SEMINAL ATTRIBUTES, PRESERVATION AND FERTILIZING ABILITY OF REF RIGERATED AND CRYOPRESERVED SEMEN OF BERARI BUCKS

T H E S I S

Submitted
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DOCTOR OF PHILOSOPHY

IN

ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BY

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled, **"SEMINAL ATTRIBUTES, PRESERVATION AND FERTILIZING ABILITY OF REFRIGERATED AND CRYOPRESERVED SEMEN OF BERARI BUCKS"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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PATIL MANJUSHA GANESHRAO has satisfactorily prosecuted her course of research for a period of not less than four semesters and that the thesis entitled, **"SEMINAL ATTRIBUTES, PRESERVATION AND FERTILIZING ABILITY OF REFRIGERATED AND CRYOPRESERVED SEMEN OF BERARI BUCKS"** submitted by her is the result of research work is sufficient to warrant its presentation to the examination in the subject of **ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS** for the award of **DOCTOR OF PHILOSOPHY** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

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This is to certify that the thesis entitled **"SEMINAL ATTRIBUTES, PRESERVATION AND FERTILIZING ABILITY OF REFRIGERATED AND CRYOPRESERVED SEMEN OF BERARI BUCKS"** submitted by **PATIL MANJUSHA GANESHRAO** to the Maharashtra Animal and Fishery Sciences University, Nagpur in partial fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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LIST OF ABBREVIATIONS

Abbreviation		***Full Form***
%	:	Per cent
*	:	Significant at 5 per cent
**	:	Significant at 1 per cent
/	:	Per
@	:	At the rate of
<	:	Less than
°C	:	Degree centigrade
µg/ml	:	Micro gram per milliliter
µmol/l	:	Micro mol per liter
A.I.	:	Artificial Insemination
a.m.	:	Ante meridium
A.V.	:	Artificial vagina
ALT	:	Alanine amino transferase
ANOVA	:	Analysis of Variance
AST	:	Asparate amino transferase
d.f.	:	Degree of freedom
E.N.	:	Eosin – Nigrosin
et al.	:	Et alia (and others)
etc.	:	Excetra
EYC	:	Egg yolk citrate
Fig.	:	*Figure*
g	:	Gram
g%	:	Gram per cent
g/dl	:	Gram per deciliter
GOT	:	Glutamic oxaloacetic transaminase
GPT	:	Glutamic pyruvic transaminase
HOST	:	Hypo osmotic swelling test
hr	:	Hour/s

i.e.	:	That is/ That means
LN	:	Liquid nitrogen
M.S.S.	:	Mean sum of squares
mEq/L	:	Milli equivalent per liter
mg%	:	Milligram per cent
mg/dl	:	Milligram per deciliter
min.	:	Minutes
ml	:	Milliliter
mOsm/L	:	Milli osmol per liter
ng/ml	:	Nenogram per milliliter
No.	:	*Number*
NS	:	Non significant
PMI	:	Plasma membrane integrity
PVC	:	Polyvinylchloride
rpm	:	Revolution per minute
S.E.	:	Standard error/s
S.M.	:	Skim milk
Tris	:	Tris-citric acid-fructose-egg yolk-glycerol dilutor
TYD	:	Tris egg yolk di-methyl sulphoxide
TYG	:	Tris egg yolk glycerol
U/L	:	Unit/ liter
viz.	:	Namely
Vs.	:	Versus
P<0.01	:	Significant at 1 per cent level
P<0.05	:	Significant at 5 per cent level

CHAPTER I

INTRODUCTION

India with 154 million goats is one of the largest goats owning country in the world and playing a significant role in livelihood and nutritional security as well as providing supplementary income to nearly 70 million farmers of over 500,000 remote villages. Goat meat production in the country has increased from 4.7 lakh tons to 5.96 lakh tons during 2002 to 2011 with an annual growth rate of 2.4%. Similarly, goat milk production in the country has also increased from 36.4 to 45.9 lakh tons during the same period with an annual growth rate of 2.6 %. Goats contribute nearly 8.5% of the total GDP from livestock sector to Indian agriculture production system. The goat husbandry also generates about 4.2% rural employment to millions of small, marginal farmers and landless laborers.

India has a range of agro climate zones and goat population is widely distributed in these varied conditions facilitating the large biodiversity. In Maharashtra, Berari was recognized 23rd goat breed of India registered as a distinct breed by the National Bureau of Animal Genetic Resources in May 2012. Berari is a local breed of goat found in the Vidarbha region of Maharashtra and in the Nimar region of Madhya Pradesh. Berari goats derive their name from the erstwhile 'Berar' region. The population of Berari goats in the Vidarbha region of Maharashtra is estimated to be about 2, 95, 408. The breed is primarily raised for meat by local farmers (Verma *et al*., 2012).

To meet requirement of milk, meat and associated by products of ever increasing human population, it is imperative to increase goat productivity. It could be possible by increasing high productive descript (33%) goat population and reducing non-descript (67%) and less productive goats by promoting scientific goat farming. AI is the most important tool contributing to the advancement of modern animal production. AI is the first generation reproductive biotechnology by which non-descript and less productive goats

with productive goat can be replaced population. AI facilitates planning of season of kidding, efficient genetic selection and storage of genetic material.

The semen of goat is preserved by refrigeration and cryopreservation techniques. The utilization of refrigerated semen is an important in a breeding programme, especially when the most of the animals live in a small geographical area and semen storage is for two to three days. The generally used media for buck semen liquid storage (4-5°C) are skimmed milk and tris egg yolk diluent (Leboeuf *et al.,* 2000). The major constraints of refrigerated semen are lesser number of does inseminated, utilization within one to two days, availability of goats in estrus during storage period. However, fertility using semen stored at 5° C is higher than when frozen semen is used (Ritar and Salamon, 1983; Roca *et al.,* 1997; Leboeuf *et al.,* 1998 and Paulenz *et al.,* 2005).

The french straw technique is the most popular method of freezing of semen. Computerized programmable freezers can also be used for freezing of goat semen which provides better control over cooling rate. The greatest problem existing with the cryopreservation of goat spermatozoa is that even with the best preservation techniques, post-thawing survival is restricted to approximately 50% of the sperm population. The number of sperms per insemination, site of deposition, type of estrus and frequency of insemination affects the fertility potential in goats. Frozen semen in cervical AI could reach acceptable fertility result in goat 55-65% (Salvador *et al.,* 2005).

Removal of seminal plasma by washing buck semen has been reported to increase the percentage of live spermatozoa and their motility during storage in egg yolk or milk diluents as well as improving spermatozoa capacity to withstand freeze thawing (Ritar and Salamon, 1983 and Leboeuf *et al.,* 2000). This is due to certain enzymes in the seminal plasma, originating from the bulbourethral gland secretion, catalyse hydrolisation of egg yolk lecithin and milk triglycerides of the extender, releasing sperm toxic products (lysolecithin and fatty acids) that lead to subsequent sperm deterioration (Pellicer-Rubio and Combamous, 1998).

Spermatozoa subjected to cryopreservation are very sensitive to a rapid reduction in temperature from 25 to 5 °C which produces cold shock, a membrane transition phase behavior exhibited by biological membranes. Cold shock results in a loss of selective permeability, integrity of the plasma membrane, release of intracellular enzymes as well as lipids, redistribution of ions, and change in the membranes of the acrosome and mitochondria, loss of motility, and diminished metabolism (Watson, 1981; White, 1993). Therefore, in vitro assays to test the effectiveness of cryopreserved semen is essential. Among these tests, sperm viability such as motility parameters, membrane integrity and acrosomal status are considered to be most valuable and useful (Amann, 1989). The extracellular release of AST and ALT enzymes have been used to test enzymes and thereby indicators of sperm cell membrane damage after freezing (Saraswat *et al*., 2010).

Several assays have been developed to evaluate sperm fitness. The standard semen analysis (SSA) relies an assessing a number of parameters such as cell concentration, motility and morphology for the assessment of male fertility. However, these parameters have limitations and cannot be used as reliable predictors of sperm fertilizing capacity (Mcniver *et al.,* 1992; Correa and Zavos, 1994). The study of sperm membrane is of particular importance since it is involved in several processes such as exchanges with the outside or recognition signals and act as a carrier to maintain the form of the cell. HOS test can be used for predicting fertilizing ability of spermatozoa and being simple and rapid, it can be used as a routine assessment technique in laboratory (Kale and Tomer*,* 2000b).

Despite success in frozen semen, cryopreservation of sperm is not without problems as the quality of sperms are significantly affected by means of ways during cryopreservation. Therefore, fertility assessment of preserved semen by recording conception rate in spontaneous as well as synchronized goat is indicative of quality of preservation. In view of this the present research work was planned to study the preservation of washed and unwashed Berari goat semen at refrigerated and ultralow temperature (-196°C)

in different extenders and conception rate after insemination in synchronized Berari goats with following objectives -

Objectives

1. To study the quality of neat semen of Berari bucks by using macroscopic, microscopic and sperm function tests.
2. To assess the freezability of washed and unwashed spermatozoa after preserving the Berari buck semen at refrigerated (4-5^{0}C) and ultra low (-196^{0}C) temperature.
3. To compare the pre-freeze quality and post-freeze quality of washed and unwashed spermatozoa using microscopic and sperm function tests at refrigerated (4-5^{0}C) and ultra low (-196^{0}C) temperature.
4. To study conception rate in goats inseminated using washed and unwashed refrigerated and cryopreserved semen of Berari bucks.

CHAPTER II

REVIEW OF LITERATURE

The literature pertaining to scrotal biometry, physical, microscopic and functional test of neat semen, freezing of washed and unwashed semen at refrigeration as well as ultra low temperature, estrus synchronization protocols and conception rate in goats is reviewed.

2.1 Scrotal Biometry

Testes are the primary sex organs of reproduction in males with major two fold functions i.e. gamatogenesis and steroidogenesis. The knowledge of the scrotal biometry is very necessary in predicting the ability of sperm production and maximum utilization of buck in A. I. programs.

Igboeli (1974) reported the scrotal length in Zambian and Boer bucks as 9.4±0.2 and 12.4±0.1 cm while scrotal circumference were 20.9±0.3 and 27.9±0.6 cm, respectively.

Parandekar (1987) recorded scrotal circumference as 27.75±0.23 cm and testicular length, breadth and width of right testicle as 8.712±0.19, 5.875±0.18 and 5.636±0.32 cm whereas left testicle as 9.312±0.13, 5.775±0.24 and 5.695±0.27 cm, respectively in Osmanabadi bucks.

Ritar *et al.* (1992) observed the mean lengths and widths of the testes of Angora bucks were 8.8±0.19 and 6.0±0.08 cm, respectively. Significant correlations were found for testicular width with testicular weight and testicular length with number of spermatozoa in testes ($P<0.01$).

Puranik *et al.* (1993) reported the average scrotal circumference as 26.675±0.537 cm and 27.25±0.297 cm in Osmanabadi bucks and crossbreed bucks, respectively. Right testicle length, breadth and thickness were 8.975±0.110, 5.775±0.228 and 5.625±0.278 cm in Osmanabadi bucks while 8.7±0.147, 5.75±0.155 and 5.95±0.086 cm in Crossbred bucks,

respectively. Left testicle length, breadth and thickness as 9.375±0.025, 5.425±0.295 and 5.525±0.390 cm in Osmanabadi bucks while 9.3±0.204, 5.9±0.177 and 6.1±0.091cm in crossbred bucks.

Ahmad and Noakes (1996) studied the testicular measurments of young (7-19 months) British bucks. The Overall mean of Sccotal Circumference (SC), testicular diameter, testis width was 24.81±0.16 cm, 56.03±0.34 mm and 92.19±0.58 mm, respectively.

Ahmed *et al.* (1997) compared the mean values of scrotal circumference of Saanen bucks in different seasons. A significantly ($P<0.01$) greater mean testicular circumference was observed in autumn (26.54 cm) than in winter (25.54 cm) and summer (23.88 cm). There was an inverse relationship between scrotal circumference and day light hours.

Oyeyemi *et al.* (2000) reported the scrotal length (cm) once weekly, twice weekly, once daily and twice daily during semen collection as 16.0±0.6, 16.4±0.8, 16.2±0.6 and 16.8±0.7. Corresponding values for scrotal circumference (cm) were 20.6±1.1, 20.4±1.2, 20.0±1.2 and 20.6±1.1 cm. Scrotal length and scrotal circumference were not affected by successive ejaculations.

Tiwari (2000) analyzed the data of growing bucks on the basis of scrotal circumference and bucks were divided into two groups on the basis having the average value as the main criteria. The averag values of scrotal circumference were as 6.16±0.18, 16.8±0.25, 19.5 ±0.19 and 20.2±0.60 cm at birth, 6 month, 9 month and 12 month, respectively in group I (having above average value) while the corresponding values in group II (having below average value) were 5.8±0.05, 13.0±0.25, 17.8±0.38 and 18.5±0.21 cm. A highly significant correlation of scrotal circumference was found with volume, seminal pH, mass motility, live sperm count and sperm concentration (0.42 to 0.62).

Ahmed *et al.* (2004) recorded the monthly body weight and scrotal circumference for a period of 1 year in mature and yearlings Damascus bucks. The mature bucks weighed significantly ($P<0.01$) more than yearlings

and had a significantly (P<0.01) larger scrotal circumference during the observation period. Month of the year significantly (P<0.01) affected the body weight and scrotal circumference.

Gbalban *et al.* (2004) evaluated influence of several factors on reproductive parameters in Damascus bucks, Scrotal circumference and semen characteristics were assessed in 38 bucks, 17 of which were mature and 21 yearlings. Body weight (BW), body condition score (BCS) and scrotal circumference were recorded monthly for a period of 1 year, together with semen collection for evaluation. Mature bucks weighed significantly (P<0.01) more than yearlings and had a significantly (P<0.01) larger scrotal circumference during the observation period. Season of year influences seminal attributes studied, indicating that optimal male performance may be obtained during the period of increasing day length.

Zamiri and Heidari (2006) reported that the mean testis length (cm) of Rayini bucks weighing 55 – 60 kg and 50 – 54 kg was 9.90±0.07 and 9.68±0.06, respectively. Corresponding values for testis width (cm) were 6.51 ±0.13 and 6.02±0.09 while that of testicular circumference (cm)were 28.23±0.69 and 25.85±0.52. Correlation coefficients of body weight with scrotal circumference, width and testis length were highly significant (P<0.01). Scrotal width was also highly correlated with the circumference. Correlation coefficient of testicular length with width and circumference was also highly significant. Scrotal circumference and width showed the largest coefficients (r = 0.8 – 0.85) with the semen volume and total sperm number in the ejaculate, followed by the number of live and normal sperm in semen (r = 0.70). Correlation coefficients of these attributes with the testicular length were lower (r = 0.45 – 0.50).

Khan *et al.* (2007) reported the average length of right testicles as 8.56±0.07 cm and 8.64±0.08 cm for left testis. The average diameter of right and left testicles were found to be 4.91±0.08 and 4.98±0.08 cm, respectively where as circumference of right testicles was 11.77±0.09 cm and left was 11.90±0.09 cm.

Kridli *et al.* (2007) evaluated monthly variation in semen characteristics in Black Bedouin and Black Bedouin×Damascus crossbred bucks. Twenty one Black Bedouin Goat bucks (BG) and 20 F1 Black Bedouin×Damascus crossbred goat bucks (CB) were used in the study. Body weight (BW), body condition score (BCS), scrotal circumference (SC) and semen samples were evaluated monthly in all bucks. Body weight, BCS and SC differed according to month of collection ($P<0.01$). Body weight and BCS were greater in the spring and summer months while SC reached their highest values during the autumn months. No differences were detected between breeds with respect to semen characteristics with the exception of ejaculate volume ($P<0.05$) and ejaculate appearance ($P=0.053$) which were greater in CB than in BG bucks. With the exception of ejaculate appearance, all semen characteristics were influenced by month of collection ($P<0.05$). Semen concentration and motility were better during the late summer and autumn months than during the spring. The percentage of abnormal spermatozoa declined from its greatest values in the spring to its lowest values in the autumn. From study it was concluded that goat breed has no effect on studied parameter. In general, most semen quality parameters were better in both breeds during the autumn than the spring months. Minimal variation in semen quality was observed between the summer and autumn months indicating the ability to use bucks for semen collection and for natural breeding during both seasons.

Gogoi *et al.* (2008) studied semen quality and their correlations with scrotal and testicular dimensions during prepubertal period and immediately thereafter in Beetal × Assam local bucks and found that the overall mean scrotal circumference increased from 16.70±0.52 cm at 13 months to 19.64±0.08 cm at 20 months. It was revealed that scrotal circumference had a significant ($P<0.01$) positive correlation with ejaculate volume, initial sperm motility, sperm concentration and live sperm count and had a significant ($P<0.01$) negative correlation with the incidences of sperm head and tail abnormalities.

Ford *et al.* (2009) studied the relationship of testicular traits (scrotal circumference-SC and testicular consistency-TC) in two breeds of goat bucks. The mean values for testicular traits were 27.3± 3.3 vs.22.8±1.03 (SC) and 4.25±0.25 vs. 3.75±0.23 (TC) for Boer or Kiko bucks, respectively.

Okere *et al.* (2011) studied the scrotal circumference (SC) and semen output in three electro-ejaculated pubertal Kiko bucks. An electro-ejaculator was used once a week for semen harvest for five consecutive weeks. Upon collection, semen samples were evaluated for ejaculate volume (ml), color, consistency, and mass activity. Mean value for scrotal circumference was 25.9±2.18 cm. Semen output was not differed throughout the five collection (1.33±0.57 ml, 1.16±1.04 ml, 1.33±0.57 1.50±0.86 ml, and 0.90±0.52 ml; P>0.05). The semen volume was not differed among bucks (1.50±0.5 ml, 1.34±0.93 ml and 0.90±0.41 ml; P>0.05). SC showed positive and moderate correlation with semen volume (r = 0.31).

Mia *et al.* (2013) estimated genetic and phenotypic parameters for semen characteristics and their relationships with scrotal circumference in Black Bengal bucks. The least-squares means of semen volume (ml/ejaculate), sperm concentration (10^9 cells/mL), mass motility (%), sperm livability (%) and normal spermatozoa (%) were 0.56, 2.55, 79.7, 86.8 and 90.5, respectively. Season of collection and scrotal circumference significantly affected all semen characteristics studied. Phenotypic correlations ranged from slightly negative (-0.001) to moderately positive (0.42).

Aliyu *et al.* (2016) determined the influence of body weight, scrotal circumference on semen characteristics and correlation between body weight and scrotal circumference in relation to semen characteristics in Red Sokoto bucks. The total eighteen (18) Red Sokoto bucks were selected and divided into three (3) groups with different number of Buck for each group: G1 with 8 animals, G2 with 4 animals and G3 with 6 animals and were grouped according to initial body weight in kilograms (KG) as; 17.00- 18.57, 18.58-20.15 and 20.16-21.73 respectively. Semen samples were collected on two weeks interval for the period of ten (10) weeks. The correlation between

body weight and scrotal circumference was positive (P<0.05) in terms of semen colour, semen motility, semen concentration and semen volume. While semen PH, live and dead ratio was negatively correlated (P > 0.05). The effect of body weight on semen parameters were significant (P<0.05) in terms of semen live and dead ratio as well as semen concentration. While semen colour, semen volume, semen pH and semen motility were not significant (P>0.05). Scrotal circumference was significant (P<0.05) in terms of semen volume and live and dead ratio while semen colour, semen PH, semen motility and semen concentration were not significant (P>0.05).

Bogra *et al.* (2016) studied biometry of testes in twelve black Bengal bucks (*Capra hircus*) of three different age groups, 0.5 to 1.0 years (group A), 1.5 to 2.0 years (group B) and 2.5 to 3.0 years (group C) to detected find out the age depended changes in the biometry of testes and their relationship with semen quality. The scrotal circumference was 17.50±0.65,21.38±0.43 and 22.88±0.66 cm in respective groups. While testicular length was 6.10±0.13 and 5.85±0.13, 7.20±0.31 and 6.90±0.33; 7.85±0.22 and 7.35±0.18 for left and right testicles. The testicular breath (cm) of left and right testicles were 3.88±0.24 and 3.75±10, 4.63±0.31 and 4.38±0.24; 4.85±0.12 and 4.68±0.12, respectively. Before slaughtering the bucks, the semen quality of the bucks of three different age groups was evaluated in terms of volume (ml), live sperm (%) and sperm concentration (billion/ml) for a period of 45 days. The semen volume and sperm concentration of age group C (0.68±0.04 ml and 3.04±0.10 billion/ml respectively) were significantly (P<0.05) higher than those of age group A (0.32±0.04 ml and 76.46±2.65 billion/ml, respectively) but no significant difference was observed with that of age group B. Whereas, live sperm percentage of age group B (85.64±0.87) was higher than those of other age groups but the difference was not significant (P>0.05). Testicular measurements were increased with the advancement of age and body weight. The size and weight of left testis were higher than right testis at same age. Semen volume and sperm concentration were highly significant (P<0.01) and positively correlated with almost all testicular measurements.

Olurode *et al.* (2018) evaluated the gross and histo-morphometry of the testes as well as the semen parameters of West African Dwarf (WAD) goat breed. Five apparently healthy WAD bucks were used for this study. The scrotal circumference showed positive relationship with both the gross and histometric parameters of the testis. However, there was no significant correlation between scrotal circumference and semen parameters.

2.2 Physical, Microscopic and Functional Tests of Neat Semen

Nimkar (1977) reported the density of Surti bucks semen as 3.64±0.08. Varshney*et al.* (1978) studied various enzymatic constituents of Barbari buck semen. The mean values of GOT and GPT in goat seminal plasma were 176.23±5.16 and 16.67±0.67 units/ml, respectively. The GOT: GPT ratio was found to be 11:1 in goat seminal plasma.

Baviskar (1985) noted the density of Osmanabadi buck semen as DDD (D) to DDDD.

Parandekar (1987) observed the density of Osmanabadi bucks semen as 3.291±0.055 (2.5 to 4) and that of crossbred bucks as 3.218±0.044 (3 to 4) in 4 point scale.

Bakshi (1991) recorded DD(D) to DDD density of semen in Osmanabadi bucks.

Roca *et al.* (1992) evaluated the type, frequency and seasonal variation of sperm abnormalities in male goats inhabiting in Mediterranean region. Distal cytoplasmic droplets and acrosomal damage were the predominant abnormalities. Increase in age (10 to 27 months) did not revealed itself as a main effect on the occurrence of sperm abnormalities. All types of sperm abnormalities studied showed significant ($P<0.01$) seasonal variation. The poorest quality semen was collected during winter and spring. In spite of a seasonal variation in sperm quality, semen of Murciano-Granadina goats in the Mediterranean area was good all year long, since the proportion of abnormal spermatozoa was well within the accepted range for normal fertility.

Antoine and Pattabiraman (1999b) studied the enzyme constituents of Tellichery buck semen. The level of GOT and GPT in seminal plasma of buck semen were reported as 117.02±1.81 and 31.99±1.81 units/ml.

Antoine and Pattabiraman (1999a) recorded value of HOST was 87.20 % in bucks.

Karagiannidis *et al.* (1999) studied the effect of photoperiod on semen production in bucks. Samples were collected with an artificial vagina and examined immediately after collection. In spite of the variation in nearly all semen characteristics studied among the 3 breeds of bucks, there was significant seasonal variation in both semen quantity (volume, concentration and total number of spermatozoa per ejaculate) and quality (percentage of motile spermatozoa, percentage of abnormal spermatozoa and rate of progressive motility). The best semen was produced during the breeding season (late summer and autumn). However, the magnitude of seasonal effects was not sufficient to prevent bucks from being used for breeding throughout the year. Individual differences in the semen quantity and quality among bucks within a breed make individual evaluation of semen necessary to select the most fertile males for breeding.

Bhuskat *et al.* (2000) reported that the average semen pH of Jamnapari, Ajmeri, Osmanabadi and Local breed as 6.68±0.02, 6.80±0.02, 6.67±0.03 and 6.65±0.04, respectively and the average semen volume of Jamnapari, Ajmeri, Osmanabadi and Local breed as 0.87±0.07, 1.07±0.07, 0.65±0.05 and 0.71±0.05 ml, respectively.

Blash *et al.* (2000) collected the epididymal sperm at necropsy from Alpine, Saanenand Toggenburg bucks and reported that the mean number of sperms × 10^9/ml were 3.7±2.5 and 3.1±1.3 and the fresh live spermatozoa were 92.0±6.7 and 86.0±9.8 % in epididymal and ejaculated sperm, respectively, with significant difference ($P<0.05$) between epididymal and ejaculated semen.

Kale and Tomer (2000a) estimated enzymatic constituent during three seasons in crossbred bucks (Alpine × Beetal and Saanen × Beetal). The mean values of GOT were reported as 1.94±0.12, 1.70±0.13 and 0.57±0.05 μmole/ml during Hot dry, Hot humid and Cold humid seasons. Corresponding values for GPT were 1.63±0.14, 1.79±0.11 and 1.48±0.09 μmole/ml, respectively. The overall mean enzyme activity of GOT and GPT in extracellular fluid were 1.40±0.42 and 1.63±0.11 μmole/ml. The season significantly ($P<0.05$) influenced GOT and GPT activity, the bucks differed significantly for GPT constituent and season × buck interaction affected all the enzymatic constituents in buck semen. The GOT activity in the seminal plasma was associated positively with GPT, dead spermatozoa percent but negatively with sperm concentration. GPT activity was negatively associated with volume and sperm number per ejaculate.

Kale and Tomer (2000b) reported 69.66% HOST percentage in buck semen.

Shamsuddin *et al.* (2000) reported the proportion of spermatozoa with abnormal acrosome, mid piece and tail and the proportion with abnormal head morphology varied between 9 to 6% and 1% respectively in Black Bengal buck semen. The total sperm concentration was reported between 4187 to 5064 $\times 10^6$/ml. The average ejaculate volume was between 267 and 342 μl.

Azeredo *et al.* (2001) evaluated the goat spermatozoa of three Saanen bucks with and without seminal plasma. The percentage of motile sperm was 85.41±4.65 and 67.50±2.47 in fresh semen with seminal plasma and without seminal plasma, respectively. The percentage of abnormal sperm was 17.73±3.31 and 14.96±2.40 in fresh semen with seminal plasma and without seminal plasma, respectively**.**HOST percentage reported 57.54% with seminal plasma

Biswas *et al.* (2002) collected the semen from three Black Bengal Buck and found the motility in fresh semen as 92±0.3 %. The motility score was 3.7±0.2, 3.8±0.2 and 3.8±0.2, the average ejaculate volume as

372±42, 433±49 and 355±45 µl/ejaculate. The total sperm concentration (10^7/ml) was reported as 249.5±8.4, 265.4±9.7 and 239.3±10.4. Sperm concentration did not differ between bucks. In fresh semen there was no significant variation on sperm motility either between bucks or within bucks.

Naskar and Nagpual (2003) studied the effect of social facilitation on seminal attributes in bucks. The mean live spermatozoa percentage in social facilitation and control groups were 75.01±1.93 and 72.06±1.45, respectively with an overall value of 73.63±1.61, abnormal spermatozoa percentages were 1.80±0.32 and 1.87±0.27, with an overall of 1.84±0.27. The sperm concentration per ejaculation were 3.11±0.24 and 2.02±0.29 billion/ml, respectively.

Sundararaman and Edwin (2003) collected 94 semen collections from two young and growing (>1 year of age) Boer Grade half-bred bucks and reported the initial motility in fresh semen as 78.19 ±0.94 %. Live sperms were 80.12±1.50 % and 84.21±0.81 % having significant difference with overall mean of 82.16±0.92 %. The sperm concentration were 2956.67±271.13 and 3875.33±169.06 million/ml having highly significant difference ($P<0.01$) between bucks and overall mean concentration of 3416.00±0.94 million/ml.

Bane *et al.* (2004) reported the mean percentage of abnormal sperm, live sperm and motile sperms in fresh semen of six local bucks was 8.04±0.65, 84.76±0.60 and 77.91±0.62

Bane *et al.* (2005) found that the percentage of abnormal spermatozoa in the fresh semen of local bucks was 3.66±0.27.

Fonseca *et al.* (2005) studied the hypoosmotic swelling test (HOST) as tool for evaluating the membrane integrity of spermatozoa of goat semen. Sodium citrate and fructose based solutions (S) with the following osmolarities (mOsm/l) were used: 50 (S1), 75 (S2), 100 (S3), 125 (S4), 150 (S5), 175 (S6), 200 (S7), 250 (S8),290 (S9) and 300 mOsm/l (S10). Twenty-eight semen samples were collected from seven mature bucks (four collections per buck) at 48 hour intervals. After macroscopic evaluation, 10µl of semen

was immediately added to 2ml of each solution and incubated for one hour at 37ºC. Sequentially, 20µl of semen diluted in HS were evaluated with oil immersion using a phase-contrast microscope. A total of 200 spermatozoa were counted in at least five different fields, and sperm tails were classified as non-coiled, coiled, and strongly coiled. The respective percentages of spermatozoa with coiled tails (coiled plus strongly coiled) in the ten solutions listed above were 34.1, 38.8, 45.3, 51.5, 46.8, 42.8, 38.2, 29.0, 19.4 and 23.1%.Percentages of strongly coiled spermatozoa were: 6.8,10.6, 21.5, 25.3, 24.3, 21.5, 19.3, 12.4, 6.4, and 7.9 for the ten solutions, respectively. According to total coiling, S4 was superior to S1, S7, S8, S9, and S10 ($P<0.05$). According to strong coiling, S4 was superior to S1, S2, S8, S9, and S10 ($P<0.05$). From study it is observed that 125 mOsm/l solution would be best for use in HOST in fresh goat spermatozoa.

Gacitua and Arav (2005) collected the semen from three Saanen bucks during the non breeding season for freezing of semen and found 88.8 % average motility before freezing with no significant difference between bucks. Further it was noted that 3.1 billion/ml sperm concentration with significant difference between bucks ($P<0.05$).

Shirbhate and Honmode (2005) collected the semen from four crossbred bucks (Ajmeri × Local) by artificial vagina method and found that the percentage progressively motile sperm in fresh semen was 77.92. It was noted percentage of live sperm in fresh semen as 83.12 and percentage of sperm abnormalities as 9.13.

Takarkhede *et al.* (2005) noted the average ejaculate volume of bucks as 0.925±0.06 ml., average abnormal sperm count as 3.17±0.54 %., average initial motility as 77.50±1.12 %.,average live sperm count as 82.67± 0.84 %.

Barbas *et al.* (2006) found average sperm concentration of Serrana buck semen as 4.04×10^9 per ml, the average mean of live sperm count as 72.20 percent, the average semen volume of Serrana buck as 1.09 ml, the normal spermatozoa 81.1 % (range 80.1 to 82.1). The individual motility

was reported as 65.1 (range 64.4 to 65.8) percent. Individual variation ($P < 0.03$) for individual motility was non-significant. The head, mid piece and tail abnormality percentage was 9.3 (range 8.6 to 9.9), 5.2 (4.6 to 5.7) and 4.4 (range 4.0 to 4.8), respectively.Individual variation ($P < 0.03$) were observed for sperm concentration, semen volume and all sperm abnormalities and also found a positive correlation between live and normal sperm count.

Zamiri and Heidari (2006) reported testicular size, semen volume, sperm concentration, percent live sperm, percent normal sperm, and total number of live-normal sperm were significantly higher during the summer months. The average semen volume, percent live sperm and percent abnormal sperm during the sampling period varied between 1 and 1.4 ml, 60 and 78%, and 7 and 13%, respectively. The total number of live and normal sperm in the ejaculate during the sampling period varied from 1000 to 2500 millions. Testicular size, semen volume and the total number of live and normal sperm were significantly greater in bucks weighing 55–60 kg as compared with 50–54 kg. The pH of semen of Rayini male goats during sampling period from July to December was between 6.0 to 6.1. Seminal fluid pH values were significantly lower from July to October (pH <6) than the values from November to December (pH >6.1).

Hidalgo *et al.* (2007) studied the seminal attributes of two Florida buck semen and found individual difference in the percentage of total sperm motility (77.04±1.31 versus 87.84±1.15) and rapid progressive motility (52.66 ±1.74 versus 60.97±1.54).

Peterson *et al.* (2007) studied the effect of semen processing techniques in Dutch AI-bucks and stated that ejaculates had 92±3 % viable spermatozoa. The concentration of spermatozoa of ejaculate was 6.2±3.9 × 10^9/ml.

Zamfirescu and Nadolu (2007) collected semen from 38 bucks of Alpine and Sannen breeds and found that the mean motility of fresh semen was 86.81±0.88 and 85±2.25% for Alpine bucks and Sannen bucks, respectively.

Bucak and Uysal (2008) collected the semen from three Saanen bucks by artificial vagina method for its freezing and reported that the motile spermatozoa, dead spermatozoa and abnormal spermatozoa were 84.25±7.19 %, 15.4±2.3 % and 8.12±0.93 %, respectively in fresh semen. The average sperm concentration was $3.6\pm0.4 \times 10^9$ per ml.

Islam *et al.* (2008) studied semen quantity and quality in relation to age, body weight and scrotal circumference (SC) of Black Bengal buck in Bangaldesh. Semen was collected from four bucks by the method of artificial vagina (AV). After collection semen evaluated for volume for ejaculate (ml), mass motility (%), individual motility (%), morphology (% normal and abnormal sperm) and concentration (million /cc) in relation to age, body weight and SC. Age, body weight and SC of four different bucks of 34 to 45 months were 20.0 to 33.6 kg and 20.1 to 21.0 cm, respectively. The mean volumes per ejaculate were 0.4±0.03 to 0.5±0.22 ml and mass motility was found 76.0±1.5 to 78.0±1.1 % .Individual motility in terms of progressive, oscillatory and rotatory were 65.7±0.1 to 68.0±1.3 %, 5.0±0.6 to 6.1±0.4 % and 4.0±0.4 to 5.4±0.3,respectively. While the (normal and abnormal sperm) and sperm concentrations were found to be 89.7±0.35 to 91.2±0.4 % and 8.8±0.4 to 10.3±0.4 % and 2434.0±52.8 to 2853.0±90.1 (million /ml), respectively.

