"INFLUENCE OF ADDITION OF GLUTATHIONE AND THEOPHYLLINE 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. PREM SHANKER

Registration No.- M/VOG/78/2007-08

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

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PATNA – 800014

2011

Dedicated

to my

beloved Parents

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to my

beloved Parents

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BIHAR VETERINARY COLLEGE, PATNA- 800 014
RAJENDRA AGRICULTURAL UNIVERSITY, PUSA (SAMASTIPUR), BIHAR

Dr. R.P. Pandey
University Professor-cum-Senior Scientist
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CERTIFICATE-I

This is to certify that the thesis entitled ***"INFLUENCE OF ADDITION OF GLUTATHIONE AND THEOPHYLLINE 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. Prem Shanker, Registration No.: M/VOG/78/2007-08** 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.

Pandey 19/4/2011

(Dr.R.P.Pandey.)
Major Advisor

Endorsed

(Chairman/ Head of Department)

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CERTIFICATE-II

We, the undersigned members of the advisory committee of **Dr. Prem Shanker, Registration No.: M/VOG/78/2007-08,** 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 the thesis ***"INFLUENCE OF ADDITION OF GLUTATHIONE AND THEOPHYLLINE ON PRESERVATION OF JAMUNAPARI BUCK SEMEN"***, may be submitted by **Dr. Prem Shanker** 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 GLUTATHIONE AND THEOPHYLLINE ON PRESERVATION OF JAMUNAPARI BUCK SEMEN"*** submitted by **Dr. Prem Shanker, Registration No.: M/VOG/78/2007-08** 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 07-07-...../2011.

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Prem

Date - 07/7/11 **Prem Shankar**

Place - Patna.

CONTENTS

CHAPTER	DESCRIPTION		PAGE NO.
1.	INTRODUCTION		1 - 4
2.	REVIEW OF LITERATURE		5-22
	2.1. Seminal attributes of neat semen of buck.	---	5-14
	2.2. Tris as extender for freezing of buck semen	---	14-16
	2.3. Semen additives	---	16-22
	2.3.1. Glutathione	---	16-20
	2.3.2. Theophylline	---	20-22
3.	MATERIAL AND METHODS		23 - 35
	3.1. Experimental animals	---	23
	3.2. Collection of semen	---	23
	3.3. Evaluations of semen	---	23
	3.3.1. Macroscopic evaluation	---	23
	3.3.2. Microscopic evaluations	---	24 - 30
	3.3.2.1. Mass activity	---	24
	3.3.2.2. Progressive motility	---	24
	3.3.2.3. Live sperm percentage	---	25

3.3.2.4. Concentration of spermatozoa --- 26

3.3.2.5. Percentage of abnormal spermatozoa --- 27

3.3.2.6. Acrosomal morphology --- 28

3.4. Preparation of Tris diluter --- 31

3.5. Dilution of semen --- 31

3.6. Processing of semen --- 31

3.7. Semen additives --- 32

3.7.1. Glutathione --- 32

3.7.2. Theophylline --- 34

3.8. Preservation of semen at refrigerated temperature --- 34

3.9. Microscopic evaluation of preserved semen samples --- 34

3.10. Statistical analysis --- 35

4. RESULTS --- 36-50

4.1. Seminal attributes of neat semen --- 36

4.1.1. Colour and consistency --- 36

4.1.2. Ejaculated volume --- 36

4.1.3. Mass motility --- 37

4.1.4. Sperm concentration --- 37

4.1.5. Live sperm percentage --- 38

4.1.6. Acrosomal abnormality	---	40
4.1.7. Head abnormality	---	40
4.1.8. Tail abnormality	---	40
Processing of semen	---	42-50
4.2.1. Progressive motility of sperm	---	43
4.2.2. Live sperm percentage	---	45
4.2.3. Acrosomal abnormality	---	46
4.2.4. Head abnormality	---	48
4.2.5. Tail abnormality	---	49
5. DISCUSSION	---	51-63
5.1. Seminal characters	---	51
5.1.1. Colour and consistency	---	51
5.1.2. Ejaculated volume	---	51
5.1.3. Mass motility	---	53
5.1.4. Sperm concentration	---	53
5.1.5. Live sperm percentage	---	54
5.1.6. Sperm abnormality percentage	---	55
5.2. Influence of additive on seminal characters at different hours of preservation.	---	56
5.2.1. Motility of spermatozoa	---	56
5.2.2. Live sperm percentage	---	58

5.2.3. Acrosomal abnormality --- 59

5.2.4. Head abnormality --- 62

5.2.5. Tail abnormality --- 63

6. SUMMARY 64-68

7. CONCLUSION 69-70

8. BIBILIOGRAPHY I – XIV

*****

LIST OF TABLES

TABLE NO.	DESCRIPTION	PAGE NO.
1.	Mean ± S.E. and C.V. % of seminal characteristics of freshly ejaculated semen.	39
2.	Analysis of variance showing the influence of buck on seminal attributes of neat semen.	39
3.	Mean ± S.E. and C.V. % of spermatozoal abnormality of freshly ejaculated semen.	41
4.	Analysis of variance showing the influence of buck on abnormality percentage of acrosome, head and tail of neat semen.	42
5.	Mean ± S.E. and C.V. % of progressive motility percentage of spermatozoa in buck semen, preserved at different time intervals.	44
6.	Analysis of variance for the effect of additive on semen quality of bucks at different hours of preservation.	44
7.	Mean ± S.E. and C.V. % of live sperm percentage in buck semen, preserved at different time intervals.	47

8. Mean ± S.E. and C.V. % of acrosomal abnormality percentage of spermatozoa in buck semen preserved at different time intervals. --- 48

9. Mean ± S.E. and C.V. % of head abnormality percentage of spermatozoa in buck semen preserved at different time intervals. --- 48

10. Mean ± S.E. and C.V. % of tail abnormality percentage of spermatozoa in buck semen preserved at different time intervals. --- 50

11. Analysis of variance for the effect additive on semen quality of bucks. --- 50

LIST OF FIGURES

FIGURE NO.	DESCRIPTION
1.	Progressive motility percentage of spermatozoa with duration of preservation.
2.	Live sperm percentage of spermatozoa with duration of preservation.
3.	Acrosomal abnormality percentage of spermatozoa with duration of preservation.
4.	Head abnormality percentage of spermatozoa with duration of preservation.
5.	Tail abnormality percentage of spermatozoa with duration of preservation.

LIST OF PHOTOGRAPHS

PHOTOGRAPHS NO.	DESCRIPTION

CHAPTER - 1

INTRODUCTION

INTRODUCTION

Goats are a renewable source of great value for our country having diverse ecological adaptability over a wide range of agro-climatic zones.

Rearing of goat is preferred over other livestock particularly in drought prone and tribal areas due to its small size, easy handling, high prolificacy, better reproductive efficiency and ability to utilize a wide range of feed and fodder resources with low initial investment and good remuneration. That's why goat is called as poor men's cow.

India ranks second in goat population at International level having 17% of total goat population. According to Animal Census report (2003), total goat population of India was 12.436 crores, which is about 25% of its total livestock population. Presently our country is having 21 well defined breeds of goats which contribute about 20 percent of the total goat population and remaining 80 percent are of non-descript type, thereby indicating a wide gap between improved and desi/non-descript type breeds.

To improve the goat population for meat, milk and wool (Pashmina) production, it is necessary to exploit the better germplasm up to maximum limits which could be achieved only through assisted

reproductive technologies such as semen preservation, A.I (Artificial Insemination), MOET (Multiple Ovulation and Embryo Transfer Technology) etc. The number of productive animal and productivity of local breed can also be increased within stipulated time through these techniques.

The semen is an important constituent and plays an important role in A.I. Studies on physical and biochemical attributes as well as dilution, addition of different chemicals and biochemical compounds (semen additives) viz:- Oxytocin (Pelaez et al. 2006, on Boar semen); Glutathione (Atessahin et al. 2008 on goat semen); EDTA (Puranik et al. 1994 on buck semen); Cysteine Hydrochloride (Raval et al. 2006 on bull semen.); Theophylline (Hoskins et al. 1974 on bull semen).were found helpful in preserving buck semen for following of insemination in time.

Several attempts have been made to improve the motility of spermatozoa as well as fertility of semen by incorporating various motility enhancing agents as additives in liquid and frozen thawed semen of different livestock and laboratory animals as well as human beings. Theophylline & Glutathione are such agents which have been tried in the recent past with variable results. The mechanism by which Theophylline stimulate sperm motility is thought to involve the inhibition of

phosphodiesterase activity & subsequent accumulation of cyclic adenosine3'-5' monophosphate (CAMP) within the sperm cell, which regulates the sperm motility by activation of CAMP dependent protein kinase / kinases. In turn the enhanced CAMP level inside the sperm cells increases their motility (Garbers et al. 1971; Schoenteld et al. 1975; Hoskins & Casillas. 1975a & Tash, 1976). On the other hand, it was also suggested by Peterson et al. (1979) that Theophylline effects the sperm plasma membrane which decreases the calcium uptake by spermatozoa since, considerable damage is inflicted on the sperm plasma membrane during cryostorage. The loss of motility observed after freezing & thawing is probably due to the inability of the damaged plasmalemma to control the levels of calcium within the sperm cells. Theophylline may compensate the resultant loss of motility by stimulating the activity of an out wardly directed plasma membrane calcium pump through the mediation of CAMP (Aitken et al. 1983).

Glutathione as a substrate of Glutathione peroxidase present in normal sperm head, decrease the peroxidation process & hence maintains the sperm motility (Christopherson, 1968).

Munsi et al. (2007) used Glutathione as additive in bull semen and recorded increased sperm

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1. Seminal attributes of neat semen of buck

Jelam and Nambiar (1965) reported that in 14 semen samples from 7 Jamunapari bucks, the average sperm motility was 72.3±3.98 percent. They also reported that when semen was diluted (1:20) in egg yolk citrate & goat milk and stored for 24 and 72 hours, the average motility was 54±2.73 and 43.6±2.69 percent respectively.

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

Mishra and Sengupta (1965) reported ejaculated volume, initial motility and sperm concentration of Jamunapari buck as 0.65±0.07ml, 2.30±0.50 and 4080±450 millions, respectively.

Patel (1967) reported the characteristics of Jamunapari buck semen and found the colour and consistency of ejaculated semen as yellowish and viscid respectively, the average volume to be 0.815 ml, motility +5, concentration 1.5 billions per ml, abnormal sperms 5 percent, pH 6.5 and live sperms 90.7 percent.

While studying the semen of Barbari Buck, Sahni and Roy (1967) reported volume, sperm motility, concentration and percentage of live sperm as 0.71 ml; 4.7; 1804 millions/ml and 82 percent, 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.

Tiwari *et al.* (1968) in an assessment on the quality of semen of Barbari and Sannen bucks recorded the sperm concentration as 2313.95±169.75 and 4652.14±205.58 millions/ml. and the percentage of live

Vinha (1975) reported that the sperm concentration was 1752380 spermatozoa /mm^3 and volume 1.68 ml. in three years aged Anglo Nubian goat.

Koh (1975) reported an average sperm concentration as $3974.8\pm186.41x10^6$ per ml. and percentage of sperm abnormalities as 3.11±0.28 in crossbred goats (Jambing x Jamunapari).

Roy (1975) studied 50 semen samples from 5 non-descript bucks and reported an average (mean) volume to be 0.46±0.01 ml, 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.

Kang and Chung (1976) reported sperm concentration as $9.86x10^8$/ml. in Korean native goats in Nov-Dec.

Patil and Raja (1978) reported an average sperm volume and concentration of Malabari bucks as 0.5 ml. and $3534x10^6$/ml. respectively.

Mohan *et al.* (1980) reported the normal values for different semen characters of Sannen, Barbari and Jamunapari x Barbari crossbred

goats and found an average sperm concentration to be 2820.96±7.427, 2117.65±32.445 and 2375.47±7.095 millions per ml. and live sperm percentage 92.46±0.449, 94.06±0.252 and 92.37±0.442 percent, respectively.

