide and was dried in the air. The smear was examined under oil immersion. Live spermatozoa were colourless, whereas dead spermatozoa took pink stain of Eosin. A total of 200

spermatozoa were counted at random in different fields of microscope and the percentage of live spermatozoa was calculated as per the formula mentioned below -

$$\% \text{ of live spermatozoa} = \frac{\text{Total no. of spermatozoa} - \text{Dead spermatozoa}}{\text{Total no. of spermatozoa}} \times 100$$

3.2.4. Concentration of spermatozoa:

The semen sample was thoroughly mixed in a watch glass to make even distribution of sperm and then it was sucked up to 0.5 mark of R.B.C. diluting pipette (containing the red bead). After that 3% Sodium Chloride solution was sucked up to 101 marks. The two ends of the pipette were closed by means of fingers and it was shaken gently just to ensure the through mixing of the semen. 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 counting chamber. Two to three 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 uniformly spread throughout the counting chamber.

Now, the number of sperms present in 5 primary squares of the counting chamber were counted under the 40 x objective of microscope. The 5 primary 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 were made as follows —

The volume of counting chamber was 1mm x1mm x 0.1mm = 0.1mm3. Let the number of sperms present in 5 primary squares be n.

Therefore nx5 will be the number of sperms present in 0.1 mm3 of diluted semen and n x 5 x 10 x 200 will be the number of sperms present in 0.1mm3 of neat semen sample.

Therefore n x 5 x 10 x 200 x 1000 will be the number of sperms present in 1ml. of neat semen samples or, in other words the total number of sperms in 1ml. of neat semen = n x 10000000.

3.2.5. Percentage of abnormal spermatozoa:

Spermatozoa abnormalities were assessed by using the slides prepared for acrosomal morphology following the procedures of Watson (1975).

3.2.6 Acrosomal morphology:

Acrosomal morphology was assessed following the procedure of Wastson (1975) by staining seminal smears with Giemsa stain.

Preparation of Giemsa stain:

The following ingredients were used:

Giemsa stain (stock solution)	3ml.
Sorenson´s 0.1 M phosphate buffer	2 ml.
Double glass distilled water	35 ml.

Giemsa stain (stock solution) was prepared as per the following formula :

Giemsa stain powder	3.8 ml.
Methonal	375 ml.
Glycerol	125 ml.

Giemsa stain powder was ground in mortar and pestle with methanol. After grinding, glycerol was added and it was finally filtered.

Sorenson's 0.1M phosphate buffer was prepared with the following compositions:

Solution A:

Na2HPO42H2O	21.68 g
H2O	500 ml.

Solution B:

KH2PO4	22.25 g
H2O	500 ml.

Stock buffer solution was prepared by adding 200 ml. of solution A to 50 ml. of solution B.

Buffered formal saline:

The solution was prepared with the following composition:

Stock buffer solution	100ml
Stock sodium chloride solution	150ml
Formalin	62.5ml
Distilled water	500ml

Stock sodium chloride solution:

The solution was prepared as follows:

Sodium chloride	9.01 g
Distilled water	500ml

Hancock's fixative:

Sodium chloride	10 gm.
Sodium bicarbonate	0.5 gm.

Formalin	125 ml.
Distilled water up to	1000 ml.

Technique of staining:

Procedure followed for staining with Giemsa stain was as follows:

a) A drop of semen was smeared on a slide which was kept at 37°C and was dried in air.

b) Smear was fixed in buffered formal saline for 15 minutes with Hancock's fixative and then washed in running tap water for 15 – 20 minutes.

c) Smear was dried in air and then stained with Giemsa stain for 90 minutes.

d) Finally, the slides was rinsed with distilled water and dried in air.

Slides were examined at a magnification of 100x, and from each slide 100 acrosome were examined. Besides acrosomal morphology, slides were utilized for assessing of sperm abnormalities.

4. 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 gm of sodium citrate

dehydrate 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 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 at its proximal end, shell membrane was removed and the white of the egg (Albumin) part was discarded.

The egg yolk was placed on a sterilized filter paper (what's man no. - 41); yolk membrane was removed carefully and yolk was collected in a measuring cylinder. One part of egg yolk and two parts of buffer solution were mixed. Then antibiotics were added as detailed below-

Benzathine Penicillin - 1000 I.U. /ml.

Streptomycin - 1000μg/ml.

5. Dilution of semen:

After initial evaluation semen samples were poured in test tube and extended with EYC. The dilutor was prepared as described earlier and kept in 10 ml. test tubes in water both at 32°C. The test tubes containing the dilutors were properly labeled. Only freshly prepared dilutor was used. Care was taken that the temperature of

dilutor and semen was maintained at 30°C at the time of dilution. The dilution was done in the ratio of 1:10 in the egg yolk citrate. The test tubes were gently rotated between palms for uniform distribution of sperms in the dilutor.

6. Processing of semen:

Semen samples showing 80 % (+4) or, more mass motility were pooled and finally diluted @ 1:10 in EYC extender.

7. Semen additives:

The tubes containing dilutor was prepared and kept in 10 ml test tubes in water bath at 32°C. The test tube containing the dilutors were properly labeled. Only freshly prepared dilutor were made. Care was taken that the temperature of dilutor and semen retained at 30°C-32°C at the time of dilution. The dilution was done in the ratio of 1:10 in Egg Yolk citrate. The test tubes were gently rotated between palms for uniform distribution of sperm in the dilutor. Subsequent to dilution of semen in egg yolk citrate, each semen sample was divided into three fractions for the addition of two additives viz. Ethylene di-amine tetra acetic acid (EDTA)and cysteine hydrochloride. Out of three fractions one fraction served as control in which no additive was added where as in two fractions each additive at the rate of 1mg/ml of extender was added separately.

Now, those three group of extenders viz:

The following combination of extenders were prepared.

EYC

EYC + EDTA

EYC + Cysteine HCl.

8. Preservation of Semen at refrigerated temperature:

Diluted semen samples were kept in clean and sterilized small test tube. Those test tube were wrapped with cotton wool and put in another big diameter test tube and were labeled for date of collection & name of additive. Those test tubes containing the diluted semen sample were placed in a beaker having 250 ml. of water at 25°C - 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 tubes were kept in the same empty beaker for preservation at 4°±1°C (Muller, 1962). The diluted semen samples were examined at zero hour and further at 24, 48 and 72 hours of preservation at refrigerated temperature.

9. Microscopic Evaluation of preserved semen samples:

The semen samples diluted with EYC and added with EDTA & cysteine HCl as well as preserved at refrigerated temperature (4°±1°C) for different durations (i.e. 0, 24, 48

& 96 hrs) were brought to room temperature & evaluated for seminal attributes viz :- progressive motility, live sperm count & abnormalities of spermatozoa as describd earlier.

10. Statistical analysis:

The data recorded were subjected to statistical analysis according to statistical book George W. Snedecor and Willium G. Cochran(1994).

Mean: Mean for different characters were calculated by using the formula of arithmetic mean as follows:

$$\bar{X} = \frac{\Sigma X}{n}$$

Where, X = Mean of any character X

ΣX = Sum of the observations of character X.

n = Total number of observations.

Standard error (S.E.) of estimate:

It was calculated according to the following formula.

$$S.E. = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}{\frac{(N-1)}{N}}}$$

$$C.V.\ \% = \frac{S.D.}{Mean \times 100} = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}{\frac{(N-1)}{Mean}}}$$

Where, X is a variable and n is number of values of that variable.

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Chapter-IV

RESULTS

RESULTS

The present study was undertaken to observe the influence of certain additives (VIZ; EDTA and cysteine HCL) in EYC diluter on preservation of Jamnapari buck semen. Neat semen samples (total 144 ejaculates) collected from 6 Jamnapari bucks were used for evaluation.Again neat semen samples were diluted in Egg Yolk Citrate (EYC) extender alone (control) and in EYC added with EDTA (1mg/ml) & cysteine HCL (1mg/ml).Diluted semen samples were evaluated for various seminal attributes. Viz: Colour & consistency, volume, mass / progressive motility, sperm concentration, live sperm percentage as well as percentage of acrosomal morphology and sperm abnormalities (Head &Tail abnormalities) at 0, 24, 48 and 72 hrs. of preservation at refrigerated temperature (4°C) and their findings are as noted below -

4.1. Seminal attributes of neat semen:

The mean, with standard error, co-variance percentage of various seminal attributes of neat semen samples collected from 6 Jamunapari bucks were represented in table -1 & 3.

4.1.1. Colour and consistency:

The colour & consistency of ejaculated semen was creamy yellowish and viscid respectively.

4.1.2. Ejaculate Volume:

The mean value for the volume of 144 semen samples collected from six Jamunapari bucks was 0.51±0.012 ml. and the separate mean values of buck no. 1 to 6 were 0.45±0.012, 0.52±0.010, 0.51±0.012, 0.42±0.012, 0.60±0.014, 0.56±0.010 ml. respectively.