Jadav *et al.* (2008) documented average mean of semen volume of Surti buck as 0.80±0.05ml with highly significant ($P<0.01$) individual variation. Negative correlation of ejaculate volume was found with abnormal sperm count and sperm concentration. The mass motility of 4.10±0.06 in the Surti buck semen. The individual buck varied non-significantly in their seminal mass motility. A highly significant ($P<0.01$) and positive correlation of mass motility was found with individual motility and live sperm count. While negative correlation observed between the mass motility and sperm concentration. The mean individual motility was 76.90±0.87 percent. Individual motility was positively and significantly ($P<0.01$) correlated with mass motility and live sperm count. A negative correlation was found between individual motility and abnormal sperm count as well as sperm concentration

per ml. The individual variation was statistically non-significant for individual motility.The mean of live sperm count was 80.14±0.88 percent. The variation between bucks was found to be non-significant. The live sperm count had shown a highly significant positive correlation with individual motility, mass motility and sperm concentration per ml and negative correlation with abnormal sperm count. The mean percentage of abnormal sperm count in semen was 4.14±0.42. A significant ($P<0.01$) variation was observed between bucks for abnormal sperm count. The abnormal sperm count was negatively correlated with volume, individual motility and live sperm count. It was stated that the mean value of total sperm concentration was 2820.10±45.54 (×106) in per ml of Surti buck semen. Individual bucks exhibited a variation in total sperm concentration. The difference was statistically non-significant. A highly significant and negative correlation of sperm concentration per ml was observed with semen volume. Sperm concentration was negatively correlated with mass motility and individual motility and positively correlated with live sperm count.

Batista *et al.* (2009) found that the mean±SEM of progressive motile sperm from five Majorera bucks were 75.3±1.8, 73.5±2.8, 72.7±3.5, 71.3±4.9 and 76.3±2.1 % and the mean±SEM of abnormal sperm from five Majorera bucks were 6.5±1.2, 3.6±0.3, 3.3±0.5, 4.0±0.8 and 3.1±0.4 % in fresh semen. The non significant difference between bucks for fresh semen was observed.The mean±SEM of sperm concentration ($\times 10^6$ / ml) of ejaculates were 4.2±0.5, 6.1±0.6, 5.6±0.3, 4.9±0.5 and 5.2±0.1 with significant difference ($P<0.05$).

Dorado *et al.* (2009) collected a total of 110 ejaculates from two mature Florida bucks by artificial vagina method and reported the average percentage of motile spermatozoa as 91.40±0.65, average percentage of abnormal spermatozoa as 28.34±2.02 with no significant difference between bucks, total volume of ejaculate as 1.20±0.0 ml in fresh semen. While assessing the goat semen freezability according to the spermatozoa characteristics reported the average sperm concentration as 3.69±0.08 $\times 10^9$/ml. The mean±SEM of the abnormal sperms of two Florida bucks during

Spring, Summer, Autumn and Winter were 31.29±4.89, 28.73±4.36, 26.36±4.13 and 27.36±2.68, respectively, with significant difference (P<0.05) between the seasons. The mean±SEM of the motile sperms during Spring, Summer, Autumn and Winter was 93.30±1.13, 92.70 ±1.09, 90.19±1.48 and 89.79±1.30, respectively, with significant difference (P<0.05) between the seasons. The individual sperm concentration, total of sperm per ejaculate, mass motility, and ejaculated volume may change with season, aspect and color of the ejaculate. The highest correlation was obtained between ejaculate volume and total number of sperm per ejaculate in both seasons. A decrease in acrosome integrity and sperm motility was found after thawing (P<0.01). Six semen parameters assessed before freezing were identified as predictors of sperm freezability (P<0.01). The percentage of morphological abnormalities (R = 0.856) and motile sperm cells (R = 0.655) in fresh semen were the best predictors to know the total post-thaw variability parameters.

Farshad *et al.* (2009b) noted 4.07±0.6 mass activity and intact acrosome sperm percentage of neat semen of Markhoz bucks was 88.51 percent.

Hidalgo and Dorado (2009) reported that intact acrosome sperm percentage of neat semen of Florida buck was 83.9 percent.

Parmar (2009) found the volume of Mehsana buck semen ranging between 0.51 to 1.61 ml. The overall mean value was observed as 0.84±0.02 ml. The individual variation among bucks was significant (P<0.05). The abnormal sperm count ranged between 2 to 10 percent with an overall mean value of 5.53±0.22 percent. The abnormalities of head, mid piece and tail were observed as 1.68±0.14, 0.82±0.10 and 3.03±0.16 percent, respectively. The individual variation in abnormal sperm count was statistically highly significant (P<0.01). The percentage of live spermatozoa was 89.17±0.51 with a range of 78 to 96.The percentage of live sperm was highly significantly (P<0.01) and positively correlated with individual sperm motility (+0.719), while negative and significant (P<0.05) correlation was found with ejaculate volume (-0.247).The mean sperm concentration was

3099.10±59.48 ($\times 10^6$/ml) with a range of 2170 to 4370 ($\times 10^6$/ml). The mean value of sperm concentration per ml of semen showed highly significant differences between bucks (P<0.01). The individual sperm motility averaged 85.73±0.43 percent in Mehsana buck semen with a range of 74 to 92 percent. The correlation between individual sperm motility and live sperm count (+0.718) and total protein (+0.405) was positive and of highly significant (P<0.01). Individual sperm motility was highly significant (P<0.01) and negatively correlated with abnormal sperm count (-0.323).Creamy semen colour was the characteristic feature throughout the study.

Santiago-Moreno *et al.* (2009) observed mean ejaculate total sperm concentration 940.21±187.58 × 10^6 /ml (range: 451.00 -1136.67 ×106/ml) from Spanish Ibex bucks collected by electro ejaculation method during outside the rutting season.

Bhoi (2010) found average sperm concentration of semen samples was 2968.22±24.54 and 2641.22±15.07×10^6 sperm /ml during breeding and non breeding season, respectively. The percentage of total abnormalities was observed to be 7.18±0.17 during breeding season whereas it increased to 10.79±0.20 during non-breeding season. Similarly the head, mid piece and tail abnormalities (3.14±0.12,1.98±0.11 and 2.05±0.10) were found to lower during breeding season than the non-breedingseason (4.82±0.20, 3.00±0.17 and 2.96±0.15 percent). The average live spermatozoa was 86.03±0.35 and 82.58±0.33 per cent during breeding and non-breeding seasons. The difference was highly significant (P<0.01) between two seasons.

Coloma (2010) studied semen attributes from Spanish Ibex bucks. Found that the percentage of motile sperms in ejaculated semen were 59.8±9.6, 75.7±4.5, 70.5±7.0 and 80.5±7.0 during winter, spring, summer and autumn, respectively, with significant difference (P<0.05) during different seasons. The percentage live sperms of ejaculated semen were 76.7±4.2, 67.8±4.0, 66.9±6.2 and 65.5±4.4 during winter, spring, summer and autumn, respectively, with no significant difference (P<0.05) during different seasons. The percentage abnormal sperms of ejaculated semen were 53.6± 10.3,

59.1±5.2, 62.6±9.1 and 22.5±4.6 during winter, spring, summer and autumn, respectively, with significant difference (P<0.05) during different seasons. The mean sperm concentration ($\times 10^6$/ml) of ejaculated semen was 1326.1±459.1, 1091.1±257.3, 1638.9±385.0 and 2592.6±639.0 during winter, spring, summer and autumn, respectively, and stated that photoperiod significantly influenced the sperm variable, with higher sperm concentrations (2230.1±389.4 $\times 10^6$/ml vs. 1081.4±218.3 $\times 10^6$/ml, P<0.05) seen during the time of decreasing photoperiod.

Kulaksiz and Daskin (2010) collected semen from four Sannen bucks during the breeding season and stated that the average spermatozoa concentration of the ejaculates was 3.62±0.41 × 10^9 /ml, motile spermatozoa were 77.0±4.83 %, the live spermatozoa were 89.10±0.88 % and abnormal spermatozoa were 19.6±2.83 %.

Ana *et al.* (2011) reported that seminal attributes were affected by many factors, including breed, body weight, age, management, climatic conditions, nutrition, method of semen collection and degree of sexual stimulation. The abnormal sperm percentage of neat buck semen in rainy and dry season was 13.13±7.13 and 17.21±9.79 percent, respectively.

Dagli (2011) noted that volume of Osmanabadi and Sirohi bucks semen was 1.7±0.11 and 1.8±0.07, abnormal sperm percentage as 13.28±1.09 and 11.78±0.90, average initial sperm motility as 86.66±1.72 and 86.13±1.33 percent, live sperm percentage as 86.05±0.41 and 84. 05±0.79, pH as 6.70±0.03 and 6.65±0.02, sperm concentration as 2616.80±39.12 and 2364.70±75.50 million /ml., acrosome intact sperm percentage as 91.94±0.75 and 90.94±1.24 percent, HOST percentage as 83.13 and 78.33 respectively. The recorded density was DDD (D) to DDDD, average mass activity in the grade of + 4 of semen from Osmanabadi and Sirohi buck. The colour of Osmanabadi buck semen as yellowish to yellowish white and Sirohi buck semen as yellowish white to creamy white. Both the breeds - Osmanabadi and Sirohi buck semen showed thick consistency.

Francisco *et al.* (2011) noted that fresh goat semen was yellowish in colour and milky in aspect.

Olayemi *et al.* (2011) noted that the initial sperm activity of semen of West African Dwarf buck was 95.00 %, mass activity was +++, sperm concentration was 2500 million per ml. and semen volume was 0.41± 0.01 ml.

Udeh and Oghenesode (2011) noted that colour of West African Dwarf buck was whitish, viscous and thick creamy. The motility was 92.00±0.86 percent, ejaculatory volume was 0.42 ml., defective acrosome sperm percentage of diluted buck semen using Tris egg yolk dilutor was 25.0±3.5 percent in semen preserved at room temperature.

Bras (2012) noted semen volume of Stud bucks as 1.1±0.4 ml. The mass activity in fresh semen was 3.9±0.1 while progressive sperm motility was 94.9±1.7 percent, sperm concentration was 2400±1.0 million per ml, the abnormal sperm percentage was 23.9±1.7, the intact acrosome sperm percentage was 99.8±0.1 percent, the live sperm count was 92.7±2.0 percent and observed yellowish colour of semen in stud buck.

Ferdinand *et al*. (2012) investigated the effects of age, temperature, diluents and storage time on WAD buck semen. The mean semen volume and concentration were 0.56±0.08 ml and 1.09±1.24x109 sperms/ml, respectively. 71.43% of ejaculate was classified as whitish, viscous and thickcreamy. The mass motility of sperms was 4.00±0.53 on a scale from 0 to 5 and classified as good for AI. The sperm morphology was acceptable in 87.00±5.71% of freshly collected sperms. The mean percentage of abnormal tails and heads was 11.58±1.56% and 1.56±0.52%, respectively. In tris-based extender, individual sperm motility declined progressively from score 4 directly after collection to score 0 on a scale from 0 to 5 after 97 h of incubation at room temperature and 105 h at 4°C. In skimmed milk-base diluent, the decrease was more rapid: from “score 3” to “score 0” in 45 h after collection at room temperature and 65 h at 4°C. In tris-based extender, the sperm motility was fairly good for AI (score 3) 36 and 45 hours after

collection at room and refrigerated temperature, respectively. In skimmed milk-based extender, this parameter was fairly good for AI. They further noted that the abnormal tails (11.58±1.56 percent) andhead(1.56±0.52 percent) of spermatozoa in the West AfricanDwarfbuck semen, the mass activity of semen was 4.00±0.53 on a scale from 0 to 5,pH of the semen as 6.73±0.25,volume as 0.56±0.08 ml.

Bezjian *et al.* (2013) observed that the colour of semen of endangered Makhor buck was white-yellow and abnormalities in the semen include bowed mid pieces, bent tail, detached heads was 11.6±3.9, 9.3±1.1 and 5.3±1.7 percent, respectively.Intact acrosome sperm percentage of fresh semen of was 66.6±3.7 percent, the progressive motility was 50.00±5.50 percent and the semen volume collected by electro ejaculation method was 1.2±0.2 ml. The total sperm concentration was 2400±0.3 million /ml.

Oliveira *et al.* (2013) evaluated the correlation between the assessment of the functional integrity of the sperm membrane by hypoosmotic swelling test using solutions with different osmolarities, and the conventional assessments of fresh semen in bucks. Samples were divided into five aliquots and subjected to hypoosmotic test using distilled water (0 mOsm/L), and sodium citrate and fructose solutions at different osmolarities (50; 100;150 and 200 mOsm/L). The 100 mOsm/L solution showed the highest percentage of reacted sperm (34.8%), but distilled water was the one with the lowest values (20.8%). No significant correlations were detected between the reacted sperm verified by the hypoosmotic swelling test and other semen characteristics ($P>0.05$). The hypoosmotic test by using a 100 mOsm/L sodium citrate and fructose solution to assess the functional integrity of the sperm membrane in caprine species was recommended.

Sultana *et al.* (2013) evaluated the performance of bucks on the basis of semen quality and fertility. Data on 162 ejaculates from five adult Black Bengal bucks (B1, B2, B3, B4 and B5) were recorded. Individual buck effect was found to be significant on semen volume ($P<0.05$), three stages of sperm motility ($P<0.01$) and percentage of live sperm ($P<0.01$) but non significant ($p>0.05$) on sperm concentration and percentage of normal sperm.

The results of semen volume per ejaculate, sperm concentration, initial sperm motility, on dilution sperm motility, post-thawing sperm motility, percentage of live sperm and percentage of normal sperm were ranged from 0.58±0.17 to 1.04±1.1 ml, 2827±0.76 to 3132±0.88 million/ml, 77.07±1.06 to 81.47±1.84%, 61.71±1.03 to 70.30±1.54%, 48.15±1.99 to 55.88±2.97%, 87.58±0.96 to 92.95±0.74% and 87.17±2.40 to 91.85±1.38%, respectively. The significantly ($P<0.05$) highest volume of semen, three stages of sperm motility and percentage of live sperm was found in buck B5 and lowest in buck B1. The significantly ($P<0.05$) highest non-return rate was found in buck B5 (87.31±7.99%) and lowest in buck B1 (63.41±672%). There was no significant ($p>0.05$) seasonal variation was observed on semen characteristics except on dilution sperm motility and post-thawing sperm motility which differed significantly ($P<0.05$). The season had non significant ($p>0.05$) effect on 25 day non-return rate of bucks. The results of the present study revealed that evaluation of breeding bucks based on semen quality is important and provides the guideline to buck evaluation for reproductive performance.

Ahmed *et al.* (2014) reported that acrosome integrity of fresh diluted semen of buck using Tris egg yolk diluter at 0 hour was 85.8±5.4 percent.

Yotov (2015) observed that the initial sperm motility of semen of Bulgarian white milk breed was 85.00±5.00 percent, the pH was 6.77±0.06, the semen concentration was 3570±0.32 million /ml.

Tahar *et al*. (2018) investigated semen parameters of Arbia breed goats living in western Algeria.Semen parameters viz.,ejaculation volume, sperm motility and sperm viability (1.1±0.39 ml; 81.79±13.42%; 73.73 ±9.07%, respectively) were high in autumn and summer and low during spring (0.11±0.14 ml, 71.07±11.28%, and 52.38±20.34%, respectively). Semen concentration followed an opposite trend to that of other parameters, it is high during winter and spring ($7.87\pm2.38 \times 10^9$ spz (spermatozoon) / ml, $7.61\pm1.81\times 10^9$ spz / ml, respectively) and low in autumn ($6.4\pm2.62 \times 10^9$ spz / ml), with no significant difference between seasons ($P > 0.05$). The seminal pH fluctuates with high values in winter and summer and low in spring and autumn.

2.3 Freezing of Washed and Unwashed Semen at Refrigeration Temperature

Salisbury and Van-Denmark (1978) reported when mammalian sperms were cooled to 5°C, causes leakage of intracellular enzymes, potassium, lipoprotein, ATP and other materials from the cells. The precise mechanism of cellular damage was unknown but presumably changes occur at unequal rates on the surface and internal portions of spermatozoa during cooling and both physical and chemical damages results.

Singh *et al.* (1985) collected the semen from Jamanapari and Barbari bucks to study the effect of preservation on live sperm percentage. The semen samples were diluted in EYC and stored at 5° C in refrigerator. The live sperm percentage was checked at every 24 hours. The live sperm percentage at 0, 24, 48, 72 and 96 hours of preservation was 89.5, 70.8, 59.45, 47.4 and 25.25 percent, respectively for Jamanapari breed and 85.0, 69.30, 51.17, 36.33 and 15.33 percent for Barbari breed.

Sinha (1988) studied the leakage of transaminases in chilled semen of Black Bengal, Jamnapari and Barbari bucks. The enzymes were estimated in diluted semen samples kept in refrigerator (4 to 8° C) for 24 hours. Mean values of activities of AST and ALT (units/10^9 sperms) in Black Bengal bucks were found to be 39.39±1.57 and 26.81±1.95, respectively. Corresponding values in Jamnapari bucks were 27.96±1.24 and 17.10±0.87, respectively and in Barbari bucks the values were 28.63±1.35 and 18.54±1.05, respectively. Higher activities of the enzymes were found in Black Bengal bucks and the effects of breed on leakage of enzymes were found to be highly significant. There was no significant difference between bucks of Jamnapari and Barbari breeds but both the breeds were significantly different from that of Black Bengal breed in respect of leakage of AST and ALT.

Singh *et al.* (1993) estimated the leakage of GOT and GPT from buck spermatozoa during freezing and refrigeration preservation. The mean (±SE) GOT release immediately after dilution, in pre-freeze and post-thaw semen and preserved in refrigerator were 6.174±0.316, 11.720±0.506,

35.101±1.420 and 21.275±0.981 µmole/litre in TEYFRAC (Tris-egg yolk-fructose-citric acid-glycerol) and 7.336±0.297, 14.851±0.572, 47.946±1.733 and 26.585±0.906 in EYECEF (Egg yolk-sodium citrate-fructose-glycerol) respectively. Corresponding values for GPT release were 0.071±0.129, 1.872 ±0.218, 7.218±0.681 and 2.977±0.287 in TEYFRAC and 0.289±0.065, 3.819 ±0.385, 12.808±1.057 and 5.864±0.525 in EYECEF, respectively. A significant difference ($P<0.01$) in GOT and GPT release at different stages of preservation was observed. Significant difference in GOT and GPT release were observed between bucks ($P<0.05$), between dilutors ($P<0.01$). The mean (±SE) release of GPT was less than of GOT at all stages of preservation, but the trend of GPT release was similar to GOT release.

Roca *et al.* (1997) conducted experiment to evaluate the viability and fertility of unwashed and washed Murciano-Granadina goat spermatozoa after dilution in Tris-egg yolk extender during storage at 5° C. The viability of spermatozoa declined with preservation time ($P<0.01$) and no differences ($P>0.01$) were found between unwashed and washed spermatozoa for either spermatozoa quality parameters evaluated.

Prasad *et al.*(1999) studied hypo-osmotic swelling test (HOST) and its response in fresh and freeze thawed semen and observed that the percentage of coiling of tail was strongly correlated to mass movement, progressive motility, live sperm count, total intact acrosome and sperm concentration.

Kale and Tomer (2000b) conducted a study on ten crossbred bucks to evaluate fertility status on the basis of semen quality, hypo-osmotic swelling test (HOST), cervical mucus penetration test (CMPT) and resistant to 1 % Nacl solution during three seasons (Hot dry, Hot humid and Cold humid). The stock solution of 0.5 g each of sodium citrate and fructose prepared in double distilled water at 150 mOsm/kg was used. The overall means of the HOS reacted (%) and HOS non reacted (%) sperms were 69.66±0.79 and 30.34±0.79, respectively. It was observed that the season, buck and season × buck interaction significantly ($P<0.01$) influenced HOS reacted and HOS non

reacted spermatozoa. Highest percentage of spermatozoa exhibited hypo-osmotic swelling during the cold humid season followed by the hot humid and hot dry season. The differences between all the seasons were significant ($P<0.01$).

Pratap *et al.* (2000) recorded the mean percentage of swollen spermatozoa as 45.0±0.44, 57.5±0.48 and 45.5±0.41 % following conventional freezing, moderate rate and slow rate of programmable, respectively.

Shamsuddin *et al.* (2000) conducted experiment in which ejaculates were collected within a 20 min. period after the smallest number of days of sexual rest. Pools of two to three ejaculates were diluted (1:5 ; semen: diluent) in splits with Glucose-citrate-egg yolk (GCEY), Tris-fructose-egg yolk (TFEY) or Skim milk (SM) and preserved at +4 to +7°C. In pooled data across the bucks, the sperm motility was better in GCEY and TFEY than in SM. The sperm motility consistently dropped after 1 day of preservation ($P<0.01$).The motility remained 50% or more up to 4 days in TFEY, 3 days in GCEY and only 2 days in SM.

Simon and Vijayakumaran (2003) studied effect of preservation at refrigeration temperature on quality of buck semen and reported that the sperm abnormalities increased with days of preservation. The mean percentage of sperm abnormalities in buck semen at 0, 24 and 48 hours of preservation were 2.97±0.37, 3.68±0.51 and 4.74±0.48, respectively.

Azeredo *et al.* (2001) evaluated the plasma membrane integrity of frozen-thawed spermatozoa of three Sannen bucks with and without seminal plasma. The percentage of spermatozoa with damaged plasma membrane was 57.54±11.43 and 60.19±12.54 in fresh semen with and without seminal plasma, respectively.

Islam and Ahmed (2003) studied the effect of seminal plasma and of its removal on the quality of goat semen. Total of 12 pooled semen samples were collected from 4 cross bred (Beetal × Assam local) bucks. One pooled semen sample was divided into two halves. One half was diluted (1:5) in warm (37°C) Tris buffer, centrifuged at 3000 rpm for 5 min. and clear

supernant was aspirated out. The sediment was extended in Tris Extender at 1:10 ration. The other half was extended in the same extender at 1:10 ration. The mean percentage of live sperm count for unwashed/washed semen was 88.00±1.26/89.67±0.61 at 0 hour which declined gradually to 65.17± 13.10/83.00±0.86 at 72 hours of preservation. The higher live sperm count maintained after 72 hours of preservation in washed semen might be due to the removal of coagulating factors from seminal plasma. The mean percentage of progressive motility for unwashed/washed semen was 84.17± 1.54/85.83±0.83 at 0 hour and 42.50±9.01/66.67±1.05 at 72 hours of preservation. The sperm motility declined gradually with the increase of preservation period.

Leboeuf *et al.* (2004) evaluated the extender composition, presence or absence of seminal plasma and dilution rate of spermatozoa for the preservation of fresh semen during three days before artificial insemination (AI). The fertility rate was decreased in both extenders tested (milk or NPPC) when the storage duration increased. It was observed that the removal of seminal plasma by centrifugation at collection does not improve the fertility rate.

Fonseca *et al.* (2005) carried the hypo osmotic swelling test in fresh goat spermatozoa to detect the best hypo osmotic solution for testing membrane integrity in fresh goat semen. The sodium citrate and fructose based solutions with the following osmolarities (mOsm/l): 50, 75, 100, 125, 150, 175, 200, 250, 290, 300 mOsm/l were used in the experiment. On the basis of reaction of spermatozoa tail to HOS, they classified the sperm tails as non-coiled and strongly coiled. It was reported that goat spermatozoa appeared to suffer increasing coiling from the 50 mOsm/l solution and reached maximum value with the 125 mOsm/l solution after which coiling began to decrease reaching minimum score with the 290 mOsm/l solution. The percentage of spermatozoa with coiled tail in the 10 solutions listed above were 34.1, 38.8, 45.3, 51.5, 46.8, 42.8, 38.2, 29.0, 19.48 and 23.1 %, while the percentage of strongly coiled spermatozoa were 6.8, 10.6, 21.5, 25.3, 29.3, 21.5, 19.3, 12.4, 6.4 and 7.9 % for the ten solutions, respectively. It was reported that according to the total coiling, solution having osmolarity of 125 mOsm/l was superior to

the solution having 50, 75, 250, 290 and 300 mOsm/l osmolarities (P<0.05). There was no interaction among solution, collection and bucks (P<0.05). The percentage of total coiling increased significantly (P<0.05) from the first to fourth semen collection and differed (P<0.05) among bucks. On the basis of results it was recommended that the 125 mOsm/l solution was best for use in HOST in fresh goat spermatozoa.

Takarkhede *et al.* (2005) studied effect of storage at refrigerator temperature on buck semen diluted in Tris egg yolk citrate and observed the mean live sperm count before storage as 81.33±1.50 percent, mean abnormal sperm percentage as 4.67±0.42 percent. The mean sperm motility before storage as 75.83±0.83 percent. The motility percent significantly (P<0.01) decreased with increase in storage period and was 54.17±1.54 by 96 hrs of storage. The live sperm percent significantly (P<0.01) decreased with increase in storage period and was 57.83±0.95 by 96 hrs of storage. The abnormal sperms increased and reached 11.5±0.99 by 96 hrs of storage.

Islam *et al*. (2006) collected total of 18 pooled semen samples from 4 healthy crossbred (Beetal×Assam Local) bucks, twice weekly for a period of 12 weeks and processed via three processing methods, viz. Method I (holding after washing: semen sample was washed twice, extended, held for 0, 1, 3 and 5 h and preserved), Method II (washing after holding: semen sample held for 0, 1, 3 and 5 h, washed twice, extended and preserved) and Method III (holding of undiluted semen: semen sample held for 0, 1, 3 and 5 h, extended and preserved). An isotonic Tris buffer was used to wash the spermatozoa with the aid of centrifugation at 3000 rotations per minute (rpm) for 10 min. A Tris–citric acid–fructose–egg yolk extender was used to extend the semen processed with all three processing methods and preserved at 5 ◦C up to 72 h. Holding of semen at 24 ◦C in each processing method caused deterioration of the semen quality during preservation, as the holding time increased from 0 (without holding), 1, 3 and 5 h. The initial sperm motility declined from 85.8 to 71.7% in Method I, from 85.8 to 21.7% in Method II and from 84.2 to 45.0% in Method III, as the holding time increased from 0 to 5 h. The corresponding values were 66.7 and 49.2% for Method I, 66.7 and 8.0% for

Method II and 42.5 and 25.8% for Method III, following 72 h of preservation. The percentage of live sperm and intact acrosomes also showed a similar decreasing trend as the holding time increased from 0 to 5 h in all processing methods as revealed in percentage of sperm motility. Washing of spermatozoa without a holding period maintained significantly higher sperm motility (66.7%), live sperm counts (83.0%) and incidence of intact acrosomes (79.3%) following 72 h at preservation, compared to the corresponding values (42.5, 65.2 and 73.3% for sperm motility, live sperm counts and intact acrosomes, respectively) recorded in unwashed semen without a holding period. The values recorded for washed semen without holding were also significantly higher than for semen held for 1, 3 and 5 h in all the three processing methods following 72 h of preservation. It was revealed that the holding of semen caused deterioration of the spermatozoa, whereas, washing without holding had a beneficial effect on the viability of spermatozoa in maintaining its storage ability for up to 72 h of preservation at refrigeration temperature (5 ◦C).

Mara *et al.* (2007) investigated the relative usefulness of SM (Skim milk), TEMPOL (4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl) and TEMPOL + hyaluronic acid (TEMPOL+HA) as extender of buck semen and found the motility rate as 51.5±2.1, 55.0±7.0 and 60.0±0.0 in SM, TEMPOL and TEMPOL+HA respectively for 24 hour storage at 4° C.

Salvador *et al.* (2007) compared the effect of three extenders for buck semen preservation ; Skimmed milk (M), Sodium Citrate (C) and Tris-based diluent (T) and washing of semen on the in vitro viability of Musciano - Granadina goat spermatozoa stored at 5° C for 72 hrs. The HOS positive sperm percentage in diluted semen preserved at 5°C was 21.0 to 31.0 percent.Semen motility and in vitro viability of spermatozoa in milk extender was significantly higher than in Citrate and Tris ($P<0.05$). Motility of diluted semen in citrate and milk extender was improved when semen was washed previously.

Sule *et al.* (2007) noted abnormal sperm count at room temperature and 52.6 and 45.0 percent the sperm cell motility at post extension of semen in dilutor containing 20% coconut milk and 80% citrate buffer in West African Dwarf bucks.

Apu *et al.* (2008) recorded that the normal spermatozoa percentages of the diluted semen varied from 88.73±0.54 to 91.91±0.78 percent the live sperm percentage of Black Bengal buck semen diluted in Egg yolk citrate dilutor was in the range of 83.73±0.94 to 89.58±0.98 percent in bucks.

Pervage *et al.* (2009) collected semen from ram using artificial vagina and was stored in a refrigerator (4^0C) for three days. The volume of semen was extended with egg yolk citrate diluter. A total of 63 ewes were inseminated with stored liquid semen collected from 15 rams by AV method. The total number of spermatozoa, live-dead, normal-abnormal, sperm motility and the pH was observed regularly. The average semen volume per ejaculate was 0.76-1.00 ml and the sperm concentration was 2.37x109 – 4.30x109 per ejaculate. The number of normal spermatozoa (95.17±2.33 percent) and the pH of fresh diluted semen of ram were almost similar irrespective of days of storage. Number of live spermatozoa (93.05±3.16 percent) and the sperm motility (78.45±3.26 percent.) of fresh diluted semen of ram were reduced with the increasing age of semen.

Ari and Daskin (2010) noted that post thaw abnormal sperm count of semen of Angora goat was dead sperm count was 54±3.8 percent, post thaw HOS positive sperm percentage was 38.0±3.8 percent, post thaw motility inTris egg yolk diluter was 25.5±2.9 percent.

Ashmawy *et al.* (2010) observed that post thaw sperm motility of 50.0 percent in Damascus and 50.0 percent Baladi cross bred bucks using 2.5, 10 and 20 percent egg yolk in Tris extender was 54.8, 50.4 and 39.0 percent, respectively.

Naing *et al.* (2010) noted that the average live sperm percentage in Boer semen before and after freezing was 77.4±1.8 and 59.5±1.7 percent, respectively. It was observed that normal sperm count in Boer buck semen at pre freezing and post freezing was 96.4±2.3 and 88.8H.6 percent, respectively. The post thaw HOS positive sperm percentage in Boer semen was 61.1±1.7 percent.

Dagli (2011) reported average abnormal sperm percentage of Osmanabadi and Sirohi buck semen diluted in Tris egg yolk glycerol dilutor at prefreezing 5°C and post freezing (24 and 72 hours) was 16.60±0.45, 18.61±0.62, 20.22±0.40 and 14.33±0.86, 17.55±1.23, 19.72±1.53, respectively. The average sperm motility was 82.22±1.73, 77.22±2.34 and 73.89±2.00 percent and 74.45±1.65, 72.76±1.02 and 72.20±0.70 percent, respectively. Average live sperm was 81.99±0.51, 78.55±0.69, 77.83±0.84and 80.44±1.26, 79.66±1.38 and 78.22 ±1.29, respectively. If was stated that the intact sperm plasma membrane integrity in Osmanabadi and Sirohi buck semen diluted in Tris egg yolk glycerol dilutor at prefreezing and post freezing (24 and 72 hours) was 72.50±1.03, 68.83±1.95, 67.27±1.16 and 71.00±1.15, 68.83±0.59, 66.33±0.72 percent, respectively.

Daskin *et al.* (2011) recorded that the post thaw intact sperm plasma membrane integrity of Angora goat semen using Bioxcell dilutor was 39±4.1 percent.

Priyadharshini *et al.* (2011) recorded 78.2±1.32 and 52.7±1.87 percent HOS positive sperm cell at prefreezing and post thawing, respectively in the Jakharana buck semen preserved using 20 percent egg yolk.

Ajala *et al.* (2012) noted that West African Dwarf buck semen was extended in 10 percent coconut milk-egg yolk citrate extender and preserved at room temperature, the motility at 0 and 12 hour was 95.0 and 85.0 percent, respectively.

Bras (2012) observed 16.2±1.9 percent post thaw average live sperm percentage, 37.6±1.4 percent abnormal sperm percentage, 32.2±2.1 percent sperm motility, 22.8±1.9 percent plasma membrane integrity of Stud

buck semen diluted in Tris Egg yolk glycerol extender. Progressive sperm motility of Stud buck semen diluted in Tris Egg yolk extender kept at room temperature was 93.8±1.7 percent.

Ferdinand *et al.* (2012) noted that semen of West African Dwarf buck preserved at room temperature in Tris based extender the sperm motility gradually declined from a score of 4.0 ("Good" class) at 0 hour to a score of 2.5 ("Fair" class) at 37 hour of storage.

Souhayla *et al.* (2012) investigated the effect of cryostorage on semen treated with modified Tris solution. The efficiency of modified Tris solution (MTS) (Tris 3.03gm+fructose 1.25gm+ citric acid 1.67gm+egg yolk) on individual sperm motility collected from buck and preserved in the refrigerator at 4-5°C was studied. The results showed no significant ($P>0.05$) changes in sperm motility after 24 to 72 hrs of cryostorage. However, there was a significant ($P<0.05$) decrease in percentage of individual motility after 96 hrs of cryostorage. Cryostorage with MTS was suitable to preserve semen of local bucks in the refrigerator at 4-5 °C for 72 hours.

Mohammed *et al.* (2012) studied the effect of semen extenders in whichGood quality semen was divided into 4 portions, each diluted with one diluent (Milk, Na.Citrate, Tris and Na.Bicarbonate). The diluted semen was packaged into 0.5ml straws then cooled to 5°C. After equilibration, straws were suspended at height 15 and 5cm of LN for 10 and 5 min, respectively before plunged into LN. Frozen semen was thawed and checked for post-thaw motility and viability. Pre-freeze semen motility was significant higher in Tris, Na.Citrate and Na.Bicarbonate than milk diluent. Post-thaw semen motility and viability were highly significant for milk and Na. Citrate than Tris and Na. Bicarbonate diluents. Milk was significantly higher than Tris and Na.Citrate diluents for fertility and fecundity. Regarding to post-thaw semen viability, fertility%, kidding%, fecundity% and prolificacy%, milk is preferable than Tris and Na.citrate diluents.