Saxena and Tripathi (1980) reported an average mass motility of 3.78±0.07 (0-5 scale), sperm concentration 4795.0±292.97 x 10^6/ml, live sperm 77.65 percent and abnormal sperm 6.84 percent in Jamunapari buck.

Mean values of sperm concentration per ml. and total sperm count per ejaculate in Pashmina bucks were 35.21±1.18x10^8 and 21.50± 0.92x10^8, respectively (Mohan *et al.*, 1980).

Sinha *et al.* (1981) reported average motility 4.02±0.14 (0-5 scale) and sperm concentration 3837.08±435.99 millions per ml in Jamunapari bucks.

The incidence of acrosomal abnormalities in Jamunapari bucks was reported as 5.23 percent by Dabas *et al.* (1982).

Singh *et al.* (1982) reported average ejaculate volume 0.86±0.09 ml. and 1.01±0.04 ml. and an average initial motility of 74.0±0.40

and 78.30±2.48 percent respectively in Jamunapari and Barbari bucks.

Sinha and Singh (1982) reported ejaculate volume 0.45 ml. and 0.75 ml; average mass motility 4.44 and 4.51 and sperm concentration 2440.15±40.885 and 2780.30±35.68 millions/ml. ($P<0.01$) interval in Black Bengal and Sannen bucks respectively on weekly collection.

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

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

Pandey *et al.* (1985) reported an average semen volume 0.96 ml. and 0.79 ml, average mass motility 89.66 and 78.85 percent, average live sperms 82.23 and 71.44 percent and sperm concentration 4503.256±114.75 and

abnormality percentage in Black Bengal bucks as 83.15±0.64, 0.03±0.03, 2.78±0.14, 0.88±0.11; in Jamunapari Bucks as 83.52± 0.48 0.06±0.04, 2.85±0.12, 1.23±0.09 and 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 the sperm concentration of Angora and 1/8 exotic Angora × 7/8 local Angora goat as 2597.98±86.38 and 2810.45±96.77×10^6/ml. and percent live spermatozoa as 86.69±1.4 and 86.68±1.10, respectively.

Mittal (1987) recorded the mean values for sperm concentration (x10^8) as 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, autumn 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 as 1 ml, with creamy consistency, pH 6.6, forward motility 80 percent and number of dead spermatozoa 20 percent.

Roca *et al.* (1992) observed 6.91±0.15 percent of spermatozoa showing acrosomal damage in the semen of Murciana Grandiana goats.

Das and Rajkonwar (1993) reported sperm concentration as 4401.05×10^6 per ml. and percentage of viable spermatozoa as 90.25 for Beetal buck.

Puranik *et al.* (1993) reported average ejaculate volume as 0.56±0.3 ml. and 0.58±0.2 ml; percentage of motile spermatozoa as 70.1±2.0 and 75.0±1.9; sperm concentration 3079±24.5 and 2763±86.3 ($\times 10^6$) per ml. for Osmanabadi and Crossbred bucks, respectively.

Singh and Raja (1995) reported percent live spermatozoa in Sirohi buck semen as 84.80±0.05 percent.

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

Noran *et al.* (1998) reported an average volume of 0.6±0.02 ml. and 0.78 ±0.02 ml. in local Katjang bucks and 0.90±0.02 ml. and 1.02±0.01 ml. in crossbred (Katjang x German) bucks for the first and second ejaculates respectively.

2.2 Tris as extender for freezing of buck semen:-

Fougner (1976) used Tris extender to freeze buck semen.

Hukeri *et al.* (1977) found Tris Fructose citric acid glycerol to be better dilutor to freeze buck semen where as Westhuysen (1978) to freeze semen of Angora buck used Tris base diluents with egg yolk and glycerol successfully.

Ritar and Salamon (1982) used Tris based diluents for freezing buck semen.

Deka (1984) diluted semen samples in four extenders namely Tris egg yolk citrate fructose

glycerol. SMEYFG, EYCFG and REYG and reported that the percentage of motile sperm varied significantly between extenders. He reported higher post- thaw motile and live sperm percentage in Tris dilutor.

Sinha (1986) successfully froze goat semen in Tris egg yolk glycerol dilutor.

Shrivastava *et al.* (1989) found that Tris base extender gave better post-thaw motility than other two extenders Glutathione and Theophylline.

Chauhan and Anand (1990) reported that egg yolk Tris was the best extender for the successful freezing of goat semen on the basis of their assessment of sperm motility, live sperm count and acrosomal damage.

Sinha *et al.* (1991) compared the effect of different dilutors on the freezing of Beetal and Crossbred buck semen. They reported higher post-thaw motility and intact acrosome percentage in Tris diluents as compared to skim milk and EYC extenders.

Puranik *et al.* (1992) conducted studies on preservation of Osmanabadi and Crossbreed buck semen at sub Zero temperature and reported that the post-thaw revival rates were

better in Tris dilutor than in laciphos. Live sperm percentage was also higher after preservation in Tris dilutor.

2.3. Semen Additives:

2.3.1. Glutathione:-

Slaweta (1973) diluted the bull semen in a yolk fructose citrate diluents containing glycerol plus 0.02 gm reduced glutathione / 100 ml of dilutor and frozen in liquid nitrogen. He reported an improvement in the motility and fertility of frozen thawed spermatozoa.

Karla *et al.* (1980) reported that the addition of 50, 75 or 100 mg of glutathione per 100 ml diluents and storage at 4-7^{0}C for 24 h had no significant effect on sperm survival.

Gupta *et* al. (1984) reported the effect of glutathione (0.125%) on room temperature (20-28^{0}c) in dilutes crossbred bull semen. They observed the sperm motility after 24 and 48 h as 58-60 vs 46%. After storage for 73 h, sperm motility was 54 Vs 44%.

Slaweta *et* al. (1987) studied the effect of glutathione (5m M) on the motility and fertility of frozen bull sperm diluted in a skim milk-egg yolk-glycerol-fructose extender. The sperm

motility after incubation were 23.3 ± 9.1 and 17.9 ± 10.3% (P<0.001) and the percentage of spermatozoa with normal acrosome was 44.9 ± 11.6 and 41.1 ± 12.4 (P<0.01) in diluents with and without glutathione respectively. The CR to 470 first inseminations with semen diluted with extender containing glutathione was 60.4% Vs 54.1 for 648 inseminations of semen without glutathione. Thus, glutathione can be incorporated as an additive in chilled and frozen bull semen to improve the motility of spermatozoa as well as to check enzyme linkage.

Nadroo *et* al. (1988) observed that the sperm motility and livability declined gradually in all the diluters during storage. In EYC, they were maintained maximally (75.8±0.8 and 77.4±1.4 at 0 hr to 59.6+1.0 and 63.9±1.5 at 72 hr respectively); closely followed by EYC + 0.125% Glutathione (75.0±0.6 and 76.2±1.6 at 0 hr to 55.8 ± 1.5 and 63.3±1.5 at 72 hr) and EYC + 1% glycine (75.4±0.7 and 77.8±1.7 at 0 hr 57.5±1.1 and 65.8±1.7 at 72 hr). They were lowest in EYC +1% glycin + 0.05% Cysteine hydrochloride + 0.125% glutathione + 0.005% EDTA diluter (73.8±0.9 and 76.8+1.5 and 0 hr to 52.5±1.9 and 65.0+1.8 at 72hr). The variations in the abnormalities of the

spermatozoa during storage with all the diluters were non significant.

Bilodeau *et* al. (2000) reported that thiols such as glutathione and cysteine prevented the loss of sperm motility in frozen-thawed bull semen. Cysteine is a low molecular weight amino acid containing thiol; it is a precursor of intracellular glutathione biosynthesis and increase the GSH level.

Foote *et* al. (2002) studied the effect of antioxidants on motility and fertility of bull sperm in whole milk extender. The motility of frozen-thawed sperm during storage at 25^{0}C or 5^{0}C after freezing was compared with duration of storage semen stored without freezing. Antioxidants were generally not beneficial, but the percentage of motile sperm was improved by 6-11 % units ($P<0.05$) when sperm were stored unfrozen or after freezing when 0.5 mM of GSH with or without SOD was added.

Sinha *et* al. (2004) reported that extent of leakage of enzymes was low in the semen extended with 5 mM glutathione. It is quite reasonable to believe that glutathione might be protecting spermatozoa from membrane damage by inhibiting the lipid peroxidation process thereby increasing sperm motility.

Funahashi et al. (2005) studied the effect of antioxidant addition to extender on viability, acrosome integrity and in vitro penetrability of boar spermatozoa preserved at 10°C. Washed spermatozoa were resuspended at 1x108 cells/ml in modified Modena solution containing 20% (v/v) boar seminal plasma and 5 mM antioxidant (glutathione, cysteine or hypotaurine). Control aliquots were the same suspension without added antioxidants. Sperm suspensions were then chilled to 10°C with a computerized cooling programme. Sperm viability after 7 and 14 day was higher in the presence of glutathione or cysteine, whereas hypotaurine did not improve the survival rate. Percentage of chlortetracycline (CTC) fluorescence pattern as intact live cells was higher in spermatozoa preserved with glutathione or cysteine at 7 and 14 day of preservation. When the preservation period was prolonged until 57 days. Survival rate was higher with cysteine than controls. Therefore, glutathione and cysteine can improve the viability and functional status of boar spermatozoa during liquid preservation and in vitro boar spermatozoa penetrated even after preservation in the presence of cysteine at 10°C for 29 days.

Uysal *et* al. (2007) reported that the antioxidants GSSH at 5 mM had a significant ($P<0.001$) effect in maintaining post-thaw sperm motility, sperm morphology, acrosome integrity, viability and membrane integrity when compared to the control group. They recorded motility 60.6±1.63 Vs 39.5±2.73 percent; total abnormality 10.9 ± 2.45 Vs 30.1 ± 5.30 percent; acrosome damage 3.0 ± 0.73 Vs13.4 ± 2.32 percent; viability 78.6 ± 4.48 Vs 50.7 ± 6.05 percent and HOST 65.2±3.81 Vs 36.9 ± 4.40 percent. Futher, they also reported that cysteine at 10 mM had a significant ($P<0.001$) effect on maintaining post-thawing sperm characteristics, when compared to the other treatments. They found the following characteristics compared to control: motility percent 59.0±4.35 Vs 39.5±2.73; total abnormality 9.8±4.05 Vs 30.1±5.30; acrosome damage 2.9±2.52 Vs 13.4 ± 2.32; viability 73.5 ± 6.72 Vs 50.7 ± 6.05 and HOST 40.7 ± 4.74 Vs 36.9 ± 4.40 percent.

2.3.2. Theophylline:-

Haesungcharern and Chulavatnatol (1973) have found the stimulatory effect of caffeine and theophylline on the motility of ejaculated human spermatozoa. They reported that caffeine

increased the motility by 8 per cent and theophylline by 10 percent.

An increase in the intrasperm levelof Camp and in the motility of immature bovine spermatozoa was reported by the addition of a Camp phoaphodiesterase inhibitor, Theophylline, (Hoskins *et* al. 1974-1975).

Hoskins and casillas (1975) reported that by incubating bovine caput spermatozoa in seminal plasma with theophylline it was possible to raise substantially their motility as well as furctolytic ability

Theophylline addition was reported to induce or improve the sperm motility in man and animals by casillas and Hoshins (1970) and Hoskins et al. (1978).

Acott and Hoskins (1978) treated the immature spermatozoa from the bovine caput epididymis with different levels of theophylline (3, 11 and 33 mM) in the presence of bovine seminal plasma and reported that the full motility was attained by theophylline in the presence of bovine seminal plasma. They also reported that the increasing concentration of theophylline markedly increased the final motility index.