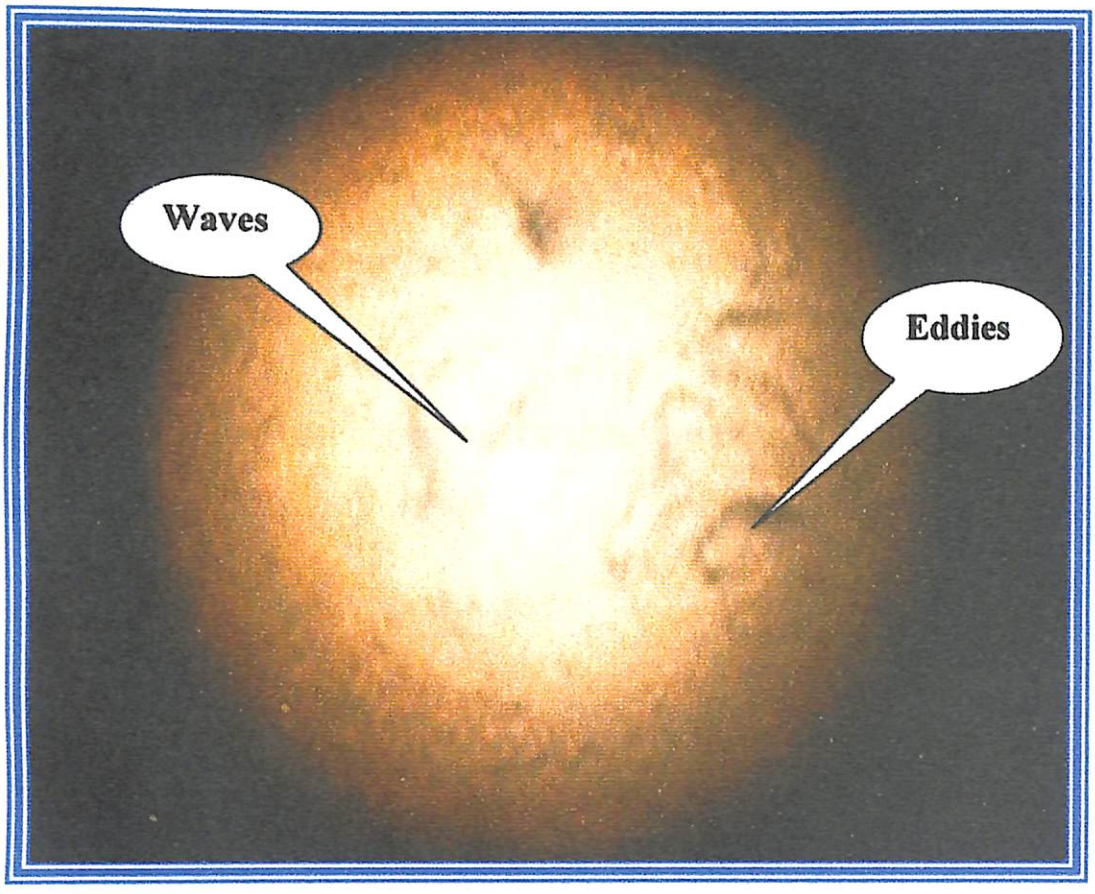
Analysis of variance (Table -2) indicated that variation of semen volume between bucks was found to be non-significant. However buck no. 05 had maximum volume (0.60ml.) & the buck no. 04 had minimum (0.42 ml.) presented in table-1.

4.1.3. Mass Motility:

The mean value of mass motility of spermatozoa of 144 ejaculates collected from 6 bucks was 81.08±3.44 percent and same values separately for buck no. 1 to 6 were found to be 80.00±0.481, 80.33±0.677, 81.42±0.458, 82.17±0.674 81.04±0.573 and 81.54±0.454 percent respectively.

Analysis of variance (Table -2) indicated that variation in mass motility between bucks was found to be non-significant. Buck no. 04 had maximum motility (82.17%) and Buck no. 01 had minimum (80.00%) presented in table -1.

Fig. III: Micro photograph showing waves and eddies in mass motility of neat semen



4.1.4. Sperm concentration:

The mean values of sperm concentration of 144 samples collected from 6 bucks was 4288.56±67.04 millions per ml. while the same values separately for buck no. 1 to 6 were 4530.62±67.04, 4168.96±86.18, 4295.25±52.56, 4300.87+93.38,4244.75±54.18 and 4190.92±66.56 respectively.

Analysis of variance (Table -2) indicated that variation of sperm concentration between bucks was found to be significant ($P<0.01$).

4.1.5. Live sperm percentage:

The mean value of live sperm percentage of 144 samples collected from 6 bucks was 80.99±3.44. Individual buck had live sperm percentage of 80.13±0.255, 80.83±0.382, 81.55±0.384, 82.63±0.566, 81.28±0.416 and 81.62±0.296 respectively from buck number 1 to 6.

Analysis of variance (Table -2) indicated that variation in live sperm percentage between bucks was found to be significant($P<0.01$). However buck no. 04 had maximum percentage of live sperm (82.63%) and buck no. 01 had minimum (80.13%) presented in table -1.

Fig. IV: Micro photograph showing Live and Dead spermatozoa

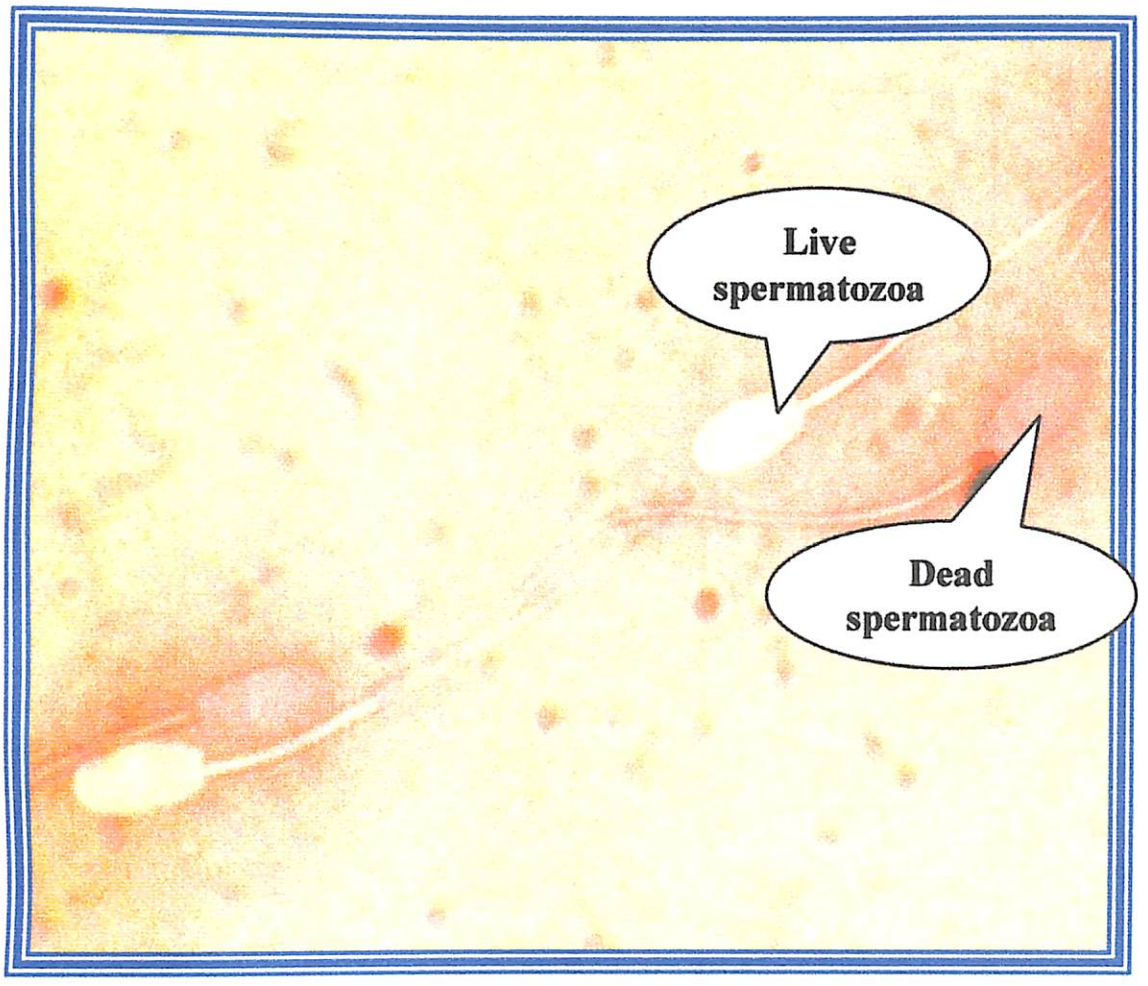


Table-1: Mean + S.E and CV percentage of Seminal characteristics of freshly ejaculated semen.