Parmar *et al.* (2012) studied the preservation of washed spermatozoa of Mehsana Bucks at refrigeration temperature.The washed spermatozoa from 78 semen ejaculates, 26 each from 3 Mehsana bucks, were preserved at 4 ± 1^{0}C temperature in SM and TCFEY diluents up to 72 hours and the physical characteristics of spermatozoa were studied to assess the suitability of diluents. The individual sperm motility (75.58±0.31 and 76.95±0.30 in TCFEY and Skim milk dilutor respectively).and live spermatozoa (78.92±0.23 and 80.02±0.22 in TCFEY and Skim milk dilutor respectively) decreased significantly whereas abnormal spermatozoa (9.25±0.77 at 24 hour and 22.41±0.57 percent at 72 hour in TCFEY dilutor), increased significantly ($P<0.05$) at each 12 hrs interval of preservation in two diluents. The effect of diluents on spermatozoan characteristics was less pronounced in SM than TCFEY diluent. Among the interaction studies, stage of preservation affected the live spermatozoa and morphology. The findings suggested superiority of SM diluent over TCFEY for preservation of Mehsana buck semen.

Beltran *et al.* (2013) recorded that the post thaw average live sperm count, abnormal sperm percentage of buck semen using Tris based extender with 5 percent Egg yolk was 59.58 and 6.49 percent respectively.

Bezjian *et al.* (2013) noted that in endangered Makhor buck semen post thaw average live sperm percentage at 0 hour using Tris egg yolk dilutor was 25.3±4.1 percent.

Kulaksize *et al.* (2013) noted that post thaw average abnormal sperm percentage of Angora, Kilis and Saanen buck semen extended with 5 percent Glycerol concentration were 34.3, 30.8 and 40.8 percent, respectively. The post-thaw motility was 51.6, 61.6 and 75.0 percent respectively.

Menon *et al.* (2013) reported that plasma membrane integrity percentage of Boer buck semen diluted in Tris dilutor before and after freezing was 71.00±1.92 and 64.54±1.80, respectively.

Ahmed *et al.* (2014) noted that the live sperm percentage in fresh diluted semen at 0 hour was 89.3±4.2 percent. The live sperm percentage of buck semen diluted in Tris egg yolk glycerol dilutor at prefreezing 5 °C and post freezing was 77.6±3.9 and 42.3±7.5 percent, respectively. Average normal sperm percentage, plasma membrane integrity of fresh buck semen diluted in Tris egg yolk dilutor at 0 hour was 96.4±5.2 percent and82.2±3.3 percent respectively. The progressive sperm motility of buck semen after dilution at room temperature was 86.0±1.4 percent. Average normal sperm percentage of buck semen diluted in Tris egg yolk glycerol dilutor at prefreezing 5°C and post freezing was 88.8±5.7 and 81.0±5.7 percent, respectively. Average sperm motility of buck semen diluted in Tris egg yolk glycerol dilutor at prefreezing 5°C and post freezing was 77.6±3.9 and42.3±7.5 percent, respectively. Sperm plasma membrane integrity of buck semen diluted in Tris Egg yolk glycerol dilutor at prefreezing 5°C and post freezing was 75.0±5.1 and 50.1±8.7 percent, respectively.

Ranjan *et al.* (2015) studied the freezability of buck semen at different levels of egg yolk during different hours of equilibration period by conventional freezing protocol.Tris citric acid fructose (TCF) diluent having 0,5,15 and 20% (v/v) egg yolk and 6% (v/v) glycerol used as a cryoprotectant. At 20 % Egg yolk level and 4 hrs equilibration period live sperm count was 38.33±2.09 % , acrosomal integrity was 35.66±2.53 %, sperm motility was 34.16±1.53, abnormal sperm count was 4.16±0.60 %, positive hypo-osmatic swelling test was 37.50±1.70 %.

Yimer *et al.* (2015) recorded 57.1±5.0 percent and 7.7±0.8 percent post thaw live sperm percentage and abnormal sperm percentage respectively of buck semen using 10 percent egg yolk.

Rasteh and Divandi (2015) evaluated the effects of different extenders on Ghezel ram spermatozoa in pre and post freezing conditions. The effects of extenders on some parameters of spermatozoa such as motility, progressive motility, viability and plasma membrane integrity, were significant ($P<0.05$), and the higher percentages of this treats were observed

in Tris–citric acid extender compared to Sodium citrate and Skim milk based extender. The effects of extenders on normal spermatozoa were not significant, but the highest abnormal of spermatozoa were obtained in Skim milk extender. In conclusion, Tris–citric acid extender appears to be a better option compared with milk and sodium citrate extenders for liquid and frozen storage of Ghezel ram semen.

Albiaty *et al.* (2016) investigated the effect of the different extenders (Tris, hydroxymethylaminoethane; Sodium citrate; and Milk) and preservation periods in some characteristics of Awassi ram semen. Significant differences ($P<0.05$) between extenders were found in progressive motility and acrosome defects, while for other characteristics (pH, dead and abnormal sperm percentages) were not significant. Tris and sodium citrate-based extenders showed superiority ($P<0.05$) in comparison with milk - based extender in progressive motility (63.87 and 61.25% vs. 39.12% respectively), in the contrast, acrosome defect values were (8.95 and 10.15% vs. 11.47% respectively). Preparation processes and storage periods showed significant ($P<0.05$) effect on all traits except dead sperm percentage. It was concluded that tris and sodium citrate extenders were preferable on milk extender. Also, preparation processes and storage periods at 5˚C affected semen quality.

Olurode and Ajala (2016) analysed that sperm motility in goat milk citrate extenders at $5^{O}C$ was significantly higher ($P<0.05$) than that stored at $28^{0}C$ and in egg-yolk citrate extenders at both investigated temperature ($P<0.05$) 48 hours post extension. Beyond 72 hours of storage, motility in both extenders and at both temperature was less than 40%. The results showed that goat milk in part can be replaced egg-yolk as a medium for semen extension. Therefore, an extender with concentration of 10-20 % heated goat milk, 70-80% sodium citrate and 10% egg-yolk could be used to store goat semen at $5^{0}C$ for 2-3 days.

Kyaw *et al.* (2017) observed the effects of seminal plasma and extenders on the motility and viability of local buck spermatozoa at 5 °C and at -20 °C. After collecting semen, the effects of seminal plasma removal were

analyzed by using two different extenders (skimmed milk and egg yolk citrate extenders). The removal of seminal plasma showed higher and significant ($P<0.01$) effect in the motility, and live spermatozoa at 5 °C and at -20 °C. For two different extenders, the differences were not observed at 5 °C and at -20 °C on the semen quality of local bucks.

Benmoula *et al.* (2018) compared the effect of eight extenders (Skim milk, INRA96®, Tris Eggs Yolk, Triladyl®, Ovipro®, Andromed®, Optixcel® and Duragen®) on ram sperm motion parameters. Sperm motility and kinematic parameters decreased significantly over time of storage in all extenders and storage temperature. The overall CASA parameters were affected by extenders in different manners depending on breed and storage temperature. Skim milk, Duragen®, and INRA96® provided the best CASA results.

Rahman *et al.* (2018) compared the preservation effects of skim milk and Tris-citrate on the quality of chilled and frozen-thawed Indigenous ram semen (n=13) during rainy monsoon season (June to October) in Bangladesh. Motility, viability and membrane integrity were significantly ($P<0.01$) higher in chilled semen samples diluted with skim milk than semen diluted with Tris-based.

2.4 Freezing of Washed and Unwashed Semen at Cryopreservation Temperature

Ritar and Salamon (1982) reported that after two fold dilution with Tris-glucose medium, buck seminal plasma was a poorer milieu than ram seminal plasma for the maintenance of viability of freshly collected spermatozoa of both species. Egg yolk (9% v/v) in the diluted buck seminal plasma caused coagulation of the medium coupled with death of spermatozoa after 2 h incubation at 37°C. Removal of seminal plasma by centrifugation (washing) of buck semen was beneficial for the survival of spermatozoa after freeze-thawing, but the effect depended on the intensity of washing. When the semen was diluted 6- or ll-fold, double washing was more effective than single washing. However, the efficiency of the latter method, after 21-fold dilution,

was similar to that of double washing at ll-fold extension. Survival of washed spermatozoa was better when the resuspending-freezing medium contained 1· 5-12 % (v/v) egg yolk than no egg yolk. Egg yolk concentrations higher than 1· 5 % (v/v) depressed the post-thawing survival of non-washed spermatozoa.

Tuli *et al.* (1991) studied the influence of thawing temperature on release of glutamic oxaloacetic transaminase in frozen semen from Boer bucks. The release of GOT was not altered significantly ($P<0.05$) by thawing temperature but showed difference between bucks ($P<0.01$). The release of GOT immediately after thawing at 70° C was not significantly different from 37 or 40° C (99.9 units/ml versus 103.6 units/ml and 104.6 units/ml). Even after 6 hr of incubation, the GOT values were still quite similar for all groups.

Singh *et al.* (1995) obtained semen samples were from 12 bucks (3 Beetal, 3 Black Bengal and 6 Beetal x Black Bengal) and 10 different extenders were constituted with varying concentrations of glycerol, DMSO, glycerol + DMSO and glycerol + lactose as the sperm cryoprotective agents. Both motility and the percentage of live spermatozoa were most affected by extenders containing only DMSO and these values improved in glycerol + DMSO extenders as the concentration of glycerol was increased while DMSO was decreased. However, the values were significantly higher in extenders containing glycerol + lactose as the cryoprotective agents, and were found to increase with increased concentration of lactose, being highest in TYGL. Acrosomal and tail abnormalities between post equilibration and post thawing stage, and were higher in extenders containing the higher levels of DMSO. Significantly ($P<0.01$) lower percentages of abnormalities were recorded in the glycerol + lactose extenders. The fertility results showed nonsignificant effect of extenders on the conception rate of does.

Sinha *et al.* (1996) studied release of various enzymes from buck spermatozoa during freezing extended semen with 10 different extenders containing glycerol, DMSO, Glycerol + DMSO and glycerol + lactose in varying concentrations as cryoprotective agents. Extracellular activities of transaminases (AST and ALT) were significantly higher in extenders

containing DMSO than lactose. Leakage of these enzymes was increased from the pre freeze to the post freeze stage.

Azeredo *et al.* (2001) evaluated plasma membrane integrity, motility, vigor and morphology of fresh and frozen goat spermatozoa with or without seminal plasma. Semen samples were diluted in Tris solution, before and after thawing, with a combination of carboxiflurescein diacetate and propidium iodide. The results showed differences ($P<0.01$) for motility and minor defects in the presence or absence of seminal plasma, for both fresh and frozen samples. Periods of collection had a significant effect on motility, probably due to changes in the photoperiod. Plasma membrane integrity was significantly reduced by the freezing process, whether seminal plasma was present or absent. In conclusion, removal of seminal plasma decreased motility and vigour rates in frozen samples.

Baruah *et al.* (2003) noted that post thaw intact acrosomal sperm percentage of buck semen using Tris-egg-yolk-citric acid -fructose glycerol diluter was 80.98±0.53.

Islam and Ahmed (2003) observed that acrosomal integrity of cross bred (Beetal Assam local buck) semen was 73.33±1.58 percent at 72 hrs of preservation at 5°C temperature.

Aboagla and Terada (2004) conducted four experiments to investigate the effects of egg yolk during the freezing step of cryopreservation (namely, the process except for the cooling step), on the viability of goat spermatozoa. The effects of egg yolk on sperm motility and acrosome integrity during the freezing step were investigated in experiment 1. Spermatozoa diluted with Tris-citric acid-glucose (TCG) solution containing 20% (v/v) egg yolk were cooled to 5^0C, washed, and then frozen in TCG with egg yolk (TCG-Y), TCG without egg yolk (TGG-NY), 0.370 Mtrehalose with egg yolk (TH-Y), or trehalose without egg yolk (TH-NY) and all extenders contained glycerol.In frozen-thawed spermatozoa, the inclusion of egg yolk in the freezing extenders increased ($P < 0:05$) percentages of motile sperm, progressively motile sperm, and the recovery rate (ratio of post-thaw to pre-

freeze values), but decreased (P < 0:05) acrosomal integrity. Moreover, extenders with trehalose had better (P < 0:05) post-thaw sperm viability. In experiment 2, the effects of egg yolk on acrosome status before and after freezing were studied. Egg yolk significantly decreased the proportion of intact acrosomes before freezing, leading to fewer (P < 0:05) intact acrosomes post-thaw and lower (P < 0:05) recovery rates for intact acrosomes. In experiment 3, including sodium dodecyl sulfate (SDS) in a diluent containing egg yolk tended to preserve the acrosome compared with the egg yolk containing diluent free of SDS, however, spermatozoa had a lower (P < 0:05) proportion of intact acrosomes than those in a yolk-free diluent. However, after cooling, spermatozoawere diluted with a glycerolated extender containing egg yolk. Therefore, the objective of Experiment 4 was to explore whether the egg yolk or glycerol was responsible for the reduced intact acrosome percentage. In this experiment, after cooling and washing the spermatozoa were diluted in TCG with glycerol and/oregg yolk. The combination of glycerol and egg yolk in the extender reduced (P < 0:05) the proportion of intact acrosomes compared with egg yolk or glycerol alone. In conclusion, the inclusion of egg yolk significantly improved sperm motility, indicating its beneficial effects during the freezing step of cryopreservation; trehalose appeared to synergistically increase its cryoprotective effects. Furthermore, although neither glycerol nor egg yolk per se affected the proportion of intact acrosomes, the combination of the two significantly reduced the proportion of acrosome intact spermatozoa.

Sinha *et al.* (2004) studied effect of glutathione on leakage of various enzymes from buck semen during freezing and found the mean values of AST and ALT during pre-freeze and post-freeze stage as 1.784±0.042 and 1.488±0.043 in Tris, 2.249±0.037 and 1.556±0.039 in Tris + 2 mm glutathione and 2.340±0.029 and 1.715±0.034 in Tris + 5 mm glutathione. The activities AST and ALT increased markly in all the extenders from the pre-freeze to post-freeze stage. However, activities of enzymes were significantly lower (P<0.05) in the presence of glutathione (5mM) at both the stages of preservation.

Cabrera *et al.* (2005) collected semen from six males was collected in spring, autumn or winter, washed or non-washed, diluted in a freezing extender with 1.5, 6 or 12% egg yolk, frozen and thawed after 2 days, 2 or 6 months of cryopreservation. The effect of egg yolk concentration in the freezing extender was far more important than the effect of washing or season on sperm cryosurvival. The quality of frozen–thawed semen improvement as egg yolk concentration increased regardless of the effects of season, washing or period of cryopreservation. Washing produced a positive effect on frozen–thawed semen collected during spring or autumn, but the difference decreased as the concentration of yolk increased. However, washing produced a negative effect on frozen–thawed semen collected during winter, diluted with either 6 or 12% egg yolk. There was no apparent seasonal effect on gross measures of sperm production but the seasonal effect was ever present and was reinforced by freezing.

Salvador *et al.* (2005) studied the effect of factors such as depth of semen deposition, inseminator skill, farm, sire and expression of estrus on pregnancy rate in Murciano–Granadina (MG) goats during non-breeding season and using frozen semen. Overall pregnancy rate was 57%. Farm and depth of semen deposition affected pregnancy rate, whereas the sire and the technician had no effect. The deeper the semen was deposited in the genital tract, the higher was the rate of pregnancy obtained, being greater when the catheter reached the uterus. In spite of the relevant difference observed (48.2% vs 59.0%), pregnancy rate of females not coming into estrus until 30 h after sponge removal was not significantly different, compared with those showing estrus during the OD procedure. AI in MG goat with frozen-thawed semen showed that post-cervical insemination presented significantly greater pregnancy rate in comparison to when semen is deposited in the vagina or in the caudal part of the cervix.

Kozdrowski *et al.* (2007) compared two protocols of goat semen cryopreservation. In protocol I, semen was centrifuged in order to remove its plasma and diluted in Tris buffer extender containing glucose, citric acid and glycerol with 20% addition of egg yolk. In Protocol II, the seminal

plasma was not removed and extender contained 1.5 % egg yolk. It was shown that the removal of semen plasma improved motility of goat spermatozoa following freezing/thawing with respect to the following motility indicators: motility, average path velocity, amplitude of lateral head displacement at $P<0.05$, and straight velocity, straightness and linearity at $P<0.01$. In conclusion, the removal of semen plasma through centrifugation improved motility properties of goat semen following the freezing/thawing procedure.

Afroz *et al.* (2008) collected semen from Black Bengal bucks to establish a cooling protocol (to -196°C) for buck semen preservation, and to study the effect of freezing on sperm motility and morphology. Semen was diluted with diluents (Triladyl andTris) and cryoprotectants, filled into straws, sealed, cooled (to 5°C) and equilibrated. After dilution, motility ranged from 75.00% to 76.67% and from 73.33% to 80.00% in Triladyl and Tris diluents, respectively. Motility of sperm after cooling to 5°C in Triladyl and Tris diluents ranged from 65.00% to 66.67% and from 63.33% to 70.00%, respectively. After equilibration in straws, the semen was subjected to a freezing protocol in a computer-controlled biofreezer CL-3000 (cooling at 10°C per minute, from 5°C to -80°C) and plunged into liquid nitrogen. Sperm motility of re-thawed semen varied from 38.33% to 43.33% and from 6.00% to-6.67% in Triladyl and Tris diluents, respectively. Sperm morphology of re-thawed semen was studied and head damage or cryoinjury was found in 2–3% of sperm in Triladyl diluents and 3–6% in Tris diluents.

Farshad *et al.* (2009a) determined the influence of different concentrations of Glycerol (1,3,5 or 7%,v/v/) in experiment 1 and DMSO (1,1.25,1.5 or 1.75 % v/v) in experiment 2 added either at 37or 5^0C. In Experiment 1, motility, progressive motility, viability of sperm were improved significantly ($P<0.05$) by increasing glycerol concentrations in the extenders, with the best results obtained with glycerol at 7% added at 37^0C.However, the rate of normal acrosome showed an opposite trend i.e the extender containing 1 % glycerol added at 5^0C showed better results ($P<0.05$). In experiment 2, the observed results showed similar tendencies to experiment 1. The data showed that the extender containing 1.75 % DMSO concentration (The highest level)

added at 37^0C was significantly (P<0.05) better than others. The percentage of intact acrosome decreased significantly (P<0.05) by increasing of DMSO concentrations, when added at 37^0C. Further the results of 1% DMSO added at 5^0C (P<0.05) better than other groups. In regard to all evaluated parameters, the observed results in experiment 3 showed that extender containing 7 % glycerol added at 37^0C was significantly (P<0.05) shared better than 7% glycerol added at 5^0C and extender containing 1.75 %DMSO added at both temperatures. In conclusion glycerol was still the cryoprotectant of choice for freezing of Markhoz goat sperm.

Ranjan *et al.* (2009) analysed that HOST proved to be a suitable technique for testing membrane status of goat spermatozoa. The concentration of 75 mOsm/lhypo osmotic solution was the most adequate for use in HOST for goat frozen-thawed goat spermatozoa and used in the routine analyses of goat semen.

Ustuner *et al.* (2009) evaluated that whether removal of seminal plasma and different egg yolk concentrations in a freezing extender reveals any effect of season on the freezability of Saanen buck semen. The semen was used in a Tris-based freezing extender with 6%, 12% or 18% egg yolk. 0.25 ml of straws were frozen in liquid nitrogen vapour. Motility, dead spermatozoa, defected acrosome, and other morphological defects (OMD) were evaluated in equilibrated at 5°C and post-thawed semen. There were seasonal differences in the semen for motility, dead spermatozoa, and OMD. For 5^0C equilibrated and post-thaw semen, the presence of buck seminal plasma had a detrimental effect on motility and dead spermatozoa. On the other hand, 18% egg yolk concentration affected post-thaw semen motility (P<0.05). In conclusion, the removal of seminal plasma improved motility following the freezing-thawing procedure in the breeding and the non-breeding seasons. The results showed that the breeding season and removal of seminal plasma had beneficial effects on the freezability of Saanen buck semen.

Ari and Daskin (2010) noted that post thaw acrosome abnormality of semen of Angora goat was 36 ±3.7 percent.

Caginand Daskin (2010) investigated necessity of separation of Angora goat seminal plasma and itsbeneficial of supplementation of bovine or ovine seminal plasma (SP) to extender prior to semen cryopreservation. The results suggested that Angora goat semen washing was beneficial and supplementation of bovine or ovine SP instead of Angora goat SP separated from semen can be used prior to freezing.

Doradoa *et al.* (2010) evaluated the effect of freeze–thawing procedure on goat sperm characteristics, and changes in sperm parameters for cryopreservation. Semen samples (n = 110) were frozen with TRIS and milk-based extenders and thawed. Sperm quality parameters (motility, morphology and acrosome) were compared between fresh and frozen–thawed samples. The ejaculates were grouped into two categories according to fertility results. In experiment 1, significant differences were found between semen extenders ($P < 0.001$), bucks ($P<0.05$) and ejaculates within the same male ($P<0.05$) in terms of sperm quality. There was no seasonal effect ($P > 0.05$) on the majority of the sperm parameters assessed after thawing. Moreover, significant differences ($P < 0.001$) in semen parameters assessed in fresh semen and frozen–thawed samples were found between groups. The effect of the freeze–thawing procedure on sperm quality parameters was also different ($P<0.05$) between extenders within the same group. The number of sperm quality parameters that had changed after cryopreservation was lower in "suitable" semen samples before and after thawing. In experiment 2, no differences ($P > 0.05$) in semen parameters assessed in fresh semen and frozen–thawed samples were found between groups. The effect of freezing and thawing on sperm quality parameters were different ($P<0.05$) between extenders within the same group. Only mean beat cross frequency (BCF) values were significantly higher ($P<0.05$) in TRIS diluted samples that led to successful pregnancies after artificial insemination. In conclusion, CASA-derived motility parameters, together with traditional semen assessment methods, give valuable information on sperm quality before and after freezing.

Naing *et al.* (2010) recorded intact acrosome sperm percentage in Boer buck semen pre freezing and post freezing was 96.4±2.3 and 88.8±1.6 percent, respectively.

Ashikin and Abdullah (2011) compared three extenders namely tris-citric acid yolk (TCAYE), yolk albumin citrate (YACE) and skimmed milk (SME) on frozen-thawed sperm characteristics of Katjang and their crossbreds; Jer-masia and Jermana goats .The Jermasia and Jermana buck sperm cryopreserved in TCAYE showed the best results in terms of motility (52.64±0.93% and 53.20±1.13%) and livability (56.47±0.96% and 57.72±1.11%), respectively. In the crossbreds, sperm motility and livability in TCAYE were found to be significantly higher compared to the sperm cryopreserved in YACE and SME. Therefore, TCAYE extender apparently was better than YACE followed by SME for the cryopreservation of goat sperm.

Bezerra *et al.* (2011) conducted study on semen dilution in two steps with a Tris–egg yolk extender containing 6% glycerol or 6% DMF, frozen in 0.50 ml straws, and stored in liquid nitrogen. There were differences ($P<0.05$) between glycerol and DMF with regard to subjective progressive motility (23.9±2.2% vs. 16.6±2.0%), objective progressive motility (3.5±0.4% vs. 1.8±0.3%), linearity (53.9±1.6% vs. 48.1±1.4%) and amplitude of lateral head (2.3±0.1 vs. 2.9±0.1 mm), which confirmed the efficiency of glycerol. In conclusion, dimethylformamidemay be used as an alternative cryoprotectant for goat semen freezing. However it was showed that no benefits were derived by using dimethylformamide to replace glycerol at an equal 6% concentration.

Dagli (2011) reported that intact acrosome sperm percentage in Osmanabadi and Sirohi buck semen diluted in Tris egg yolk glycerol dilutor evaluated at prefreezing and post freezing (24 and 72 hours) was 86.89±1.00, 85.00±1.25, 83.61±1.30 and 85.99±1.98, 82.89±0.73, 83.05±1.51 percent, respectively.

Daskin *et al.* (2011) recorded that the post thaw intact acrosome sperm percentage of Angora goat semen using Bioxcell dilutor was 32.00±3.10 percent.

Naing *et al.* (2011) carried out three experiments to improve semen quality during cryopreservation process. Total motility, forward motility, acrosome integrity, live spermatozoa, and normal spermatozoa were measured as semen quality. In Experiment 1, the effects of seminal plasma removal were analyzed by using two different extenders (GE and FE). The removal of seminal plasma gave higher and significant ($P<0.05$) effect in the total motility, forward motility, and live spermatozoa after cryopreservation. For two different extenders, however, the differences were not observed on the semen quality. In Experiment 2, three different washing solutions (namely, phosphate buffered saline, normal saline and Tris-based extender) were tested to evaluate the effects of semen quality after cryopreservation. Tris-based extender (TCG) conferred the highest ($P<0.05$) sperm quality values in the total motility, forward motility, and live spermatozoa after cryopreservation. In Experiment 3, the effects of different centrifugation regimes (3000 × g for 3 min, 1600 × g for 10 min, 800 × g for 15 min) were evaluated on Boer semen quality. Semen quality parameters (namely, total motility, forward motility, acrosome integrity, and live spermatozoa) were significantly ($P<0.05$) higher for cryopreserved spermatozoa centrifuged with 3000 × g for 3 min than the others. In conclusion, the removal of seminal plasma, washing solution TCG, and the use short-term centrifugation with a relative high g-force could contribute to the increased Boer semen quality after cryopreservation.

Priyadharshini *et al.* (2011) noted that intact acrosome sperm percentage pre-freezing and post thawing in Jakharana buck semen preserved using 20 % egg yolk was 91.3±0.69 and 32.5±2.59 percent respectively.

Bras (2012) observed the post thaw intact acrosome sperm percentage of Stud buck semen diluted in Tris egg yolk glycerol extender was 99.4±0.1 percent.

Beltran *et al.* (2013) recorded the post thaw intact acrosome sperm percentage of buck semen using Tris based extender with 5 % Egg yolk was 99.27 percent.

Bezjian *et al.* (2013) determined the reproductive seasonality of four captive Markhor goats (Capra falconeriheptneri), to characterize semen collected by electroejaculation, and to compare extenders and processing techniques for semen cryopreservation. Post thaw evaluation revealed no significant differences between centrifuged and non centrifuged samples. Sperm in Tris 5% and 15% egg yolk displayed higher total motility at 0, 3, and 6 hr post thaw and higher progressive motility post thaw compared with sperm in Bioxcell ($P<0.05$). Sperm in Bioxcell displayed higher viability than spermin both Tris–egg yolk extenders ($P<0.01$), more intact acrosomes than sperminTris–15% egg yolk ($P<0.05$), and a tendency for more intact acrosomes than sperm in Tris–5% egg yolk ($P <0.10$). Sperm in Tris–5% egg yolk showed a higher percentage of morphologically normal sperm compared with Bioxcell ($P < 0.10$). From the study it was observed that Markhor goats exhibit seasonality in scrotal circumference and testosterone levels and that centrifugation may be eliminated from the processing of Markhor semen. The post thaw intact acrosome sperm percentage using Tris egg yolk dilutor was 26.0±7.8 percent.

Sharma *et al.* (2013) assessed seminal plasma transaminases (GOT and GPT) in diluted (Tris-egg yolk-citric acid-fructose glycerol extender 1:4) and thawed (40^0C for 15 seconds) semen of Sirohi bucks (control group). Diluted, cooled (from 25^0C up to 5^0C) and equilibrated (at 5^0C for 2 hours) semen straws were frozen @ 15, 20, 25 and 300C/minute for M1, M2, M3 and M4 groups, respectively. These frozen straws were stored separately in LN2. Effect of freezing rates on leakage of tranferases was studied by assessing GOT and GPT in seminal plasma of thawed semen from M1, M2, M3 and M4 groups. The Mean±S.E. (C.V.%) values of transferases (GOT : GPT) in M1, M2, M3, M4 and control group were 313.00±8.40 (6.58)

:20.5±0.43 (5.12), 272.5±6.68 (6.01) : 16.33±1.02 (15.33), 255.00±10.1 (9.7) : 14.5±0.67 (11.33), 292.0±9.67 (8.09) : 19.67±0.70 (8.99) and 147.67±10.96 (18.17) ; 9.5±0.62 (15.96) units per 0.92 x 109 spermatozoa respectively. Least square analysis of variance revealed highly significant (P<0.01) rise in the seminal plasma GOT enzyme levels in frozen thawed semen as compared to that in fresh diluted, cooled and equilibrated semen. Among the freezing mode group slowest GOT and GPT values in seminal plasma was observed in M3 followed by M2, M4 and M1 groups in increasing order.

Ahmed *et al.* (2014) investigated the effect on buck sperm during different stages of semen preparation including dilution, cooling, equilibration and freeze-thawing. Mean percentage motility after dilution (86.0±1.4%) was reduced significantly (P<0.05) due to cooling and equilibration (77.6±1.3% and 74.6±1.4% respectively); furthermore, it decreased significantly (P<0.05) after freezing and thawing (42.3±2.5%). Mean percentage of live sperm was higher (P<0.05) after dilution (89.3±1.4%) compared with cooling (84.8±1.8%) and equilibration (80.2±2.5%) and further reduced (P<0.05) after freezing and thawing (56.0±3.4%). Sperm morphology dropped significantly (P<0.05) from 96.4±0.3% after dilution to 88.8±1.3% at cooling and further decreased (P<0.05) after freezing and thawing (81±1.9%). Mean percentage of sperm with normal plasma membrane after dilution (82.2±1.1%) was significantly reduced (P<0.05) at cooling or equilibration (73.8±1.8) and further decreased (P<0.05) after freezing and thawing (50.1±2.9%). The percentage of sperm with normal acrosomes did not differ significantly due to dilution, cooling or equilibration (85.8±1.7%, 83.2±1.6%, 81.7±1.8%) but was significantly reduced after freezing and thawing (45.2±2.8%). Intact acrosome sperm percentage of buck semen diluted in Tris Egg yolk glycerol dilutor at prefreezing 5°C and post freezing stage was 83.2±4.8 and 45.2±8.4 percent, respectively. In conclusion, frozen thawed sperm showed maximum damage to motility, morphology, plasma membrane and acrosome integrity following cooling.

Baghel *et al.* (2014) compared the effectiveness of different egg yolk concentration with and without sperm washing on plasma membrane integrity following cryopreservation of the Barbari buck semen. Ejaculates were collected from five fertile Barbari bucks using artificial vagina. Samples were pooled and divided into three fractions. Each fraction was diluted in a tris-citric acid-fructose extender containing 3% egg yolk (group I), 20% egg yolk (group II) and 20% egg yolk after sperm washing (group III) later subjected to cryopreservation. Diluted samples were cooled slowly to 5°C over 2 h and equilibrated at that temperature for 2 h. Aliquots of samples were loaded into 0.25 ml straws and frozen in liquid nitrogen vapour for 15 min and stored in liquid nitrogen. The sperm plasma membrane integrity was evaluated using hypo-osmotic swelling test (HOST) at each step involved during cryopreservation i.e just after dilution, at equilibration and post thaw. The observed mean (±S.E.) values of per cent HOST positive spermatozoa in groups I was 67.70±0.69, 62.10±0.63, 41.30±1.02, group II was 72.20±0.72, 66.50±0.82, 51.87±0.77 while in group III was 70.10±0.75 64.50±0.75, 47.20±0.80. The results indicates that the semen extender with 20% egg yolk without sperm washing was best suited for cryopreservation of buck semen for better fertility.

Buyukleblebici *et al.* (2014) evaluated glycerol (G), ethylene glycol (EG) and dimethylsulfoxide (DMSO) which were used two different doses on in vitro semen parameters, after the freeze-thaw process in Angora goat semen. Each pooled ejaculate was splited into 6 equal aliquots and diluted with tris base extenders supplemented with two different doses of cryoprotectants (G 3%, 6%; EG 3%, 6%; DMSO 3%, 6%). G 3% and 6% was added as a cryoprotectant had better CASA motility ($P<0.01$) and progressive motility ($P<0.001$) values when compared to EG and DMSO groups. On the other hand, EG 6% showed the best values of preserved membrane integrity ($P<0.01$). The evaluation of CASA sperm motions parameters, adverse effects were procured in the groups with DMSO groups when compared to the other

groups (P<0.05; P<0.001). In conclusion, no advantages were found in using EG or DMSO to replace G for freezing of Angora goat sperm.

Ranjan *et al.* (2014) assessed viability and acrosomal integrity individually as well as simultaneously in the same slide using 12 frozen semen samples from adult Jamunapari bucks. The mean per cent live and acrosome intact (A), live and acrosomenon-intact (B), dead and acrosome intact (C) and dead and acrosome non-intact (D) spermatozoain dual staining technique (Eosin-Nigrosine and Giemsa staining) were 49.13±0.59, 1.16±0.08, 1.77±0.04 and 47.94±0.50, respectively. The mean per cent live sperm count, dead sperm count, acrosome intact spermatozoa and acrosome non-intact spermatozoa in dual staining technique were 50.29±0.56 (A+B), 49.71±0.56 (C+D), 50.90±0.70 (A+C) and 49.10±0.70 (B+D), respectively. The corresponding figures in normal staining procedure were 49.27±0.63, 50.73±0.63, 49.99±0.55 and 50.01±0.55, respectively. The results obtained in the study did not differ significantly (P<0.05) between dual and normal staining technique.