Brandt *et* al. (1978) studied the synergistic effect of theophylline with the forward motility protein of seminal plasma and reported that the forward motion was evoked in 40 to 50 per cent of bovine caput spermatozoa in vitro.

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CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. 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.

3.2. 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.

3.3. Evaluation of semen:

The seminal ejaculates were evaluated for the following attributes:

3.3.1. Macroscopic evaluations:

Volume, Colour & consistency of ejaculated semen were observed and recorded.

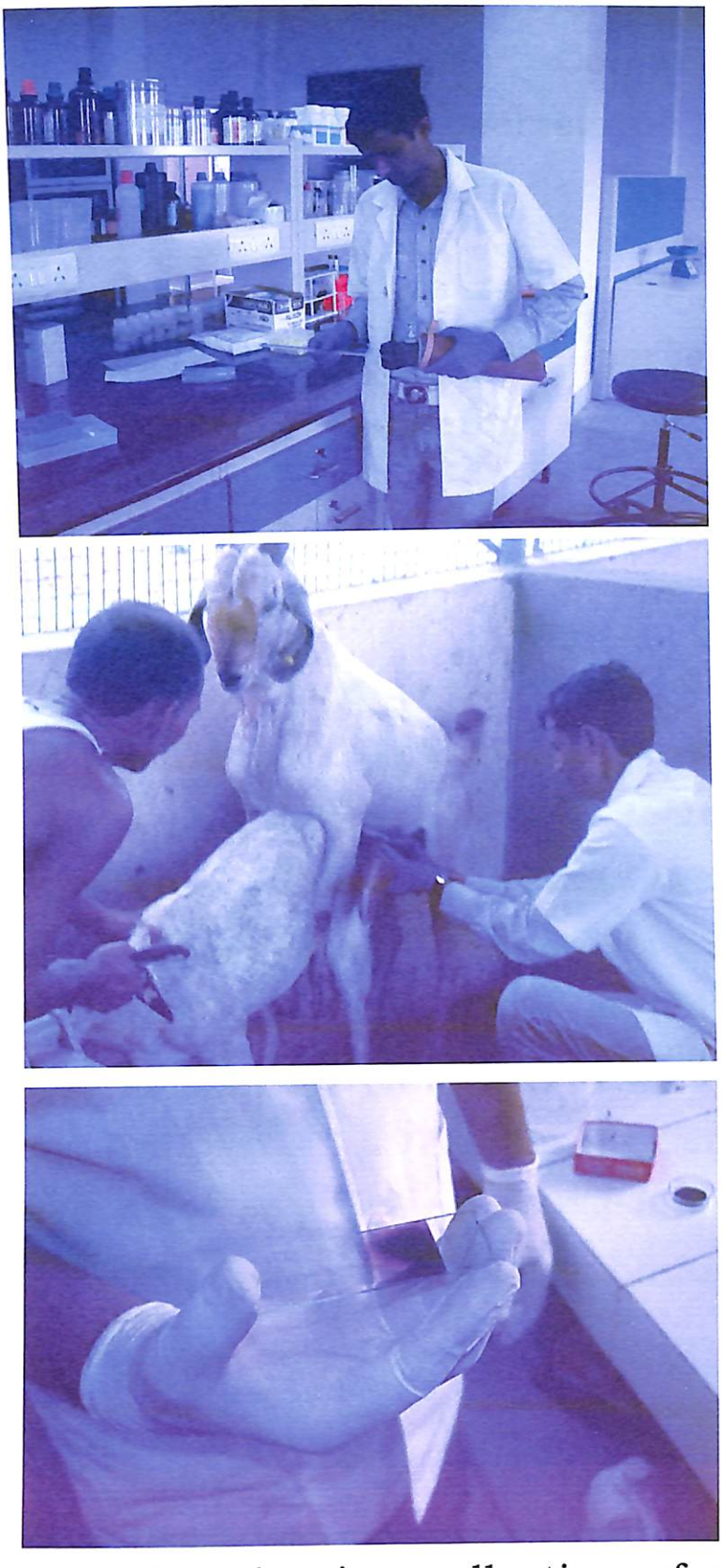

Fig. I: Photographs showing collection of semen and preparation of Tris.

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

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

3.3.2.1. 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 N.S.Tomar, 1976. Semen samples showing mass activity of grade +4 (80%) and above were included for further processing i.e. dilution & preservation.

3.3.2.2. 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 i.e., 2.9% sodium citrate dehydrate solution was used for dilution of semen. Total non-motile spermatozoa where counted in the chamber. Then the same charge haemocytometer 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-

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

3.3.2.3. 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.

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

3.3.2.4. 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 numbers 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.

3.3.2.5. Percentage of abnormal spermatozoa:

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

3.3.2.6 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.
Methanol	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:

KH_2PO_4	22.25 g
H_2O	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.

3.4. Preparation of tris dilutor:

$NH_2C(CH_2OH)3$	12.1 gm
Fructose	5.0 gm
Citric acid	6.8 gm

Glycerol	52.0 ml
Double glass distilled water	369.0 ml
Stock Tris buffer	400.00 ml

3.5. Dilution of semen:

After initial evaluation semen samples were pulled in test tube and extended with Tris. The dilutor was prepared as described earlier and kept in 10 ml. test tubes in water bath at 32°C. The test tubes containing the dilutors were properly labeled. Only freshly prepared dilutor was utilized. 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 Tris. The test tubes were gently rotated between palms for uniform distribution of sperms in the dilutor.

3.6. Processing of semen:

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

3.7. Semen additives:

Subsequent to dilution of semen with Tris dilutor, each semen samples was divided into five fractions for the addition of different levels of two motility enhancing agents. Out of five fractions one fraction

served as control in which no additive was mixed whereas in other four fractions different concentration of two motility enhancing agents were added.

3.7.1. Glutathione – Glutathione was added in extended semen samples in two concentrations viz. 2 mM and 5mM.

To achieve these concentrations (i.e. 2 mM and 5mM) equal volume of diluted semen and standard solution of glutathione (4 mM and 10mM) were mixed together.

Standard solution containing 2 mM and 5 mM glutathione were prepared in Tris dilutor as detailed below:-

a) Glutathione 2 mM:

Glutathione	-	12.292mg
Tris dilutor	-	10 ml

b) Glutathione 5 mM:

Glutathione	-	30.730 mg
Tris dilutor	-	10ml

3.7.2. Theophylline – Theophylline was also added in two concentrations viz., 2mM and 5mM in extended semen samples.

To obtain these concentrations (2 mM and 5mM) equal volume of diluted semen and standard solutions of theophylline (4 mM and 10mM) were mixed together.

Standard solutions containing 4 mM and 10mM theophylline were prepared Tris dilutor as detailed below:

a) Theophylline 4 mM :

Theophylline	-	7.205mg
Tris dilutor	-	10ml

b) Theophylline 10 mM :

Theophylline	-	18.02 mg
Tris dilutor	-	10ml

3.8. 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 additives. 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.

3.9. Microscopic Evaluation of preserved semen samples:

The semen samples diluted with Tris and added with Glutathione and Theophylline as well as preserved at refrigerated temperature ($4^0\pm1^0$C) for different durations (i.e. 0, 24, 48 & 72 hrs) were brought to room temperature & evaluated for seminal attributes viz progressive motility, live sperm count & abnormalities of spermatozoa as detailed earlier.

10. Statistical analysis:

The data recorded were subjected to statistical analysis according to statistical book George W. Snedecor and William 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; Glutathione and Theophylline) with Tris dilutor on preservation of Jamunapari buck semen. Altogether 144 semen ejaculates, collected from 6 Jamunapari bucks were utilized. Freshly ejaculated semen samples were evaluated to access the spermatozoan characteristics viz. mass motility, sperm concentration, live sperm percentage, abnormalities of spermatozoa and sperm acrosomal abnormalities.

4.1. Seminal attributes of neat semen:

Mean with standard error and co-variance percentage of various seminal attributes of neat semen samples have been represented in table -1 & 3.

4.1.1. Colour and consistency:

The colour and consistency of ejaculated semen samples were creamy yellow and viscid respectively.

4.1.2. Ejaculate Volume:

The mean value of ejaculated semen samples collected from six Jamunapari bucks was 0.51±0.012 ml, where as buck wise mean of ejaculated volume were 0.45±0.012, 0.52±0.010,

0.51±0.012, 0.42±0.012, 0.60±0.014 and 0.56±0.010 ml. respectively in utilized buck 1 to 6.

Analysis of variance (Table -2) indicated that variation of semen volume among bucks was non-significant. Buck wise comparison (table-1) showed that buck no. 05 had the maximum volume (0.60ml.) & the buck no. 04 had the minimum (0.42 ml.).

4.1.3. Mass Motility:

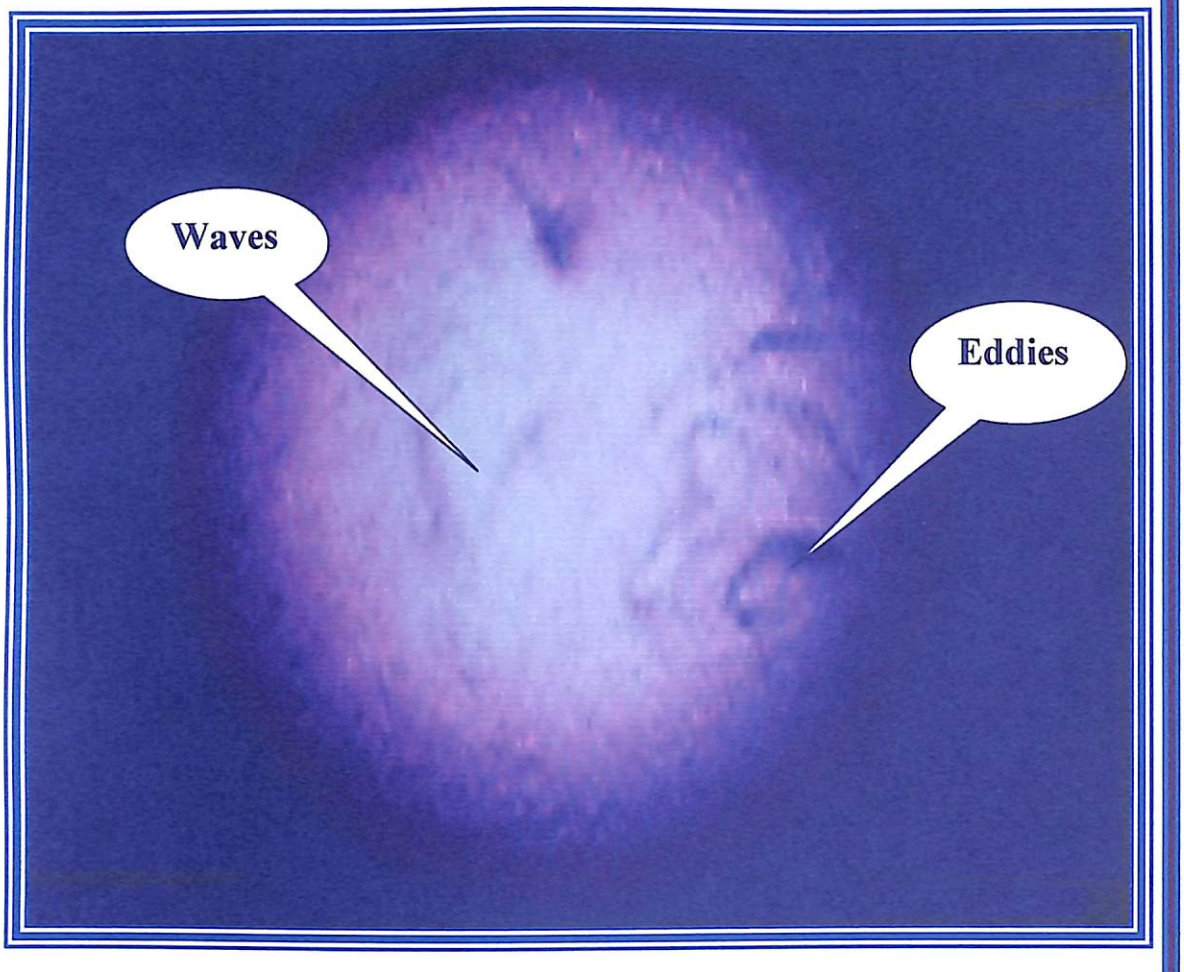
The mean value of mass motility of spermatozoa of 144 ejaculates, collected from 6 bucks was 81.08±3.44 percent. Separately buck wise value for buck no. 1 to 6 were found to be 80.00±0.481, 80.33±0.677, 81.42±0.458, 82.17±0.674 81.04±0.573 and 81.54±0.454 percent respectively.