Buck No.	Volume (ml.)	Mass motility (Percent)	Live Sperm (Percent)	Sperm Concentration (*Millions*)
	Mean ± S.E & CV%	Mean ± S.E & CV%	Mean ± S.E & CV%	Mean ± S.E & CV%
01.	$0.45^{a}\pm0.012$ (8.384)	$80.00^{ab}\pm0.481$ (3.020)	$80.13^{a}\pm0.255$ (1.594)	$4530.92^{a}\pm67.04$ (7.249)
02.	$0.52^{a}\pm0.010$ (7.082)	$80.33^{b}\pm0.677$ (4.340)	$80.83^{a}\pm0.382$ (2.37)	$4168.96^{b}\pm83.18$ (10.127)
03.	$0.51^{a}\pm0.012$ (8.786)	$81.42^{ac}\pm0.458$ (6.330)	$81.55^{a}\pm0.384$ (2.392)	$4295.25^{ac}\pm52.56$ (5.599)
04.	$0.42^{a}\pm0.012$ (7.905)	$82.17^{cde}\pm0.674$ (4.120)	$82.63^{a}\pm0.416$ (2.593)	$4300.87^{c}\pm93.38$ (10.636)
05.	$0.60^{a}\pm0.014$ (9.234)	$81.04^{dce}\pm0.573$ (3.460)	$81.28^{a}\pm0.566$ (3.541)	$4244.75^{c}\pm54.18$ (6.253)
06.	$0.56^{a}\pm0.010$ (5.294)	$81.54^{dc}\pm0.454$ (2.790)	$81.62^{a}\pm0.296$ (1.836)	$4190.92^{b}\pm66.56$ (7.781)
Mean	0.51	81.08	81.34	4288.56

Column - wise means with similar superscripts (a,b,c,d,e) didn't differ significantly.

Table-2: Analysis of variance showing the influence of buck on seminal attributes of neat semen.

Source of variation	d.f	Volume		Mass motility%		live %		Concentration (10^6)	
		MSS	F	MSS	F	MSS	F	MSS	F
Between Bucks	5	0.002	0.625^{NS}	1.0272	0.868^{NS}	67.518	8.93^{**}	405839.8	0.33^{**}
Error	138	0.0032		1.197		7.56		123077.19	

NS = Non significant ** = Significant at $P < 0.01$

4.1.6. Acrosomal abnormality:

The mean value of abnormal acrosome of 144 samples collected from 6 bucks was 0.815±0.007 and same for individual buck was 0.816±0.003, 0.812±0.010, 0.813±0.004, 0.817±0.011, 0.809±0.003 and 0.824±0.011 of buck number 1 to 6 respectively for buck no. 1 to 6. (Table -3).

Analysis of variance(Table -4) indicated that variation in acrosomal abnormality percentage between bucks was found to be non-significant.

4.1.7. Head abnormality:

The mean value of abnormal head of 144 samples collected from 6 bucks was 0.085±0.003 and same for individual buck was 0.084±0.003, 0.086±0.003,

0.086±0.004, 0.085±0.002, 0.086±0.001 and 0.081±0.005 from buck number 1 to 6 respectively. (Table -3)

Analysis of variance indicated that variation in head abnormality percentage between bucks was found to be non-significant.

4.1.8. Tail abnormality:

The mean value of abnormal tail of 144 samples collected from 6 bucks was 4.498±0.021 and same for individual buck (1 to 6) was 4.488±0.028, 4.502±0.063, 4.492±0.003, 4.512±0.012, 4.501±0.007 and 4.496±0.008. (Table -3)

Analysis of variance indicated that variation in tail abnormality percentage between bucks was found to be non-significant.

Table-3: Mean+S.E and CV percentage of Spermatozoal abnormality of freshly ejaculated semen.

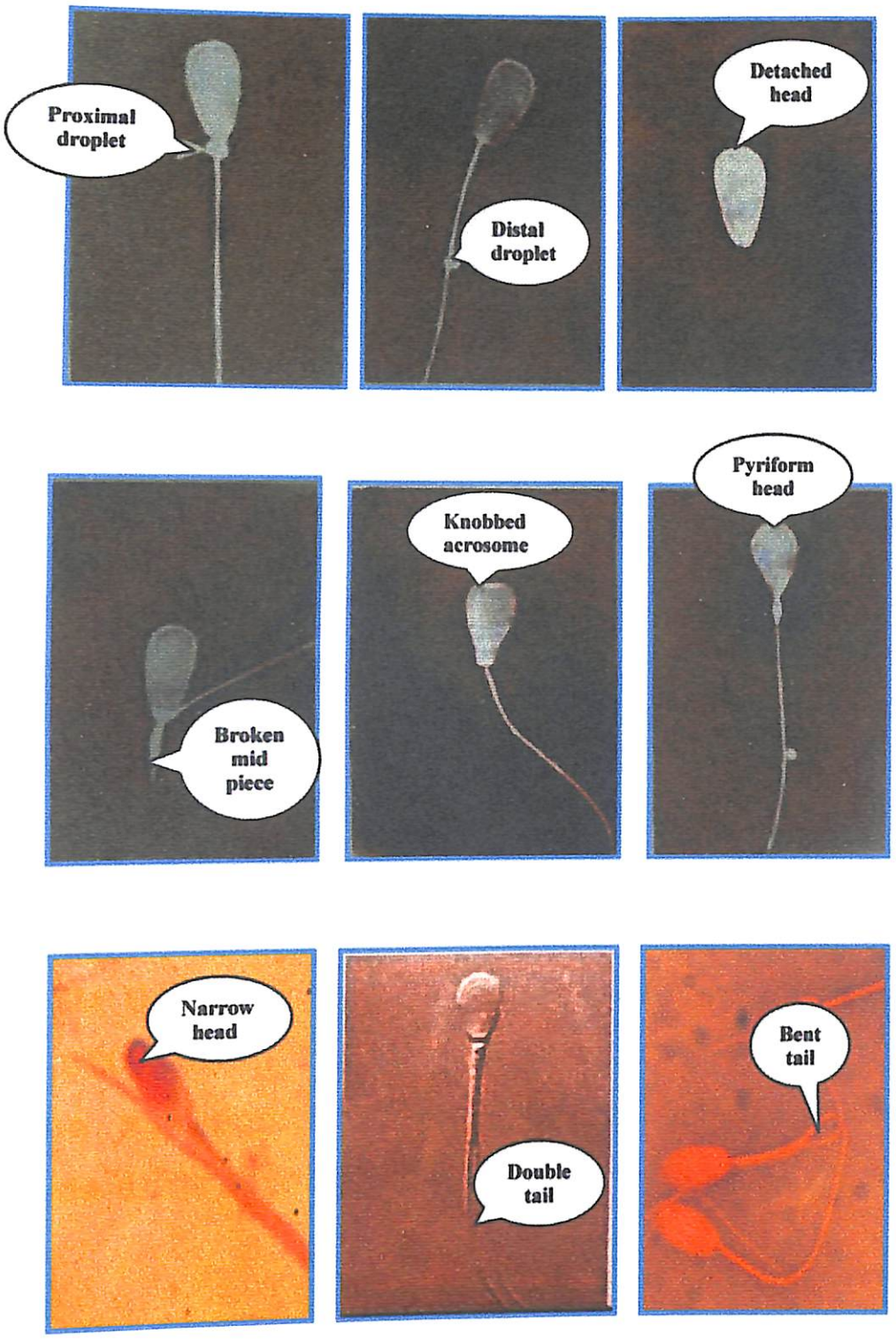
Buck No.	Acrosomal abnormality (Percent) Mean ± S.E & CV%	Head abnormality (Percent) Mean ± S.E & CV%	Tail abnormality (Percent) Mean ± S.E & CV%
01.	0.816±0.003 (0.163)	0.0845±0.003 (5.67)	4.488±0.028 (4.315)
02.	0.812±0.010 (0.331)	0.086±0.003 (5.43)	4.502±0.063 (5.032)
03.	0.813±0.004 (2.124)	0.0857±0.004 (4.22)	4.492±0.003 (5.064)
04.	0.817±0.011 (0.011)	0.0855±0.002 (3.76)	4.512±0.012 (4.282)
05.	0.809±0.003 (0.425)	0.0862±0.001 (5.27)	4.501±0.007 (4.491)
06.	0.824±0.011 (1.351)	0.0812±0.005 (2.84)	4.496±0.008 (2.674)
Mean	0.815±0.007	0.0848±0.003	4.498±0.021

Table-4: Analysis of variance showing the influence of buck on abnormality percentage of acrosome, head and tail of neat semen.

Source of variation	d.f	Acrosomal abnormality (Percent)		Head abnormality (Percent)		Tail abnormality (Percent)	
		MSS	F	MSS	F	MSS	F
Between Bucks	5	0.014	0.55^{NS}	0.005	0.28^{NS}	1.734	0.81^{NS}
Error	138	0.026		0.018		2.134	

NS = Non significant

Fig.VI: Micro photographs showing different abnormalities of spermatozoa.



4.2. Seminal characteristics of EYC diluted semen added with EDTA & Cysteine HCL at different hours of preservation.

4.2.1 Progressive motility of sperm :

The progressive sperm motility percentage with EYC dilutor (control) at 0, 24, 48 and 72 hours of preservation was recorded as 77.61±0.12, 70.31±0.11, 64.71±0.14 and 55.09±0.22 percent; EYC added with EDTA 78.02±0.12, 72.00±0.25, 65.21±0.11, 56.21±0.13 percent, and EYC added with cysteine HCl 78.70±0.10, 73.44±0.12, 67.07±0.14, 57.79±0.17 respectively (Table -5).