Ramachandran *et al.* (2015) evaluated the effect of different equilibration periods on the post thaw seminal traits of buck semen collected at weekly intervals from the adult Jamunapari bucks. The pre-freeze and post-thaw progressive motility at 1, 2, 3 and 4 h of equilibration periods were 83.24±1.05 and 30.59±2.42, 81.76±0.85 and 31.47±1.76, 74.71±1.39and 33.24±2.10, 70.59±1.28 and 28.82±1.99%, respectively. The respective values for live sperm count were 88.44±1.21 and 46.02±2.83, 80.85±5.36 and 41.95±1.56, 81.90±2.06 and 43.57±3.53,80.37±2.32 and 41.01±3.02%. The total abnormal sperm ranged from 0.91 to1.74% before freezing and 2.18 to 2.84% after freezing at different hours of equilibration periods. The pre-freeze progressive motility up to 2 hrs of equilibration period varied significantly (P<0.05) with and 4 hrs of equilibration periods. The remaining characteristics did not show any significant variation between different hours of equilibration periods. Therefore, equilibration period of 3 hrs may be followed in the

freezing protocol used for buck semen cryopreservation as equilibration period of 2 or 3 hrs showed superiority over 1 or 4 hrs in terms of post-thaw spermatozoan characteristics in frozen-thawed Jamunapari buck semen.

Sikarwar *et al.* (2015) used total 196 ejaculates from 8 adult Sirohi bucks to find out the freezability of buck semen at different levels of two cryoprotectants. The ejaculates were extended to maintain sperms concentration approximately 100 million per dose (0.25 ml) containing Tris-Citric acid- Fructose (TCF) with different concentrations of glycerol and DMSO viz. 4%, 6% and 8% (v/v) as a cryo-protecting agent. Filling and sealing of straws were done at 5ºC in cold handing cabinet after 4 hr of equilibration period, then straws were vapor frozen for 10 minutes above 2 cm of liquid nitrogen and finally put in to liquid nitrogen. Post thaw motility, live sperm count, acrosomal integrity was conducted to know freezability. The results showed that the 6% glycerol level significantly improved sperm post thaw progressive motility.

Ustuner *et al.* (2015) reported the post thaw defective acrosome sperm percentage of buck semen in Tris egg yolk dilutor was 51.9±3.7 percent.

Sikarwar *et al.* (2016) studied that 6 % glycerol significantly ($P<0.05$) improved sperm post thaw progressive motility (41.13±0.28), live sperm count (77.70±0.53) and acrosomal integrity (84.50 ± 0.47) and effectively protects the sperm damages during cryopreservation.

Singh *et al.* (2016) assessed the physical and morphological characteristics of fresh, pre-freeze and post-thaw black Bengal buck (*Capra hircus*) semen processed in Tris-egg yolk-citrate-fructose-glycerol (TEYCFG) extender containing 4 different concentrations (2.5, 5, 7.5 and 10%) of egg-yolk. Best values for all the semen parameters were obtained using extender with 2.5% egg-yolk. Of all egg-yolk concentrations, the 10% egg-yolk supplement caused the highest percentage of abnormal sperm, which was

significant (P<0.05), compared to 2.5% and 5% egg-yolk containing extenders, but non-significantly (P>0.05) different compared with 7.5% egg-yolk containing extender. Only progressive motility was significantly different (P<0.05) within extenders containing 2.5%, 5%, 7.5% and 10% egg-yolk. It was concluded that Black Bengal buck semen can be cryopreserved effectively with tris-egg yolk-citrate-fructose-glycerol extender containing 2.5% egg yolk (V/V).

Sundaraman *et al.* (2016) evaluated the structural and functional integrity of spermatozoa cryopreserved buck semen. Individual genotypes have shown significant variations (P<0.01) for PTM. Significant variations (P<0.01) were seen between bucks and between ejaculations of two Tellicherry bucks for hypo osmotic reacted spermatozoa. The differences in mean values for hypo osmotic reacted spermatozoa between I and II ejaculations of Tellicherry bucks were significant (P<0.01). Significant variations (P<0.05) were also observed for hypo osmotic reacted spermatozoa between I ejaculations of Tellicherry and Boer bucks. The variations in means of intact acrosome percent between I and II ejaculations of Tellicherry bucks was significant (P<0.05). Besides post thaw motility, incorporation of structural and functional integrity tests like HOST and acrosome integrity in semen evaluation protocol added value to quality assurance of frozen buck semen.

Anand *et al.* (2017) evaluated the effect of egg yolk concentration and washing on sperm quality in cryopreserved Barbari buck semen at higher dilution (100×10^6 cells/ml). Five healthy Barbar bucks of similar age and weight were selected as semen donor. Six ejaculates were collected from each buck during the experiment. Collected semen samples were pooled and diluted with Tris-based semen extender containing 6% glycerol and egg yolk according to the treatments – T-1, that is, 3%egg yolk, T-2, that is, 20% egg yolk and T-3, that is, 20% egg yolk after washing and

cryopreserved using the slow freezing technique. Semen was evaluated at three different steps, namely, after dilution,after equilibration and after freeze–thawing. Significantly ($p \leq .01$) higher values for motility and viability were observed in T-3 just after dilution while significantly ($p \leq .01$) higher values for acrosomal integrity and pattern F (uncapacitated sperm) was observed in T-2. Significantly ($p \leq .01$) higher values were observed in T-2 after equilibration and thawing. It may be concluded that incorporation of 20% egg yolk compared to 3% egg yolk or 20% egg yolk after washing in extender confer better cryoprotection to Barbari buck spermatozoa as reflected in semen quality parameters studied.

Daramola (2017) removed seminal plasma from semen by centrifugation (0 centrifugation, 1 centrifugation, 2 centrifugations, 3 centrifugations) and preservation in two different tris extenders viz., avocado seed milk (ASM) and soy bean milk (SBM) based extenders for their ability to support motility, in vitro capacitation and acrosome reaction of spermatozoa obtained from West African Dwarf (WAD) goat bucks during cryopreservation. Semen samples collected with the aid of artificial vagina were centrifuged for one, two and three times. The centrifuged samples were diluted with the two tris extenders each containing 20 ml of avocado seed milk and soybean milk and cryopreserved for 30 days. The results showed higher ($P<0.05$) sperm motility ($P<0.05$) with increased centrifugation times. Spermatozoa that were centrifuged had higher ($P<0.05$) percentage of acrosome reaction and capacitation with increased centrifugation times compared to the control. Optimal improvement in parameters was obtained with increased centrifugation times. The findings revealed that removal of seminal plasma by centrifugation improved sperm quality of WAD goat bucks during cryopreservation and optimum improvement was achieved consistently with 3 centrifugations.

Jha *et al.* (2018) examined the seminal attributes, effectiveness of cooling process and post thawed semen quality of a Nepalese Indigenous Khari bucks. The mean semen volume, semen color, sperm mass activity, sperm motility, sperm viability, sperm concentration, abnormal acrosome, mid piece and tail, and abnormal head were 0.73 ml, 3.10, 3.80, 80.9%, 94.6%, 2597.0×10^6 ml-1, 10.7% and 5.00%, respectively. Further, 12 ejaculates (6 ejaculates) from each buck (only having ≥0.5ml semen volume, ≥80% sperm motility, ≥90% sperm viability and ≥2500×106 ml-1 sperm concentration) were considered for cooling, freezing and post thawed semen quality. Tris-egg-yolk citrate extender was used for semen dilution. The mean sperm motility and viability of post diluted semen after 90 minutes of cooling were 73.8% and 88.1%, respectively Similarly, the mean sperm motility and viability of post diluted semen after 210 minutes of cooling were 69.2% and 85.0%, respectively. The mean post thaw sperm motility and viability were 49.0% and 81.2%, respectively. It was concluded that the seminal attributes and results of post thaw semen quality are acceptable and indicated the feasibility of cryopreserving Khari buck semen.

2.5 Synchronization of Estrus

Greyling *et al.* (1983) divided 32 cycling Boer goats into 4 treatment groups (n=8). The sponges containing medroxy-progesterone acetate 60 mg were inserted intra-vaginally for 12 ,14, 16, and 18 days in group 1, 2, 3 and 4, respectively. All the does were injected subcutaneously with eCG @ 300 IU after sponge withdrawal. All the does were tested at 8 hourly intervals for estrus response with the aid of vasectomized bucks. The number of does exhibiting estrus were 7 (87.50%), 8 (100%), 7 (87.5%) and 5 (62.5%) in 1^{st}, 2^{nd}, 3^{rd} and 4^{th} group, respectively. The average interval from cessation of treatment to onset of estrus was 63±19, 81±17, 74±18 and 52±15 hrs in group 1 to 4, respectively. Number of follicles more than 0.2 cm at the time of ovulation was 5.7±1.4, 5.1±2.0, 4.9±1.7 and 5.8±6 in group 1 to 4,

respectively. The interval from sponge withdrawal to estrus was significantly longer in group 2, 14-day treatment B ($P<0.01$) and in group 3, 16 day ($P<0.5$) treatment when compared to 18 day in 4th group. Ovulation occurred in all treatment groups on an average 31.0 hrs after onset of estrus. Further it was reported that no significant difference was observed in estrus response, follicular activity, ovulation rate, and time of ovulation between the different periods of intravaginalprogestagen treatment.

Menegatos *et al.* (1995) induced synchronization in six Saanen goats during the breeding season by insertion of intravaginal sponges (60 mg medroxyprogesterone acetate) for 17 days followed by intramuscular injection of 500 IU PMSG, 2 days before sponge withdrawal. Does were inseminated intracervically using fresh semen at 24 and 48 hrs after sponge removal. Goats were checked for estrus, twice daily by using teaser bucks from the day of sponge removal until 60 hrs later and progestrone was estimated from day 0 (day of sponge insertion) until day 25 after sponge removal. All the six goats (100%) exhibited estrus between 36 and 48 hrs after sponge removal.

Freitas *et al.* (1996) conducted experiment during anestrus season on Saanen (n=53) dairy goats. The goats were allocated to 1 of 3 treatments. Group I, received a vaginal sponge impregnated with 45 mg of fluorogestone acetate (FGA) on day 0, Group II, received sponge on day 0 plus a second sponge on day 7, Group III, received a sponge on day 0 plus a second sponge on day 9. The sponges were removed on day 11. All goats received 400 or 500 IU eCG and 50 µg $PGF_2\alpha$ analogue 48hrs prior to sponge removal and inseminated with frozen thawed semen 24hrs after the onset of estrus. The percentage of goats in estrus within 60 hrs after sponge removal were 100, 100 and 94.7 per cent while interval from sponge removal to onset of estrus was 29.2±6.8, 29.9±8.4 and 35.1±10.6 hrs, respectively in group 1 to 3. The kidding rate was 58.8, 52.9 and 52.6 per cent in group 1, 2 and 3, respectively.

Romano (1996) synchronized forty eight Nubian does either by using fluorogestone acetate (FGA-30 mg) or medroxyprogesterone acetate (MAP, 60 mg) intravaginalpessaries which were left in the vagina for 12 or 14 days. All the does were lactating and between 2-5 years old, natural prostaglandin $F_2\alpha$ (lutalyse) 5 mg was injected to all does intramuscularly at the time of pessary removal. Estrus was detected once a day during pessary treatment and at 6 to 4 hrs interval (at 6.00, 12.00, 18.00 and 24.00 hrs) during the 5 days after pessary removal with the help of vasectomised buck. Each doe was inseminated with refrigerated semen twice at 12 and 24 hrs after estrus detection. Estrus was initiated at 53.0±14.9 and 41.5±8.1 hrs after sponge removal. Estrus duration was 30±7.9, 35.0±14.3 hrs in MAP and FGA treatment groups respectively. Out of 24 goats, 16 (66.7%) and 18 (75%) were conceived in MAP and FGA treatment groups, respectively.

Gardon and Simonetti (1997) synchronized estrus in thirteen cyclic goats withintravaginallyMAP-impregnated pessaries. After 14 days treatment, sponges were removed. Forty eight hours post-withdrawal, goats which exhibited estrus were artificially inseminated. The percentage of estrus synchronization was 92.31% and the pregnancy rate was 69.23%.

Wildeus (2000) reported that estrus synchronization in goats and sheep is achieved by control of luteal phase of estrous cycle, either by providing exogenous progesterone or by inducing premature luteolysis. It was also pointed that the latter approach is not applicable during seasonal anestrus, whereas exogenous progesterone in combination with gonadoropin can be used to induce and synchronize estrus in anovularatory does and ewes. The traditional product of choice for estrus synchronization in goats and sheep was the intravaginal sponges impregnated with progestagens. (e.g. flurogestone acetate or methylacetoxyprogestrone) for 9 to 19 d followed by eCG injected 48 to 0 hrs from sponge removal. The alternative choices of progesterone/ progestagen have been controlled internal drug release (CIDR) devices, supplying natural progesterone, norgestomet implants and orally active

melengestrol acetate. Other products used alone or in conjunction with progestagens are $PGF_2\alpha$or an analogue (Cloprostenol), a combination of eCG/hCG and zeranol.

Leboeuf *et al.* (2003) synchronized the Alpine and Saane goat by using Flurogestone acetate (20 mg) during 12 days + eCG (500 IU) + cloprostenol (50 µg) (i.m., 2 days before sponges withdrawal); TAI : 43±1 hrs and observed 100% estrus. He recorded 69.1% (67/97) pregnancy rate.

Pierson *et al.* (2003) conducted experiment to study the effect of exogenous gonadotropin releasing hormone (GnRH) on the synchrony of estrus, the LH surge, ovulation following estrus synchronization in Dwarf goats, and to assess the effect of season on the these parameters. In January and June, estrus was synchronized in 12 Pigmy and Nigerian Dwarf goats by means of intra-vaginal sponges (Veramix) impregnated with 60 mg MAP inserted for 10 days, coupled with 125 µg cloprostenol (Estrumate) i. m. 48 hrs before sponge removal and 300 I.U. eCG (Equinex) at the time of sponge removal. Six of the 12 goats were also given 10 µg GnRH (Factrel) i.m. 24 hrs after sponge removal. Onset of estrus was monitored by using two males. The effect of administration of 10 µg GnRH and the season on the onset of estrus following the sponge removal for the goats in which estrus was synchronized in January and June were 25.8±1.22 and 39.3±3.76 hrs respectively. Further it was observed that the administration of GnRH had no significant effect on onset of estrus; however, it reduced the interval from sponge removal to the LH surge and improved the synchrony of the LH surge ($P<0.05$).

Dogan *et al.* (2004) treated forty three lactating Saanen does for 11 days with 60 mg MAP (n=19) or 40 mg FGA (n=24) sponges. All does received intramuscular injections of 750 IU pregnant mare serum gonadotrophin (PMSG) and cloprostenol 125 µg ($PGF_2\alpha$) 48 hrs prior to sponge removal. Cervical artificial insemination (AI) with fresh diluted semen was performed at a fixed time (36 and 48 hrs) after progestagen withdrawal.

The two different progestagen treatments showed no significant difference in estrus response (100% for both MAP and FGA groups), mean time interval to the onset of estrus was 15.8±0.9 and 15.0±0.6 hrs duration of estrus 30.5±1.9 and 34.0±1.4 hrs and cessation of estrus 42.32±1.6 and 43.25±1.3 hrs for MAP and FGA treatment protocols respectively. The pregnancy rates were 52.63 and 50.0% observed in MAP and FGA with A.I, respectively. However, the difference was non-significant.

Whitley and Jackson (2004) studied ten-day MAP (60 mg) treatment during the breeding season in West African Dwarf goats in combination with gonadotropin and PG or an additional GnRH treatment (24 hrs after sponge removal) for estrus synchronization. The GnRH co-treatment being more successful in inducing estrus by 74 hrs after sponge removal. Out of six does which were treated with GnRH, five does exhibited estrus and in untreated does only three does exhibited estrus within 72 hrs.

Ataman *et al.* (2006) performed the experiment at two periods, during the breeding and the anestrus seasons, on two different groups of 30 ewes. During each period, the ewes were randomly allocated to two groups of 15 ewes, which received a short term (7 days) or a long term (12 days) progesterone treatment, respectively. The progesterone treatment consisted of a vaginal sponge containing 30 mg fluorogestone acetate (FGA) inserted into the vagina of the ewes for 7 or 12 days. Triaprosttromethamine, an analogue of $PGF_2\alpha$, was intramuscularly administered to all ewes at the moment of the sponge withdrawal. Afterwards, 400 IU of PMSG was intramuscularly administered to all the ewes. The percentage of estrus, pregnant and lambing sheep was 100%, 86.7 % and 80% in both the short term and the long term treated groups, during the breeding season. The mean litter size did not differ between the short and the long term treated groups (1.8 *vs*1.7). During the anestrus season, the percentage of estrus, pregnant, lambing sheep and mean litter size were 86.6%, 76.9%, 61.5% and 1.5 in the long term treated group and 93.3%, 85.7%, 71.4% and 1.5 in the short term treated group, respectively.

Menchaca and Rubianes (2007) synchronized the Alpine, Saanen and Anglo Nubian lactating goats by using Medroxi progesteronaceatate (60 mg) during 5-6 days + eCG (250 IU) + Delprostenate (160 µg) (i.m., 2 days before sponges withdrawal); TAI : 54 hrs and observed 91.7% (154/168) estrus. He recorded 63.7% (107/168) pregnancy rate.

Azawii and Almola (2010) investigated the effect of GnRH administration on super-ovulatory response in Awassi ewes in two groups (n= 6). Each ewe was treated with progesterone impregnated intra-vaginal sponge for 12 days. Each of group received eCG @ 1200 IU i. m. once as intramuscular injection 48 hrs prior to sponge withdrawal and after 12 hrs of sponge removal ewes were injected with GnRH @ 8 µg. Ewes standing to be mounted were recorded as in estrus. It was observed that ewes were treated in breeding season the number of corpora lutea were significantly higher ($P<0.05$) in eCG ± GnRH (7.33 ± 0.54) than eCG alone (4.33±0.39). However, they observed there was no significant difference in the number of corpora lutea in non-breeding season when ewes treated either with eCG alone or eCG+GnRH. Number of embryos recovered from ewes treated with eCG + GnRH and eCG differ significantly ($P<0.05$) as 4.32±0.56 and 2.66±0.66, respectively, in breeding season. While no significant difference was observed when these hormones used for superovulation in the non-breeding season. Further it was concluded that administration of GnRH 24 hrs after sponge removal increased ovulation rate in Awassi ewes treated with eCG for superovulation in breeding season.

Widayati (2010) synchronized estrus in fifteen Etawah crossbred does of 2 to 3 years age with normal estrus cycle and given at least once birth. Synchronization was conducted by implanting intravaginal CIDR for 10 days while dose of $PGF_2\alpha$ 125 µg was given on day 8. Estrus was detected every 4 hrs till 60 hrs after CIDR removal. All the does were intravaginally inseminated with frozen semen at 24 hrs after the onset of estrus. The estrus response was 100 per cent and no pessaries were lost during

this research. The initiation of estrus following CIDR removal was 26.59±0.98 hrs, duration of estrus was 35.6±2.6 hrs and pregnancy rate was 73.33 per cent.

Amle (2011) conducted experiment of estrus synchronization in Sangamneri and Osmanabadi goats by using different types of hormonal protocols and reported that estrus response was 100% for three treatment protocols in Sangamneri and Osmanabadi goats subjected to estrus synchronization using progestagen-impregnated intra-vaginal sponges + eCG on day of sponge removal, progestagen-impregnated intra-vaginal sponges + $PGF_2\alpha$ + eCG on day of sponge removal, progestagen-impregnated intra-vaginal sponges + GnRH 24 hrs before sponge removal and progestagen-impregnated intra-vaginal sponges + GnRH on day of sponge removal. These four types of estrus synchronization protocols used in these trials were equally efficient and resulted in synchrony of estrus in goat of both breeds. The longer interval to onset of estrus in both breeds of goats when exposed to the treatment of progestagen-impregnated intra-vaginal sponges + GnRH, 24 hrs before sponge removal and progestagen-impregnated intra-vaginal sponges + GnRH, on the day of sponge removal was observed the higher conception rate was recorded in Sangamneri goats (88.89%) when exposed to synchronization treatment of progestagen-impregnated intra-vaginal sponges + GnRH 24 hrs before sponge removal followed by conception (72.73%) in goats provided with progestagen-impregnated intra-vaginal sponge + eCG administered on the day of sponge removal and (72.73%) conception in progestagen impregnated intra-vaginal sponges + GnRH administered on the day of sponge removal in Osmanabadi goats. Amongst treatment groups, highest conception (100%) was recorded in goats treated with synchronization treatment of progestagen + GnRH administered on day of sponge removal, followed by conception (71.43%) in goats provided with progestagen + GnRH administered 24 hrs before sponge removal. The use of GnRH with shorter

progestagen treatment (intra-vaginal sponges) had a positive effect on the fertility parameters viz. conception rate in both breeds of goats.

Martemucci and Alessandro (2011) synchronized the Indigenous dairy goats by using Fluorogestone acetate (45 mg) + during 5 days + eCG (300 IU) + cloprostenol (50 µg) (i.m., at sponges withdrawal); Natural mating and observed 78.3 % (18/ 23) estrus. He recorded 60.9% (14/23) pregnancy rate.

Mehmood *et al.* (2011) introduced artificially inseminated (A.I) goats at farmer level with chilled semen in Pakistan. The estrus was synchronized in does (n=18) with progesterone impregnated vaginal sponges (60 mg Medroxyprogesterone acetate MAP) for 11 days. At 48 hrs prior to removal of the sponges, intramuscular injection of 400 IU equine chorionic gonadotropin (eCG) and cloprostenol (0.075 mg) was given. Fixed time vaginal insemination (43-45 hrs after sponge removal) was done twice (at 12 hrs interval) in 17 does with chilled Beetal buck semen (4°C) extended with Tris citric acid (TCA) or skim milk (SM) based extender (75 x 10^6 sperm/ml). Pregnancy test was performed at 45 days post insemination through ultrasonography. An overall 94.5% (17/18) of does showed heat signs and 78% of them were detected in heat between 12 - 24 hrs after sponge removal. An overall 29.4% (5/17) pregnancy rate was recorded. Higher pregnancy rate (44.4%) was obtained in does inseminated with SM extended semen as compared to 12.5% for TCA extended semen. It was indicated that feasibility of using synchronization and fixed time AI during low breeding season to enhance the reproductive efficiency in local goats.

Pawashe (2012) carried out the study on estrus synchronization in goats using intravaginal progestagen sponges supplied by CIRG. The does were randomly divided into two groups (n=12). In group - I selected does were inserted with intravaginal progesterone sponges (CIRG) and injection CIRG 1 ml was administered at the day (D=0) of sponges insertion. On 10^{th} day sponges were removed and injection cloprostenol sodium 263 µg was

administered intramuscularly to experimental goats. Similar of group – I, group – II does were inserted with CIRG intravaginalprogestogen sponges (CIRG) and injection CIRG 1 ml was administered I.M on day zero (D=0). Further on 9^{th} day injection eCG 400 I.U. was administered and on 10^{th} day sponges were removed and injection cloprostenol sodium 263 µg intramuscularly administered. In both the groups intravaginal sponges were retained till the sponges were removed i.e. 100% retention observed. From both groups all the does exhibited the estrus synchronization 100%, the average time interval for onset of estrus from sponge removal in synchronized goats was 20.91 ± 0.27 and 17.41 ± 1.31 hrs in group – I and II, respectively. The difference was statistically significant ($P<0.05$). The pregnancy rate was 33.33 and 25.00 per cent in group – I and II in natural mating, respectively.

Bagwan (2014) studied efficacy of intravaginal sponges for estrus synchronization to assess the conception in synchronized goats as well as to detect the early pregnancy through transrectal and transabdominal ultrasonography in goats. The selected 21 goats (group A, B and C) were synchronized estrus by using progesterone containing sponges supplied by CIRG, Makhdoom, Mathura. In Group: A, CIRG sponges were kept for 10 days and injection PG on day 9 and bred during the estrus period. Group: B similar protocols to group A was followed, however injection GnRH was given on day of removal of sponges. Group: C was kept control. Retention of intravaginal was 100% in group A and B without any signs of infection after removal of sponges and onset of estrus was shortest in Group B (35.14 to 2.42 hrs) as compared to Group A (48.85 to 2.42 hrs) and group C (49.71 to 1.10) and found significantly different among the groups ($P<0.01$). All does in group B exhibited estrus at earlier hours as compared to Group A and C.

Zarazaga *et al.* (2014) synchronized the 32 Blanca Andaluza goats by using Fluorogestone acetate (20 mg) during 11 days + eCG (450 IU) + luprostiol (6 mg) (i.m., 2 days before sponges withdrawal) and observed 92 % estrus.

Dogan *et al.* (2016) evaluated the duration of four progestagen treatments on estrus synchronization and pregnancy rate in non-lactating Anatolian black goats during the natural breeding season. All does were divided into four groups according to progestagen treatment duration using intravaginal sponges (60 mg MAP): group 1, 13 days (n = 23), group 2, 11 days (n = 25), group 3, 9 days (n = 25) and group 4, 6 days (n = 25). In addition, 24 h before sponges removal, each doe was injected with 0.075 mg of cloprostenol (PGF2α) and 500 IU eCG. The goats within the same group were naturally mated at fixed time 40 h following progestagen removal, using the same breed fertile bucks (1:5 mating ratio). The total estrus response for the first 12 h, total estrus response within 60 h, time to onset of the induced estrus, duration of the induced estrus and pregnancy rate were 3.0, 96.9%, 26.5 ± 0.7 h, 22.6 ± 0.8 h and 92.0%, respectively.

2.6 Conception Rate

Ritar and Salamon (1983) reported that the mean rate of kidding for double insemination with frozen-thawed buck semen was 53.3 percent.

Restall *et al.* (1987) reported 43.2 percent conception rate using frozen semen of the Anglo-Nubian goat.

Restall *et al.* (1988) inserminated The native goats with fresh native breed semen (cervical insemination) or frozen Anglo-Nubian semen (intra-uterine insemination) at natural estrus during a 25 day period, with or without a prior 21 day contact with bucks. With fresh native breed semen fertility (82.1%) and kidding percentage (132%) to a single insemination were high, but were much reduced for frozen Anglo-Nubian semen (43.2% and 64%).

Chauhan and Anand (1990) obtained 81.00 percent fertility rate in trial conducted with frozen semen from Jamnapari bucks.

Sinha *et al.* (1995) reported 50 to 60.20 percent fertility rate with Tris based extender semen in Black Bengal, Beetal and their cross breed goats.

Greyling and Nest (2000) recorded 74.2 percent pregnancy rate following Al in Boar and Indigenous goats.

Leboeuf *et al.* (2004) recorded 71 percent average kidding rate using milk and native P and B extender using semen preserved at refrigeration temperature 4°C for 4 hours.He evaluated the extender composition, presence or absence of seminal plasma and dilution rate of spermatozoa for the preservation of fresh semen during three days before artificial insemination (AI). The percentage of kidding was influenced by the duration of storage: 4 h = 71%; 28 h = 61%; 52 h = 39%; 76 h = 28%. The fertility rate decreased in both extenders tested (milk or NPPC) when the storage duration increased. Evidence was given that the removal of seminal plasma by centrifugation at collection does not improve the fertility rate. Using the same number of total spermatozoa per straw (100 x 10^6), but with a variable inseminated volume of 0.20 ml or 0.40 ml per straw, AI results showed that the reduction of the concentration of spermatozoa in the AI dose did not improved the fertility rate after AI, whatever the storage duration.

Salvador *et al.* (2005) studied the effect of factors such as depth of semen deposition, inseminator skill, farm, sire and expression of estrus on pregnancy rate in Murciano–Granadina (MG) goats during non-breeding season and using frozen semen. Overall pregnancy rate was 57%. Farm and depth of semen deposition affected pregnancy rate, whereas the sire and the technician had no effect. The deeper the semen was deposited in the genital tract, the higher was the rate of pregnancy obtained, being greater when the catheter reached the uterus. In spite of the relevant difference observed (48.2%vs 59.0%), pregnancy rate of females not coming into estrus until 30 h after sponge removal was not significantly different, compared with those

showing estrus during the OD procedure. AI in MG goat with frozen-thawed semen showed that post-cervical insemination presented significantly greater pregnancy rate in comparison to when semen is deposited in the vagina or in the caudal part of the cervix.

Dogan *et al.* (2008) investigated the efficiency of cronolone sponges in combination with either pregnant mare serum gonadotrophin (PMSG) or cloprostenol (PGF2α) for inducing and synchronizing the estrus cycle in Turkish Saanen does during the transition from non-breeding to breeding season. All does (n=80) were treated with 20 mg cronolone sponges for 11 days and divided into 4 equal groups. In addition, each doe received an intramuscular injection of either 1.5 ml sterile saline solution, 0.075 mg PGF2α, 500 IU PMSG or 500 IU PMSG and 0.075 mg PGF2α, 24 h before the sponge removal. Cervical artificial insemination (AI) with frozen-thawed semen was performed once 16 h after the detection of the first accepted mount. The total estrus response for the first 24±4 h, total estrus response within 96 h, time to onset of the induced estrus, duration of the induced estrus and pregnancy rate was found to be 75.0%, 97.5%, 31.4±1.2 h, 29.3±1.2 h, and 33.3%, respectively. There were significant differences between the first two groups and the last two groups in terms of the onset of induced estrus and estrus response at the first 24±4 h ($P<0.05$). These results indicate that the use of cronolone/PMSG was more effective than cronolone/PGF2α in the attainment of early and compact induction of estrus in Turkish Saanen does.

Mara *et al.* (2007) recoded that at day 50 from AI the percentages of pregnant goats were 71.4 % (30/42) with skim milk, 61.4 % (27/44) with TEMPOL and 48.8 % (22/45) with TEMPOL +HA, with significant differences between skim milk and TEMPOL + HA.

Pervage *et al.* (2009) collected semen from ram using artificial vagina and was stored in a refrigerator (4^0C) for three days. The volume of semen was extended with egg yolk citrate diluter. A total of 63 ewes were

inseminated with stored liquid semen collected from 15 rams by AV method. The total number of spermatozoa, live-dead, normal-abnormal, sperm motility and the pH was observed regularly. The average semen volume per ejaculate was 0.76-1.00ml and the sperm concentration was $2.37x10^9$ – $4.30x10^9$ per ejaculate. The number of normal spermatozoa and the pH was almost similar irrespective of days of storage. Number of live spermatozoa and the sperm motility were reduced with the increasing age of semen. The average conception rate (%) was obtained as 63.61, 61.90, 52.38 and 47.61 with sperm in zero, 1st, 2nd and 3rd day storage, respectively.

Ashmawy *et al.* (2010) reported 50.0, 42.10 and 31.57 percent conception rate using 50 % Damascus and 50 % Baladi cross bred bucks pelleted frozen semen.

Dagli (2011) reported that conception rate obtained by using Osmanabadi and Sirohi buck semen diluted in Tris Egg yolk glycerol dilutor and preserved at ultra low temperature was 33.33 and 30.55 percent, respectively.

Arrebola *et al.* (2012) synchronised 3,941 goats using intravaginal progesterone sponges and inseminated 46.0±0.5 h. after sponge removal. Pregnancy was diagnosed by ultrasonography 42-46 days after AI, obtaining an average pregnancy rate of 48.7%.

Bhattacharyya (2012) determined fertility of local goats of Kashmir using frozen pellet semen of Boer bucks during breeding season. Overall pregnancy rate (PR) found was 71.43% with kidding rate of 1.27.

Mohammed *et al.* (2012) designed experiment to study the effect of bucks breeds, semen extenders and freezing regimens on post-thaw semen motility and viability index. The effect of semen diluents used for AI on the reproductive performance; the fertility rate were 45.21% (33/73), 34.40% (215/625) and 57.76% (134/232) for does that had been inseminated with Tris, sodium citrate and milk diluted semen, respectively.

Ajala *et al.* (2012) artificially inseminated West African Dwarf Goats using an extender containing 10% coconut milk, 10% egg yolk and 80% tri sodium citrate dehydrate. Buck semen was safely extended in 10% coconut milk-egg yolk – citrate extender and used fresh for artificial insemination with approximately 100% conception rate and kidding rate.

Arrebola *et al.* (2013) determined the effect of different factors on pregnancy rate after artificial insemination (AI) with refrigerated and frozen semen in Payoya goats. The factors like year, farm, month of AI, age of the goat at AI, dose of progestagen, eCG dose, buck, type of semen (refrigerated or frozen), technician, number of goats to be inseminated (group size), incidences at AI (problems, no problems, trans-cervical insemination), distance from semen collection to farm, number of previous kidding, kidding–AIinterval, and milk production were considered. Mean fertility was 59%. Six (farm, age, group size, transcervical AI, number of kidding and milk production) of the 15 factors studied presented a significant ($P<0.05$) effect on fertility after AI. It was concluded that it is difficult to control all the critical aspects of AI programs at the farm level.

Beltran *et al.* (2013) achieved 70.50 percent conception rate using frozen-thawed buck semen using Tris citric acid fructose with 5 percent egg yolk.

Kharche *et al.* (2013) observed fertility following AI using frozen semen of Jamunapari bucks. Out of 32 Jamunapari goats 17 become pregnant with a pregnancy rate of 53.12%. The pregnancy rate based on actual kidding was recorded to be 44.44% in goats restrained manually for insemination (group 1) whereas it was non significantly higher (64.28%) in goats restrained using specially designed AI crate.

Yotov (2015) evaluated the effect of semen extender with soybean lecithin and/or low concentration of glycerol on the quality of goat chilled-stored semen. Group I (n=6) was inseminated with 0.5 ml (400×106

sperm/ml) chilled-stored semen diluted with Ext. 1 and group II (n=6) received the same dose, but diluted with Ext.2. On day 20 after artificial insemination, the pregnancy rate in the first and the second groups was 83.3%s and 50%, respectively.

Hashemi and Safdarian (2017) recorded 84.2 % fertility rate by using CIDR for 16 days without administration of eCG for estrus synchronization in fixed-time artificial insemination programs in Persian downy does.

CHAPTER III

MATERIALS AND METHODS

The present study on the "Seminal attributes, preservation and fertilizing ability of refrigerated and cryopreserved semen of Berari bucks" was carried out at the Department of Animal Reproduction, Gynaecology and Obstetrics, PGIVAS Akola. Ten Berari bucks were selected and trained for three months to donate the semen. Out of ten trained bucks, six well trained bucks were used for the present study.The study was undertaken from November 2016 to March 2018.