Analysis of variance (Table -2) indicated that variations in mass motility among bucks were non-significant. Buck no. 04 had maximum motility (82.17%) and Buck no. 01 had minimum (80.00%) as presented in table -1.

4.1.4. Sperm concentration:

The mean value of sperm concentration of 144 samples collected from 6 bucks was 4288.56±67.04 millions per ml. while the mean values separately for buck no. 1 to 6 were 4530.62±67.04, 4168.96±86.18,

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



4295.25±52.56, 4300.87+93.38, 4244.75±54.18 and 4190.92±66.56 millions/ml respectively.

Analysis of variance (Table -2) indicated that variation of sperm concentration among 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 -wise mean live sperm percentages were 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). Buck-wise comparison showed that buck no. 04 had the maximum percentage of live sperm (82.63%) and bucks no. 01 had the minimum (80.13%) as presented in table -1.

Fig. IV: Micro photograph showing Live and Dead spermatozoa

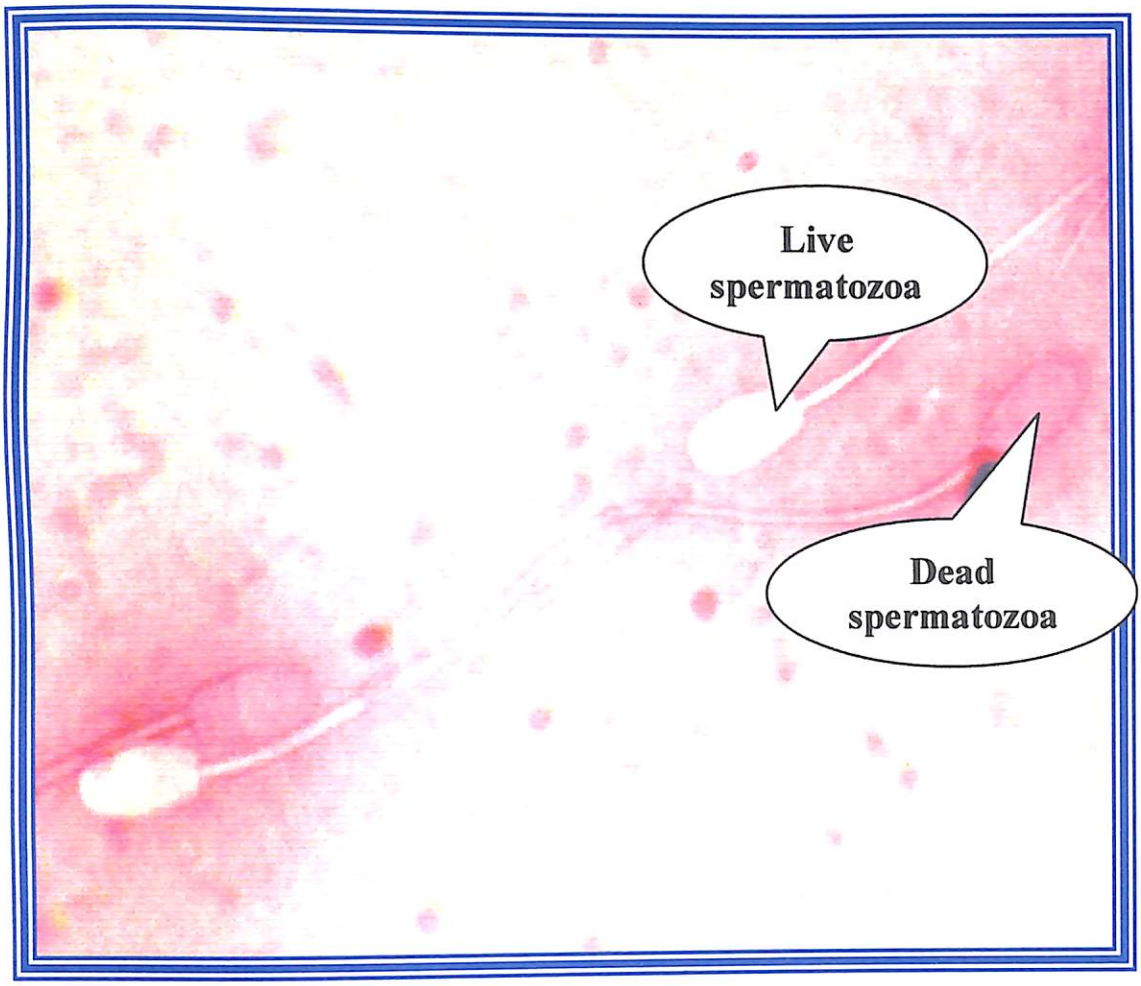


Table-1: Mean±S.E and CV percentage of Seminal characteristics of freshly ejaculated semen.

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}±0.012 (8.384)	80.00^{ab}±0.481 (3.020)	80.13^{a}±0.255 (1.594)	4530.92^{a}±67.04 (7.249)
02.	0.52^{a}±0.010 (7.082)	80.33^{b}±0.677 (4.340)	80.83^{a}±0.382 (2.37)	4168.96^{b}±83.18 (10.127)
03.	0.51^{a}±0.012 (8.786)	81.42^{ac}±0.458 (6.330)	81.55^{a}±0.384 (2.392)	4295.25ac±52.56 (5.599)
04.	0.42^{a}±0.012 (7.905)	82.17^{cc}±0.674 (4.120)	82.63^{a}±0.416 (2.593)	4300.87^{c}±93.38 (10.636)
05.	0.60^{a}±0.014 (9.234)	81.04d^{cc}±0.573 (3.460)	81.28^{a}±0.566 (3.541)	4244.75^{c}±54.18 (6.253)
06.	0.56^{a}±0.010 (5.294)	81.54^{dc}±0.454 (2.790)	81.62^{a}±0.296 (1.836)	4190.92^{b}±66.56 (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 (106)	
		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. Whereas, mean abnormal acrosomes on individual buck were 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. (Table -3).

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

4.1.7. Head abnormality:

The mean value of abnormal sperm head in 144 semen samples collected from 6 bucks was 0.085±0.003 and the mean value of abnormal sperm head in individual bucks were 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 in buck number 1 to 6, respectively. (Table -3)

Analysis of variance showed non-significant variation in head abnormality percentage among bucks.

4.1.8. Tail abnormality:

The mean value of abnormal sperm tail in 144 semen samples collected from 6 bucks was

4.498±0.021 and the same for individual buck (1 to 6) were 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, respectively (Table -3)

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

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

Buck No.	Acrosomal abnormality (Percent)	Head abnormality (Percent)	Tail abnormality (Percent)
	Mean ± S.E & CV%	Mean ± S.E & CV%	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.

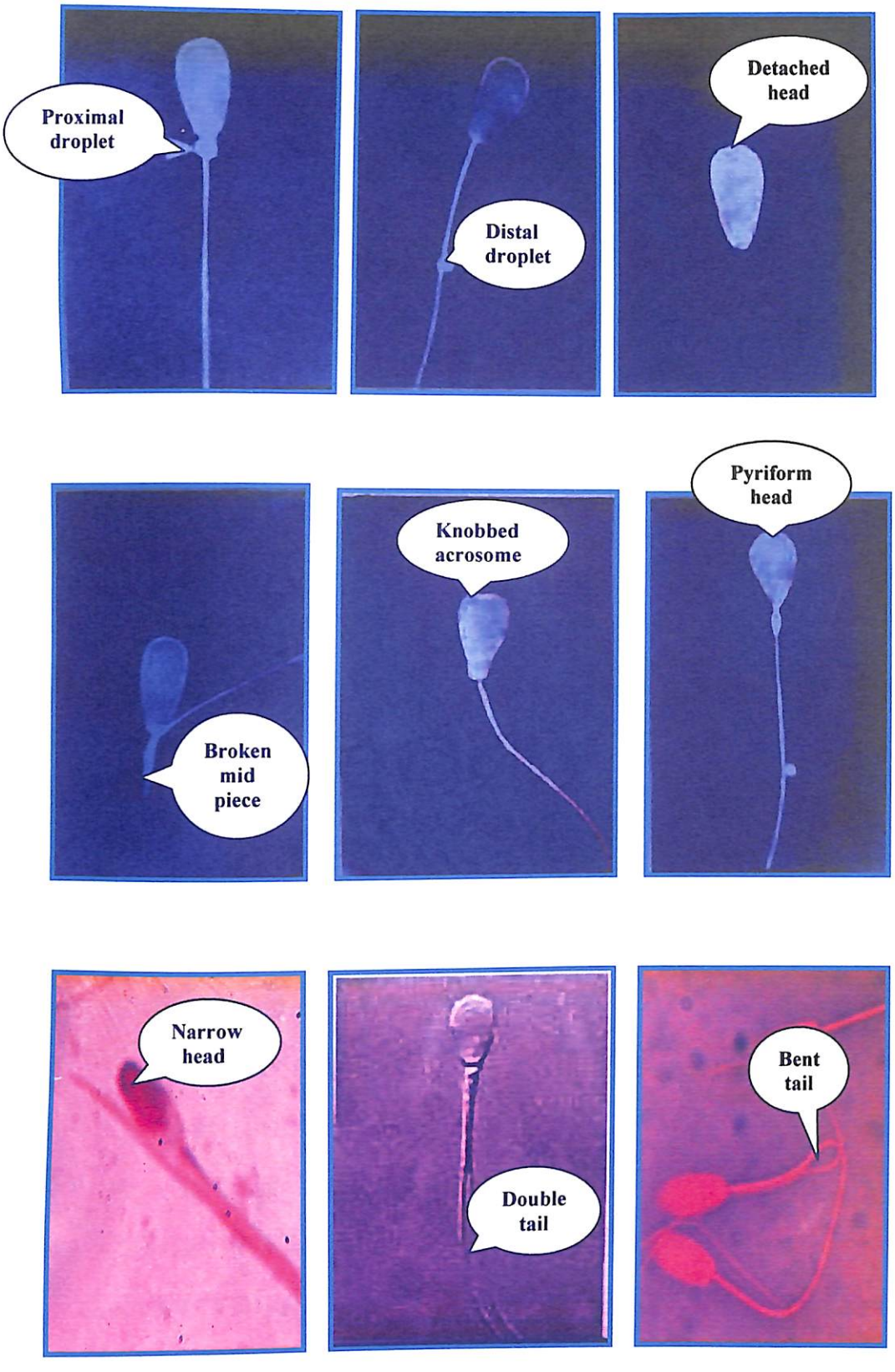
Source of variation	d.f	Acrosomal abnormality (Percent)		Head abnormality (Percent)		Tail abnormality (Percent)	
		MSS	F	MSS	F	MSS	F
Between Bucks	5	0.014	0.55^{NS}	0.005	0.28^{NS}	1.734	0.81^{NS}
Error	138	0.026		0.018		2.134	

NS = Non significant

4.2. Processing of the semen:

Semen samples showing 80 percent or more mass motility of spermatozoa were diluted in Tris extender alone (control) and in glutathione (2mM,5mM) & Theophylline added (4mM,10mM) Tris. Diluted semen samples were preserved at refrigerated temperature up to 72 hrs and were evaluated for various seminal attributes. Viz: Colour & consistency, volume, 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 and their findings were recorded as follows.