Analysis of variance(Table-6) showing the effect of additives on progressive motility percentage was found to be significant($p<0.01$) at different hours of preservation.

Critical difference test indicated that all the our of preservation the highest motility percentage was observed in EYC+Cysteine HCl and lowest in EYC extender. At 0 hrs the difference in motility percentage between EYC, EYC+EDTA and EYC +cysteine HCl was found to be non significant. However at 24,48,72, hrs of preservation differ significantly from each other.

Table-5: Mean + S.E and CV percentage of Progressive motility percentage of spermatozoa preserved at different time interval.

Period	Control (EYC)	EYC+ EDTA	EYC+ Cysteine HCL
	Mean ± SE & CV%	Mean ± SE & CV%	Mean ± SE & CV%
0 hr	$77.61^{aA} \pm 0.12$ (1.049)	$78.02^{aA} \pm 0.12$ (1.044)	$78.70^{aA} \pm 0.10$ (0.812)
24 hrs	$70.31^{aB} \pm 0.11$ (1.315)	$72.00^{bB} \pm 0.25$ (1.117)	$73.44^{cB} \pm 0.12$ (0.706)
48 hrs	$64.71^{aC} \pm 0.14$ (1.844)	$65.21^{bC} \pm 0.11$ (1.801)	$67.07^{cC} \pm 0.14$ (2.439)
72 hrs	$55.09^{aD} \pm 0.22$ (6.221)	$56.21^{bD} \pm 0.13$ (2.196)	$57.79^{cD} \pm 0.17$ (3.251)

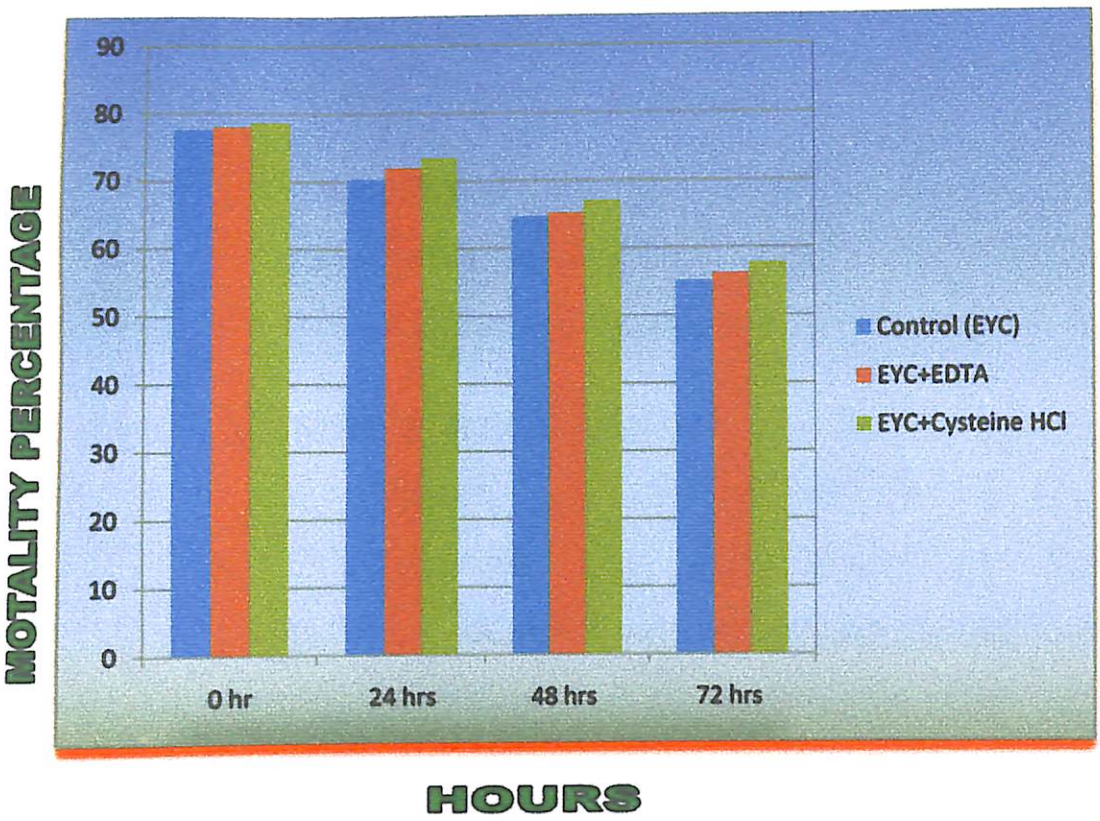
Mean with similar with super scripts (row–wise: abc & Column–wise ABC did not differ significantly.

Table-6: Analysis of variance showing the influence of additives at different hours of preservation on live sperm percentage and progressive motility percentage.

Source of variation	d.f.	Live %		Progressive motility %	
		MSS	F	MSS	F
Between treatments	2	214.61	247.88**	1899.83	1809.36**
Between periods	3	12229.46	2498.81**	79709.68	75913.98**
Error	570	4.9		1.05	

** = Significant at $P < 0.01$

Fig.1: Motility percentage of spermatozoa.



4.2.2. Live sperm percentage:

The Percentage of live spermatozoa diluted with EYC (control), EYC + EDTA & EYC + Cysteine HCL recorded at the time of dilution (0 hr.) were 79.07±0.19, 79.90±0.199 and 80.10±0.192 percent respectively followed by 72.36±0.11, 73.51±0.246 and 74.87± 0.245 at 24 hours 63.09±0.198, 65.72±0.182 and 69.97±0.184 at 48 hours 52.97±0.175, 57.90±0.183 and 58.90±0.185 at 72 hrs. respectively (Table -7).

Analysis of variance (Table -6). showing the effect of additives on live sperm percentage was found to be significant($p<0.01$) at different hours of preservation.

Critical difference test indicated that all the hour of preservation the highest live sperm percentage was observed in EYC+Cysteine HCl and lowest in EYC extender. At 0 hrs the difference in live sperm percentage between EYC, EYC+EDTA and EYC +cysteine HCl was found to be non significant. However at 24,48,72, hrs of preservation differ significantly from each other.

Table -7: Mean + S.E. and CV percentage Of Live sperm percentage of spermatozoa preserved at different time interval.

Period	EYC (Control)	EYC+ ETDA	EYC+ Cysteine HCl
	Mean ± SE & CV%	Mean ± SE & CV%	Mean ± SE & CV%
0 hr	$79.07^{aA} \pm 0.19$ (1.693)	$79.90^{aA} \pm 0.199$ (1.752)	$80.10^{aA} \pm 0.192$ (1.668)
24 hrs	$72.36^{aB} \pm 0.11$ (2.326)	$73.51^{bB} \pm 0.246$ (2.323)	$74.87^{cB} \pm 0.24$ (2.271)
48 hrs	$63.09^{aC} \pm 0.198$ (2.174)	$65.72^{bC} \pm 0.182$ (1.914)	$69.97^{cC} \pm 0.184$ (1.818)
72 hrs	$52.97^{aD} \pm 0.175$ (2.286)	$57.90^{bD} \pm 0.183$ (2.181)	$58.90^{cD} \pm 0.185$ (2.081)

Mean with similar superscripts (row -wise: abc & Column - wise: ABCD) did not differ significantly.

Fig. 2: Live sperm percentage of spermatozoa.