3.1 Selection of Bucks and Management

For the present research work six (6 Berari bucks) mature, healthy bucks of one year age were selected. The animals were fed pelleted feed @ 500 g/animal/day having DCP 13% and TDN 69%. The animals were fed 1 kg green fodder/animal /day besides 5-7 hours of grazing. A fixed schedule of watering and feeding was practiced during experimentation. The does were housed in temporary shed and the flooring was kept dry. The does were sent out for grazing daily from 9 am to 4 pm. Drinking water was available *ad-libitum.*

3.2 Semen Collection

3.2.1 Training of bucks

Semen was collected from well trained bucks (n=6) at weekly interval, for twelve weeks duration. Semen was collected aseptically and hygienically, by Artificial Vagina (8 Inch AV, 12 Inch Liner and 6 Inch Cone) method and it was maintained at 37^0C in water bath for further evaluation. Three collections from each buck were obtained for analysis.

3.2.2 Sterilization of articles

All the glass articles used during collection, evaluation and preservation of semen were sterilized in hot air oven at 180° C for 60 minutes. All the rubber articles including artificial vagina and buffer solutions were autoclaved at 115.6°C temperature and 15 lb. pressure for 10 minutes. The sterilized articles were kept in an air-tight instrument cabinet and buffer solutions were transferred to a refrigerator till use.

3.2.3 Semen collection by AV method

Semen was collected aseptically and hygienically, by Artificial Vagina (AV) method. AV was filled with warm water for providing an internal temperature of approximately 37˚C and air was insufflated with pressure bulb to create appropriate pressure. Collection cup was fitted to artificial vagina and internal later liner was lubricated with liquid paraffin.The dummy was restrined and the buck was allowed to serve the artificial vagina after two false movements. The penis of buck was directed towords A.V. by grasping the sheath of propuce with gloved hands. After ejaculation, the A.V. was turned up and semen in collection up was shifted immediately to laboratory.Ejaculates obtained were used for further evaluation. Semen was collected from each buck at weekly interval, in morning hours.

The study of refrigerated semen and cryopreserved semen was conducted for 3 months each, thus 12 ejaculates were collected from each buck. Hence from six selected Berari bucks 72 semen samples were collected for refrigerated and cryopreserved group each. For both the groups total 144 semen samples were analysed. (Plate 4)

3.3 Evaluation of Semen

Semen was evaluated by using various tests immediately after semen collection, macroscopic (ejaculate volume, colour, consistency, density and pH and microscopic (mass motility, initial motility, total sperm concentration, percentage of live spermatozoa, abnormal sperm percentage) and sperm function tests (Hypo-osmotic swelling test – HOST, Acrosome intactness test, Enzyme leakage) were carried out for evaluation of it's quality.

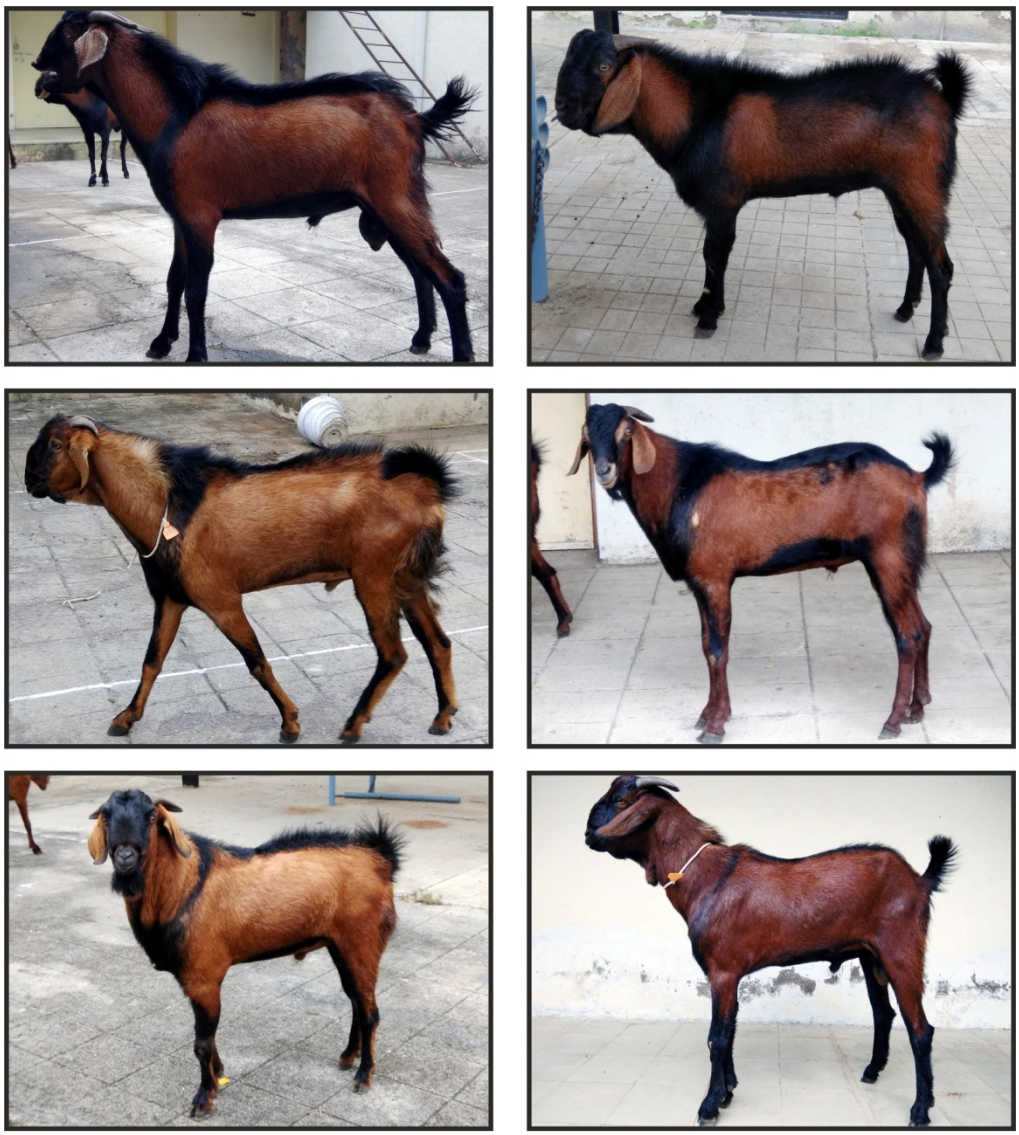

Plate 3.1. Berari bucks used in research

Plate 3.2. Measurement of scrotal circumference

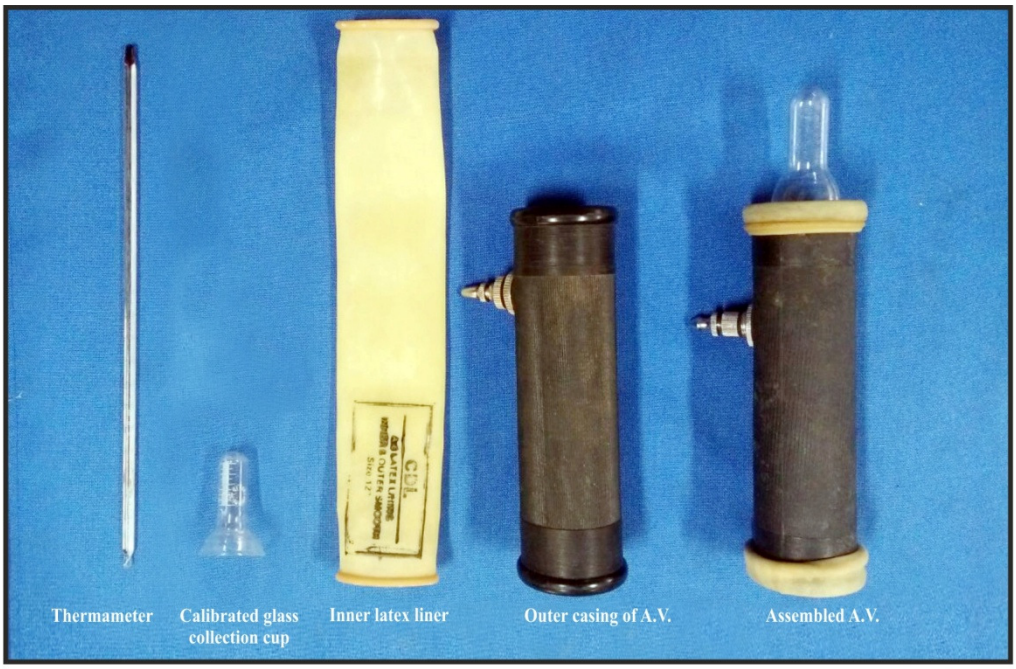

Plate 3.3. Different equipments for A.V. preparation and assembled for A.V.

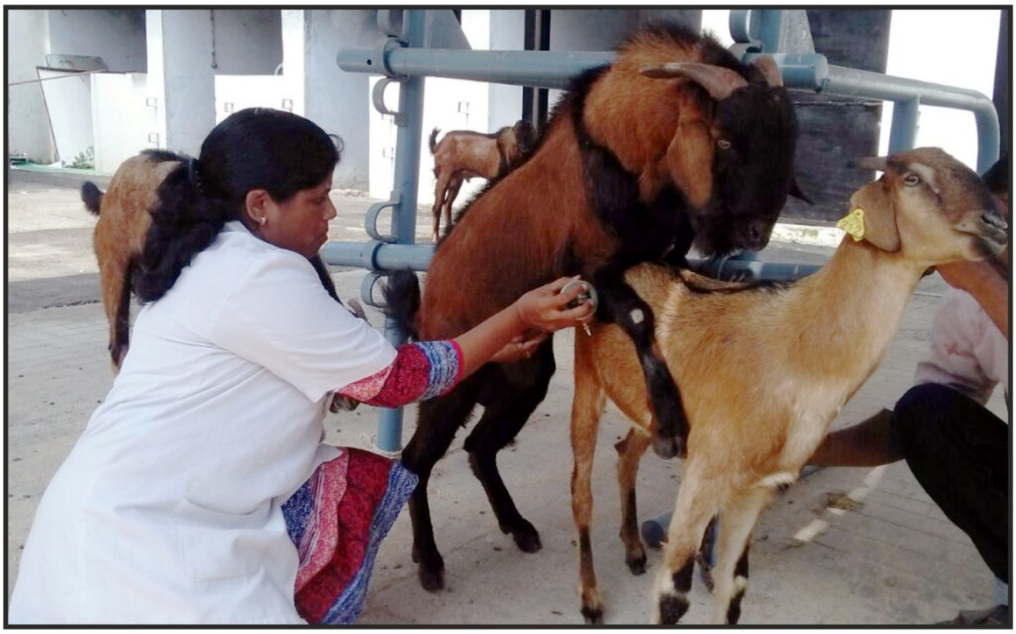

Plate 3.4. Semen collection by artificial vagina method from Berari buck

3.3.1 Macroscopic Evaluation of Semen

a) Ejaculate volume

It was recorded in millilitre and measured directly from calibrated collection cups of 5ml capacity having 0.1 ml calibration.

b) Colour

The colour score for each ejaculate was recorded as creamy, milky, cloudy and watery,respectively.

c) Consistency

The consistency score for each ejaculate was recorded as thick, medium and thin consistency, respectively.

3.3.2 Microscopic evaluation of semen

In microscopic evaluation the semen parameters such as mass motility, initial motility, total sperm concentration, percentage of live spermatozoa, abnormal sperm percentage were analysed.

a) Mass motility

Mass motility of semen was recorded by placing a small drop of freshly collected semen on a pre-warmed clean glass slide, examined without cover slip under the low power magnification (10X). Depending upon the intensity of wave formation score was assigned to each sample in between 0 to 5 and it was graded as +,++,+++ (Salisbury and Van Demark,1978). Semen ejaculates with mass activity greater than (+3) were processed further.

b) Initial motility

Initial motility of semen was measured by covering the semen drop with a thin cover glass ensuring that no air bubbles was formed and was observed under phase contrast microscope having high power magnification (40X), maintained at 35-37^{0}C. The motility of sperm was graded as percentage of progressively motile spermatozoa from 0 to 100. Observations on semen movement as progressive, oscillatory, reverse was noted.

c) Total sperm concentration

The concentration of the sperm cells was counted by using a haemocytometer (Neubar's chamber). The semen samples were diluted 1000 times with 2 % Na saline solution (first dilution, 0.1 ml neat semen + 0.9 ml saline solution; second dilution, 0.1 ml first diluted semen + 0.9 ml saline solution; third dilution, 0.1 ml second diluted semen + 0.9 ml saline solution). A pinch of eosin was added to the saline solution for easy countability of the sperm cells. A haemocytometer cover slip was placed over the ruled field of the Neubar's chamber and 10µl diluted semen was allowed to run under the cover slip without floating. It was then allowed to settle for one minute. The spermatozoa in the four corner squares were counted under 40x magnification of a phase contrast microscope.

The sperm concentrations were calculated as follows:

Area of one primary square	= 1mm X 1mm X1mm= 1 sq.mm
Volume of one primary square	= 1mmX1mm X0.1mm= 1 cu.mm
Volume of five secondary square	= (0.1/25) x 5=1/50 cu.mm
Number of sperms counted in 1/50 cu.mm	= n
Number of sperms counted in 1 cu, mm	= n x 50
Dilution rate	= 1 : 1000
Therefore, 1 cu.mm of neat semen	= n x 50 x 1000
1 cu.cm of neat semen	= n x 50 x 1000 x 1000
Sperm concentration in the semen sample	= n x 50 million sperms / ml

d) Percentage of live spermatozoa

The eosin-nigrosin statin was used to detect the live spermatozoa as per Campbell *et al* (1952). The composition of the statin included Eosin-Yellow 1.67 gm and 10 gm of Nigorsin in 100ml of 2.90% sodium citrate buffer. The stain was ripened and then used. One drop of semen was mixed with three drops of Eosin – Nigrosin stain and was kept for 2 minutes. Thin smear was prepared on the slide and slide was air dried. The slide was observed under oil immersion (100X) and 100 spermatozoa were

Plate 3.5. Semen ejaculate

Plate 3.6. Semen evaluation under phase contrast microscope

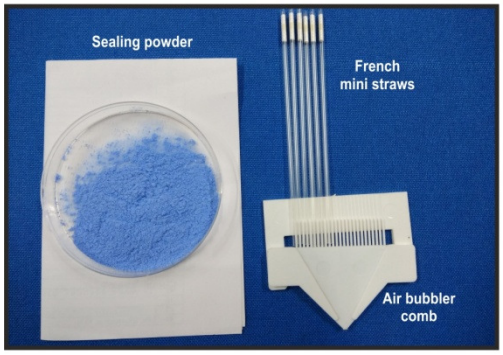

Plate 3.7. Semen freezing equipments and materials

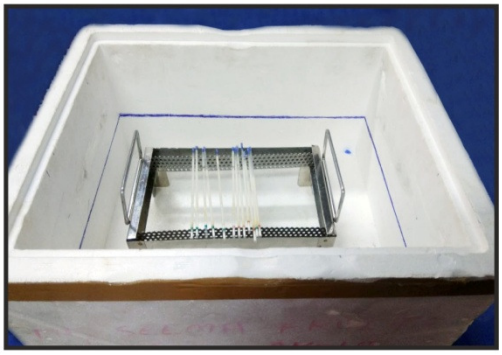

Plate 3.8. The semen filled straws arranged and kept horizontally during semen freezing

counted. The unstained spermatozoa was counted as live and pinkish eosinophilic or partially stained spermatozoa was classified as dead.

$$\text{Live Spermatozoa (\%)} = \frac{\text{Number of unstained spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100$$

e) Abnormal sperm percentage

The slide prepared for live and dead spermatozoa count were observed under oil immersion lens (100 x) using phase contrast microscope for enumerating abnormalities of the sperms abnormalities like detached head or tail, microhead, macrohead, bent tail, coiled tail etc were observed. About 200 sperms were counted at different field and the percentage of the head, mid-piece, tail and total abnormal spermatozoa were noted.

The percent abnormal sperm count was calculated as follows.

$$\text{Abnormal spermatozoa (\%)} = \frac{\text{Number of abnormal (head, mid-piece, tail and total) spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100$$

3.3.3 Sperm function tests

In sperm function tests, hypo-osmotic swelling test (HOST), acrosome intactness test and enzyme leakage test were performed.

a) Hypo-osmotic swelling test (HOST)

1. Two hypo-osmotic solutions were prepared as follows
   a. 2.7% aqeous solution of fructose (1.351 gm) and
   b. 1.47% aqeous solution of sodium citrate (0.735 gm)
2. Equal volumes of both solutions were mixed and incubated at 37^0C for 10 mins.
3. In above hypo-osmotic solution, 0.1 ml of semen was added and incubated at 37^0C for 30 min.
4. 10 µl of this mixture was taken on glass slide, covered with coverslip and observed under 40 X.

5. Total 100 sperms were counted including spermatozoa showing swollen head and coiled tail indicating sperm with intact plasma membrane and were expressed in percentage.

$$\text{HOST positive spermatozoa (\%)} = \frac{\text{No. of spermatozoa with swollen head and coiled tail}}{\text{Total number of spermatozoa counted}} \times 100$$

b) Acrosome intactness test

Acrosomal intactness of spermatozoa was assessed by using Giemsa stain.

Stock Solution

Giemsa stain	1 gm
Methanol	66 ml
Glycerol	60 ml

Working solution

Stock solution	3 ml
Distilled water	47 ml

A thin smear of semen was prepared on a clean glass slide. The smear was then air dried and kept in 5 % formaldehyde solution for 30 minutes at 37^0C temperature. The slide was washed in distilled water and air dried.

The air dried slides of semen were kept in the staining jar containing working staining solution for 3 hours at 37^0C. The stained slides were washed with distilled water and air dried. Hundred spermatozoa were counted from each slide under 100X. Spermatozoa with head stained purple were counted as intact acrosome and spermatozoa with head unstained and observed as white were counted as non-intact spermatozoa and were expressed in percentage.

$$\text{Intact acrosome spermatozoa (\%)} = \frac{\text{Number of spermatozoa with purple stainedhead stained}}{\text{Total number of spermatozoa counted}} \times 100$$

c) Enzyme leakage test

The activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were estimated in the seminal plasma of neat, pre-freeze and post-freeze semen samples employing standard diagnostic kits by Clinical autoanalyzer. The kits used for estimating AST (Aspartate amino transferase) and ALT (Alanine amino transferase) enzymes were AUTOSPAN® (Manufactured by ARKRAY Healthcare Pvt. Ltd. Plot NO.336, 338, 340 Road No 3, G.I.D.C., Sachin 394 230, Surat, India).

3.4 Preservation of Semen

3.4.1 Seminal plasma separation and washing of spermatozoa

The collected semen was divided in two equal parts,one part was washed for removing the traces of seminal plasma, while the other part was kept unwashed. For washing of semen, washing solution (Krebs-Ringer-Phosphate-Glucose solution) was added at the rate of 1: 9 (1 ml semen: 9 ml washing solution) and centrifuged at 3000 rpm for 5 min to remove supernatant. Sperm pack and unwashed semen sample were diluted in Tris based dilutor and preserved at refrigeration and ultralow temperature.

Krebs-Ringer-Phosphate-Glucose solution (Chemineau *et al.,* 1991)

Initial solution (Final concentration in bi-distilled water)	Volume of initial solution in final solution (ml)
0.9% NaCl	100.0
1.15% KCl	4.0
1.22% $CaCl_2$	3.0
2.11% KH_2PO_4	0.4
3.82% $MgSO_4,7H_2O$	1.0
Phosphate buffer pH 7.4	12.0
5.34% anhydrous glucose	4.5

Phosphate buffer – Dissolved 35.81 g of Na_2HPO_4, $12H_2O$ in 20ml of HCl/N (Completed 1000ml buffer by bi Distilled Water)
HCl/N - 50ml of bi Distilled Water + 84.7ml HCl + necessary volume of water to obtain 1000 ml of final solution.

3.4.2 Dilution of semen for refrigeration preservation

The two dilutors i.e. Tris – citric acid – fructose – egg yolk and Skim milk were used for the refrigerator preservation of semen. Semen sample of each buck was diluted with dilutors separately at the ratio of 1:10 (1ml semen: 10ml dilutor). The details of dilutors are given below:

a) Tris – citric acid – fructose – egg yolk (TCFEY) dilutor

It was prepared as per Deka and Rao (1986) and kept in refrigerator till use -

Tris (Hydroxy methylamine methane)	: 2.422 g
Citric acid	: 1.34 g
Fructose	: 1.0 g
Egg yolk	: 20 ml
Distilled water	: 80 ml
Penicillin	: 100000 IU
Streptomycin	: 100 mg

b) Skim milk dilutor

It was prepared as per Corteel *et al.*(1974) as given below-

Skimmed milk powder	: 10g
Distilled water	: 100ml
D-Glucose	: 0.2g
Penicillin	: 50,000IU
Streptomycin	: 50mg

Heated in water bath at 92^0C for 10 minutes, cooled at room temperature and kept in refrigerator till use.

3.4.3 Refrigeration of semen

a) Preservation Technique for Refrigeration of semen:

The dilutors were heated up to 37^0C temp. in water bath before diluting the semen. After the final dilution, both the samples (Washed and Unwashed) were preserved at refrigeration temperature. After dilution semen

samples were kept in refrigerator. The semen was diluted making approximately 300 X 10^6 sperm concentration in 1 ml of diluted semen.The goats were inseminated with 1ml of diluted semen sample.

3.4.4 Dilution of semen for cryopreservation

The dilutors used for cryopreservation of semen were Tris Egg yolk Glycerol (TYG) dilutor and Tris-Dimethyl sulphoxide (DMSO) dilutor (TYD).

a) Tris-citric acid-furctose-egg yolk (TCFEY) dilutor

It was prepared as per Sinha *et al.* (2004) and kept in refrigerator till use-

Tris-(Hydroxy methylamine methane)	: 2.422 g
Citric acid	: 1.34 g
Fructose	: 1.0 g
Egg yolk	: 20ml
Distilled water	: 80ml
Penicillin	: 1,00,000 IU
Streptomycin	: 100 mg

Glycerol to make final concentration of 6 percent.

The required quantity of dilutor buffer thus prepared, exercising all aseptic measures, just overnight before the actual use, was then added with the antibiotics. Egg yolk was added in appropriate quantity just before the use.

b) Tris egg Dimethyl Sulphoxide (DMSO) dilutor

Tris (Hydroxy methylamine methane)	: 2.422 g
Citric acid	: 1.34 g
Fructose	: 1.0 g
Distilled water	: 90ml
Penicillin	: 100000 IU
Streptomycin	: 100 mg
Fresh egg yolk	: 05 ml
Dimethyl Sulphoxide (DMSO)	: 05 ml

Dilutors were prepared by standard procedure.

Tris-citric acid – fructose- egg yolk diluter was used for the cryopreservation of semen. Semen sample of each buck was diluted with dilutor kept in a water bath at the dilution rate of 1:3 (diluter without glycerol). After initial dilution, the semen samples were kept in the cold handling cabinet for cooling of semen to 4˚C. After reaching at 4˚C temperature, further dilution of semen with the glycerolated diluter was done to make the final dilution of 1:6. The addition of the glycerolated diluter was performed slowly and in three fractions at the interval of 10 minutes each. The final concentration of sperm was adjusted approximately 150 X 10^6/ straw.

3.4.5 Freezing technique for cryopreservation of semen

After the final dilution, both the semen samples (washed and unwashed) were filled in precooled 0.5 ml polyvinyl chloride (PVC) medium straws by manual filling and sealing . The semen straws were labelled as washed and unwashed semen. The straws were then placed in cold handling cabinet at 4˚C temperature for 3 hours for equilibration.

At the end of the equilibration period, the straws were collected and were subjected for -140˚C temp. These straws were spread in the freezing rack and ramp. The freezing racks and ramps were kept in LN_2 vapour (horizontal vapour freezing) 2cm above the surface of LN_2 in a thermacol box for 10 minutes.

After 10 minutes, the frozen straws were collected in goblets filled with liquid nitrogen and the goblets were transferred into liquid nitrogen container for storage. After 24 hours of cryopreservation the straws were thawed in water bath at 37°C for 30 seconds to assess the post thaw motility and damage to spermatozoa due to cryopreservation.

3.4.6 Thawing of cryopreserved semen straws

The semen sample stored in 0.5ml straws was taken out from LN_2 container after 24 hours of storage and thawed in water at 37^0C for 30 seconds. The straws were then dried using tissue / filter paper. Both the ends

of the straws were then cut and the samples decanted in a test tube kept in water bath at 37^0C for post-thaw evaluation.

3.4.7 Pre – freeze and post-freeze motility of preserved semen

a) Pre – freeze and post-freeze motility of refrigerated semen

The semen sample, just before freezing (post-diluted) and 4 hours of equilibration were used for assessing the pre-freeze / equilibration motility. 10 µl of diluted semen was placed on warm (37^0C) clean grease free slide and covered with microscopic cover glass in such a way that a fresh thin film without air bubbles was formed. The subjective evaluation of the progressive pre-freeze and post-freeze sperm motility was examined under high power objective (40 x) of the phase contrast microscope.

b) Pre – freeze and post-freeze motility of cryopreserved semen

The straws, just before freezing (post-diluted), 4 hours of equilibration and post thaw were cut and a drop of semen (10 µl) was used for assessing the post diluted /post- equilibrated / post thaw motility. 10 µl of diluted semen was placed on warm (37^0C) clean grease free slide and covered with microscopic cover glass in such a way that a fresh thin film without air bubbles was formed. The subjective evaluation of the progressive pre-freeze (post-diluted, post equilibrated) and post-thaw sperm motility was examined under high power objective (40 x) of the phase contrast microscope.

3.4.8 Evaluation of refrigerated and cryopreserved semen

The semen samples were evaluated for the following criteria using same methods as described earlier in refrigerated semen [pre-freeze (post diluted), post-freeze (post-equilibrated)], in cryopreserved semen [post diluted, post equilibrated and post thaw] stages (24 hours of preservation).

a. Microscopic Evaluation

1. Individual sperm motility
2. Live sperm count and
3. Abnormal sperm count using the same methods as described earlier.

b. Spermatozoa acrosomal abnormalities

c. Plasma membrane integrity test

The percentages of spermatozoa with intact plasma membrane were evaluated to know plasma membrane integrity by using hypo osmatic swelling test (Pant *et al*., 2002).

d. Leakage of Enzymes

1. Asparate AminoTransferase (AST)
2. Alanine AminoTransferase (ALT)

3.5 Study of Conception Rate

3.5.1 Selection of goats

The experimental does of two to six years of age having approximate body weight ranging from 20 to 35 kg, first to fourth parity, apparently healthy and who have kidded at least once before the commencement of experiment and on the basis of history which have completed 45 days after kidding were selected for the study (Plate 11). The gynaecological examination was carried out with the help of ultrasonographic machine.

Non-pregnant does with normal and disease free genital tract irrespective of ovarian cyclicity were selected for this study. All the non-pregnant females were separated, special care taken. Bucks were kept away from them and again after one month, USG examination was carried out and those does which were found non pregnant included in experiment.Before conducting the study, each doe was dewormed and submitted to a general physical examination and vaginal inspection.

3.5.2 Hormonal protocol for estrus synchronization

Thirty two Berari goats (n=32) were divided randomly into 2 groups. The treatment for estrus synchronization was performed using CIRG, intravaginalprogestagen sponges for upto 10 days. Intravaginal sponges designed for goats (Plate 3.9) were made available from Central Institute for Research On Goats (CIRG) Makhdoom, Farah, Dist. Mathura (U.P.) India. Synchronization of estrus was carried out by using different hormonal protocol as described below.

Plate 3.9. CIRG Intravaginal progestogen sponges used for oestrus synchronization

Plate 3.10. Berari does selected for oestrus synchronization

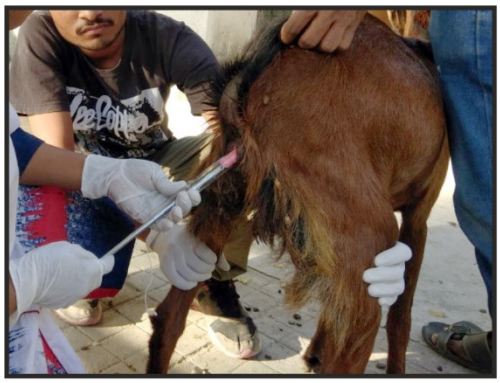

Plate 3.11. Sponge insertion in Berari goat

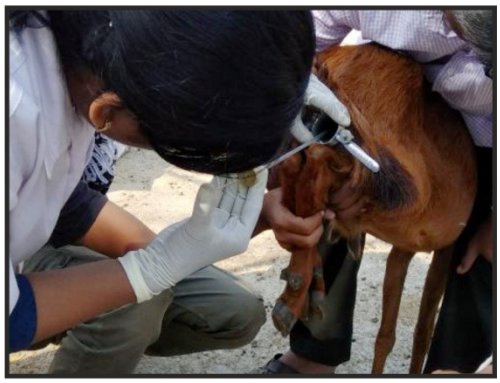

Plate 3.12. Artificial insemination in Berari goat

a) CIRG intra-vaginal sponge + $PGF_2\alpha$ + GnRH :

CIRG Intra-vaginal sponges were kept for 10 days + Inj. Cloprostenol (Inj. Pregma®) @ 125 µg I.M were injected on day 9 + GnRH (Inj. Gynarich®) @ 10 µg I.M on day 10 , thesponges were removed and on day 12 does exhibited estrus. FTAI were done twice at 12 hrs interval on the same day i.e 12^{th} day.

(Inj. Pregma® – Brand of Intas Pharmaceuticals Ltd., Ahmedabad, containing Cloprostenol 250 mcg/ml)

Inj. Gynarich®- Brand of Intas Pharmaceuticals Ltd., Ahmedabad, containing Buserelin 4 mcg/ml)

Synchronization protocol : CIRG intra-vaginal sponge + $PGF_2\alpha$ + GnRH

CIRG Intra-vaginal sponges were kept for 10 days + Inj. Cloprostenol @ 125 µg I.M were injected on day 9^{th} + Inj. Buserelin acetate (GnRH) @ 10 µg I.M on day 10, the sponge removed and day 13, does exhibited estrus and FTAI were done twice at 12 hrs interval.

a. All the does were watched carefully four times a day for behavioural estrus signs starting from the day of intravaginal sponge removal.

b. Out of thirty two synchronized Berari goats, eight (n=8) goats were inseminated with washed refrigerated semen while remaining eight goats (n=8) were inseminated with unwashed refrigerated semen.

c. Out of thirty two synchronized Berari goats, eight (n=8) goats were inseminated with washed cryopreserved semen while remaining eight goats (n=8) were inseminated with unwashed cryopreserved semen.

d. The inseminated goats were checked ultrasonographically on 30 days post insemination. The conception rate was studied in all the four groups.

3.5.3 Artificial Insemination (A.I.)

Artificial insemination (A.I.) was done at 48 ±1.0 and 60 ±1.0 hours interval from the withdrawal of sponges.Previously frozen semen from selected bucks was thawed. In selected goats Artificial Insemination was done

using frozen thawed semen. At the time of insemination hind limbs of goat were lifted from behind, then cervix was located with the help of vaginal speculum, after that semen was deposited by passing one fold of cervix with the help of French insemination gun without harming the cervical tissues. Goat number and details of straws used were recorded.

All does were inseminated intracervically with refrigerated / cryopreserved semen using a speculum (Plate12). Pregnancy diagnosis was done on day 30 following AI by using transrectal USG (Plate 17) and pregnancy was confirmed.

3.6 Statistical Analysis

The data of this investigation was analyzed by employing suitable statistical design as recommended by Snedecor and Cochran (1994).

CHAPTER IV

RESULTS AND DISCUSSION

The present work on "Seminal attributes, preservation and fertilizing ability of refrigerated and cryopreserved semen of Berari bucks" was carried out at the Department of Animal Reproduction, Gynaecology and Obstetrics, PGIVAS, Akola (Maharashtra). The research was planned to record the scrotal biometry, physical characteristics, functional tests of washed neat semen as well as unwashed semen of Berari buck preserved by refrigeration and cryopreservation technic. The conception rate with washed and unwashed refrigerated and cryopreserved semen in Berari goats was studied.

The results obtained on different aspects of the present investigation are presented in tabular form and discussed under following heads and their respective subheads.

1) Scrotal biometry of Berari bucks
2) Physical and functional tests of neat semen
4) Refrigeration preservation semen
5) Cryopreservation of semen.
6) Conception rate in refrigeration and cryopreservation groups.

4.1 Scrotal Biometry

The scrotal biometry of the Berari buck included the measurement of scrotal circumference, testicular width and testicular length is presented in Table 4.1 and correlation of scrotal biometry with physical and microscopic characteristics of neat semen is presented in Table 4.2.

4.1.1 Scrotal circumference

The overall mean of scrotal circumference was 24.62±0.51 cm in Berari buck. It was observed that the individual variation among bucks was significant ($P<0.05$) (Table 4.1A).

The result of present study for scrotal circumference is in close agremment with Ahmed and Noakes (1996) and Okere *et al.* (2011).

However, much lower values were reported by Olurode *et al.* (2018), Khan *et al.* (2007) and Oyeyemi *et al.* (2000), Khan *et al.* (2007) and Gogoi *et al.* (2008) in Beetal × Assam buck.

Table 4.1. Scrotal biometry (Mean±S.E.) of Berari bucks

Sr. No.	Buck No.	Scrotal circumference (cm)	Testicular length (cm)	Testicular width (cm)
1	1 (n = 24)	$25.99^{b}\pm 0.06$	$8.33^{b}\pm 0.05$	$5.41^{b}\pm 0.03$
2	2 (n = 24)	$26.36^{a}\pm 0.06$	$8.53^{a}\pm 0.08$	$5.68^{a}\pm 0.03$
3	3 (n = 24)	$24.35^{c}\pm 0.05$	$7.28^{d}\pm 0.05$	$5.21^{d}\pm 0.03$
4	4 (n = 24)	$23.97^{d}\pm 0.05$	$7.93^{c}\pm 0.04$	$5.31^{c}\pm 0.03$
5	5 (n = 24)	$23.65^{e}\pm 0.05$	$7.37^{d}\pm 0.05$	$4.48^{e}\pm 0.03$
6	6 (n = 24)	$23.36^{f}\pm 0.05$	$6.56^{e}\pm 0.05$	$4.12^{f}\pm 0.03$
Overall mean (n = 144)		24.62 ± 0.51	7.67 ± 0.30	5.04 ± 0.23

Note : Superscripts are to be read column-wise.