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



4.2.1 Progressive motility of sperm:

The progressive sperm motility percentages with Tris dilutor (control) were recorded as 78.17±0.123, 75.05±0.17, 61.18±0.154 and 56.71±0.281 percent respectively at 0, 24, 48 and 72 hours of preservation. whereas the corresponding values Glutathione (2mM) added Tris dilutor were 78.46±0.41, 76.27±0.10, 62.04±0.16, 57.94±0.15 percent, Glutathione (5mM) added Tris dilutor were 78.58±0.17, 77.76±0.15, 64.23±0.24, 60.61±0.16 percent, Theophylline (4mM) added Tris dilutor were 78.85±0.16, 77.96±0.15, 64.61±0.16, 60.96±0.23 and Theophylline (10mM) added Tris dilutor were 78.35±0.42, 77.96±0.41, 63.08±0.18, 59.64±0.66 percent 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 showed that highest motility percentage was observed in Theophylline added Tris diluter and the lowest in Tris extender at all stages of preservation. The difference in motility percentage among Tris, Tris + Glutathione and Tris + Theophylline was found to be non significant at 0 hrs of preservation. However the sperm motility

differed significantly from each other at different duration of preservation.

Table-5: Mean ± S.E. and CV percentage of progressive motility percentage of spermatozoa in buck semen preserved at different time intervals.

Period	Control	Glutathione		Theophylline	
	Mean ± SE & CV%	Mean ± SE & CV %		Mean ± SE & CV %	
		2mM	5mM	4mM	10mM
0 hr	78.17^{aA}±0.123 (1.051)	78.46^{aA}±0.41 (3.719)	78.58^{aA}±0.17 (1.490)	78.85^{aA}±0.16 (1.353)	78.35^{aA}±0.42 (3.659)
24 hr	75.05^{aB}±0.17 (1.535)	76.27^{bB}±0.10 (0.931)	77.76^{bB}±0.15 (1.347)	77.96^{cB}±0.15 (1.347)	77.96^{cB}±0.41 (3.723)
48 hr	61.18^{aC}±0.154 (1.745)	62.04^{bC}±0.16 (1.720)	64.23^{bC}±0.24 (2.694)	64.61^{cC}±0.16 (1.766)	63.08^{cC}±0.18 (1.959)
72hr	56.71^{aD}±0.281 (3.200)	57.94^{bD}±0.15 (1.7740	60.61^{bD}±0.16 (1.907)	60.96^{cD}±0.23 (2.650)	59.64^{cD}±0.66 (7.529)

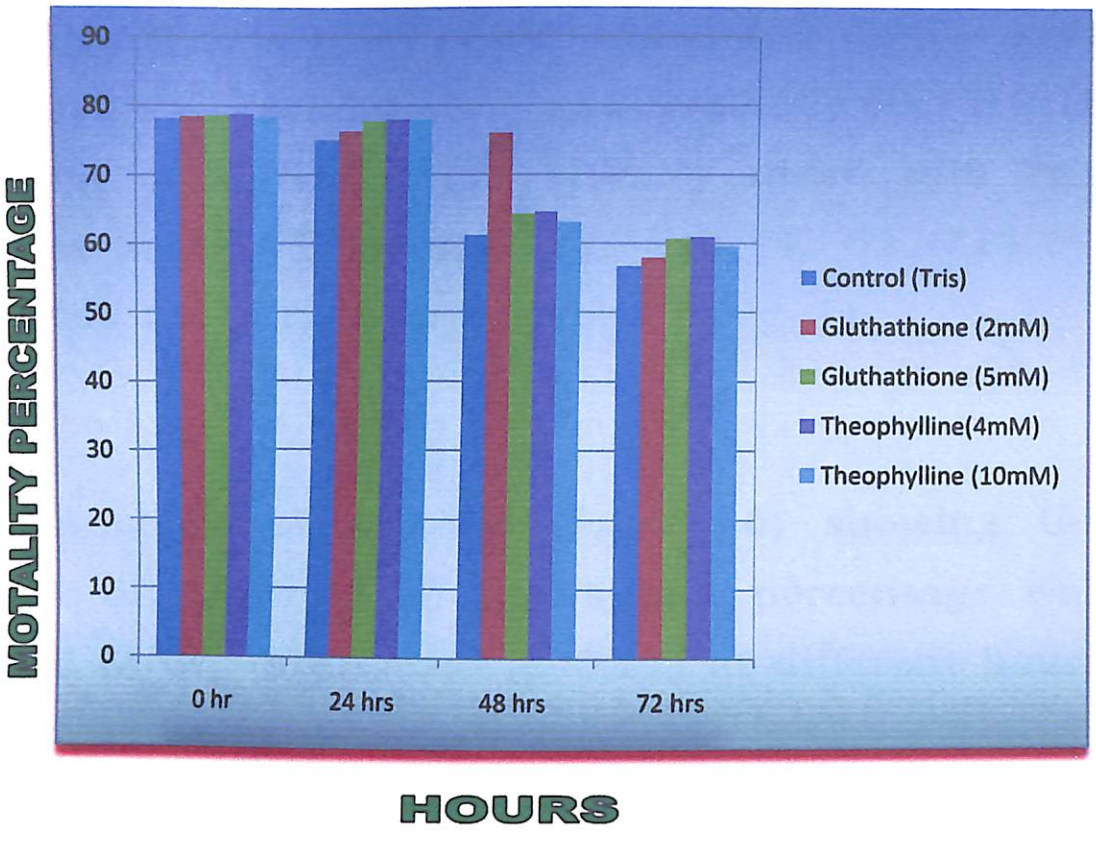
Mean with similar superscripts (row-wise: abc & Column – wise: ABC) did not differ significantly.

Table-6: Analysis of Variance for the effect of additive on semen quality of bucks at different hours of preservation.

Source of variation	d.f.	Live %		Progressive motility%	
		MSS	F	MSS	F
Between Treatment	4	77.06	40.34**	184.13	1.187^{ns}
Between periods	3	27500.37	14398.1**	31334.87	201.98**
Error	952	1.91		155.137	

Ns- Non significant **= Significant at P<0.01

Fig.1: Motility percentage of spermatozoa.



4.2.2. Live sperm percentage:

The Percentage of live spermatozoa with Tris dilutor (control) at 0, 24, 48 and 72 hours of preservation were recorded as 79.56±0.10, 76.22±0.10, 62.97±0.10 and 58.18±0.07 percent. The corresponding values of live sperm with Glutathione (2mM) added Tris dilutor were 79.88±0.05, 77.46±0.06, 62.11±0.18, 58.01±0.15 percent; Glutathione (5mM) added Tris dilutor were 80.10±0.12, 78.04±0.15, 63.32±0.16, 60.86±0.15 percent; Theophylline (4mM) added Tris dilutor were 80.19±0.15, 78.17±0.17, 63.69±0.17, 60.75±0.18 percent and Theophylline (10mM) added with Tris dilutor were 79.98±0.14, 77.46±0.13, 62.97±0.14, 59.70±0.15 percent 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 the highest live sperm percentage with Tris + Theophylline and the lowest in Tris extender alone at all stages of preservation. The difference in motility percentage among Tris, Tris + Glutathione and Tris + Theophyline was found to be non significant at 0 hrs

of preservation. However at 24, 48, 72, hrs of preservation the percentage of live sperms differed significantly from each other.

Table -7: Mean ± S.E. and CV percentage of live sperm percentage in buck semen preserved at different time intervals.

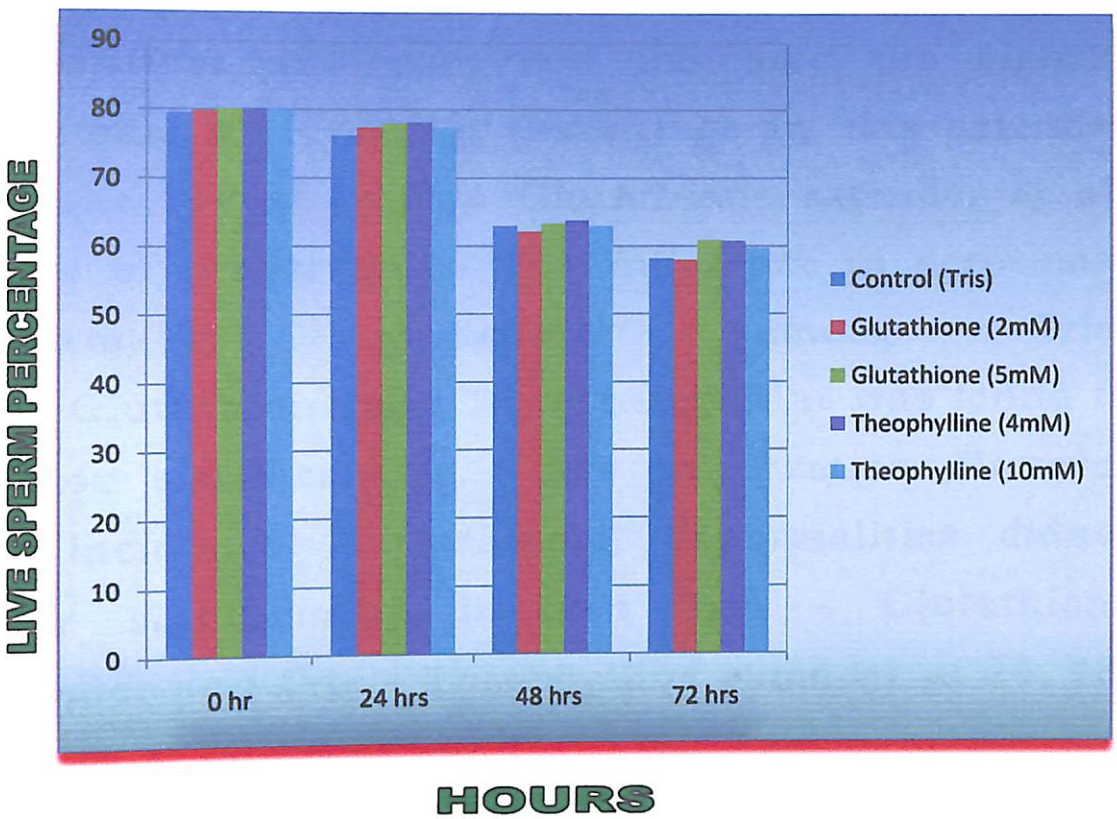
Period	Control	Glutathione		Theophylline	
	Mean ± SE & CV %	Mean ±SE & CV %		Mean ± SE & CV %	
		2mM	5mM	4mM	10mM
0 hr	79.56^{aA}±0.10 (0.882)	79.88^{cA}±0.05 (0.476)	80.10^{Ac}±0.12 (1.053)	80.19^{bA}±0.15	79.98^{cA}±0.14 (1.241)
24 hr	76.22^{aB}±0.10 (0.939)	77.46^{cB}±0.06 (0.614)	78.04^{Bb}±0.015 (1.325)	78.17^{bB}±0.17 (1.511)	77.46^{bB}±0.13 (1.227)
48 hr	62.97^{aC}±0.105 (1.164)	62.11^{dC}±0.18 (2.108)	63.32^{Cc}±0.16 (1.789)	63.69^{bC}±0.17 (1.905)	62.97^{cC}±0.14 (1.590)
72hr	58.18^{aD}±0.07 (0.868)	58.01^{aD}±0.15 (1.888)	60.86^{Dc}±0.15 (1.816)	60.75^{bD}±0.18 (2.089)	59.70^{cD}±0.15 (1.715)

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

4.2.3. Acrosomal abnormalities:

Change in acrosomal morphology was studied on 18 semen samples. The acrosomal abnormalities percentage with Tris dilutor (control) at 0, 24, 48 and 72 hours of preservation were recorded as 0.841±0.004, 1.752±0.004, 3.136±0.006 and 4.013±0.06 percent with Glutathione (2mM) added Tris dilutor 0.826±0.001, 1.597±0.002, 2.282±0.003, 3.369±0.006 percent; The corresponding values with Glutathione (5mM) added Tris dilutor where 0.812±0.001, 1.501±0.001, 2.420±0.006, 3.060±0.005 percent; with Theophylline (4mM) added Tris dilutor 0.824±0.001, 1.592±0.003, 2.754±0.008, 3.058±0.01 percent and with

Fig. 2: Live sperm percentage of spermatozoa.