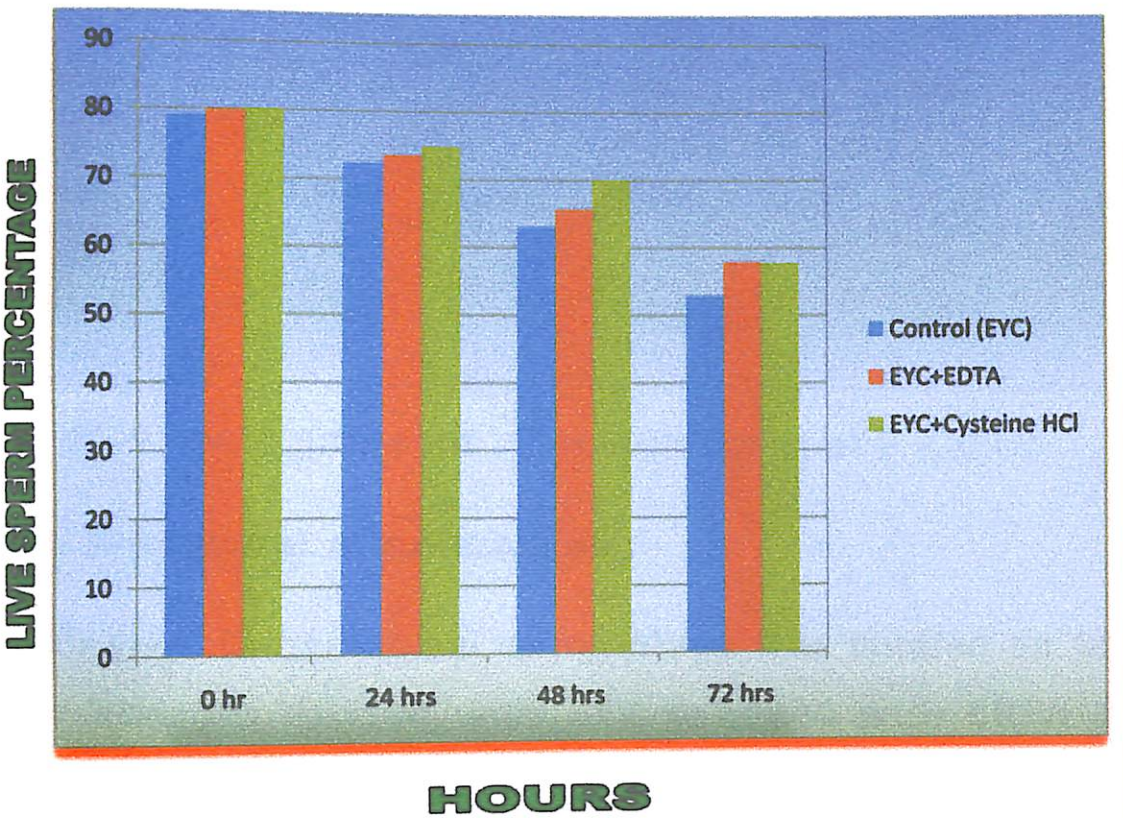


Table-8: Analysis of variance showing the influence of additives on the acrosomal, head & tail abnormality percentage of spermatozoa at different hours of preservation.

Source of variation	d.f.	Acrosomal abnormality (Percentage)		Head abnormality (Percentage)		Tail abnormality (Percentage)	
		MSS	F	MSS	F	MSS	F
Between treatments	2	3.57	137.31**	0.005	0.21NS	3.89	155.6**
Between periods	3	109.32	4204.6**	0.008	0.33NS	27.92	1116.8**
Errors	210	0.026		0.024		0.025	

NS = Non significant ** = Significant at $P < 0.01$

4.2.3. Acrosomal abnormalities:

Change in acrosomal morphology was studied on 18 semen samples diluted with EYC & added with EDTA & Cysteine HCL at 0, 24, 48 and 72 hours of post preservation at refrigerated temperature (Table -9). The acrosomal abnormalities percentage at 0, 24, 48 and 72 hours of post preservation were 0.839±0.003, 1.8±0.011, 3.14±0.012 and 4.55±0.048 with EYC extender (control). The corresponding values with EYC+ EDTA were 0.828±0.002, 1.60±0.056, 2.84±0.004 and 4.03±0.046 and with EYC+ Cysteine HCL were 0.811±0.001, 1.50±0.007, 2.54±0.005 and 3.68±0.027 respectively. There was decreasing trend of acrosomal abnormality percentage with time as compare to control.

Analysis of variance (table -8) showing the effect of additives on acrosomal abnormality was significant($p<0.01$) at different hours of preservation.

Critical difference test indicated that all the hour of preservation a higher acrosomal abnormality percentage was observed in EYC extender and lowest in EYC+ Cysteine HCl extender. At 0 hrs, the difference in acrosomal abnormality percentage between EYC, EYC+EDTA and EYC +cysteine HCl was found to be non significant. However at 24,48,72, hrs of preservation differ significantly from each other.

Table -9: Mean + S.E and CV percentage of Acrosomal abnormality percentage of spermatozoa preserved at different time interval.

Period	EYC (Control)	EYC+ EDTA	EYC+ Cysteine HCL
	Mean ± SE & CV%	Mean ± SE & CV%	Mean ± SE & CV%
0 hr	$0.839^{aA} \pm 0.003$ (1.628)	$0.828^{aA} \pm 0.002$ (1.358)	$0.811^{aA} \pm 0.001$ (0.992)
24 hrs	$1.81^{aB} \pm 0.003$ (0.715)	$1.60^{bB} \pm 0.056$ (0.857)	$1.50^{cB} \pm 0.007$ (2.157)
48 hrs	$3.14^{aC} \pm 0.012$ (5.200)	$2.84^{bC} \pm 0.004$ (0.560)	$2.54^{cC} \pm 0.005$ (1.793)
72 hrs	$4.55^{aD} \pm 0.048$ (4.514)	$4.03^{bD} \pm 0.046$ (4.887)	$3.68^{cD} \pm 0.027$ (3.118)

Mean with similar superscripts (row-wise: abc & Column - wise: ABCD) didn't differ significantly.

ph showing Acrosomal

Fig. V: Micro photograph showing Acrosomal morphology.

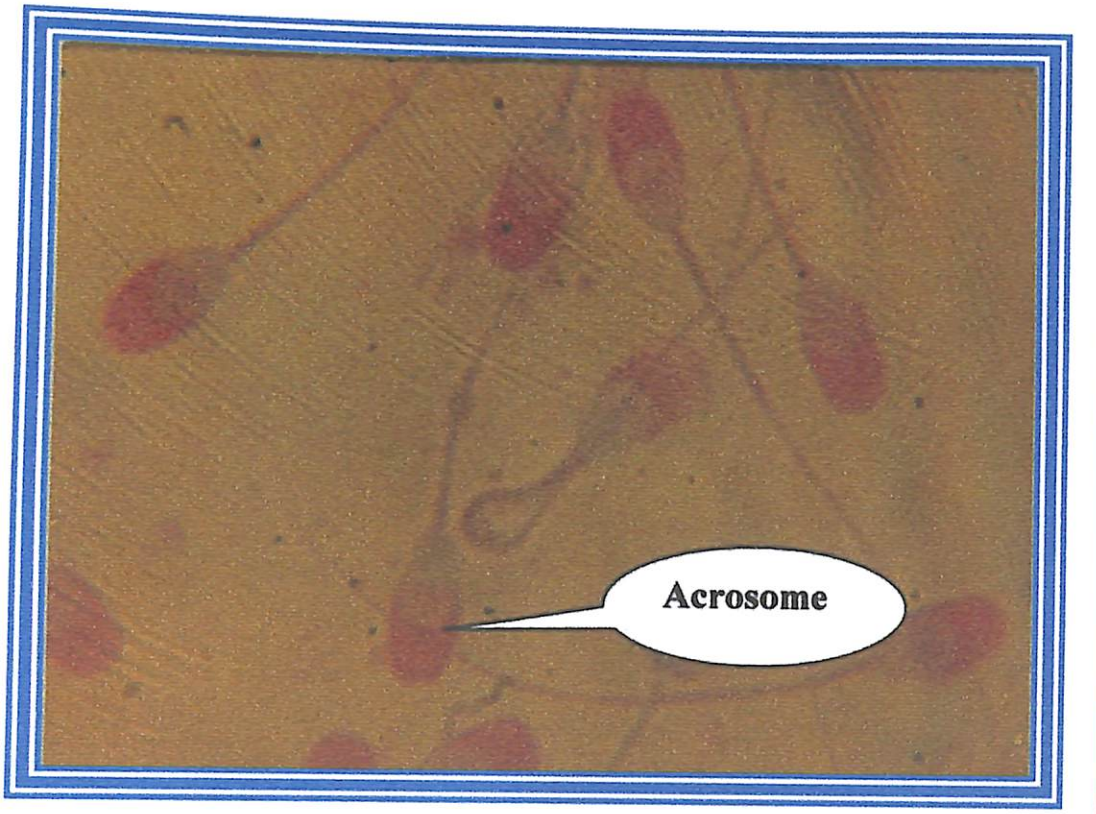
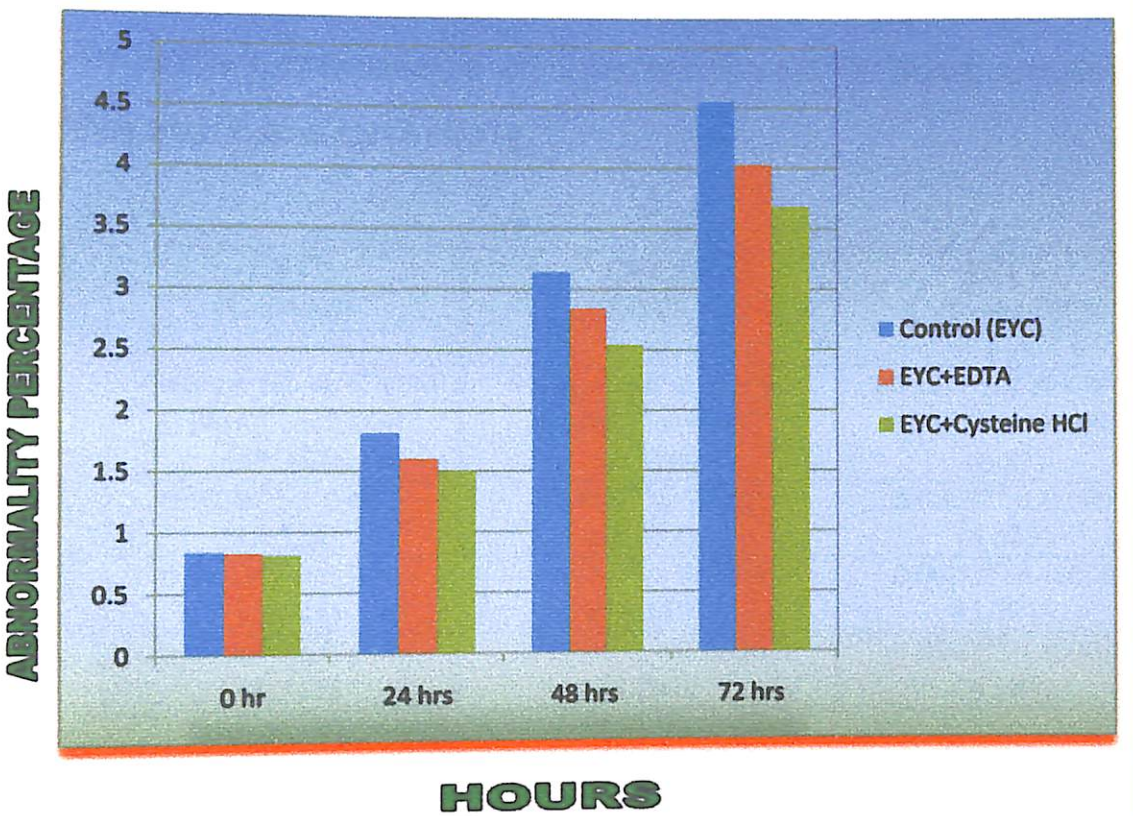