Means bearing different superscripts differ significantly (P<0.05).

Table 4.1A. ANOVA for the scrotal biometry of Berari bucks

Source Table	d.f.	Scrotal circumference		Testicular length		Testicular width	
		M.S.	Cal. F value	M.S.	Cal. F value	M.S.	Cal. F value
Bucks	5	37.98	519.58**	13.13	188.51**	8.63	427.16**
Error	138	0.07		0.07		0.02	
Total	143						
CD (5%)		0.15		0.14		0.08	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Table 4.2. Correlation of scrotal biometry with physical and microscopic characteristics of Berari buck semen in refrigerated group

	Scrotal circumference	Testicular length	Testicular width
Scrotal circumference	1		
Testicular length	0.859*	1	
Testicular width	0.812*	0.861*	1
Ejaculate volume	0.581*	0.433*	0.440*
Seminal pH	0.176*	0.133^{NS}	0.069^{NS}
Mass motility	0.423*	0.370*	0.319*
Individual motility	0.337*	0.330*	0.314*
Live sperm count	0.420*	0.422*	0.390*
Sperm concentration	0.433*	0.397*	0.282*

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Higher values were documented by Zamiri and Heidari (2006) in Rayini bucks, Ghalban *et al.* (2004) in Damascus bucks.

The correlation coefficient of the scrotal biometry with different semen characteristics is depicted in Table 4.2. The scrotal circumference was found to be highly significantly (P<0.01) and positively correlated with testicular length (0.859), testicular width (0.812), ejaculate volume (0.581), seminal pH (0.176), mass motility (0.423), Individual motility (0.337), live sperm count (0.420) and sperm concentration (0.433).

Aliyu *et al.* (2016) reported significant and positive correlation of scrotal circumference was found with semen colour, volume, semen motility and live and dead ratio while negative correlation with semen pH and semen concentration.

Positive correlation of scrotal circumference with semen volume and sperm concentration, mass motility, sperm livability and percent normal spermatozoa was recorded by Mia *et al.* (2013).

A highly significant correlation of scrotal circumference was reported with volume, seminal pH, mass motility, live sperm count and sperm concentration in different breed of goat. (Zamiri and Heidari, 2006 and Gogoi *et al.*, 2008).

Zamiri and Heidari (2006) reported the significant correlation of testicular length with scrotal circumference, testicular width, live sperm count, sperm concentration and ejaculate volume.

In present study, scrotal circumference was significantly and positively correlated with semen concentration. On the contrary to the findings Aliyu *et al.* (2016) recorded the negative correlation with semen pH and semen concentration. These differences in results may be due to difference in breed, age, season and other factors. Testicular length was significantly varied between individuals.

4.1.2 Testicular length

The overall mean of testicular length was measured as 7.67 ± 0.30 cm in Berari buck. The individual variation among bucks was significant ($P<0.05$). (Table 4.1A).

The results of testicular length (cm) recorded in Berari buck are in agreement with Boagra *et al.* (2016) (6.90±0.33) Khan *et al.* (2007) (8.56±0.07), however the higher values were reported by Parandekar (1987) 8.71±0.19, Ritar *et al.* (1992) 8.8±0.19 in Angora bucks and Puranik *et al.* (1993) 8.97±0.11 in Osmanabadi and 8.7±0.14. Zamiri and Heidari (2006) reported that the mean testis length of Rayini bucks weighing 55 – 60 kg and 50 – 54 kg was 9.90 ± 0.07 and 9.68 ± 0.06 cm, respectively.

A significant and positive correlation of testicular length with testicular width (0.861), ejaculate volume (0.433), mass motility (0.370), live sperm count (0.422) and sperm concentration (0.397) was observed. Correlation of testicular length with scrotal pH was statistically non-significant.

Present findings corroborates with the findings of Zamiri and Heidari (2006) who reported the significant correlation of testicular length with testicular width, scrotal circumference, live sperm count, the sperm concentration and ejaculate volume.

4.1.3 Testicular width

The mean of testicular width of Berari buck was 5.04 ± 0.23 cm. The individual variation among bucks was significant (P<0.05) (Table 4.1A)

The result found in present investigation is in agreement with Khan (2007) 4.91±0.08, Boagra *et al* (2016) value 4.38±0.24 in blackbucks and Zamiri and Heidari (2006) who reported that the mean testicular width of Rayini bucks weighing 55-60 kg and 50-54 kg were 6.51 ± 0.13 and 6.02 ± 0.09 cm, respectively. The higher values of testicular width (cm) were observed by Parandekar (1997) 5.63±0.32, Ritar *et al* (1992) 6.0±0.08 and puranik *et al*., (1993) 5.77±0.22 in Osmanabadi buck 5.75±0.15 in crossbreed buck, Ahmad and Noakes (1996).

Testicular width was significantly (P<0.05) and positively correlated with ejaculate volume (0.440), mass motility (0.319), individual motility (0.314), live sperm count (0.319) and sperm concentration (0.282) whereas positively and non-significantly correlated with seminal pH (0.069).

The testicular width was significantly differed among bucks. Highly significant and positive correlation of testicular width with scrotal circumference, testicular length, live sperm count, the sperm concentration and ejaculate volume is in close agreement with the reports of Zamiri and Heidari (2006).

4.1.4 Seminal attributes of Berari buck neat semen in refrigerated and cryopreserved group

Physical characteristics of Berari buck semen viz. semen colour, volume, pH, mass motility, individual motility, live sperm count, abnormal sperm count, sperm concentration and damaged acrosome were analysed from the 72 semen ejaculates obtained from six Berari bucks. These biophysical attributes are delineated as below and mentioned in Table 4.3 and

4.4 (refrigerated group) as well as in Table 4.6 and 4.7 (cryopreserved group). Correlation of physical characteristics, HOST and enzymes of neat semen is depicted in Table 4.5 (refrigerated group) and Table 4.8 (cryopreserved group).

a) Physical characteristics of neat semen in refrigerated group and cryopreserved group

i. Colour

The colour of Berari buck semen was found creamy in all the semen samples during research work.

Creamy colour of buck semen was reported by Olurode *et al.* (2018) and Dagli (2011) in West African Dwarf and Sirohi bucks respectively. However, some researchers reported yellowish (Bezjian *et al.*, 2013) and yellowish white (Bras, 2012) colour of goat semen. The colour pattern of the neat semen is the species specific and is also dependent on the sperm concentrations and presence of pigmented proteins and caratinoids in the seminal plasma.

ii. Ejaculate volume

The overall mean volume of Berari buck neat semen was observed as 1.84 ± 0.06 ml (Range :0.80 ml to 1.60 ml) and 1.22 ± 0.04 ml (Range : 0.90 to 1.60 ml) in refrigerated and cryopreserved group, respectively. The individual variation among bucks was highly significant ($P<0.01$) in refrigerated group and significant ($P<0.05$) in cryopreserved group.

The results are in concurrence with other Indian breeds reported by Dagli (2011) in Osmanabadi and Sirohi , Islam *et al.* (2008) in Black Bengal, Bhuskat *et al.* (2000) in Jamnapari and Ajmeri breeds of buck. Similar findings were also reported by Mahmood *et al.* (1988), Kale *et al.* (1998), Barbas *et al.* (2006) and Jadav *et al.* (2008) in different goat breeds.

Table 4.3. Seminal attributes (Mean ± S.E.) of Berari buck neat semen in Refrigerated Group

Buck No.	Volume (ml)	pH	Mass Motility (0-5 scale)	Individual Motility (Percent)	Live sperm (Percent)	Abnormal sperm (Percent)	Sperm Conc (10^6 /ml)	Damaged Acrosome (Percent)
1 (n=12)	1.38^{a}±0.04	6.81 ± 0.03	4.42^{a} ± 0.15	87.50^{a}± 1.31	89.67^{a} ± 0.99	4.50 ± 0.40	3579.17^{a} ± 18.93	2.25^{c} ± 0.18
2 (n=12)	1.29^{ab}±0.04	6.80 ± 0.02	4.08^{ab} ± 0.15	85.83^{b}± 1.93	88.17^{ab} ± 1.07	4.17 ± 0.42	3450.00^{b} ± 23.03	2.58^{bc} ± 0.23
3 (n=12)	1.23^{b}± 0.03	6.74 ± 0.02	3.75^{bc} ±0.13	85.00^{c}± 1.51	86.17^{bc} ± 1.25	5.50 ± 0.48	3379.17^{bc} ± 37.16	2.75^{abc} ± 0.25
4 (n=12)	1.03^{c} ±0.04	6.79 ± 0.03	3.58^{c} ± 0.15	84.17^{d}± 1.49	86.00^{bc} ±1.08	5.08 ± 0.38	3341.67^{c} ± 43.01	2.92^{abc} ± 0.23
5 (n=12)	1.05^{c}± 0.04	6.77 ± 0.02	4.08^{ab} ± 0.08	82.50^{e}± 1.31	84.58^{c}± 1.03	5.42 ± 0.40	3354.17^{c} ± 36.65	3.42^{a} ± 0.26
6 (n=12)	1.12^{c}± 0.02	6.79 ± 0.03	3.58^{c} ± 0.15	81.67^{f}± 2.07	83.92^{c} ± 1.61	5.83 ± 0.51	3400.00^{bc} ± 22.19	3.17^{ab} ± 0.27
Overall Mean (n=72)	**1.18 ± 0.06**	**6.78 ± 0.01**	**3.92 ± 0.14**	**84.44 ± 0.88**	**86.42 ± 0.88**	**5.08 ± 0.26**	**3417.36 ± 35.93**	**2.85 ± 0.17**

Means bearing different superscripts differ significantly in a column (P<0.05).

Table 4.4. HOST and enzyme profile (Mean + S.E.) of Berari buck neat semen in Refrigerated Group

Buck No.	Host (Percent)	AST (IU/L)	ALT (IU/L)
1 (n=12)	81.92^{a}±0.48	55.42^{bc}±0.96	11.83^{c}±0.69
2 (n=12)	81.25^{ab}±0.48	52.83±0.79	15.25^{a}±0.41
3 (n=12)	80.33^{bc}±0.48	55.17^{c}±0.34	13.17^{b}±0.52
4 (n=12)	79.75^{bc}±0.49	58.75^{a}±0.57	14.75^{a}±0.30
5 (n=12)	80.00^{bc}±0.33	57.17^{ab}±0.72	11.00^{c}±0.39
6 (n=12)	79.17^{c}±0.81	57.50^{a}±0.60	12.00^{bc}±0.41
Overall Mean (n=72)	80.40±0.41	56.14±0.86	13.00±0.70

Means bearing different superscripts differ significantly in a column (P<0.05).

Table 4.5. Correlation of physical characteristics, HOST and enzymes of Berari buck neat semenin Refrigerated Group

-	Volume	pH	Mass Motility	Individual Motility	Live Sperm Count	Abnormal Sperm Count	Sperm Conc	Damage Acrosome	HOST	AST	ALT
Volume	1	0.19^{NS}	0.29*	0.36*	0.43*	-0.23^{NS}	0.56*	-0.37*	0.40*	-0.41*	-0.04^{NS}
pH		1	0.21^{NS}	0.12^{NS}	0.19^{NS}	-0.20^{NS}	0.20^{NS}	-0.31*	0.09^{NS}	-0.16^{NS}	-0.03^{NS}
Mass Motility			1	0.38*	0.45*	-0.32*	0.28*	-0.26*	0.36*	-0.24*	-0.05^{NS}
Individual Motility				1	0.95*	-0.32*	0.18^{NS}	-0.61*	0.76*	-0.12^{NS}	-0.09^{NS}
Live Sperm Count					1	-0.34*	0.27*	-0.66*	0.79*	-0.12^{NS}	-0.09^{NS}
Abnormal Sperm Count						1	-0.25*	0.23*	-0.25*	0.17^{NS}	-0.09^{NS}
Sperm Concentration							1	-0.28*	0.25*	-0.28*	-0.14^{NS}
Damaged Acrosome								1	-0.57*	0.23^{NS}	0.19^{NS}
HOST									1	-0.18^{NS}	-0.09^{NS}
AST										1	0.02^{NS}
ALT											1

* :Significant at 5% level NS: Non Significant

Table 4.6. Seminal attributes (mean + S.E) of Berari buck neat semen in Cryopreservation Group

Buck No.	Volume (ml)	pH	Mass Motility (0-5 scale)	Individual Motility (Percent)	Live sperm (Percent)	Abnormal sperm (Percent)	Sperm Conc (10^6 /ml)	Damaged Acrosome (Percent)
1 (n=12)	$1.34^{a} \pm 0.06$	6.81 ±0.03	4.42^{a} ±0.15	$88.33^{a} \pm 1.12$	$91.67^{a} \pm 0.45$	2.92^{c} ±0.19	$3766.67^{a} \pm 25.62$	2.25^{b}±0.18
2 (n=12)	$1.28^{ab} \pm 0.04$	6.81 ±0.03	4.33^{ab} ±0.14	86.67^{a} ±1.42	90.83^{ab} ±0.95	3.58^{abc} ±0.29	$3683.33^{ab} \pm 33.33$	2.33^{b}±0.14
3 (n=12)	1.28^{ab} ±0.02	6.79 ±0.03	4.08^{ab} ±0.15	85.83^{ab} ±1.49	91.33^{a} ±0.38	3.50^{bc} ±0.23	$3641.67^{b} \pm 46.40$	2.33^{b}±0.14
4 (n=12)	1.18^{c} ±0.04b	6.74 ±0.02	4.00^{abc} ±0.17	85.83^{ab} ±1.49	90.17^{abc} ±1.36	4.00^{ab}±0.25	$3620.83^{b} \pm 33.97$	3.00^{a} ±0.21
5 (n=12)	1.13^{c} ±0.03	6.80 ±0.02	3.58^{c} ±0.19	82.50^{b} ±1.31	87.83^{c} ±1.25	4.25^{a} ±0.28	$3616.67^{b} \pm 30.98$	3.08^{a}±.026
6 (n=12)	1.13^{c} ±0.03	6.77 ±0.02	3.92^{bc} ±0.15	82.50^{b} ±1.79	88.25^{bc}±1.31	4.08^{ab}±0.26	$3658.33^{b} \pm 30.05$	3.33^{a}±0.38
Overall Mean (n=72)	**1.22 ±0.04**	**6.79 ±0.01**	**4.06 ±0.12**	**85.28 ±0.95**	**90.01 ±0.66**	**3.72 ±0.20**	**3664.58 ± 22.76**	**2.72 ±0.19**

Means bearing different superscripts differ significantly in a column (P<0.05).

Table 4.7. HOST and enzyme profile (mean + S.E.) of Berari buck neat semen in Cryopreserved Group

Buck No.	Host (Percent)	AST (IU/L)	ALT (IU/L)
1 (n=12)	81.75 ±0.75	40.50^{c} ±0.97	9.00 ±0.17
2 (n=12)	82.25 ±0.51	43.50^{ab} ±0.58	8.50 ±0.34
3 (n=12)	81.42 ±0.90	41.00^{c} ±0.44	9.33 ±0.54
4 (n=12)	81.50 ±0.57	40.17^{c} ±0.66	9.33 ±0.59
5 (n=12)	81.33 ±0.51	42.00^{c} ±0.84b	9.33 ±0.40
6 (n=12)	80.92 ±0.54	44.08^{a} ±0.47	8.83 ±0.24
Overall Mean (n=72)	81.53 ±0.18	41.88 ±0.66	9.06 ±0.14

Means bearing different superscripts differ significantly in a column (P<0.05).

Table 4.8. Correlation of physical characteristics, HOST and enzymes of Berari buck neat semen in Cryopreserved Group

-	Volume	pH	Mass Motility	Individual Motility	Live Sperm Count	Abnormal Sperm Count	Sperm Concentration	Damaged Acrosome	HOST	AST	ALT
Volume	1	0.29*	0.28*	0.23^{NS}	0.371*	-0.24*	0.32*	-0.29*	0.40*	-0.27*	0.09^{NS}
pH		1	0.12^{NS}	0.07^{NS}	0.21^{NS}	-0.16^{NS}	0.32*	-0.10^{NS}	0.21^{NS}	0.14^{NS}	0.02^{NS}
Mass Motility			1	0.22^{NS}	0.33*	-0.02^{NS}	0.32*	-0.21^{NS}	0.38*	-0.09^{NS}	0.01^{NS}
Individual Motility				1	0.47*	-0.43*	0.15^{NS}	-0.37*	0.08^{NS}	-0.20^{NS}	-0.08^{NS}
Live Sperm Count					1	-0.53*	0.22^{NS}	-0.55*	0.27*	-0.23^{NS}	0.12^{NS}
Abnormal Sperm Count						1	-0.09^{NS}	0.56*	-0.06^{NS}	0.06^{NS}	-0.13^{NS}
Sperm Concentration							1	-0.18^{NS}	0.38*	-0.13^{NS}	-0.03^{NS}
Damaged Acrosome								1	-0.21^{NS}	0.02^{NS}	0.10^{NS}
HOST									1	-0.06^{NS}	0.01^{NS}
AST										1	-0.06^{NS}
ALT											1

* :Significant at 5% level NS : Non Significant

However, a wide variation in the volume of the neat semen has been reported for few other breeds viz. West African Dwarf buck (Olurode *et al.,* 2018), Black Bengal (Bogra *et al.,* 2016) volume 0.68. Individual variation in the seminal volume was recorded to be significant in the present studies. Contradictory to the present findings Biswas *et al.* (2002) reported the non-significant differences between bucks. However, the volume of semen collected is influenced by large number of factors such as the age of animal, the method of semen collection, frequency of semen collection and the season of semen collection.

The correlation of coefficient is depicted in Table 4.5 (refrigerated group). The ejaculate volume was found to be significantly ($P<0.05$) and positively correlated with mass motility (0.293), individual motility (0.36), live sperm count (0.426), sperm concentration (0.560) and HOST (0.396). The negative significant ($P<0.05$) correlation of ejaculate volume was found with the damaged acrosome (-0.367) and AST (-0.410). Whereas, the correlation of ejaculate volume with abnormal sperm count (-0.228) and ALT (-0.036) were negative and statistically non-significant.

The correlation of coefficient is depicted in Table 4.8 (cryopreserved group). The ejaculate volume was found to be significantly ($P<0.05$) and positively correlated with seminal pH (0.29), mass motility (0.28), live sperm count (0.371), sperm concentration (0.32) and HOST (0.40). The negative significant ($P<0.05$) correlation of ejaculate volume was found with abnormal sperm count (-0.24), damaged acrosome (-0.29) and AST (-0.27). The correlation of ejaculate volume with individual motility (0.23) and ALT (0.09) were positive but was statistically non-significant.

Similarly to the present observations Chandler *et al.* (1988) and Singh and Raza Nasir (1995) also reported significant correlation between semen volume and sperm concentration and Jadav *et al.* (2008) reported negative correlation of ejaculate volume with abnormal sperm count. Singh and Raza Nazir (1995) obtained a positive correlation of ejaculate volume with individual motility. Contrary to these results, negative correlation of

ejaculate volume with sperm concentration (Jadav *et al*., 2008) have also been observed. Further, Kale *et al*., (1998) reported the significant correlation between volume and mass motility, which was in accordance with the present study.

iii. Seminal pH

The mean pH value of Berari buck semen was noted as 6.78 ± 0.01 (Range: 6.60 to 7) in refrigerated group while 6.79 ± 0.01 (Range: 6.70 to 7.00) in cryopreserved group and the difference is non-significant among both groups

The studies on Rayini (Zamiri and Heidari, 2006) has shown semen pH below 6.60. The studies carried out on Bulgarian White milk (Yotov, 2015), West African Dwarf (Ferdinand *et al.*2012), Osmanabadi and Sirohi buck (Dagli, 2011), Jamnapari, Ajmeri, Osmanabadi and Local (Bhuskat *et al.,* 2000) breeds of buck have reported seminal pH in the range of 6.6 to 7.00. However, slightly higher value of 7.15 in Angora bucks has been recorded by Tekin *et al.* (1996), where semen was collected by electroejaculatry method. The individual bucks had shown non- significant variation in the seminal pH. These findings are in agreement with Mahmood *et al.* (1988) who has not observed significant difference among bucks.

In refrigeration group, the correlation of seminal pH was non significantly and positively correlated with mass motility (0.205), individual motility (0.121), live sperm count (0.189), sperm concentration (0.196) and HOST (0.095). The negative significant ($P<0.05$) correlation of ejaculate volume was found with damaged acrosome (-0.308). The correlation of seminal pH with abnormal sperm count (-0.196), AST (-0.163) and ALT (-0.029) were negative and statistically non-significant.

In cryopreserved group, Seminal pH was significantly and positively correlated with sperm content (0.32). The seminal pH was significantly and positively correlated with mass motility (0.12), individual motility (0.07), live sperm count (0.21), HOST (0.21), AST (0.14) and ALT

activity (0.02) and negatively correlated with abnormal sperm count (-0.16), damage acrosome (-0.10).

iv. Mass motility

Mass motility of Berari buck semen ranged between +3 to +5 with a mean value of 3.92 ± 0.14 in refrigerated and 4.06 ± 0.12 in cryopreserved group. The individual variation among bucks was highly significant (P<0.01).

These findings are in agreement with the observation of Olayemi *et al.* (2011), Ferdinand *et al.* (2012) in West African Dwarf buck semen , Farshad *et al.* (2009b) in Markhoz bucks, Dagli (2011) in Osmanabadi and Sirohi buck , Jadav *et al.* (2008) in Surti buck. However, much lower value of mass motility 2.72 ± 0.11 was recorded by Mahmood *et al.* (1988) in Changthangi breed. Significant individual variation among bucks was found in present investigation was in agreement with Joseph and Nair (1991) and Kale *et al.* (1998) whereas and Jadav *et al.* (2008) found varied findings. The differences could be attributed to the difference in the breeds and climatic condition under which experimental animals were reared.

In refrigerated group mass motility was significantly (P<0.05) and positively correlated with individual sperm motility (0.383), live sperm count (0.451), sperm concentration (0.280) and HOST(0.362). Negatively significant correlation of the mass motility was observed with the abnormal sperm count (-0.319), damaged acrosome (-0.259) and AST (-0.240). However ALT (-0.046) alone was observed negatively and non-significantly correlated with mass motility.

In cryopreserved group, mass motility was significantly (P<0.05) and positively correlated with live sperm count (0.33), sperm concentration (0.32), HOST (0.38) and non-significantly and negatively correlated with abnormal sperm count (-0.02), damage acrosome (-0.21), AST (-0.09). The correlation of mass motility with ALT (0.01) was positive but non-significant.

In present study, mass motility is significant and positively correlated with individual sperm motility, which supports the findings of Ahmed *et al.* (1997), Jadav *et al.* (2008) and Parmar (2009). Significant positive correlation of mass motility with ejaculate volume was in agreement with Kale *et al.* (1998). However contrary to findings of present study, Ahmed *et al.* (1997) documented non-significant correlation between mass motility and volume. Bera and Singh (1999) and Jadav *et al.* (2008) reported a significant and positive correlation between mass motility and live sperm count. Similarly in present study positive correlation was found between mass motility and live sperm count. Mass motility has a great bearing and is either directly or indirectly related with the other semen characters.

v. Individual sperm motility

The individual sperm motility averaged 84.44 ± 0.88 percent in refrigerated group while 85.28 ± 0.95 percent in cryopreserved group in Berari buck semen with a range of 70 to 90 percent. The individual variation among bucks was non-significant.

The present findings of individual sperm motility corroborates with the observations of Singh *et al.* (1993) in Indigenous bucks (70.75 ± 0.714 % to 79.82 ±1.47 %), Farshad *et al.* (2009b) in Morkhoz , Dagli (2011) in Osmanabadi and Sirohi,. Yotov (2015) in Bulgarian white milk breed, Tahar *et al.* (2018) in Arbia buck semen have reported the individual motility in the range of 70 to 90 percent. Whereas, higher individual motility was also reported in exotic breed by Olayemi *et al.* (2011), Bras (2012) in Stud breed. However, lower value of 40-80% was recorded in Malabari bucks by Patil and Raja (1978), 52.00 -68.0 % observed by Mohan *et al.* (1980) in Pashmina bucks, 50.00 ± 5.50 % was recorded by Bezjian *et al.* (2013) in Makhor buck. The individual motility of Berari buck semen seems to be more nearer to the exotic breeds as compared to some of the Indian breeds. Significant individual variation in present study varies the observation of Mohan *et al.* (1980). Chavan and Kale (2002), Barbas *et al.* (2006) and Jadav *et al.* (2008). The

individual motility seems to be a specific breed character However, Kale *et al.* (1998) reported the significant variation among bucks for individual motility.

In the refrigerated group, the correlation between individual sperm motility and live sperm count (0.949) and HOST (0.764) was positive and significant ($P<0.05$). The correlation of individual sperm motility with sperm concentration (0.182) was positive but non-significant. The values of abnormal sperm count (-0.322), damaged acrosome (-0.610) were negatively correlated with motility. The individual motility was negatively and significantly correlated with abnormal sperm count (-0.322) and damaged acrosome (-0.610). A statistically non-significant and negative correlation of individual motility with AST (-0.117) and ALT (-0.088) was observed.

In the cryopreserved group, the correlation between individual sperm motility and live sperm count (0.47) was positive and significant. Individual sperm motility was positive with the sperm concentration (0.15) and HOST (0.08) but stastically non-significant. The negative and significant correlation was recorded with the abnormal sperm count and damaged acrosome (-0.37). Individual motility was found to be negative with both the enzymes AST (-0.20) and ALT (-0.08) but they differ non-significantly.

In present investigation significant and positive correlation of individual motility was found with live sperm count and mass motility and negative correlation with abnormal sperm count. Similar findings were also reported by Deshpande (1989), Kale *et al.* (1998) and Jadav *et al.* (2008). Singh and Raza Nasir (1995) reported positive significant correlation between individual motility and semen volume.

vi. Live sperm count

The percentage of live spermatozoa was 86.42 ± 0.88 (Range: 74 to 93) and 90.0 ± 0.66 (Range : 81 to 95) in refrigerated and cryopreserved groups, respectively. The differences between the bucks were found to be highly significant ($P<0.01$) in refrigerated while significant in ($P<0.05$) in cryopreserved group.

The earlier reporters documented by Jadav *et al.* (2008) in Surti, Kulaksiz and Daskin (2010) in Sannen, Dagli (2011) in Osmanabadi and Sirohi have reported above 80 percent live sperm count which is slightly lower than the result of present study. Bras (2012) in Stud, Olayemi *et al.* (2011) in West African Dwarf bucks have reported 90 percent and above normal live sperm count which is higher than the results of the present study. However, much lower value of 48.9 ± 6.0 was recorded by Bezjian *et al.* (2013) in Makhor buck. Highly significant individual variation in present study supports the findings of Barbas *et al.* (2006), Mohan *et al.* (1980). Whereas Jadav *et al.* (2008) and Mittal (1982), Sundararaman and Edwin (2003) in Boer grade half breed (Boer X local) found non-significant individual variation among bucks for live sperm count. The variation in the live sperm count may be due to seasonal fluctuation or ambient temperature existing in the goat shed.

In refrigerated group, the percentage of live sperm was significantly and positively correlated with sperm concentration (0.0266) and HOST (0.788) while negative and significant ($P<0.05$) correlation was found with abnormal sperm count (-0.337) and damaged acrosome (-0.661). A statistically non-significant and negative correlation of AST (-0.124), ALT (-0.085) enzymes were also observed.

In cryopreserved group, the percentage of live sperm was highly significantly and positively correlated with HOST (0.27) while positive and non-significant correlation was found with abnormal sperm concentration (0.22) and ALT (0.12).Negative and non-significant correlation of the live sperm count with abnormal sperm count (-0.53) and damaged acrosome (-0.55) was observed. A statistically non - significant negative AST (-0.23) enzyme correlation of live sperm was also observed.

A highly significant and positive correlation of live sperm count with individual sperm motility during present investigation is in close agreement with the reports of Deshpande (1989), Kale *et al.* (1998) and Jadav *et al.* (2008). A significant and positive correlation of live sperm count with mass motility and negative correlation with abnormal sperm count have been

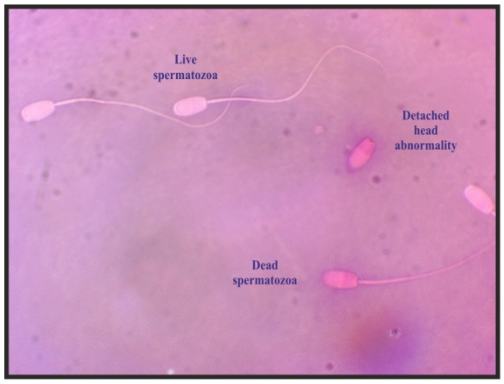

Plate 4.1. Live, dead spermatozoa and detached head abnormality of spermatozoa

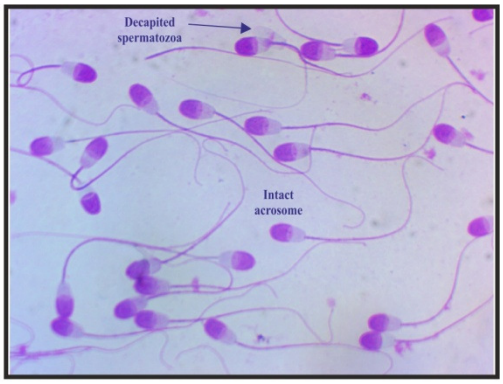

Plate 4.2. Intact and non intact acrosame of spermatozoa

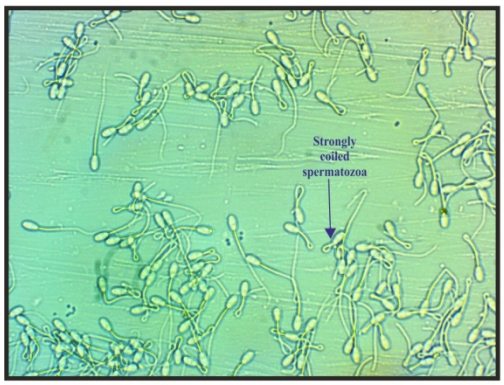

Plate 4.3. Coiling of sperm tail in hypo-osmotic swelling test

Plate 4.4. Coiling of sperm tail in hypo-osmotic swelling test of post thawed semen

documented by Jadav *et al.* (2008) which were in accordance to the present study. Contrary to present results, Deshpande (1989) reported a highly significant and negative correlation between live sperm count and semen volume.

vii. Abnormal sperm count

In refrigerated group, the abnormal sperm count in Berari buck semen ranged between 3 to 8 percent with an overall mean value of 5.08 ± 0.26 percent. The individual variation in abnormal sperm count was non-significant. While in cryopreserved group, the abnormal sperm count in Berari buck semen ranged between 2 to 5 percent with an overall mean value of 3.72 ± 0.20 percent. The individual variation in abnormal sperm count was statistically significant ($P<0.05$).

The abnormal sperm count in present result is in correlation with Bhuskat *et al.* (2000) in Jamnapari and Puranik *et al.* (1993) in crossbred buck. Sundararaman and Edwin (2003) in Boer grade half bred buck (8.43%) reported the mean abnormalities of sperms in the range of 5.0 to 5.91 percent. However, Bras (2012) in Stud, Dagli (2011) in Osmanabadi and Sirohi, Kulaksiz and Daskin (2010) in Sannen reported much higher sperm abnormalities. Non significant variation among bucks for abnormal sperm count found in present study agreed the findings of Mahmood *et al.* (1988), Batista *et al.* (2009). However Saxena and Tripathy (1980) in Jamnapari bucks, Chavan and Kale (2002), Barbas *et al.* (2006) and Jadav *et al.* (2008) reported significant variation for the same trait among the bucks. The variation in the abnormal sperm count may be due to semen volume (Chung and Kang,1976), pH of dilutor, age of the buck, season (Vinha,1980) and frequency of semen collection.

In refrigerated group, the correlation revealed that the abnormal sperm count was significant and positively correlated with damage acrosome (0.234) and negatively with sperm concentration (-0.245) and HOST (-0.248). The correlation of abnormal sperm count with AST (0.097) was negative but was statistically non-significant.

In cryopreserved group, the statistical results for the correlation revealed that the abnormal sperm count was significant (P<0.05) and positively correlated with damaged acrosome (0.56). However the correlation of abnormal sperm count with AST enzyme activity (0.06) was positive but statistically non-significant. The correlation of abnormal sperm count with sperm concentration (-0.09), HOST (-0.06) and ALT (-0.13) was negative and statistically non-significant.

In present investigation, abnormal sperm count was significantly and negatively correlated with individual motility. Similar findings were also reported by Saxena and Tripathi (1980), Hafez (1993), Kale *et al.* (1998), Jadav *et al.* (2008) .Negative correlation of abnormal sperm count was found with live sperm count supports the findings of Barbas *et al.*, (2006) and Jadav *et al.* (2008). A significant and negative correlation between abnormal sperms and mass activity was present, which was in harmony with Deshpande (1989).So higher sperm abnormalities might be one of the reasons of low sperm motility Saxena and Tripathi (1980).

viii. Sperm concentration

The mean sperm concentration of Berari buck semen in refrigerated group was 3417.36 ± 35.93 × 10^6/ml with a range of 3000 ×10^6/ml to 3700 ×10^6/ml while in cryopreserved group was 3664.58 ± 22.76 × 10^6/ml with a range of 3500 ×10^6/ml to 3950 ×10^6/ml. The mean value of sperm concentration per ml of semen showed highly significant differences between bucks (P<0.01).