Theophylline (10mM) added Tris dilutor 0.804±0.001, 1.44±0.008, 2.526±0.007, 4.026±0.006 percent respectively (Table-8). A decreasing trend in acrosomal abnormality percentage with increase in duration of preservation in additives added dilutor was recorded as compared 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 the highest acrosomal abnormality percentage in Tris extender and the lowest in Tris+Glutathione extender at all hours of preservation. The difference in acrosomal abnormality percentage among Tris, Tris+Glutathione and Tris+Theophyline was found to be non significant at 0 hrs preservation. However the incidence of acrosomal abnormalities didnot differ significantly between Tris – Glutathione extender and Tris – Theophylline extender at 24, 48, 72, hrs of preservation.

Fig. V: Micro photograph showing Acrosomal morphology.

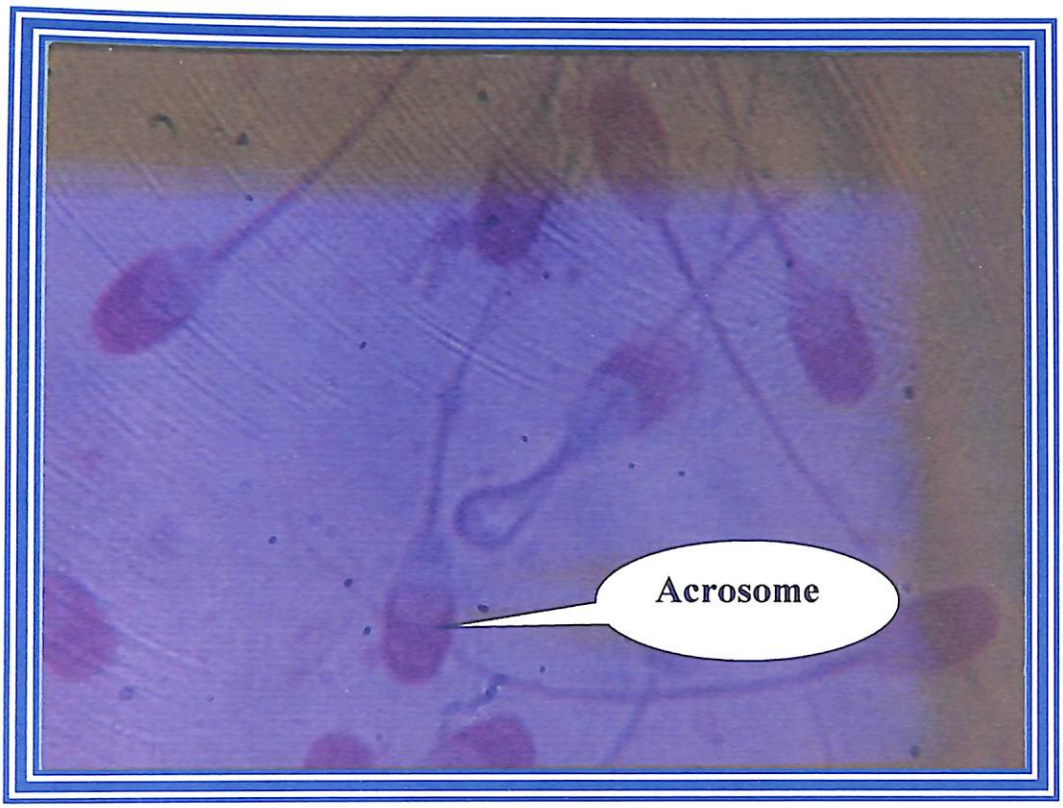


Table -8: Mean + S.E. and CV percentage of acrosomal abnormality percentage of spermatozoa in buck semen preserved at different time intervals.

Period	Control	Glutathione		Theophylline	
	Mean±SE & CV%	Mean ± SE & CV %		Mean ±SE & CV %	
		2mM	5mM	4mM	10mM
0hr	$0.841^{aA}\pm0.004$ (2.018)	$0.826^{aA}\pm0.001$ (0.5293)	$0.812^{aA}\pm0.0006$ (1.1797)	$0.824^{aA}\pm0.0009$ (0.474)	$0.804^{aA}\pm0.000$
24 hr	$1.752^{aB}\pm0.00471$ (1.41)	$1.597^{abc}\pm0.0028$ (0.774)	$1.501^{abB}\pm0.0009$ (1.8065)	$1.592^{abB}\pm0.0037$ (0.987)	$1.44^{bB}\pm0.008$ (2.1465)
48 hr	$3.136^{aC}\pm0.00676$ (0.915)	$2.282^{dC}\pm0.0032$ (0.485)	$2.420^{cC}\pm0.006$ (1.532)	$2.754^{bdC}\pm0.008$ (1.245)	$2.526^{abC}\pm0.007$ (1.1915)
72 hr	$4.013^{aD}\pm0.0612$ (7.100)	$3.369^{aD}\pm0.0063$ (0.7984)	$3.060^{cD}\pm0.0051$ (0.0907)	$3.058^{bD}\pm0.0103$ (1.09430	$3.026^{aD}\pm0.007$ (0.8991)

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

4.2.4. Sperm head abnormality:

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

Table -9: Mean ± S.E. and CV percentage head abnormality percentage of spermatozoa in buck semen preserved at different time intervals.

Period	Control	Glutathione		Theophylline	
	Mean±SE & CV%	Mean ± SE & CV %		Mean + SE & CV %	
		2mM	5mM	4mM	10mM
0 hr	$0.084^{A}\pm0.000636$ (3.215)	$0.083^{A}\pm0.0008$ (4.0278)	$0.082^{A}\pm0.006$ (3.074)	$0.83^{A}\pm0.00066$ (3.3547)	$0.084^{A}\pm0.000$ (2.4966)
24 hr	$0.118^{B}\pm0.001010$ (3.959)	$0.104^{B}\pm0.0009$ (3.775)	$0.093^{A}\pm0.0010$ (4.6246)	$0.094^{B}\pm0.00441$ (7.3561)	$0.122^{B}\pm0.000$ (3.1069)
48 hr	$0.156^{C}\pm0.0007343$ (1.997)	$0.136^{C}\pm0.0008$ (2.6331)	$0.127^{B}\pm0.0006$ (1.909)	$0.126^{C}\pm0.0008$ (2.6875)	$0.133^{C}\pm0.000$ (2.302)
72hr	$0.175^{D}\pm0.00090$ (2.187)	$0.144^{D}\pm0.0010$ (3.0781)	$0.133^{C}\pm0.0005$ (1.5915)	$0.132^{C}\pm0.0224$ (7.9521)	$0.138^{C}\pm0.021$ (8.35210

Fig. 3: Acrosomal abnormality percentage of spermatozoa.

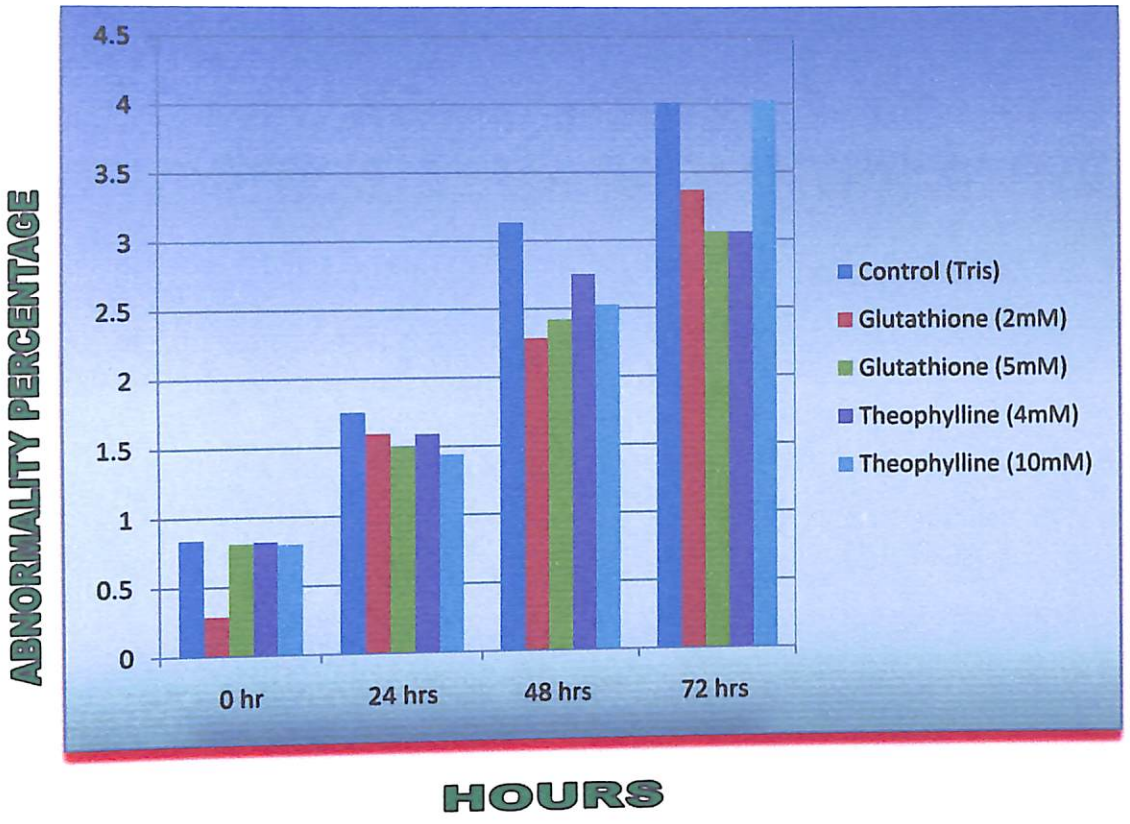
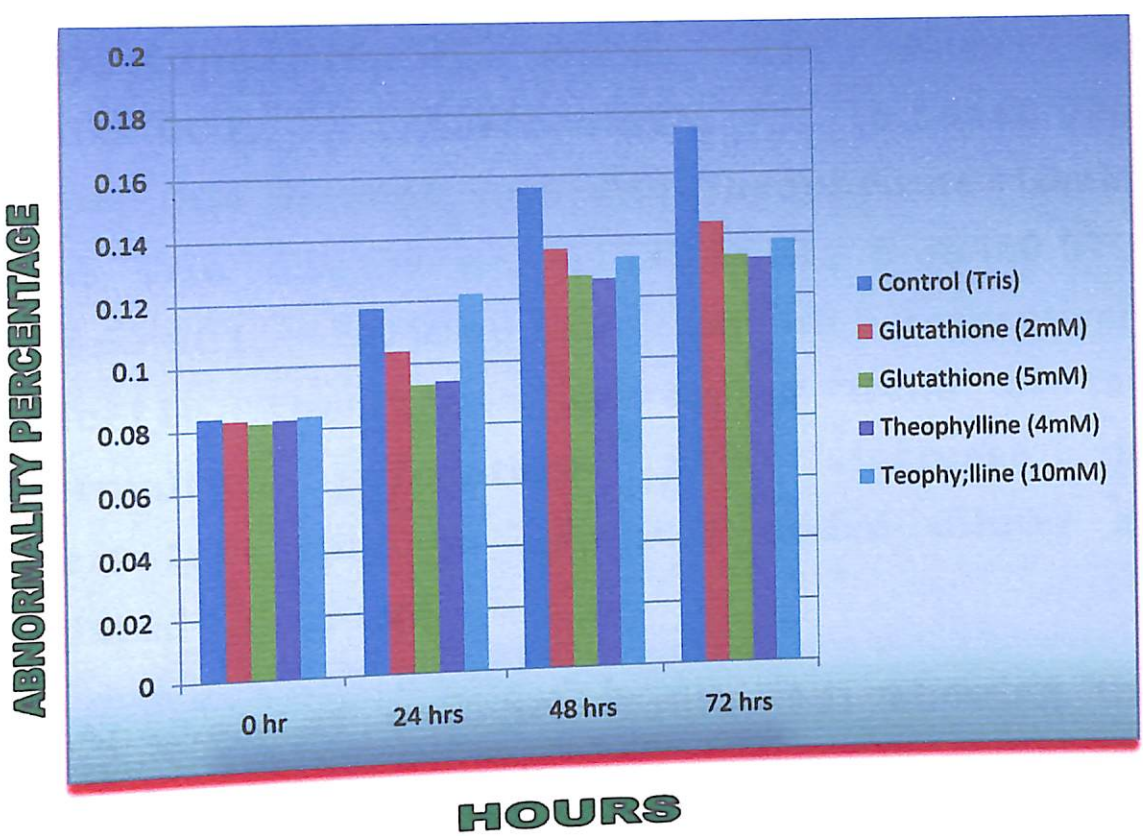


Fig. 4: Head abnormality percentage of spermatozoa.