Fig. 3: Acrosomal abnormality percentage of spermatozoa.



4.2.4. Sperm head abnormality:

Analysis of variance showing the mean value of head abnormality with both additives at different hours of preservation varied non -significantly which is presented in Table -10.

Table -10: Mean + S.E. and CV percentage of Head Abnormality percentage of spermatozoa in Buck Semen at Different time intervals.

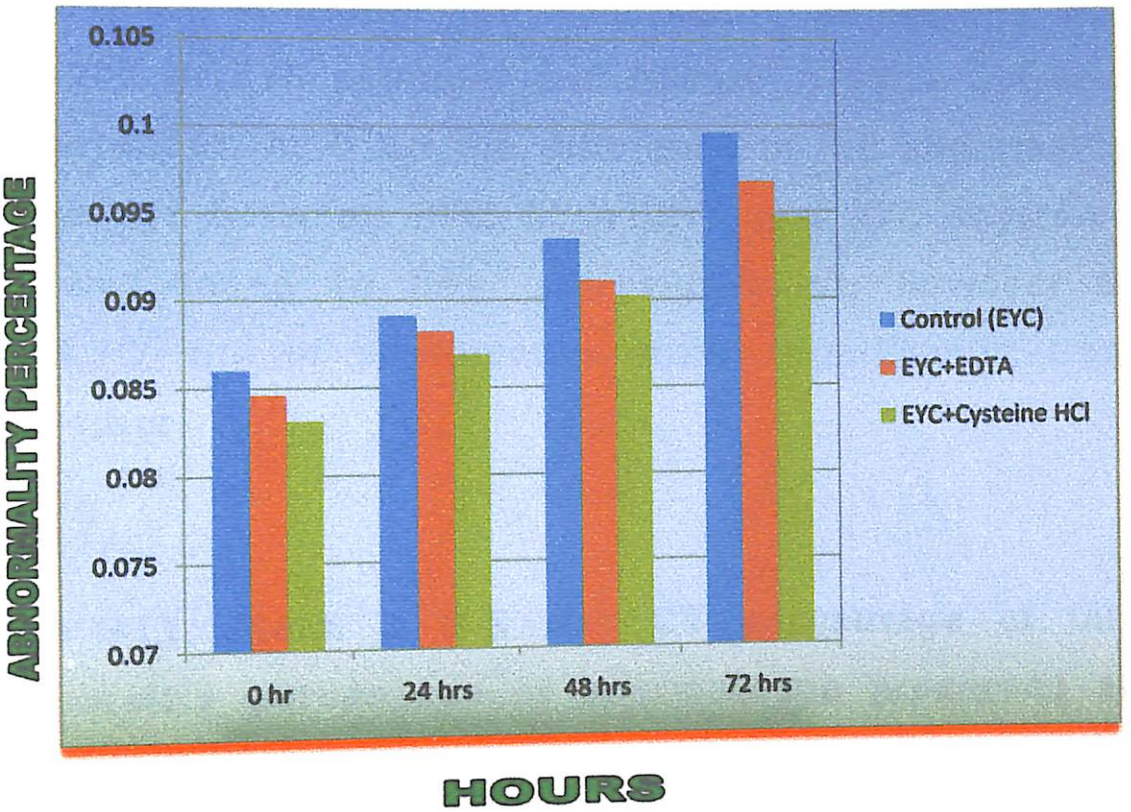
Period	EYC (Control)	EYC+ EDTA	EYC+ Cysteine HCL
	Mean ± SE & CV%	Mean ± SE & CV%	Mean ± SE & CV%
0 hr.	$0.0860^{aA} \pm 0.004$ (21.752)	$0.0846^{aA} \pm 0.002$ (6.320)	$0.0831^{aA} \pm 0.0008$ (6.82)
24 hrs.	$0.0891^{aA} \pm 0.003$ (12.222)	$0.0882^{aA} \pm 0.002$ (9.773)	$0.0869^{aA} \pm 0.001$ (4.679)
48 hrs.	$0.0935^{aA} \pm 0.006$ (15.401)	$0.0911^{aA} \pm 0.0084$ (13.119)	$0.0902^{aA} \pm 0.006$ (5.771)
72 hrs.	$0.0996^{aA} \pm 0.007$ (18.341)	$0.0968^{aA} \pm 0.0053$ (7.291)	$0.0947^{aA} \pm 0.001$ (4.186)

Mean with similar superscripts (row -wise: abc & Column - wise: ABCD) didn't differ significantly.

4.2.5. Sperm tail abnormality:

The mean value of tail abnormality with EYC dilutor (control) at 0, 24, 48, 72 hours of preservation were 4.495±0.028, 5.523±0.064, 5.994±0.034 and 6.021±0.027 respectively. The corresponding values with EYC+ EDTA were 4.221±0.018, 5.392±0.06, 5.578±0.03, and 5.869±0.02 and EYC+ Cysteine HCL the

Fig. 4: Head abnormality percentage of spermatozoa.



value were 4.037±0.023, 5.173±0.017, 5.455±0.049 and 5.615±0.022 respectively (Table no.-11).

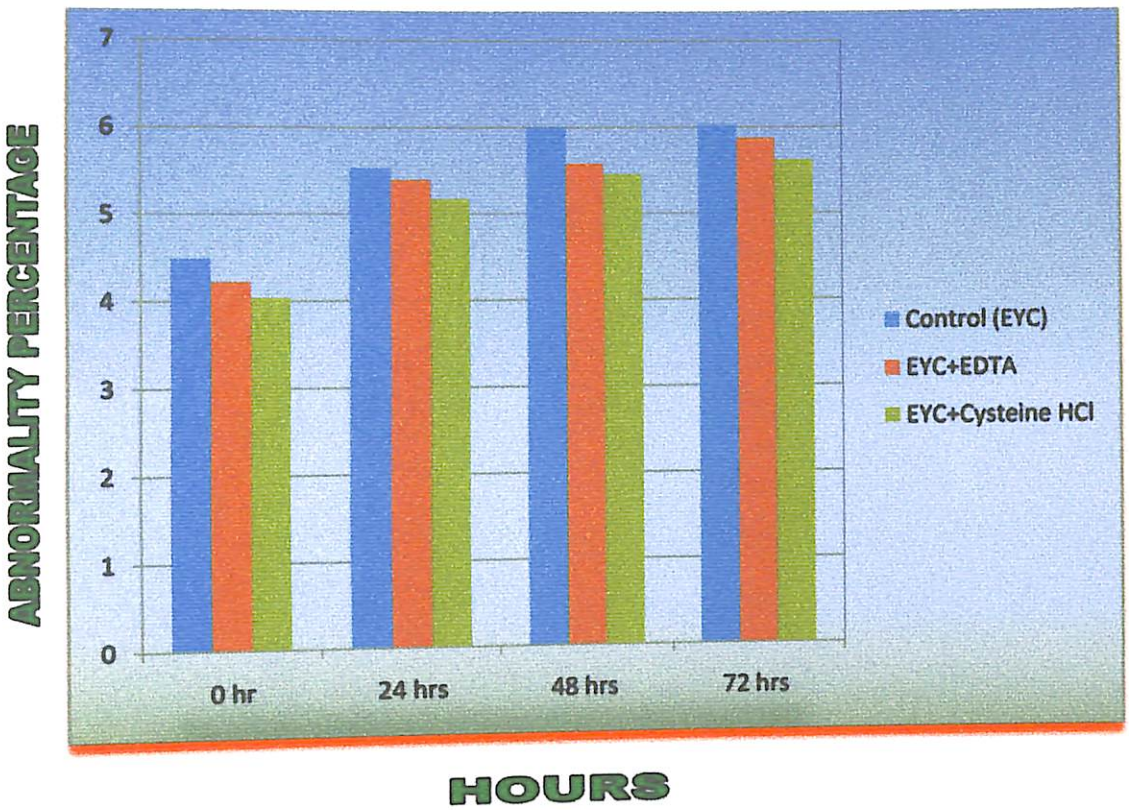
The analysis of variance (table-8). showing the effect of additives on tail abnormality percentage was significant($p<0.01$)) at different hours of preservation.