The value recorded in the present study was in agreement with those reported for various goat breeds by Yotov (2015) in Bulgarian White milk breed, Sundararaman and Edwin (2003) in Boer Grade half-bred bucks and Gacitua and Arav (2005) in Saanen bucks. Higher value of sperm concentration was reported by Barbas *et al.* (2006) in Serrana buck semen. Lower sperm count than results in the present study was reported by Batista *et al.* (2009) in Majorera , Dagli (2011) in Osmanabadi and Sirohi , Olayemi *et al.* (2011), Bras (2012) in Stud, Bezjian *et al.* (2013) in Makhor, Jadav *et al.*

(2008) in Surti,.Tahar *et al.* (2018) in Arbia breed. The frequency of semen collection, the age of the buck and season affected the sperm concentration in goat (Vinha,1980). Gacitua and Arav (2005) and Batista *et al.* (2009) reported the significant variation among bucks for sperm concentration.

In refrigerated group, sperm concentration showed significant and positive correlation with HOST (0.250). The correlation between sperm concentration and damage acrosome (-0.28) and AST (-0.28) enzyme activity was negative and statistically significant. The sperm concentration exhibited negative and non-significant correlation with the ALT (-0.139) enzyme activity.

In cryopreserved group, sperm concentration showed significant ($P<0.05$) and positive correlation with HOST (0.38). The correlation between sperm concentration and damaged acrosome (-0.18), AST (-0.13) and ALT (-0.03) was negative and non-significant.

However Parmar, (2009) found highly significant correlation between sperm concentration and seminal enzymes.

b) Sperm Function Tests

i. Damaged acrosome

The percentage of damaged acrosome recorded was 2.85 ± 0.17 (Range: 2.00 to 5.00 percent) and 2.72 ± 0.19 (Range:1.00 to 7.00 percent) in refrigerated and cryopreserved group, respectively. The differences between bucks were found to be highly significant ($P<0.01$).

The result obtained was similar to that reported by Dorado *et al.* (2010) in Florida buck semen. Contrary to the present findings some workers reported higher acrosomal damaged spermatozoa count (Batista *et al.*, 2009; Dorado *et al.*, 2009; Farshad *et al.*, 2009b; Coloma , 2010; Kulaksiz and Daskin, 2010) and other reported lower acrosomal damage count (Bucak and Uysal, 2008). Significant difference between bucks observed for the acrosomal damaged sperm percent in present study compared well with the reports of Coloma (2010) and Dorado *et al.* (2010). On the contrary, Dorado *et*

al. (2009) has not observed buck variation in percentage of acrosomal abnormalities of sperm.

In refrigerated group, the positive non-significant correlation was noted with both the enzymes i.e. AST (0.231) and ALT (0.194) while the percentage of damaged acrosome was significant and negatively correlated with HOST (-0.568).

In cryopreserved group, the percentage of damaged acrosome was positively correlated with AST (0.02), ALT (0.10) enzymes and negatively correlated with HOST (-0.21) but non-significant.

ii. Hypo-osmatic swelling test (HOST)

In refrigerated group, the average percentage HOST was 80.40 ± 0.41 with a range of 74 to 83 percent. The differences between the bucks were found to be highly significant ($P<0.01$) while in cryopreserved group the overall mean percentage of HOST was 81.53 ± 0.18 with a range of 78 to 85 percent. The differences between the bucks were found to be non-significant.

The results of present investigation were in agreement with the reports of Dagli(2011) in Osmanabadi (83.13%) and Sirohi (78.33%) bucks ,Antonie and Pattabiraman (1999a) (87.20 %). Higher values of HOST reacted spermatozoa in neat semen were obtained by Ahmed *et al.*(2014) of fresh semen of bucks, while the lower values i.e 69.66% and 57.54% were reported by Kale *et al.*(2000b) and Azeredo *et al.*(2001), respectively.

Season has a great influence on the percentage HOST reacted spermatozoa in the semen (Kale *et al.*, 2000b and Coloma, 2010) and reported that the highest number of spermatozoa exhibited hypo-osmotic swelling during the cold humid season followed by the hot humid and hot dry season with a significant difference between all the seasons.

In refrigerated group, the correlation of HOST with both the enzymes i.e AST (0.177), ALT (0.088) were negative and statistically non-significant.

In cryopreserved group the percentage of HOST reacted sperm was positively correlated with ALT activity (0.01) while negatively correlated with AST (-0.06) activity but are non-significant

The present study revealed that percentage HOST had a positive significant correlation with live sperm count, sperm motility and a negative correlation of HOST with abnormal sperms was found. This was in concurrence with previous work by Jeyendran (1984) Prasad *et al.*, (1999), Neild *et al.*, (1999), Fonseca *et al.*, (2005) and Lodhi *et al.*, (2008). While Oliveira *et al.* (2013) reported that no significant correlations were detected between the reacted sperm verified by the hypoosmotic swelling test and other semen characteristics (P>0.05). HOS sperm percentage variation may be due to breed, climatic condition and managemental practice.

iii. Aspartate Aminotransferase (AST)

The overall mean AST activity (IU/L) observed in seminal plasma of Berari buck was 56.140 ± 0.86 (Range : 49 IU/L to 62 IU/L) and 41.88 ± 0.66 (Range : 35 IU/L to 46 IU/L) in refrigerated and cryopreserved groups, respectively. The individual variation among bucks was highly significant (P<0.01)

The values found in present investigation was lower than recorded by Dagli (2011)in Osmanabadi (82.55 ± 6.95 IU/L) and Sirohi bucks (73.61 ± 4.37 IU/L) and by Tuli and Holtz (1994) in Boer goats (82.5 ± 3.0 units/L) in which these AST values were increased after freezing.

Much higher values than the present investigation were recorded by Singh *et al.*, (2005) in Sirohi bucks (149.2 ± 6.25 units/ml), in Marwari bucks (150 ± 5.89 units/ml),in Jakhrana bucks (135 ± 9.77 units/ml).

Whereas, Kale and Tomer (2000) in crossbred bucks (Saanen × Beetal and Alpine × Beetal) and Kapila (1992) in Jamnapari bucks found lower value of 1.40 ± 0.42 µmole/ml and 0.36 ± 0.04 units/10^9 sperm, respectively. Significant variation among bucks in present study was similarly

to the finding of Tuli *et al.* (1991) who reported a highly significant difference in enzyme leakage among Boer goat bucks.

In refrigerated group the AST activity was positively correlated with ALT activity (0.017) with non-significant difference while in cryopreserved group it was negatively (-0.06) and non-sigificantly correlated with ALT activity.

In present study, a positive correlation found between AST and ALT and negative correlation of AST with ejaculate volume and sperm concentration is in accordance to reports of Kale and Tomer (2000a) in crossbred bucks. Further, positive correlation of AST with abnormal sperm ,damaged acrosome count was observed in present study. This confirmed the findings of Shelke and Dhami (2002) who reported that leakage of enzyme was proportional to the damage of spermatozoa.

iv. Alanine Aminotransferase (ALT)

In refrigerated group the ALT activity of seminal plasma of Berari buck ranged between 9 IU/L to 17 IU/L with an overall mean value of 13.00 ± 0.70 IU/L. The individual variation among bucks was highly significant ($P<0.01$).

In cryopreserved group the overall mean value of ALT enzyme activity of seminal plasma of Berari buck neat semen in cryopreserved was 9.06 ± 0.14 with a range of 7 IU/L to 14 IU/L. The individual variation among bucks was insignificant.

The observations recorded by Dagli (2011) are slightly higher than results of present observations 16.94 ± 0.84 and 16.06 ± 0.81 IU/L ALT release in Osmanabadi and Sirohi buck semen which increased after freezing.

The values found in present investigation are in concurrence with reports of Singh *et al.* (2005) in Sirohi bucks (14.6 ± 0.73 units/ml), in Marwari bucks (13.6 ± 0.93 units/ml),in Jakhrana bucks (13.4 ± 0.84 units/ml).

It is envisaged from the results of physical characteristics of semen and the plasma membrane integrity of spermatozoa that a wide variation in the results of the present study and the findings of the other workers were discernible which is likely to be expected on account of breed, age, management, nutrition and climate of the region apart from subjective technique of semen evaluation.

4.3 Refrigeration of Semen

A total of 72 ejaculates (collected once in a week) from each of 6 bucks were utilized with two different dilutors viz., Tris – citric acid – fructose – egg yolk (TCFEY) and Skim milk (SM). After evaluation of neat semen, washing and dilution of semen was carried out and washed and unwashed semen samples were preserved at refrigerator temperature (4±1°C). The preserved semen samples were examined at different preservation stages (Pre-freeze Stage and Post-freeze Stage) in different dilutors for individual sperm motility, live sperm count and morphologically abnormal sperm count damaged acrosome and HOST. Seminal plasma was collected at Pre-freeze (Post –diluted) and Post freeze (Post-Refrigeration) stages of preservation for leakage of enzymes (Aspartate Aminotransferase and Alanine Aminotransferase).

4.3.1 Refrigeration of washed spermatozoa of Berari buck

a) Physical characteristics of washed spermatozoa of Berari buck

i. Individual sperm motility

The mean percent individual motility of washed buck spermatozoa in different dilutors at different stages of preservation are shown in Table 4.9. The effect of dilutor stages of preservation was found to be highly significant ($P<0.01$). The values of percent motility decreased significantly at different stages in both dilutors.

Table 4.9. Individual Motility percent (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	75.00 ± 0.81	69.86 ± 0.90	72.43^{I} ± 2.57
TCFEY	73.06 ± 1.90	68.19 ± 1.19	70.63^{II} ± 2.43
Mean ± SE for stages	74.03^{A} ± 0.97	69.03^{B} ± 0.83	

Mean bearing different superscript differ significantly

Table 4.9A. ANOVA for Individual Motility of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	69.41	1.95	
Stage	1	1800.00	50.69**	1.38
Dilutor	1	234.72	6.61**	1.38
Stage × Dilutor	1	1.39	0.04^{NS}	1.95
Error	213	35.51	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The individual sperm motility (percent) during preservation at Pre-freeze and Post-freeze stages in SM dilutor were 75.00 ± 0.81, and 69.86 ± 0.90 respectively. Whereas, the corresponding values for the TCFEY dilutor were 73.06 ± 1.90 and 68.19 ± 1.19, respectively. The sperm motility was higher initially and reduced gradually during preservation in both the dilutors.

The overall means of percent motility in the semen dilutors in present study revealed that the best motility was in skim milk dilutor whereas; the lower motility was observed in TCFEY dilutor. The interaction between

buck and dilutor, buck and stage and dilutor and stage were found to be statistically non-significant (Table 4.9A).

The effect of stages of preservation was found to be significant. The values of percent motility decreased significantly at each interval of 12 hours. In close agreement to present study Islam and Ahmed (2003), Islam *et al.* (2006), opined that the sperm motility decreased significantly with the increase in storage period during refrigeration preservation. The sperm motility was higher initially and reduced gradually during preservation in both the dilutors. An estimation of critical difference in the overall means of percent motility between the two semen dilutors under study revealed that the best motility was for skim milk dilutor whereas lower motility was in TCFEY dilutor at each stage of evaluation. A significant difference was reported between two dilutors. The observations found in this study are in agreement with that of Salvador *et al.* (2006), Parmar *et al.* (2012) who also reported the significant difference between extenders at 4-5°C of preservation.

In controversy to this study Deka and Rao (1985) reported that the percentage of motile sperm in frozen semen did not vary significantly between TEYCAFG and SMEYFG dilutors. Similar to present findings they also reported the higher percentage of motile sperm in SMEYFG than in EYCFG dilutor.

In present group dilution of semen was carried out after washing of semen and sperm motility in SM and TCFEY dilutor at 24 hrs was found as 69.86 % and 68.19 %. Islam and Ahmed (2003) reported 69.58 % sperm motility in washed semen samples at 72 hrs of preservation which is in concurrence with result of present study. Islam *et al.* (2006)also reported higher value 85.8% and 78.3% of mean percentage motile sperm in Tris extender at 0 hrs and 24hrs of preservation at 5°C.Parmar *et al.* (2012) reported sperm motility in SM and TCFEY dilutor at 24 hrs was found as 76.95 % and 75.58% in washed semen samples at 24 hrs of preservation which is slightly higher than result of present study.

ii. Live sperm count

The mean percent live sperm of washed semen for different dilutors and stages are shown in Table 10.

Table 4.10. Live Sperm percent (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	79.65 ± 0.80	75.33 ± 0.72	77.49^{II} ± 2.16
TCFEY	81.15 ± 0.29	76.60 ± 1.00	78.88^{I} ± 2.28
Mean ± SE for stages	80.40^{A} ± 0.75	75.97^{B} ± 0.63	

Mean bearing different superscript differ significantly

Table 4.10A. ANOVA for Live Sperm of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	18.19	1.67	
Stage	1	1417.78	130.08**	0.76
Dilutor	1	137.50	12.62**	0.76
Stage × Dilutor	1	1.00	0.09^{NS}	1.08
Error	213	10.90	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of live sperm at pre-freeze and post freeze stage was 79.65 ± 0.80 and 75.33 ± 0.72 in SM dilutor and 81.15 ± 0.29 and 76.60 ± 1.00 in TCFEY dilutor, respectively. The percent live sperm count was declinined at each stage of preservation.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between dilutors and stages of preservation. The stage X dilutor interaction was non-significant (Table 4.10A).

The percent live sperm count was decline regularly at each stage of preservation in both diluent. Misra *et al.* (1993) and Islam *et al*. (2006) also reported that the live sperm count decreased significantly with increase in storage period during preservation. Whereas, a significant variation was observed among the two dilutors for live sperm count, being apparently higher in SM dilutor than TCFEY dilutor. In support of present observation Parmar *et al.* (2012) observed significant difference between two dilutors and the percentage of live spermatozoa in milk dilutor remained at a significantly higher level at all periods of refrigeration preservation than tris dilutor.

Salvador *et al.* (2006) recoded live sperm count 73% and 60% at 0 hrs and 24 hrs in SM dilutor during storage at 5°C which is slightly lower than the result of present study. Parmar *et al.*(2012) found 80.02 % and 78.92% in SM and TCFEY dilutor at 5°C are in agreement with present study reports . While much higher values i.e 89.7 % and 86.8% at 0 hrs and 24 hrs in washed semen was found by Islam *et al*. (2006) both in 20 % egg yolk containing tris dilutor. Similarly, Islam and Ahmed (2003) also recorded 89.67 % and 83.00 % live spermatozoa at 0 hrs and 72 hrs in washed semen at 5°C, preservation with tris diluent. Much lower values were reported by Misra *et al.* (1993) 59.98 % at 72 hrs in 20 % egg yolk containing tris dilutor.

iii. Abnormal sperm count

The mean percent morphological abnormalities of washed semen for different dilutors and stages are shown in Table 4.11.

Table 4.11. Abnormal Sperms percent (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	5.47^{c}± 0.16	6.01^{b}± 0.43	5.74^{II} ± 0.27
TCFEY	5.99^{b}± 0.24	7.17^{a}± 0.30	6.58^{I} ± 0.58
Mean ± SE for stages	5.73^{B} ± 0.26	6.59^{A} ± 0.58	

Mean bearing different superscript differ significantly

Table 4.11A. ANOVA for Abnormal Sperm of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	1.97	2.39	
Stage	1	53.39	64.70**	0.21
Dilutor	1	50.00	60.59**	0.21
Stage × Dilutor	1	7.35	8.90**	0.30
Error	213	0.83	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of abnormal sperms at pre-freeze and post freeze stages was 5.47 ± 0.16 and 6.01 ± 0.43 in SM dilutor and 5.99 ± 0.24 and 7.17 ± 0.30 in TCFEY dilutor, respectively. The values of percent live sperm count decreased significantly at subsequent stage of preservation in both dilutors.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation, different dilutors and stage X dilutor interaction. (Table 4.11A).

Stage of preservation had also a marked effect on the mean percent morphological abnormalities. The abnormal sperm count was lower initially and increased gradually during stages of preservation. Parmar *et al.* (2012) also found that the sperm abnormalities increased significantly at each interval of 12 hours in both dilutors.

Parmar *et al.* (2012) recorded percent sperm abnormalities 7.96 % and 9.25% at 24 hrs of refrigeration storage in SM and TCFEY diluent, whereas in present study sperm abnormalities at 24 hrs of preservation were recorded as 6.01 percent in SM dilutor and 7.17 percent in TCFEY dilutor, respectively. Simon and Vijaykumaran (2003) also reported much lower values (4.74 %) at 48 hrs of preservation than present record.

b) Sperm function tests of washed spermatozoa of Berari buck

i. Damaged acrosome percentage

The mean percent of damaged acrosome of washed buck spermatozoa for different dilutors and stages are shown in Table 4.12.

Table 4.12. Damaged Acrosome percent (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	7.07 ± 0.46	9.18 ± 0.27	8.13^{II} ± 1.06
TCFEY	8.04 ± 0.40	10.50 ± 0.24	9.27^{I} ± 1.23
Mean ± SE for stages	7.56^{B} ± 0.49	9.84^{A} ± 0.66	

Mean bearing different superscript differ significantly

Table 4.12A. ANOVA for Damaged Acrosome of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	2.33	1.66	
Stage	1	375.84	267.99**	0.27
Dilutor	1	94.53	67.41**	0.27
Stage ×Dilutor	1	2.17	1.55^{NS}	0.39
Error	213	1.40	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percent of damaged acrosome at pre-freeze and post freeze stage was 7.07 ± 0.46 and 9.18 ± 0.27 in SM dilutor and 8.04 ± 0.40 and 10.50 ± 0.24 in TCFEY dilutor, respectively. The values of percent damaged acrosome were increasing significantly (P<0.05) at stage of preservation.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation and different dilutors. The stage X dilutor interaction was non-significant (Table 4.12A).

Perusal of data on refrigeration preservation of washed buck semen revealed that damaged acrosome percentage increased significantly as the stages of preservation progressed, which is in agreement with Raval and Dhami (2007),Singh *et al* (1993).The values of intatact acrosome in washed semen reported by Islam (2006) were 85.0 % and 83.2% at 0 hrs and 24 hrs both in 20 % egg yolk containing tris dilutor. Singh *et al* (1993) reported 88.76 % in prefreez to 68.90% in post thaw semen in TRIS diluter.

Washing of sperm are known to cause damage to the plasma membrane and the acrosome (Jones and Holt, 1974; Lenz *et al.*, 1977). Mathur (2003) concluded that irrespective of thaw temperature, percentage of intact

acrosome was not affected by extenders which is not in relation with observation of present study.

ii. Hypo-osmatic swelling test

The mean percent of actual HOST reacted washed buck spermatozoa during pre-freeze and post-freeze stages of preservation in different dilutors are shown in Table 13.

Table 4.13. HOST percent (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	72.50 ± 0.41	55.17 ± 1.02	63.83^{I} ± 8.67
TCFEY	70.21 ± 0.35	54.17 ± 0.87	62.19^{II} ± 8.02
Mean ± SE for stages	71.35^{A} ± 1.15	54.67^{B} ± 0.50	

Mean bearing different superscript differ significantly

Table 4.13A. ANOVA for HOST of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	1.79	1.02	
Stage	1	20050.03	2320.81**	0.68
Dilutor	1	195.03	22.58**	0.68
Stage × Dilutor	1	30.03	3.48^{NS}	0.96
Error	213	8.64	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of HOST positive spermatozoa at pre-freeze and post freeze stage was 72.50 ± 0.41 and 55.17 ± 1.02 in SM dilutor and 70.21 ± 0.35 and 54.17 ± 0.87 in TCFEY dilutor, respectively. The values of percent HOST reacted spermatozoa decreased significantly (P<0.01) at stage of preservation.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation and both dilutors. The stage X dilutor interaction was non-significant (Table 4.13A).

The membrane integrity (HOST) was higher initially and reduced gradually during preservation in both the dilutors. An estimation of critical difference between the overall means of membrane integrity (HOST)between the two semen dilutors under study revealed that the best membrane integrity (HOST) was for skim milk dilutor whereas; lower motility was in TCFEY dilutor at each stage of evaluation. A significant difference was reported between two dilutors. The observations found in this study are in agreement with that of Salvador *et al.* (2006) who reported the significant different between extenders at 5°C of preservation.

Compared to result of present study Salvador *et al.* (2006) reported the less percentage of membrane integrity (HOST) 48% and 30 % in SM dilutor at 0 hrs and 24 hrs during preservation at 5°C. However, Salvador *et al.* (2006) in Murciano-Granadina bucks observed 37 % HOS positive sperm percentage in washed semen stored at 5°C, which was much lower than present observations and reported that washing of semen might be affecting the plasma membrane integrity.

iii. Leakage of enzymes

The overall mean activity of AST enzyme of washed buck spermatozoa observed was 80.88 ± 1.26 and 139.57 ± 1.78 IU/L in SM dilutor. 86.03 ± 1.36 and 153.64 ± 1.16 IU/L in TCFEY dilutor at pre-freeze and post-freeze stages respectively.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of preservation, dilutors, stage X dilutor interaction. (Table 4.14A and 4.15A).

At refrigerator temperature (4 to 8°C), the sperms are exposed to cold shock and the leakage of transaminases, consequent upon membrane damage was evident. The precise mechanism of cellular damage is unknown, but presumably changes occur at unequal rates on the surface and internal portions of spermatozoa during cooling and both physical and chemical damages result (Salisbury and Van Denmark *et al.*,1978). Higher and lower values for transaminase activity in different extenders have been reported to reflect the protective properties of the ingredients added to the extenders and the inherent quality of the spermatozoa to with stand cooling (Bhatt and Chauhan, 1984).

Table 4.14. AST (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	AST (IU/L) at Pre-freeze stage	AST (IU/L) at Post-freeze stage	Mean ± SE for Dilutors
SM	80.88^{d} ± 1.26	139.57^{b} ± 1.78	110.22^{II} ± 29.35
TCFEY	86.03^{c} ± 1.36	153.64^{a} ± 1.16	119.83^{I} ± 33.81
Mean ± SE for stages	83.45^{B} ± 2.58	146.60^{A} ± 7.03	

Mean bearing different superscript differ significantly

Table 4.14A. ANOVA for AST of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	26.96	1.50	
Stage	1	287155.68	16001.90**	0.98
Dilutor	1	6650.89	370.62**	0.98
Stage × Dilutor	1	1431.13	79.75**	1.38
Error	213	17.95	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Table 4.15. ALT (Mean ± SE) of Washed Spermatozoa at pre-freeze and post - freeze stages in refrigerated group of Berari buck

Stage of Freezing / Dilutors	ALT (IU/L) at Pre-freeze stage	ALT (IU/L) at Post-freeze stage	Mean ± SE for Dilutors
SM	$19.21^{d} \pm 0.27$	$24.85^{b} \pm 0.70$	$22.03^{II} \pm 2.82$
TCFEY	$20.08^{c} \pm 0.20$	$29.07^{a} \pm 1.05$	$24.58^{I} \pm 4.49$
Mean ± SE for stages	$19.65^{B} \pm 0.44$	$26.96^{A} \pm 2.11$	

Mean bearing different superscript differ significantly

Table 4.15A. ANOVA for ALT of Washed Semen of Berari buck during refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	7.94	1.37	
Stage	1	3850.03	666.78**	0.56
Dilutor	1	467.67	80.99**	0.56
Stage × Dilutor	1	201.67	34.93**	0.79
Error	213	5.77	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

A significant difference found at different stages of refrigeration preservation as well as dilutor X stage interaction observed in present study is in harmony to the findings of Parmar *et al.* (2012).

The AST activity was lower initially and increased gradually during preservation. Leakage of AST in SM dilutor was significantly lower than TCFEY dilutor at stage of preservation. The SM dilutor showed the lower mean value of AST whereas, TCFEY dilutor showed the higher mean value of AST in the present study.

The differences in the values of AST activity in seminal plasma samples diluted and preserved at 0 and 24 hours were significant for two dilutors studied. Parmar *et al.* (2012) found AST level as 81.71± 0.35 IU/ L and 97.25 ± 1.10 IU/L at 24 hrs of refrigeration preservation in SM and TCFEY dilutor. Whereas, in present investigation such values were recorded to be 139.57 ± 1.78 IU/ L and 153.64 ± 1.16 IU/L at 24 hrs stage in same dilutor, respectively.

The overall mean activity of ALT enzyme of washed buck spermatozoa observed was 19.21 ± 0.27 and 24.85 ± 0.70 IU/L in SM dilutor. 20.08 ± 0.20 and 29.07 ± 1.05IU/L in TCFEY dilutor in pre-freeze and post-freeze stages, respectively. The value of AST and ALT enzyme leakage increased significantly ($P<0.01$) at each stage of preservation (Table 4.14 and 4.15), respectively.

Leakage of ALT was significantly increased in both the dilutors as period of preservation increased. A significant variation was observed among the two semen dilutors for ALT activity, being apparently lower in SM dilutor followed by TCFEY dilutor. In present investigation, values of ALT activity were recorded as 24.85 ± 0.70 IU/ L and 29.07 ± 1. 05 IU/L at 24 hrs stage in SM and TCFEY dilutor, respectively. Whereas, Parmar *et al.* (2012) had found ALT level as 41.38 ± 0.82 IU/ L and 47.23 ± 0.83 IU/L at 24 hrs of refrigeration preservation in same dilutor which are higher than results of present study.

The plasma membrane integrity of washed buck spermatozoa was detoriated significantly at different stages of preservation as detected by leakage of enzymatic constituents in seminal plasma.

4.3.2 Refrigeration of unwashed spermatozoa of Berari buck

a) Physical characteristics of unwashed spermatozoa of Berari buck

i. Individual Sperm motility

The mean percent individual sperm motility during pre-freeze and post –freeze stages of preservation in SM and TCFEY dilutors are shown in Table 16.

Table 4.16. Individual Motility percent (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post - freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	71.11 ± 2.44	67.08 ± 1.13	69.10^{I} ± 2.01
TCFEY	70.14 ± 1.79	65.28 ± 0.70	67.71^{II} ± 2.43
Mean ± SE for stages	70.63^{A} ± 0.49	66.18^{B} ± 0.90	

Mean bearing different superscript differ significantly

Table 4.16A. ANOVA for Individual Motility of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	65.71	1.99	
Stage	1	1422.22	43.11**	1.33
Dilutor	1	138.89	4.21*	1.33
Stage × Dilutor	1	12.50	0.38^{NS}	1.88
Error	213	32.99	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of individual sperm motility at pre-freeze and post freeze stage was 71.11 ± 2.44 and 67.08 ± 1.13 in SM dilutor and 70.14 ± 1.79 and 65.28 ± 0.70 in TCFEY dilutor, respectively. The values of percent individual motility decreased significantly at each stage of preservation.

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of preservation and significant ($P<0.05$) difference between dilutors. The stage X dilutor interaction was non-significant (Table 4.16A).

The effect of stages of preservation was found to be significant. The values of percent motility decreased significantly at each interval of 12 hours is in close agreement to present study, Kulaksiz *et al.* (2012), Benmoula *et al.* (2018) and Rahman *et al.* (2018) also opined that the sperm motility decreased significantly with the increase in storage period during refrigeration preservation.

The sperm motility was higher initially and reduced gradually during preservation in both the dilutors. An estimation of critical difference between the overall means of percent motility between the two semen dilutors under study revealed that the best motility was for Skim milk dilutor whereas; lower motility was in TCFEY dilutor at each stage of evaluation. A significant difference was reported between two dilutors. The observations found in this study are in agreement with that of Benmoula *et al.* (2018), Rahman *et al.* (2018), Olurode and Ajala (2016) and Kulaksiz *et al.* (2012) who also reported the significant different between extenders at 4-5°C of preservation. Kulaksiz *et al.* (2012) reported that milk extender is better than tris, sodium citrate and Bioxcell ® extenders.

Authors like Stefanov *et al.* (2015), Bohlooli *et al.* (2012), Paulenz *et al.* (2002) reported that extender components affect the progressive motility and other characteristics of the spermatozoa. Gundogan *et al.* (2011) opined that major differences in the sperm motility were raised due to qualified components of the extender especially the lipoproteins, glucose and

egg yolk which had positive impact on the spermatozoa. The present findings noted the superiority of milk dilutor over egg yolk citrate for preservation of buck semen.

However, Shamsuddin *et al.* (2000) and Ferdiand *et al.* (2012) recorded higher motility when buck semen was preserved in Tris- Fructose-egg yolk citrate and Glucose-citrate-egg yolk than in skim milk dilutor. Rasteh and Divandi (2015) reported that the higher percentage of motility and progressive motility were observed in Tris-citric acid extender compared to sodium citrate and skim milk based extender. Rather *et al.* (2017) also noted contrary findings and found that Tris citric acid fructose egg yolk was the best in maintaining the quality of ejaculated ram spermatozoa during preservation for 72 h at 4°C.

In present group, dilution of semen was carried out after unwashing of semen and sperm motility in SM and TCFEY dilutor at 24 hrs was found as 67.08 % and 65.28 %. Similarly, Rahman *et al.* (2018) reported 61.8 % and 58.8 % and Benmoula *et al.* (2018) reported 61.78 % and 36.96 % sperm motility in SM and TCFEY dilutor at 24 hrs respectively in unwashed semen samples which is slightly lower than the result of present study. However Ranjan *et al.* (2009) observed higher value of 69.17 % in tris. Meyers (2005) pointed the reason for motility decline that the osmotic regulation in lower temperatures affects motility and intact membrane of the spermatozoa. Therefore the significant difference in motility and acrosome defect percentage between the extenders may due to the difference in osmotic regulation capacity of milk and Tris-citrate. Rota *et al.* (2001) reported that skim milk with egg yolk showed better post-thaw semen parameters compared to tris based buffer, may be due to caseins, the proteins of milk and appear to be responsible for the protective effect of milk ions, semen pH and may also chelate any heavy metal.

ii. Percentage of live spermatozoa

The mean percent of live sperm of unwashed buck spermatozoa for different dilutors and stages are shown in Table 17.

Table 4.17. Live Sperm percent (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post - freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	76.99 ± 1.06	71.44 ± 0.55	74.22^{I} ± 2.77
TCFEY	75.28 ± 0.70	70.28 ± 0.90	72.78^{II} ± 2.50
Mean ± SE for stages	76.13^{A} ± 0.85	70.86^{B} ± 0.58	

Mean bearing different superscript differ significantly

Table 4.17A. ANOVA for Live Sperm of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	20.10	1.84	
Stage	1	2000.28	182.63**	0.76
Dilutor	1	148.78	13.59**	0.76
Stage × Dilutor	1	5.28	0.48^{NS}	1.08
Error	213	10.95	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of live sperm at pre-freeze and post freeze stage was 76.99 ±1.06 and 71.44 ± 0.55 in SM dilutor and 75.28 ± 0.70 and 70.28 ± 0.90 in TCFEY dilutor, respectively. The values of percent individual motility decreased significantly at each stage of preservation.

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation and dilutors. The stage X dilutor interaction was non-significant (Table 4.17A).

The percent live sperm count was declined at each stage of preservation in both diluent. Takarkhede *et al.* (2005) also reported that the live sperm count decreased significantly with increase in storage period during preservation. Whereas, a non-significant variation was observed among the percentage of live spermatozoa in two dilutors and stages for live sperm count, being apparently higher in SM dilutor than TCFEY dilutor which is in support to observations of present study Rahman *et al.* (2018) observed non-significant difference between the percentage of live spermatozoa in two dilutors at 0 hrs and 24 hrs of refrigeration preservation.

Rahman *et al.* (2018) reported the percentage of live spermatozoa 77.8 and 64.4 in SM dilutor and 75.0 and 62.8 in Tris at 0 hrs and 24 hrs during preservation at 4°C. The present findings of superiority of skim milk dilutor over egg yolk citrate for preservation of buck semen is in concurrence with Rahman *et al.* (2018) and contrary with Rasteh and Divandi (2015) who reported mean live sperm percent 63 % and 78.3 % in SM and Tris dilutor respectively.

Higher values of live sperm spermatozoa i.e 88.0 % and 65.17% at 0 hrs and 72 hrs of preservation with tris diluent in unwashed semen at 5°C were noted by Islam and Ahmed (2003) and 86.17 % in tris was noted by Ranjan *et al.* (2009). Albiaty *et al.*(2016) reported 14.60 % dead sperm in Milk dilutor and 14.12 % in Tris dilutor.

iii. Abnormal sperm Percentage

The mean percent of abnormal sperm of unwashed buck spermatozoa for different dilutors and stages are shown in Table 4.18.

The percentage of abnormal sperm at pre-freeze and post freeze stage was 6.61 ± 0.06 and 15.68 ± 0.09 in SM dilutor and 8.07 ± 0.19 and 17.07 ± 0.12 in TCFEY dilutor, respectively. The values of percent abnormal sperm increased significantly at each stage of preservation.

Table 4.18. Abnormal Sperms percent (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post - freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	6.61± 0.06	15.68 ± 0.09	11.15^{II} ± 4.53
TCFEY	8.07± 0.19	17.07 ± 0.12	12.57^{I} ± 4.50
Mean ± SE for stages	7.34^{B} ± 0.73	16.38^{A} ± 0.69	

Mean bearing different superscript differ significantly

Table 4.18A. ANOVA for Abnormal Sperm of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	0.72	1.47	
Stage	1	5877.09	11961.25**	0.16
Dilutor	1	145.92	296.99**	0.16
Stage × Dilutor	1	0.09	0.18^{NS}	0.23
Error	213	0.49	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between dilutors and stages of preservation. The stage X dilutor interaction was non-significant (Table 4.18A).

Stage of preservation had also a marked effect on the mean percent morphological abnormalities. The abnormal sperm count was lower initially and increase gradually during stages of preservation.

Takarkhede *et al.* (2005) recorded 4.67% and 11.5 % sperm abnormalities at 0 hrs and 96 hrs of refrigeration storage in Tris diluent

respectively and reported sperm abnormalities increased significantly at each interval of 12 hrs in Tris dilutor.

Simon and Vijarykumar (2003) reported much lower values (4.74 %) at 48 hrs of preservation than present record.

b) Sperm function tests of unwashed spermatozoa of Berari buck

i. Damaged acrosome percentage

The mean percent of damaged acrosome of unwashed buck spermatozoa for different dilutors and stages are shown in Table 4.19.