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 percentage with Tris dilutor (control) at 0, 24, 48 and 72 hours of preservation were recorded as 4.492±0.116, 50523±0.017, 5.986±0.017and 6.021±0.02 percent; with Glutathione (2mM) added Tris dilutor 4.221±0.004, 5.389±0.001, 5.574±0.001, 5.866±0.001 percent, with Glutathione (5mM) added Tris dilutor as 4.215±0.002, 5.086±0.011, 5.547±0.002, 5.767±0.011percent and with Theophylline (4mM) added Tris dilutor as 4.116±0.001, 5.076±0.021, 5.544±0.006, 5.759±0.004 percent, and with Theophylline (10mM) added Tris dilutor as 4.114±0.001, 5.394±0.079, 5.554±0.001, 5.791±0.002 percent respectively (Table-11). There was decreasing trend of tail abnormality percentage with increase in preservation time in additives added diluter as compared to control.

Analysis of variance (table -11) showing the effect of additives on tail abnormality was significant ($p<0.01$) at different hours of preservation.

Critical difference test indicated that the highest tail abnormality percentage was observed

with Tris extender and the lowest with Tris+Theophyline extender at all hours of preservation. The difference in tail abnormality percentage among Tris, Tris+Glutathione and Tris+Theophyline was found to be non significant at 0 hrs of preservation. However the occurrence of tail abnormalities at 24, 48, 72, hrs of preservation differed significantly from each other.

Table -10: Mean±S.E. and CV percentage of tail abnormality percentage of spermatozoa in buck semen preserved at different time intervals.

Period	Control	Glutathione		Theophylline	
	Mean±SE & CV %	Mean + SE & CV %		Mean + SE & CV %	
		2mM	5mM	4mM	10mM
0 hr	4.492^{aA}±0.1160 (8.956)	4.221^{aA}±0.0042 (0.4249)	4.215^{bA}±0.0028 (0.28250	4.116^{bA}±0.000808 (0.833)	4.114^{bA}±0.0008 (0.0834)
24hr	5.523^{aB}±0.01732 (3.076)	5.389^{bB}±0.0006	5.086^{bB}±0.0113 (0.8910	5.076^{bB}±0.02154 (1.6937)	5.394^{cB}±0.0794 (6.6442)
48 hr	5.986^{aC}±0.01732 (3.015)	5.574^{bC}±0.0014 (0.10830	5.547^{bC}±0.0022 (0.1665)	5.544^{bC}±0.00678 (0.5194)	5.554^{bC}±0.0009 (0.0795)
72hr	6.021^{aC}±0.00249 (2.89610)	5.866^{bD}±0.0011 (0.08160	5.767^{bD}±0.0113 (0.1533)	5.759^{bD}±0.00420 (0.30940	5.791^{bD}±0.0029 (1.3561)

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

Table -11: Analysis of Variance for the effect additive on semen quality of bucks.

Source of variation	d.f.	Acrosome abnormality %		Head abnormality %		Tail abnormality	
		MSS	F	MSS	F	MSS	F
Between Treatment	4	1.89	2.4*	00.00315	0.48 NS	1.5	8.52**
Between periods	3	130.42	166.032**	0.005	0.77**	46.22	262.61**
Error	352	0.7855		0.0065		0.176	

**= Significant at P<0.01

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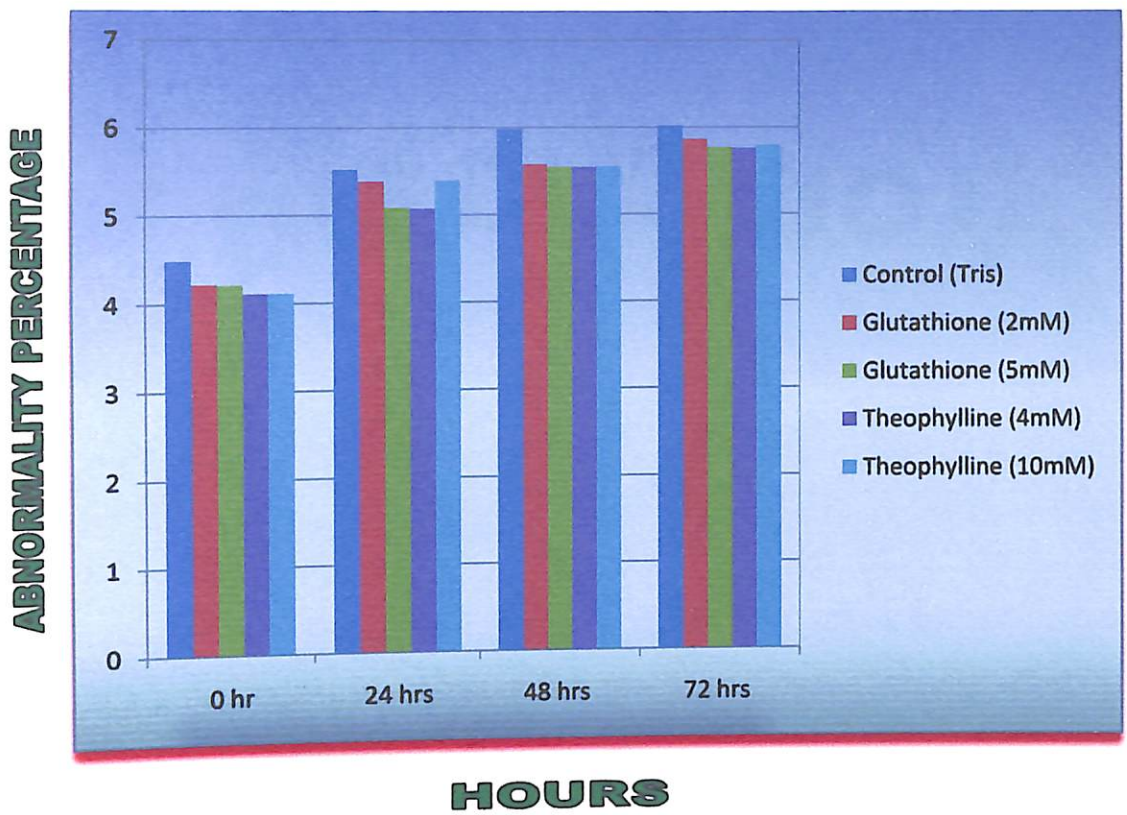
Fig. 5: Tail abnormality percentage of spermatozoa.



CHAPTER - V

DISCUSSION

DISCUSSION

In this study, attempts have been made to assess the effect of additives viz.Glutathione and Theophylline on preservability of Jamunapari buck semen. Evaluation of the neat semen as well as diluted semen samples added with Glutathione and Theophylline and preserved up to 72 hours (0, 24, 48 and 72 hours) were assessed on the basis of motility, live sperm percentage, and spermatozoa abnormalities.

5.1. Seminal characters:

5.1.1. Colour and consistency:

The colour and consistency of Jamunapari buck semen was found to be creamy 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 reported to be influenced with its consistency (Tomar, 1976).

5.1.2. Ejaculate volume:

The mean volume of seminal ejaculates of different bucks (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

almost similar semen volume (0.5ml) in Malabari buck.

Sahni and Roy (1967) and Ahmed et al. (1997) found the average ejaculated volume of 0.71ml and 0.77 ml. in Barberi and Sannen bucks respectively which are almost in agreement with present findings. However Pandey et al. (1985) observed semen volume in Sannen and Barbari buck as 0.96 ml. and 0.79 ml. respectively. Roca et al. (1992) found the average semen volume as 1.05±0.1 ml. in Murcica-Granada goats; Results of above workers were higher than that of present studies (0.53 ml.) which might be due to variation in breed as well as of bucks. In another study Patnaik et al. (1991) observed semen volume in Ganjan bucks as 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 (Mittal 1987). 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) who found the average initial motility as 78.30±2.48 percent in Barbari bucks. Sinha et al. (1981) found the average motility percentage as 80.04±14 in Jamunapari bucks. Where as Ahmed et al. (1997) observed the average mass activity as 60.38 percent in sannen buck which were lower than the present finding. However Traldi (1983) reported the average sperm motility of Moxoto bucks as 90.6±6.14 percent which is higher than present investigation (81.83±44).

Motility is greatly influenced by breed, individual secretion of accessory sex glands, season, and techniques of collection, handling of semen and this 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±61.53X10^6 /ml. (Table-1). The sperm concentration of bucks used in present investigation was within the normal limits as reported by previous workers in other breeds of buck. Koh (1975) found the average sperm

concentration to be 3974.8±186.41x10^6 per ml.; Pandey et al. (1985) observed semen concentration as 4503.26±14.75 millions per ml in sannen bucks, Das and Rajkonwar (1993) reported 4401.05x10^6 sperm per ml. in Beetal buck semen, Ahmed et al. (1997) reported average 2.77 billion per cmm. in Sannen bucks under tropical climate. Puranik et al. (1993) obtained 3079x10^6±24.5 and 2763x10^6±86.3 per ml. for Osmanabadi and crossbred bucks respectively which are lower than the present finding. Sharma and Arora (1970) reported average sperm concentration as 5166.277±176.53 million per ml. in Betal buck and Saxena and Tripathi (1979) reported average sperm concentration as 4795.0x10^6 per ml. in Jamunapari bucks which are higher than present investigation (4210.51±61.53x10^6).

This variation in present investigation might be due to effect of climate, season, nutrition, breed, weight of animal 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) who recorded the live sperm percentage as 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 82.23 and 71.44 percent live spermatozoa in Sannen and Barbari bucks respectively. The present finding of live spermatozoa are in agreement with the finding of above workers. However some workers reported more live sperm percentage than present investigation (81.83±3.44). Roy (1975) reported 87.81±1.32 percent of live spermatozoa in nondescript breed of bucks. Igboeli (1974) reported 87.2±10.0 and 87.7±1.0, percent of live spermatozoa in native Zembian and Boer bucks respectively.

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

5.1.6. Sperm abnormality:

The percentage of acrosomal, head and tail abnormalities in Jamunapari buck recorded in the present study were 0.815±0.007, 0.0848±0.003 and 4.498±0.021 respectively (table-3) which are within normal range and in close agreement with the findings of Koh, (1975); Patnaik et al. (1991); and Mittal (1987).However 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 those additives were found highly significant ($P<0.01$) on sperm motility percentage in jamunapari buck semen during preservation up to 72 hours

The present observation in Tris extender is comparable with the finding of Borgohain et al (1985) and Mohanty et al (1993). However, higher sperm motility percentage at 24 hrs of preservation and lower sperm motility percentage at 72 hrs of preservation with Tris extender alone were recorded by Puranik et al. (1994) and Deka & Rao (1986) respectively. The present finding showing the influence of glutathione during liquid preservation of buck semen was directly uncomparable being avialibility of limited references. However, the present findings of higher sperm motility, during the course of preservation with glutathione added Tris diluter are in close agreement with findings of Nadroo et al. (1988) in bovine semen and Sinha et al. (2004) in buck semen, Bilodean et al. (2004) had also reported that the loss of ram sperm motility

could be prevented by addition of thiole viz. Glutathione. However, Karla et al. (1980) did not find significant effect on sperm survival during storage of semen at 4 to 7°C for 24hrs in Glutathione added extender.