Critical difference test indicated that all the our of preservation a higher tail abnormality percentage was observed in EYC extender and lowest in EYC+ Cysteine HCl extender. At O hrs,the difference in tail abnormality percentage between EYC, EYC+EDTA and EYC +cysteine HCl was found to be non significant. However at 24,48,72, hrs of preservation differ significantly from each other.

Table -11: Mean ± S.E and CV percentage of tail abnormality percentage of spermatozoa preserved at different time interval.

Period	EYC (Control)	EYC+ EDTA	EYC+ Cysteine HCL
	Mean ± SE & CV%	Mean ± SE & CV%	Mean ± SE & CV%
0 hr	$4.495^{aA} \pm 0.028$ (2.616)	$4.221^{aA} \pm 0.018$ (1.859)	$4.037^{aA} \pm 0.023$ (2.454)
24 hrs	$5.523^{aB} \pm 0.064$ (4.898)	$5.392^{aB} \pm 0.06$ (4.701)	$5.172^{aB} \pm 0.017$ (1.422)
48 hrs	$5.994^{aC} \pm 0.034$ (2.434)	$5.578^{aC} \pm 0.03$ (2.263)	$5.455^{cC} \pm 0.049$ (3.864)
72 hrs	$6.021^{aD} \pm 0.027$ (1.903)	$5.869^{bD} \pm 0.02$ (1.652)	$5.615^{cD} \pm 0.022$ (1.711)

Fig. 5: Tail abnormality percentage of spermatozoa.



Mean with similar superscripts (row -wise: abc & Column - wise: ABCD) didn't differ significantly.

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Chapter-V

DISCUSSION

DISCUSSION

In this study, attempts have been made to assess the effect of additives viz., EDTA and Cysteine Hcl on preservability of Jamunapari buck semen. Evaluation of the neat semen was also done and diluted semen samples added with EDTA and Cysteine HCl preserved upto 72 hours and evaluation was done for 0, 24, 48 and 72 hours and assessed on the basis of motility, live sperm percentage, and spermatozoal abnormalities.

5.1. Seminal characters:

5.1.1. Colour and consistency:

The colour and consistency of Jamunapari buck semen was found to be whitish yellow and viscid respectively which is in agreement with the finding of Patel (1967). In non-descript breed, Roy (1975) also reported similar colour. Colour of semen is greatly influenced with its consistency (Tomar, 1976).

5.1.2. Ejaculate volume:

The mean volume of different bucks of seminal ejaculates (Table-I) was found to be 0.51±0.012. The finding is in close agreement with the findings of Patil and Raja (1978) who observed semen volume in Malabari buck was 0.5 ml.

Peskovastsov (1985) reported ejaculated volume of buck, 0.71 ml. at the frequency of 3 colletions weekly. Sahni and Roy (1967) studied the volume of Barbari bucks (Av. 0.71 ml). Ahmed et al. (1997) who found the average ejaculated volume as 0.77 ml. in Sannen bucks. Pandey et al. (1985) observed semen volume of buck in Sannen and Barbari buck were 0.96 ml. and 0.79 ml. respectively. Roca et al. (1992) who found that average semen volume was 1.05±0.1 ml. in Murcica-Granada goats, Results of above workers were higher than that of present studies (0.53 ml.). In another study Patnaik et al. (1991) who observed semen volume in Ganjan bucks was 0.44±0.05 ml. which is lower than present studies (0.53 ml.).

Ejaculate volume has been found to be affected by many intrinsic and extrinsic factors viz. agro-climatic conditions, age, body weight, feeding, management, plane of nutrition and functional status of the accessory sex glands (Prasad et al. 1980). This might be the reason for variation in ejaculate volume, recorded during the study.

5.1.3. Mass motility:

The mean percentage of mass motility has been presented in Table-1. These observations are in close agreement with the findings of Singh et al. (1982) average initial motility was 78.30±2.48 percent for

Barbari bucks. Sinha et al. (1981) studied that average motility percentage was 80.04±14 for Jamunapari bucks. Another workers reported lower value of initial motility percentage viz; Ahmed et al. (1997) observed that the average mass activity was 60.38 percent. Traldi (1983) reported that average sperm motility of Moxoto bucks was 90.6±6.14 percent which is higher than present investigation (81.83±44).

Motility was greatly influenced by breed, individual secretion of accessory sex glands, season, and techniques of collection, handling of semen which might be the reason for variation in sperm motility recorded in the present findings.

5.1.4. Sperm concentration:

The average sperm concentration in neat semen was found to be 4210.51±61.53X106 /ml. (Table-1). The sperm concentration of bucks used in present investigation was within the normal limits as reported by some workers in other breeds of buck. Koh (1975) found the average sperm concentration to be 3974.8±186.41x106 per ml.; Pandey et al. (1985) observed that in Sannen buck was 4503.26±14.75 millions per ml, Das and Rajkonwar (1993) reported 4401.05x106 sperm per ml. for Beetal buck semen. Other scientist reported lower concentration than that of present investigation (4210.51±61.53x106). Ahmed et al.

(1997) reported average 2.77 billion per cmm. in Sannen bucks under tropical climate. Puranik et al. (1993) obtained 3079x106±24.5 and 2763x106±86.3 per ml. for Osamnabadi and crossbred bucks, respectively. Sharma and Arora (1970) reported 5166.277±176.53 million per ml., Saxena and Tripathi (1979) reported 4795.0x106 per ml. average sperm concentration in Jamunapari bucks which is higher than present investigation (4210.51±61.53x106).

This variation in present investigation might be due to effect of climate, season, nutrition, breed, weight of animal, individual collection and frequency of collection.

5.1.5. Live sperm percentage:

The mean percentage of live sperm has been presented in Table-1. The live sperm percentage recorded in this study is in close agreement with the finding of Mittal (1987) 80.0, 80.8, 78.2 82.3 and 79.1 during spring, summer, monsoon, autumn and winter season respectively in indigenous and crossbred goats. Pandey et al. (1985) observed that percent live spermatozoa were 82.23 and 71.44 in Sannen and Barbari bucks, respectively. The present investigation for live spermatozoa was in agreement with above mentioned values. Some other workers reported more live percentage than present investigation (81.83±3.44). Roy (1975) reported 87.81±1.32 percentage in nondescript

breed of bucks. Igboeli (1974) reported 87.2±10.0 and 87.7±1.0, respectively in native Zembian and Boer bucks.

Variation in present investigation might be due to effect of age, season, climate, method of semen collection, frequency of collection and breed.

5.1.6. Sperm abnormality:

The acrosomal, head and tail abnormalities percentage in Jamunapari buck as reported in the present study in table -3 (i.e. 0.815±0.007, 0.0848±0.003 and 4.498±0.021 respectively) are within normal range and in close agreement with the findings of Koh, (1975); Patnaik et al. (1991); and Mittal (1987). Baviskar (1985) reported total sperm abnormality as 7.85±0.34 which is higher than the present study. Sinha (1986) reported head, tail and acrosomal abnormality percentage as 0.06±0.04, 2.85±0.12 and 1.23±0.09 respectively in Jamunapari buck.

5.2. Influence of additives on seminal characters at different hours of preservation:

5.2.1. Motility of spermatozoa:

Mean motility percentage at different hours of preservation in various additives added extender have been presented in Table-5. The influence of various additives was found highly significant ($P<0.01$) decline upon preservation upto 72 hours in jamunapari buck.

The present observation in EYC extender is comparable with the finding of Borgohain et al (1985) and Mohanty et al (1993). At 24 hours of preservation in EYC extender it is higher than the values reported by Puranik et al (1994). At 72 hours of preservation in EYC extender, the present finding is lower than the finding of Deka and Rao (1986)

Reports regarding the effect of EDTA and Cysteine HCl on post preservation of buck semen seems to be apparently lacking.

However, the result of the present study are comparable with the earlier reports on bovine chilled semen (Popov, 1968; Sengupta et al 1969; Shannon and Curson, 1983; Singh et al 1989; Dhami and Sahni 1994; and Kumar et al 1996a and 1996b) where higher motility percentage in EDTA and Cysteine HCl treated extender were recorded.

The beneficial effect of EDTA and Cysteine HCl in sperm preservation was shown to be due to chelation of most noxious mineral elements, protection of sulph-hydryl containing substances in reduced environment and its restrictive effect on aerobic metabolism and stimulation of anaerobic glycolysis by bovine spermatozoa (Sengupta et al 1969; Abdou and Guindi, 1977).

5.2.2. Live sperm percentage:

The live sperm percentage at different hours of preservation indicated that there was decrease in percentage of live sperms with increase in duration of preservation (Table-7). However the highest mean live sperm percentage was present at 0 hour which was followed by 24,48,and 72 hours of preservation. Highly significant ($P<0.01$) effect of hours of preservation on this character with both additives. However EYC+Cysteine HCl had better result in comparison to EYC+EDTA at this particular concentration of both additives. Available literatures on live sperms percentage after addition of EDTA and Cysteine HCl at different hours of preservation at refrigerator tempreture were scanty. However percentage of live spermatozoa obtained during the present study were higher at all the hours of preservation when EDTA and Cysteine HCl were added with EYC extender which support the reports of Saxena and Tripathi, (1984) in rams; Nadroo et al. (1988);Singh et al. (1989); Dhami and Sahni, (1994) and Kumar et al. (1996b) in bovines. overall percentage of live sperm obtained during the present study in EYC dilutor as compared with reports of Deka (1984) who recorded the percentage of live sperm as 82.21±1.08 with Tris dilutor.

Thus, it can be inferred that incorporation of EDTA and Cysteine HCl with EYC extender improved the post-

preservation longivity of life of spermatozoa in comparison to without additives added EYC extender.

5.2.3. Acrosomal abnormality:

It is well known that during storage and freezing of spermatozoa, sperm phospholipids undergo peroxidation which leads to formation of toxic fatty acids peroxides. The peroxides further lead to the structural damage to the sperm cell accompanied by lower motility and enzyme leakage from acrosome (Jones and Mann, 1977 and Mann et al., 1980). The acrosome and its enzyme play essential role in the penetration and fertilization of mammalian ova (Bedford, 1968; Stambaugh and Buckley, 1969).

Reports on effects of EDTA and Cysteine HCl on acrosomal damage in caprines are apparently lacking. Acrosomal abnormality varies from 0.839±0.003 to 4.55±0.048 percent (Table no.-9) at different hours of preservation which was lower than that of Deka (1984) whose figures ranged from 5.07±0.77 to 11.87±1.94 after 5 hours of equilibration in different extenders. The acrosomal abnormality result was in close conformity with Sinha (1986) who found the acrosomal abnormality range between 1.58 and 5.17 percent.

The acrosomal abnormalities values were found significantly ($P<0.01$) lower in EYC extender having Cysteine HCl followed by EDTA. However, significant effects on acrosomal damage were observed due to the

addition of EDTA and Cysteine HCl in the EYC extender. In present study additives influenced significantly the acrosomal abnormality which supports the finding of Singh (1992), Singh et al. (1995).

Studies pertaining to the effect of additives on the acrosomal morphology in buck semen are very scanty and thus the results of the present study can not be compared in lack of information on this aspect.

5.2.4. Head abnormalities:

The incidence of head abnormality with different additives at 0, 24, 48 and 72 hours of preservation have been presented in table-10 . Non-significant effect of additives at different hours of preservation was observed (Table - 10).

Percentage of head abnormality remained appreciably stable in both additives. The percentage of head abnormality which remained fairly stable and did not vary significantly in EYC+EDTA and EYC+Cysteine HCl. Sinha (1986); Sinha (1992) reported head abnormality remains stable in both additives.

5.2.5. Tail abnormality:

Perusal of Table-11 indicates that percentage of tail abnormalities varied from 4.495 to 5.615 with different additives added extenders at different hours of preservation. There was noticeable change between

control and EYC+EDTA, control and EYC+Cysteine HCl at all hours of preservation except at zero hour. It was highest in control and lowest in EYC+CysteineHCl. It was from 4.495±0.028 to5.615±0.022 which was lower to the reports of Sinha (1986) who recorded the values vary from 5.83±0.38 to 7.33±0.33 percent.The present finding on tail abnormality are in close accordance with the reports of Singh and Purbey (1996) who observed 3.88±0.09 and 5.67+0.024 percent in tail abnormality in Tris and EYC extenders respectively.

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Chapter-VI

SUMMARY

SUMMARY

A study was conducted on 144 semen samples of 6 Jamunapari breed of bucks maintained at the I.C.A.R Eastern Zone, Patna under the standard feeding and managemental conditions. A schedule of twice weekly collection of semen per buck was followed and collection was made with artificial vagina technique. Ejaculates having more or, equal to 70 percent initial motility was finally diluted with EYC extender in 1:10 ratio containing additives viz. Cysteine Hcl and EDTA separately at the rate of 1 mg/ml. respectively. Extended semen samples were preserved in refrigerator at 40C and evaluated for the various sperm characteristics viz., progressive motility, live sperm percentage, sperm abnormality and acrosomal morphology at 24 hours interval up to 72 hours of preservation. Data were statistically analysed for assessing their preservability.

The mean value for different seminal attributes viz., mean volume (ml.), mass motility (%), sperm concentration (millions/ml.), live sperm percentage, acrosomal abnormality percentage, head abnormality percentage and tail abnormality percentage in neat semen were observed 0.51±0.012, 81.08±0.46, 4288.56±61.53 , 81.34±0.38, 0.815+0.07, 0.0848+0.003 4.498+0.021 respectively.

There was non-significant difference in the sperm volume, percentage of live spermatozoa between bucks but there was significant difference in mass motility and concentration between jamunapari bucks.

Sperm motility and live percentage of spermatozoa declined gradually in both additives added extender (EYC) during preservation but remained higher than control. Their maximum level for motility was with Cystine Hcl at 0 hour were recorded 78.70 percent and live percentage with Cysteine Hcl was 80.10 percent. After 72 hours of preservation progressive motility percentage was 57.79 percent and live percentage was 58.90 percent. Further, it was observed that the values of these characteristics were found lowest with extender EYC without additives, for motility 77.61 percent and for live percentage 79.07 percent at 0 hour and 55.09 and 52.97 at 72 hours of preservation respectively. Effects of both, hours of preservation and additives on these seminal attributes were found to be significant ($P<0.01$), except at zero hour in EYC added with EDTA & Cysteine Hcl.

The variations in the head abnormalities of the spermatozoa during preservation with both additives were found non - significant, which was ranging from 0.0860 at 0 hour to 0.0947 percent at 72 hours of preservation. The incidence of tail abnormality was recorded 4.495 at 0 hour which increases to 6.021 at 72 hours of preservation. The influence of additives on tail

abnormalities were recorded significant ($P<0.01$) at different hours of preservation except at zero hour. It was found significantly lower with Cysteine Hcl as compared to EDTA.

The percentage of acrosomal abnormality varied significantly ($P<0.01$) between additives added extender at different hours of preservation. The acrosomal abnormality were found significantly lowest in EYC extender with Cysteine Hcl 0.811 at 0 hour and 3.68 at 72 hours in comparison to EDTA 0.828 at 0 hour and 4.03 at 72 hours; in compared to EYC 0.839 at 0 hour and 4.55 at 72 hours of preservation respectively.

Thus, on the basis of evaluation of post preservation seminal characteristics, it could be concluded that the semen quality of preserved bucks semen can be maintained better with additives added extender viz. Cysteine Hcl and EDTA up to 72 hours of preservation. Additives-wise comparison showed that maintaining the progressive motility and live percentage of spermatozoa was better with Cysteine Hcl.

The effect of additives on seminal attributes can be judged more accurately on the basis of conception rate. It requires further more study.

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Chapter-VII

CONCLUSION

CONCLUSION

The following conclusion could be drawn from the present study:

1. A decline trend in motility and livability were observed with the increase in duration of preservation.

2. EDTA and Cysteine Hcl added in EYC extender @ 1mg/ml each respectively were found to improve the quality of buck semen at different hours (0, 24, 48 & 72 hrs) of preservation at 4°C.

3. The progressive motility and live sperm percentage were significantly ($P<0.01$) higher in EYC extender added with Cysteine Hcl as compared to EDTA added extender upto 72 hours of preservation.

4. The acrosomal abnormality was lower in Cysteine Hcl added extender (EYC) as compared to EYC. added with EDTA at different hours of preservation.

5. Head abnormality did not vary significantly between additives as well as different hours of preservation.

6. Significant ($p<0.01$) effects of additives on tail abnormality were recorded at different hour of

preservation. Its incidence was significantly lower in Cysteine Hcl added EYC extender compered to EDTA added EYC extender.

7. Incorporation of additives such as Cysteine Hcl and Ethylene di-amine tetra acetic acid (EDTA) in EYC extender improved the post preservation quality of Jamunapari buck semen.

8. Overall influence of Cysteine Hcl was found to be better additive in terms of progressive motility, live percentage, acrosomal abnormality and tail abnormality in comparision to EDTA.

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Chapter-VIII

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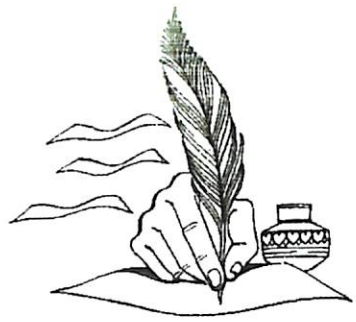

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