The motility percentage of spermatozoa in Glutathione added extender at different stages of preservation were recorded higher in the present studies which might be due to elimination of free radicals, produced during the course of preservation. The sperm plasma membrane is rich in polyunsaturated fatty acid and is therefore susceptible to peroxidation damage with consequent loss of membrane integrity and decrease sperm motility (Alvarez et al. 1987 and Sinha et al. 1996). This might be the reason for higher sperm motility percentage of buck semen during preservation with Glutathione added extender.

Though the progressive motility of spermatozoa declined with increase in duration of preservation in those two groups of Glutathione added extender (TG_2 & TG_5) and two groups of Theophylline added extender (TT_4 & TT_{10}), the motility did not differ significantly among those additive added extenders at similar stage of preservation. The highest mortality percentage was observed with 4mM Theophylline added Tris extender at both 24hrs of

preservation (77.96±0.15) and at 72hrs of preservation 80.96±0.23).

Theophylline is inhibitor of cyclic AMP phosphodiesterase which leads to inhibition of phosphodiesterase activity and resultant rise in intracellular cyclic AMP level (Bhatnagar et al. 1979).The elevated cyclic AMP level increases energy production by accelerating glycolysis and tricarboxylic acid cycle. The energy, thus produced is utilized by the motile apparatus of the spermatozoa (Haesungeharern and chulavatanatol, 1973) and so, this might be a reason to enhance the sperm motility by Theophylline as recorded in the present findings.

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). Highly significant effect of duration of preservation on live sperm percentage in buck semen by Sikarwal et al. (2005) and Sengar et al. (2005) had been recorded which are in close agreement with the present findings.

The influence of additive viz. Theophylline and Glutathione on the live sperm percentage during preservation were found significant. Significantly

higher percentage of live sperm in 4mM Theophylline added Tris diluter as 77.96±0.15, 64.61±0.16 and 69.96±0.23 were recorded at 24, 48 and 72hrs of preservation in comparison of Tris dilutor. However, difference in percentage of live sperms among four additive added Tris extender did not differ significantly. The positive influence of both Theophylline and Glutathione on sperm live percentage, recorded durng the present studies corroborates with the observations of Gupta et al. (1984), Dhami and Sahni (1994) and Shukla et al. (2005). However, Sinha et al. (1994) could not observe significant difference in live sperm percentage due to addition of additives in the extender. Variable findings recorded by different worker might be due variation in type of utilized additives and mode of preservation.

Thus, it could be inferred on the basis of present finding that incorporation of Theophylline and Glutathione with Tris dilutor in concentration of 4mM and 5mM respectively improved the motility as well as livability of spermatozoa in preserved buck semen.

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).

The effect of Theophylline, an inhibitor of cyclic AMP phosphodiesterase and Glutathione, an antioxidant with Tris extender on the incidence of Acrosomal abnormality during different stages of preservation, as recorded in the present findings (Table -8) showed significant effect in comparison of Tris dilutor alone. Glutathione, as a substrate in peroxidase reductase system, can help to stabilize the lipid membrane of spermatozoa and maintain their viability (Bortoff, 1956). This might be possible reason for lower Acrosomal damage in Glutathione added extender during different duration of preservation. Slaweta et al. (1987) have recorded higher conception rate (60.04%) with Glutathione added semen dilutor in comparison of without Glutathione added extender (54.2%). Sinha et al (2004) also reported low extent of enzyme leakage in the semen, extended with 5mM glutathione added Tris extender in frozen thawed buck semen.

As studies pertaining to the effect of Glutathione during liquid preservation of buck semen are scanty, the present finding could not be compared directly but indirectly corroborates with the findings of Slaweta et al. (1987) and Sinha et al. (2004).

The effect of Glutathione added Tris extender on the occurrence of Acrosomal abnormalities showed lesser percentage of abnormal sperm at 24hrs, 48 hrs and 72hrs of preservation in comparison of Tris extender alone. The present findings are in accordance to the observations of Sinha et al (2004) who reported lower enzyme leakage with 5mM Glutathione. This is quite reasonable to believe that Glutathione, acting as membrane binder, might be protecting spermatozoa from membrane damage and thus showing lesser occurrence of acrosomal abnormalities as well as lesser extent of intracellular enzyme leakage.

The non significant difference in acrosomal abnormalities between Tris – Glutathione and Tris – Theophylline extender at different stages of preservation might be due to favorable effect of both additives. Brandt et al. (1978) reported the synergistic effect of Theophylline with forward motility protein of seminal plasma which prevents head to head agglutination of motile spermatozoa

and also prevent the reverse movement of immature sperm. Though the direct effect of Theophylline on occurrence of Acrosomal abnormality have not been documented, a probable reason to maintain lesser occurrence of acrosome abnormal sperms at those three durations of preservation with Theophylline added extender, as recorded in the present finding, might be due to checking of reversal movement of immature sperms.

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). As the available documented references pertaining to influence of Glutathione and Theophylline on occurrence of head abnormalities are very scanty, the present findings were compared with similar type of additives and found that the present finding with respect to occurrence of head abnormalities with and without additives added Tris extender are in agreement with the findings of Saxena et al. (1985), Sinha et al. (1992) and Singh et al. (2002).

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. The effect of additives with different concentration in Tris diluter on occurrence of Tail abnormalities was found highly significant ($P<0.01$) at different hours of preservation except freshly diluted semen. Raval and Dhami (2006) had recorded lower occurrence of tail abnormalities with additives added extender during refrigeration storage of bull semen. Though, there was non significant effect of those additives added diluents on percentage of tail abnormalities. The percentage of tail abnormalities showed an increasing trend with increase in duration of preservation in all five types of extenders as recorded in the present findings, are in agreement with the findings of Singh et al. (2002) and Raval and Dhami (2006).

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SUMMARY

The present study was conducted on semen samples collected from six Jamunapari bucks on twice a week collection schedule by artificial vaginal technique. All together, 144 semen ejaculates were utilized. Freshly ejaculated semen samples were evaluated to assess the different sperms characteristics. Ejaculates having 70 percent or more initial motility of spermatozoa were finally diluted with 5 extenders namely Glutathione (2mM) added Tris extender, Glutathione (5mM) added Tris extender, Theophylline (4mM) added Tris extender, Theophylline (10mM) added Tris extender and Tris extender alone in the ratio of 1:10. Those extended semen samples were preserved at 4°C for 72 hrs and were evaluated for sperm progressive motility, live sperm percentage, sperm abnormalities and acrosomal morphology at 24 hrs interval. Those observations were statistically analyzed by following the standard method.

The mean ejaculated semen volume was 0.51±0.012 ml. The colour and consistency were recorded creamy yellow and viscid respectively. The mean value of 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 recorded as 0.51±0.012, 81.08±0.46, 4288.56±61.53, 81.34±0.38, 0.815±0.007, 0.0848±0.003 4.498±0.021 respectively.

There was non-significant difference in the sperm volume and mass motility where as there was significant difference in live sperm percentage and concentration of spermatozoa among Jamunapari bucks.

The mean value of sperm concentration in those utilized buck semen ejaculates was 4228.56±67.04 million per ml. Significant variation in concentration of spermatozoa was also recorded among the bucks. The mean value of live sperm percentage in freshly ejaculated semen samples of buck was 80.99±3.44 was found to be significant among bucks. The abnormal acrosomal spermatozoon in freshly ejaculated buck semen sample was found to be 0.815±0.007 without significant variation among the bucks. The other sperm abnormalities viz. head abnormality and tail abnormality in freshly ejaculates were recorded as 0.085±0.003 and 4.498±0.021 respectively. The head abnormality showed non significant variations among bucks where as a significant variation in occurrence of tail

abnormality percentage was recorded with among utilized bucks.

Sperm motility and live percentage of spermatozoa though found declined gradually in both additives added Tris extenders of different concentration during preservation; the motility of spermatozoa remained higher in additives added Tris extender in comparison of control i.e. Tris extender alone. The maximum motility of spermatozoa was recorded with Theophylline added Tris extender at 24hrs (77.96 %). Likewise, the live sperm percentage with Theophylline added Tris extender was also recorded the maximum (77.96%) at 24hrs of preservation. The progressive motility percentage and live sperm percentage at 72 hrs of preservation were recorded 60.96 and 60.86 percent respectively.

The variations in the head abnormalities of the spermatozoa during preservation with both additives were found non - significant, ranging from 0.084 at 0 hour to 0.133 percent at 72 hours of preservation. The incidence of tail abnormality was recorded 4.492 at 0 hour which was found increased to 5.791 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. The occurrence of tail abnormality was also

found significantly lower with Theophylline as compared to Glutathione.

The percentage of acrosomal abnormality varied significantly ($P<0.01$) among those five utilized extenders at different hours of preservation. The acrosomal abnormality were found the lowest at different stages of preservation with Theophylline added Tris extender (1.44 at 24hrs, 2.526 at 48hrs and 3.026 at 72hrs) in comparison of Tris diluter alone at the corresponding stages of preservation (1.752 at 24hrs, 3.136 at 48hrs, 4.013 at 72hrs). The occurrence of acrosomal abnormalities was also found lower with glutathione added Tris extender at all stages of presentation in comparison of Tris extender alone but there was no significant difference between those Theophylline added Tris extender and glutathione added Tris extender. It indicates the positive action of those additives on maintaining the spermatozoal integrity and thus minimizing the occurrence of acrosomal abnormality.

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. Glutathione and Theophylline up to 72 hours of preservation. Additives-wise

comparison showed that maintaining the progressive motility and live percentage of spermatozoa was better with Theophylline.

Thus, it could be inferred on the basis of present finding that incorporation of Theophylline and Glutathione with Tris dilutor in concentration of 4mM and 5mM respectively improved the motility as well as livability of spermatozoa in preserved buck semen.

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 declining trend in motility and livability of spermatozoa in liquid buck semen were observed with the increase in duration of preservation.

2. The progressive motility and live sperm percentage were significantly ($P<0.01$) higher in Tris extender added with Theophylline as compared to Glutathione added extender up to 72 hours of preservation.

3. The acrosomal abnormality was lower in Glutathione (5mM) added Tris extender as compared to other utilized diluters at different hours of preservation.

4. Head abnormality did not vary significantly between additives added extender at different hours of preservation.

5. Significant ($p<0.01$) effects of additives on tail abnormality were recorded at different hour of preservation. Its incidence was significantly lower in Theophylline added Tris extender as compared to Glutathione added Tris extender.

6. Incorporation of additives such as Theophylline and Glutathione in Tris extender improved the post preservation quality of Jamunapari buck semen.

7. Overall influence of Theophylline was found to be better additive in terms of progressive motility, live percentage and tail abnormality in comparison to Glutathione, where as Glutathione was found more effective in minimizing the occurrence of acrosomal abnormalities.

Though addition of Theophylline @ 4mM & 10mM and Glutathione @ 2mM & 5mM with Tris extender were found to improve the preserving quality of buck semen at 4°C, the addition of Glutathione and Theophylline @ 5mM & 10mM respectively were found more superior among others.

Thus, on the basis of present findings it could be inferred that incorporation of Theophylline @ 4mM and Glutathione @ 5mM with Tris extender could be practiced / used for liquid preservation of buck seminal ejaculates upto 72 hours of preservation.

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CHAPTER - VIII

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