Table 4.19. Damaged Acrosome percent (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post - freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	$4.90^{d} \pm 0.08$	$9.68^{b} \pm 0.11$	$7.29^{II} \pm 2.39$
TCFEY	$6.90^{c} \pm 0.16$	$10.83^{a} \pm 0.06$	$8.87^{I} \pm 1.97$
Mean ± SE for stages	$5.90^{B} \pm 1.00$	$10.26^{A} \pm 0.58$	

Mean bearing different superscript differ significantly

Table 4.19A. ANOVA for Damaged Acrosome of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	2.50	1.53	
Stage	1	1365.03	833.39**	0.29
Dilutor	1	178.92	109.24**	0.29
Stage × Dilutor	1	12.92	7.89**	0.42
Error	213	1.64	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of damaged acrosome at pre-freeze and post freeze stage was 4.90 ± 0.08 and 9.68 ± 0.11 in SM dilutor and 6.90 ± 0.16 and 10.83 ± 0.06 in TCFEY dilutor, respectively. The values of percent individual motility increased significantly at each stage of preservation. (Table 4.19).

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between dilutors, stages of preservation and stage X dilutor interaction. (Table 4.19A).

It is noted in the present investigation that stage of preservation had also a marked effect on the mean damaged acrosome percentage. The damaged acrosome percentage was lower initially and increased gradually during stages of preservation. These observations are also in agreement with Albiaty *et al.* (2016) and recorded damaged acrosome percentage 11.47 % and 8.95% at refrigeration storage in SM and TCFEY diluent. They further reported the damaged acrosome percentage 8.16 % and 10.25% at 0 hrs and 24 hrs of refrigeration storage in TCFEY dilutor. El-kon *et al.* (2010) recorded 91.00 % intact acrosome in Damascus bucks semen after dilution. The acrosome intactness observed by Eiman and Takato (2004) in Japanese buck spermatozoa was 78.0 percent. Variation in damaged acrosome percent in buck semen may be due to breeds, species, dilutors, age of bucks etc.

ii. Hypo-osmatic swelling test

The mean percent of actual HOST reacted unwashed buck spermatozoa during pre-freeze and post-freeze stages of preservation in different dilutors are shown in Table 4.20.

The percentage of HOST positive spermatozoa at pre-freeze and post freeze stage was 69.54 ± 0.79 and 53.53 ± 0.94 in SM dilutor and 64.93 ± 0.60 and 52.25 ± 0.44 in TCFEY dilutor, respectively. The values of percent HOST reacted spermatozoa decreased significantly (P<0.01) at each stage of preservation. (Table 4.20)

Table 4.20. HOST percent (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post - freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	Pre-freeze	Post-freeze	Mean ± SE for Dilutors
SM	$69.54^{a} \pm 0.79$	$53.53^{c} \pm 0.94$	$61.54^{I} \pm 8.01$
TCFEY	$64.93^{b} \pm 0.60$	$52.25^{d} \pm 0.44$	$58.59^{II} \pm 6.34$
Mean ± SE for stages	$67.24^{A} \pm 2.31$	$52.89^{B} \pm 0.64$	

Mean bearing different superscript differ significantly

Table 4.20A. ANOVA for HOST of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	22.45	2.50	
Stage	1	14820.68	1650.97**	0.69
Dilutor	1	624.22	69.54**	0.62
Stage × Dilutor	1	200.00	22.28**	0.98
Error	213	8.98	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between dilutors, stages of preservation and stage X dilutor interaction (Table 4.20A).

Compared to result of present investigation Salvador *et al.* (2006) in Murciano-Granadina bucks observed 28 % HOS positive sperm percentage in unwashed semen stored at 5^{0}C, which was much lower than present observations. Washing of semen might be affecting the plasma membrane integrity. (Salvador *et al.*, 2006)

The membrane integrity (HOST) was higher initially and reduced gradually during preservation in both the dilutors. An estimation of critical difference between the overall means of membrane integrity (HOST)between the two semen dilutors under study revealed that the best membrane integrity (HOST) was observed in skim milk dilutor whereas; lower motility was in TCFEY dilutor at each stage of evaluation. A significant difference was reported between two dilutors. The observations found in this study are in agreement with that of Salvador *et al.* (2006) reported the significant difference between extenders at 5°C of preservation.

iii. Leakage of enzymes

The overall mean activity of AST enzyme of unwashed buck spermatozoa observed was 77.79 ± 1.33 and 159.72 ± 1.37 IU/L in SM dilutor while 72.58 ± 1.44 and 167.57 ± 0.93 IU/L in TCFEY dilutor in pre-freeze and post-freeze stages respectively.

A significant difference found at different stages of refrigeration preservation in present study was also in harmony to the findings of Singh *et al.* (1993).

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between both stages of preservation, dilutors and stage X dilutor interaction (Table 4.21A and 4.22A).

Table 4.21. AST (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	AST (IU/L) at Pre-freeze stage	AST (IU/L) at Post-freeze stage	Mean ± SE for Dilutors
SM	77.94^{c} ± 1.33	159.72^{b} ±1.37	118.83^{II} ± 40.89
TCFEY	72.58^{d} ± 1.44	167.57^{a} ± 0.93	120.08^{I} ± 47.49
Mean ± SE for stages	75.26^{B} ± 2.68	163.65^{A} ± 3.92	

Mean bearing different superscript differ significantly

Table 4.21A. ANOVA for AST of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	49.16	3.21	
Stage	1	562418.50	36733.32**	0.90
Dilutor	1	111.25	7.27**	0.90
Stage × Dilutor	1	3140.28	205.10**	1.28
Error	213	15.31	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Table 4.22. ALT (Mean ± SE) of Unwashed Spermatozoa at pre-freeze and post - freeze stages in Refrigeration Group of Berari buck

Stage of Freezing / Dilutors	ALT (IU/L) at Pre-freeze stage	ALT (IU/L) at Post-freeze stage	Mean ± SE for Dilutors
SM	$16.54^{d} \pm 0.91$	$32.38^{b} \pm 0.84$	$24.46^{II} \pm 7.92$
TCFEY	$17.61^{c} \pm 0.89$	$41.15^{a} \pm 0.46$	$29.38^{I} \pm 11.77$
Mean ± SE for stages	$17.08^{B} \pm 0.53$	$36.76^{A} \pm 4.39$	

Mean bearing different superscript differ significantly

Table 4.22A. ANOVA for ALT of Unwashed Semen of Berari buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	33.37	2.70	
Stage	1	27907.03	2253.49**	0.81
Dilutor	1	1745.42	140.94**	0.81
Stage × Dilutor	1	1069.53	86.37**	1.15
Error	213	12.38	-	
Total	287	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The AST activity was lower initially and increased gradually during preservation. Leakage of AST in SM dilutor was significantly lower than TCFEY dilutor at stage of preservation. The SM dilutor showed the lower mean value of AST whereas, TCFEY dilutor showed the higher mean value of AST.

The differences in the values of AST activity in seminal plasma samples diluted and preserved at 0 and 24 hours were significant for two dilutors studied. Singh *et al.* (1993) had found AST level as 21.27 and 26.58 µmole/litre at 24 hrs of refrigeration preservation in TEYFRAC and EYECEF dilutor. Whereas, in present investigation such values were recorded to be 139.5 IU/ L and 153.64 IU/L at 24 hrs stage in SM and TCFEY dilutor, respectively. At the above stage of preservation Sinha *et al.* (1988) found the AST values in Tris diluent as 39.39, 27.96 and 28.63µmole/litre in Black Bengal, Jamnapari and Barbari bucks. Similar to the findings of Singh *et al.* (1993) in present study dilutor × stage of preservation interaction were significant.

The overall mean activity of ALT enzyme of unwashed buck spermatozoa observed was 16.54 ± 0.91 and 32.38 ± 0.84 IU/L in SM dilutor while 17.61 ± 0.89 and 41.15 ± 0.46 IU/L in TCFEY dilutor in pre-freeze and post-freeze stages, respectively. The value of AST and ALT enzyme leakage increased significantly ($P<0.01$) at each stage of preservation (Table 4.21 and 4.22) respectively.

Similar to the result of present findings Singh *et al.* (1993) also found significant difference between dilutors as well as stages of preservation. The dilutor × stage interaction was found to be significant. Similar findings were also reported by Singh *et al.* (1993).

Leakage of ALT was significantly increased in both the dilutors as period of preservation increase. A significant variation was observed among the two semen dilutors for ALT activity, being apparently lower in SM dilutor followed by TCFEY dilutor. Singh *et al.* (1993) had found ALT level as 2.98 ± 0.29 and 5.86 ± 0.52 µmole/liter at 24 hrs of refrigeration preservation in

TEYFRAC and EYECEF dilutor. At the above stage of preservation Sinha *et al.* (1988) found the AST values in Tris diluent as 26.81 ± 1.95, 17.10 ± 0.87 and 18.54 ± 1.05 µmole/litre in Black Bengal, Jamnapari and Barbari bucks.

The plasma membrane integrity of unwashed buck spermatozoa was detoriated significantly at different stages of preservation as detected by leakage of enzymatic constituents in seminal plasma in the present study.

4.3.3 Comparison between washed and unwashed refrigerated buck spermatozoa

Comparison between washed and unwashed refrigerated buck spermatozoa were done to judge the effect of washing on pre-freeze and post-freeze quality of Berari buck spermatozoa during refrigeration.

a) Physical Characteristics of washed and unwashed refrigerated buck spermatozoa

i. Individual sperm motility

The mean percent individual sperm motility of washed and unwashed buck spermatozoa during pre-freeze and post –freeze stages of preservation in SM and TCFEY dilutors are shown in Table 23 and results have been graphically presented in Fig. 4.1.

Data pertaining to preservation of washed and unwashed buck spermatozoa showed non significantly higher sperm motility (75.00 ± 0.81 and 69.86 ± 0.90) at pre freeze and post freeze stage in washed semen, as compared to the corresponding values of unwashed spermatozoa (71.11 ± 2.44 and 67.08 ± 1.13) in SM dilutor. Higher sperm motility (73.06 ± 1.90 and 68.19 ± 1.19) at pre-freeze and post-freeze stage in washed semen as compared to corresponding values of unwashed spermatozoa (70.14 ± 1.79 and 65.28 ± 0.70) in TCFEY dilutor but the difference was non-significant.

Table 4.23. Individual Motility percent (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	Pre-freeze		Post-freeze		Mean ± SE for Dilutors	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed		
SM	75.00 ± 0.81	71.11 ± 2.44	69.86 ± 0.90	67.08 ± 1.13	**SM** 70.76^{I} ± 1.64	**Washed** 71.53^{i} ± 1.54
TCFEY	73.06 ± 1.90	70.14 ± 1.79	68.19 ± 1.19	65.28 ± 0.70	**TCFEY** 69.17^{II} ± 1.64	**Unwashed** 68.40^{ii} ± 0.90
Mean ± SE for Stages	72.33^{A} ± 1.08		67.60^{B} ± 0.96			

Mean bearing different superscript differ significantly

Table 4.23A. ANOVA for Individual Motility of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	103.86		
Stage	1	3211.11	94.94**	0.95
Condition	1	1406.25	41.58**	0.95
Dilutor	1	367.36	10.86**	0.95
Stage X Condition	1	11.11	0.33^{NS}	1.34
Stage X Dilutor	1	2.78	0.08^{NS}	1.34
Condition X Dilutor	1	6.25	0.19^{NS}	1.34
Stage X Condition X Dilutor	1	11.11	0.33^{NS}	1.90
Error	497	33.82	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The analysis of variance for the individual sperm motility percentage of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between washed and unwashed spermatozoa at each stage of preservation, washing condition, and dilutors. A non-significant difference was observed between stage X condition X dilutor interaction (Table 4.23A).

Some enzymes in seminal plasma, originating from bulbourethral gland secretion, catalyse the hydrolysation of egg yolk lecithin and milk triglycerides of the extender, releasing sperm toxic substances (lysolecithin and fatty acids) that lead to sperm damage (Roy, 1957; Pellicer Rubio and Combamous, 1998). The improvement in sperm quality in the present study indicated the beneficial effects of separating sperm by centrifugation from deleterious effects of coagulating enzyme such as phospholipase A from bulbourethral gland before dilution and preservation. In addition, different findings indicated that removal of the seminal fluid of chilled semen increases the sperm survival and motility (Kozdrowski *et al.*, 2007).

Goat semen differs from other mammalian species due to the presence of enzymes in the seminal plasma that react with egg yolk and milk, resulting in damaged spermatozoa following production of toxic compounds (Pellicer Rubio and Combarnous, 1998). Furthermore, the lifespan of stored sperm is increased by centrifugation (Pellicer Rubio *et al.*, 1997). Centrifugation gave optimal sperm motility in equine as found by Waite *et al.* (2008) supporting thus the results of the present study. The results in present study are further supported by the work of Marti *et al.* (2006) who showed that centrifugation yielded higher percentages of capacitated spermatozoa.

Islam *et al.* (2006) reported beneficial effect of removal of seminal plasma on the quality of goat semen at 5^0C in a 20% egg-yolk-containing Tris extender. On the contrary, statistical differences in the semen quality, either in the presence or absence of seminal plasma at 5^0C, in either

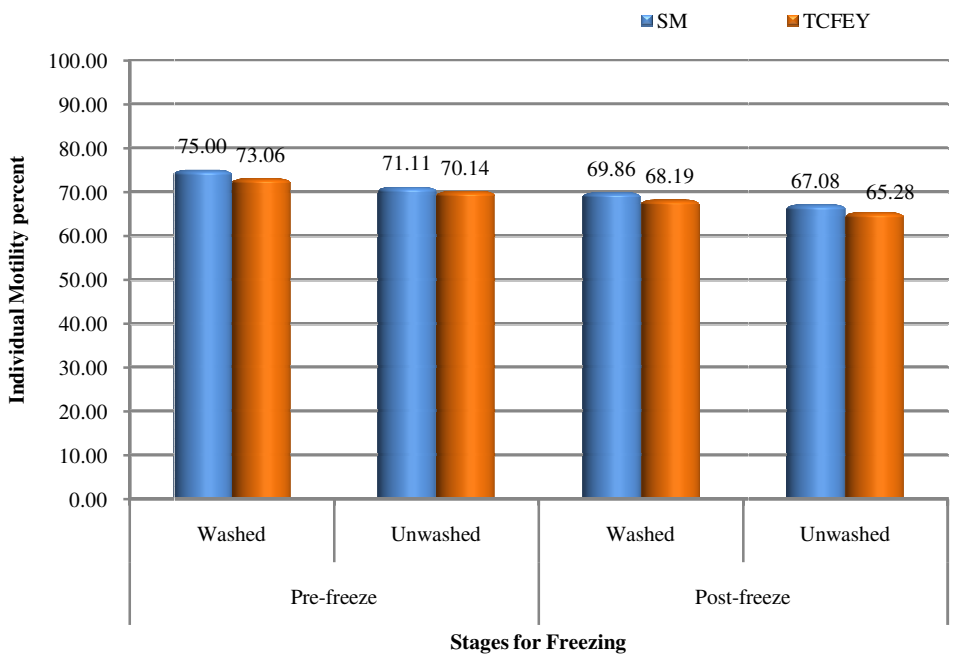

Fig. 4.1. Individual Motility percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor spaces at pre-freeze and post-freeze stages in Refrigeration group

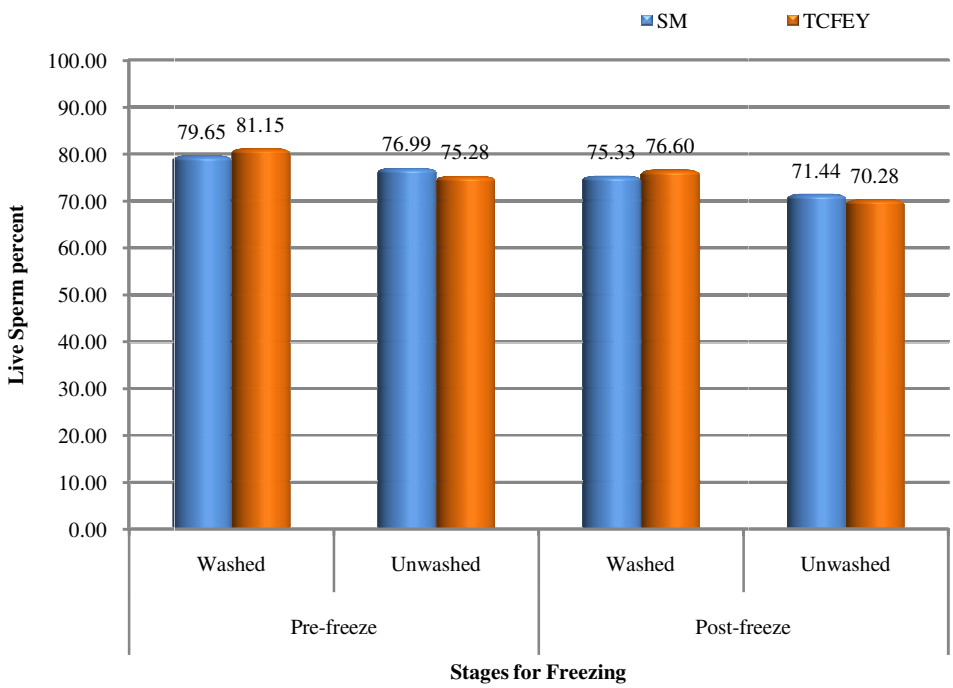

Fig. 4.2. Live Sperm percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at pre-freeze and post-freeze stages in Refrigeration group

season, except for motility of diluted in 18% egg yolk in the non-breeding season has not observed in present study.

On the contrary, Azeredo *et al.* (2001) reported that sperm motility showed a significant increase when the seminal plasma was present ,both for fresh and frozen semen and the motility was influenced by the increase of photoperiod. Roca *et al.* (1997) stated that motility and percentage of dead spermatozoa at 5^0C were affected by seminal plasma.

ii. Live sperm percent

The mean percent of live sperm of washed and unwashed buck spermatozoa during pre-freeze and post –freeze stages of preservation in SM and TCFEY dilutors are shown in Table 24 and results have been graphically presented in Fig. 4.2.

Table 4.24. Live Sperm percent (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	**Pre-freeze**		**Post-freeze**		**Mean ± SE for Dilutors**	**Mean ± SE for Conditions**
Condition / Dilutors	**Washed**	**Unwashed**	**Washed**	**Unwashed**		
SM	79.65 ± 0.80	76.99 ± 1.06	75.33 ± 0.72	71.44 ± 0.55	**SM** 75.85 ± 1.72	**Washed** 78.18^{i} ± 1.34
TCFEY	81.15 ± 0.29	75.28 ± 0.70	76.60 ± 1.00	70.28 ± 0.90	**TCFEY** 75.83 ± 2.24	**Unwashed** 73.50^{ii} ± 0.58
Mean ± SE for Stages	78.26^{A} ± 1.32		73.41^{B} ± 1.51			

Mean bearing different superscript differ significantly

Table 4.24A. ANOVA for Live Sperm of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	23.39		
Stage	1	3393.06	295.21**	0.55
Condition	1	3164.06	275.29**	0.55
Dilutor	1	0.11	0.01^{NS}	0.55
Stage X Condition	1	25.00	2.18^{NS}	0.78
Stage X Dilutor	1	0.84	0.07^{NS}	0.78
Condition X Dilutor	1	286.17	24.89**	0.78
Stage X Condition X Dilutor	1	5.44	0.47^{NS}	1.11
Error	497	11.49	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Data pertaining to preservation of washed and unwashed buck spermatozoa showed non significantly higher live sperm percentage (79.65 ± 0.80 and 75.33 ± 0.72) at pre freeze and post freeze stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (76.99 ± 1.06 and 71.44 ± 0.55) in SM dilutor. A non significantly higher live sperm percent was observed (81.15 ± 0.29 and 76.60 ± 1.00) at pre-freeze and post-freeze stages in washed semen as compared to corresponding values of unwashed spermatozoa (75.28 ± 0.70 and 70.28 ± 0.90) in TCFEY dilutor.

The analysis of variance for the live sperm percentage of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between washed and unwashed spermatozoa at each stage of preservation, washing condition, and washing condition X dilutor interaction while a non-significant difference was observed between stage X condition X dilutor interaction.(Table 4.24A).

Ejaculated semen contains aging spermatozoa, defects, leukocytes and particle debris that can reduce sperm survival. Therefore, the separation of mammalian spermatozoa from seminal plasma by centrifugation is preferred and supported by several studies in order to increase motility and fertility after freeze thaw procedure (Kozdrowski *et al.*, 2007). Leboeuf *et al.* (2000) reported that phospholipase A coagulates egg yolk extender and reduces the viability of sperm cells. In contrast, some findings indicated positive results without centrifugation (Cabrera *et al.*,2005; Ari and Daşkin, 2010). Roca *et al.* (1997) stated that motility and percentage of dead spermatozoa at 5^0C were affected by seminal plasma.

iii. Abnormal sperm percent

The mean abnormal sperm percent of washed and unwashed buck spermatozoa during pre-freeze and post –freeze stages of preservation in SM and TCFEY dilutors are shown in Table 4.25 and results have been graphically presented in Fig. 4.3.

Table 4.25. Abnormal Spermspercent (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	**Pre-freeze**		**Post-freeze**		**Mean ± SE for Dilutors**	**Mean ± SE for Conditions**
Condition / Dilutors	**Washed**	**Unwashed**	**Washed**	**Unwashed**		
SM	5.47^{g} ± 0.16	6.61^{e} ± 0.06	6.01^{f} ± 0.43	15.68^{b} ± 0.09	**SM** 8.44^{I} ± 2.42	**Washed** 6.16^{i}± 0.36
TCFEY	5.99^{f} ± 0.24	8.07^{c} ± 0.19	7.17^{d} ± 0.30	17.07^{a} ± 0.12	**TCFEY** 9.57^{II} ± 2.53	**Unwashed** 11.86^{ii}± 0.69
Mean ± SE for Stages	6.53^{A} ± 0.56		11.48^{B} ± 2.85			

Mean bearing different superscript differ significantly

Table 4.25A. ANOVA for Abnormal Sperm of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	1.84		
Stage	1	3525.39	5128.34**	0.13
Condition	1	4675.14	6800.86**	0.13
Dilutor	1	183.38	266.76**	0.13
Stage X Condition	1	2405.09	3498.64**	0.19
Stage X Dilutor	1	2.92	4.25*	0.19
Condition X Dilutor	1	12.54	18.25**	0.19
Stage X Condition X Dilutor	1	4.52	6.57**	0.27
Error	497	0.69	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly lower abnormal sperm percentage (5.47 ± 0.16 and 6.01 ± 0.43) at pre freeze and post freeze stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (6.61 ± 0.06 and 15.68 ± 0.09) in SM dilutor. A significantly lower abnormal sperm percentage was observed (5.99 ± 0.24 and 7.17 ± 0.30) at pre-freeze and post-freeze stages in washed semen as compared to corresponding values of unwashed spermatozoa (8.07 ± 0.19 and 17.07 ± 0.12) in TCFEY dilutor.

The analysis of variance for the abnormal sperm percentage of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between washed and unwashed spermatozoa at each stage of preservation, washing condition, dilutors, stage X condition interaction, condition X dilutor interaction, stage X condition X dilutor interaction while a non-significant difference observed between stage X condition X dilutor interaction (Table 4.25A).

Removal of seminal plasma is usually carried out in artificial insemination programme in order to reduce its adverse effect on spermatozoa and improve sperm quality (Marti *et al.,* 2006).

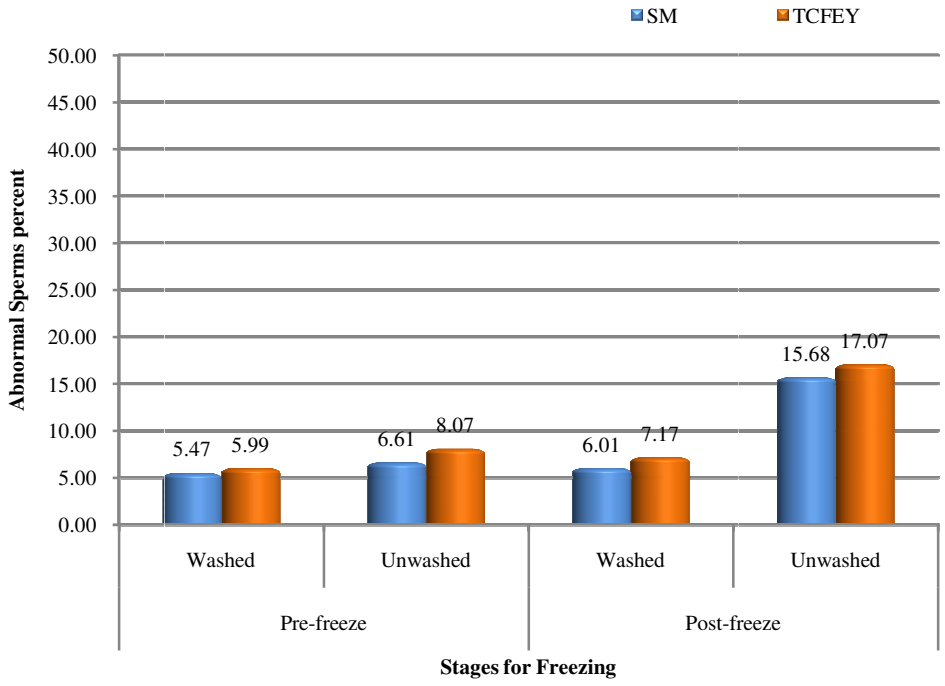

Fig. 4.3. Abnormal Sperms percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at pre-freeze and post-freeze stages in Refrigeration group

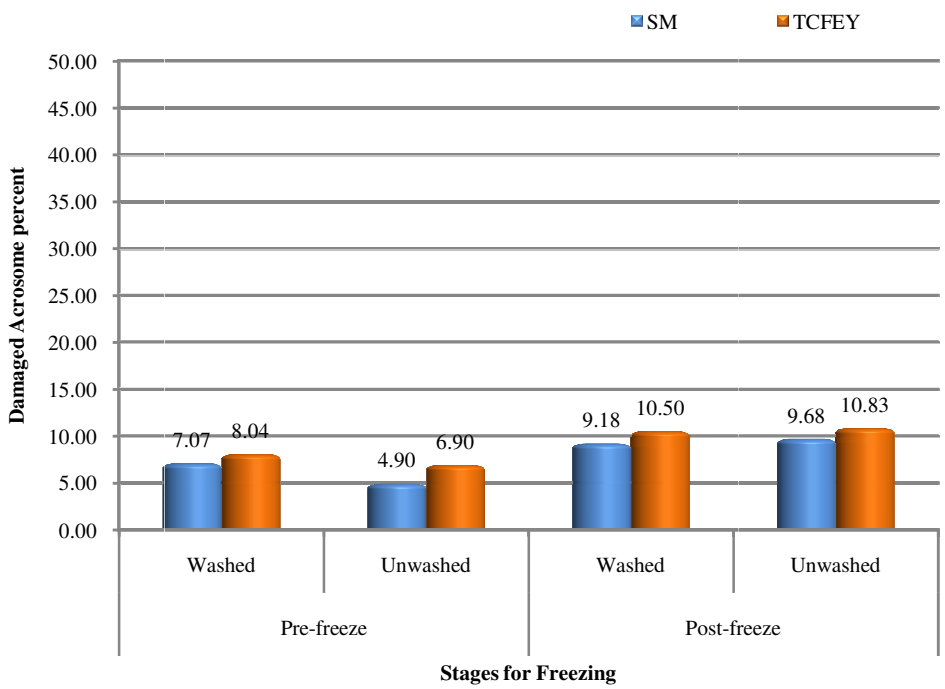

Fig. 4.4. Damaged Acrosome percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at pre-freeze and post-freeze stages in Refrigeration group

b) Sperm function tests of washed and unwashed refrigerated buck spermatozoa

i. Damaged acrosome percent

The mean percent of damaged acrosome of washed and unwashed buck spermatozoa during pre-freeze and post –freeze stages of preservation in SM and TCFEY dilutors are shown in Table 4.26 and results have been graphically presented in Fig. 4.4.

Data pertaining to preservation of washed and unwashed buck spermatozoa at pre freeze stage showed significantly higher damaged acrosome percentage (7.07 ± 0.46 and 8.04 ± 0.40 in SM and TCFEY dilutor, respectively) in washed semen than the unwashed semen (4.90 ± 0.08 and 6.90 ± 0.16 in SM and TCFEY dilutor, respectively). At post freeze stage the values of acrosomal damaged were having an apparent difference between washed semen (9.18 ± 0.27 and 10.50 ± 0.24 in SM and TCFEY dilutor, respectively) and unwashed semen (9.68 ± 0.11 and 10.83 ± 0.06 in SM and TCFEY dilutor respectively).

Table 4.26. Damaged Acrosome percent (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	**Pre-freeze**		**Post-freeze**		**Mean ± SE for Dilutors**	**Mean ± SE for Conditions**
Condition / Dilutors	**Washed**	**Unwashed**	**Washed**	**Unwashed**		
SM	7.07^{e} ± 0.46	4.90^{f} ± 0.08	9.18^{c} ± 0.27	9.68^{b} ± 0.11	**SM** 7.71^{I} ± 0.66	**Washed** 8.70^{i} ± 0.74
TCFEY	8.04^{d} ± 0.40	6.90^{e} ± 0.16	10.50^{a} ± 0.24	10.83^{a} ± 0.06	**TCFEY** 9.07^{II} ± 0.95	**Unwashed** 8.08^{ii} ± 0.58
Mean ± SE for Stages	6.73^{A} ± 0.66		10.05^{B} ± 0.38			

Mean bearing different superscript differ significantly

Table 4.26A. ANOVA for Damaged Acrosome of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	2.65		
Stage	1	1586.69	982.56**	0.21
Condition	1	55.01	34.06**	0.21
Dilutor	1	266.78	165.20**	0.21
Stage X Condition	1	154.17	95.47**	0.29
Stage X Dilutor	1	2.25	1.39^{NS}	0.29
Condition X Dilutor	1	6.67	4.13*	0.29
Stage X Condition X Dilutor	1	12.84	7.95**	0.42
Error	497	1.62	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Analysis of variance for the damaged acrosome percent value of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stage of preservation, washing condition, dilutors, stage X condition, stage X condition X dilutor interactions (Table 4.26A).

Seminal plasma protects the sperm from damage by oxidative stress (Saleh *et al.*,2002).The beneficial effect of removal of seminal plasma on the quality of goat semen during preservation observed in the present study was in agreement with the earlier reports (Memon *et al.*, 1985; Deka and Rao, 1986; Misra *et al.*, 1993).

ii. Hypo-osmatic swelling test

The mean percent of HOST reacted washed and unwashed buck spermatozoa during pre-freeze and post-freeze stages of preservation in different dilutors are compared and depicted in Table 4.27 and results have been graphically presented in Fig. 4.5.

Table 4.27. HOST percent (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	Pre-freeze		Post-freeze		Mean ± SE for Dilutors	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed		
SM	72.50^{a} ± 0.41	69.54^{b} ± 0.79	55.17^{d} ± 1.02	53.53^{e} ±0.94	**SM** 62.68^{I} ± 4.86	**Washed** 63.01^{i}± 4.84
TCFEY	70.21^{b} ± 0.35	64.93^{c} ± 0.60	54.17^{e} ± 0.87	52.25^{f} ±0.44	**TCFEY** 60.39^{II} ± 4.30	**Unwashed** 60.06^{ii}± 0.64
Mean ± SE for Stages	69.30^{A}± 1.59		53.78^{B} ± 0.61			

Mean bearing different superscript differ significantly

Table 4.27A. ANOVA for HOST of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	22.21		
Stage	1	34673.54	3922.56**	0.49
Condition	1	1251.39	141.57**	0.49
Dilutor	1	758.54	85.81**	0.49
Stage X Condition	1	197.17	22.31**	0.69
Stage X Dilutor	1	192.52	21.78**	0.69
Condition X Dilutor	1	60.71	6.87**	0.69
Stage X Condition X Dilutor	1	37.52	4.24*	0.97
Error	497	8.84	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

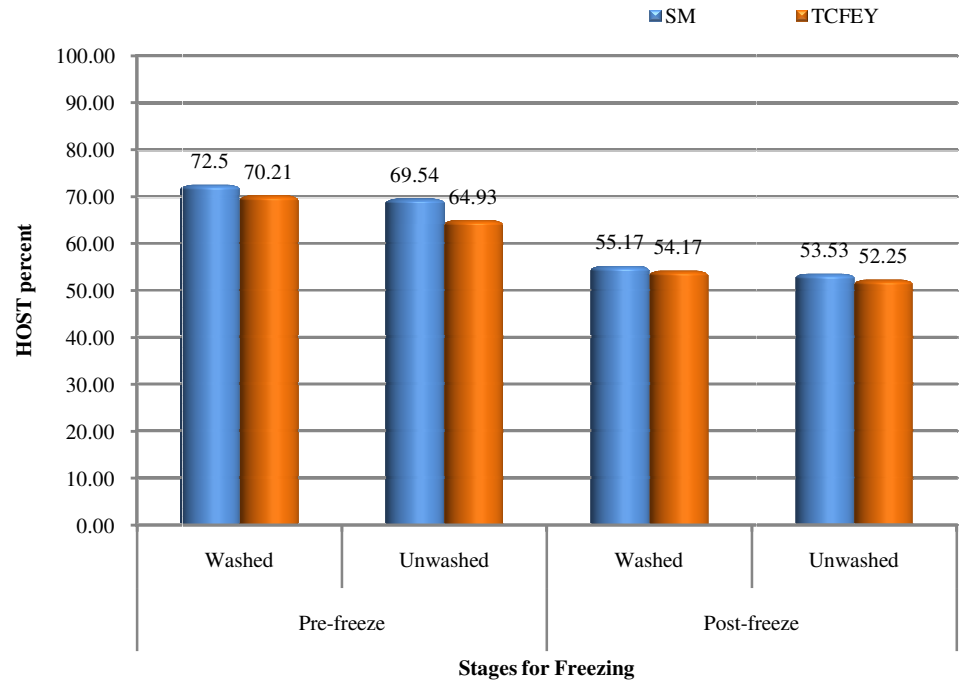

Fig. 4.5. HOST percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at pre-freeze and post-freeze stages in Refrigeration group

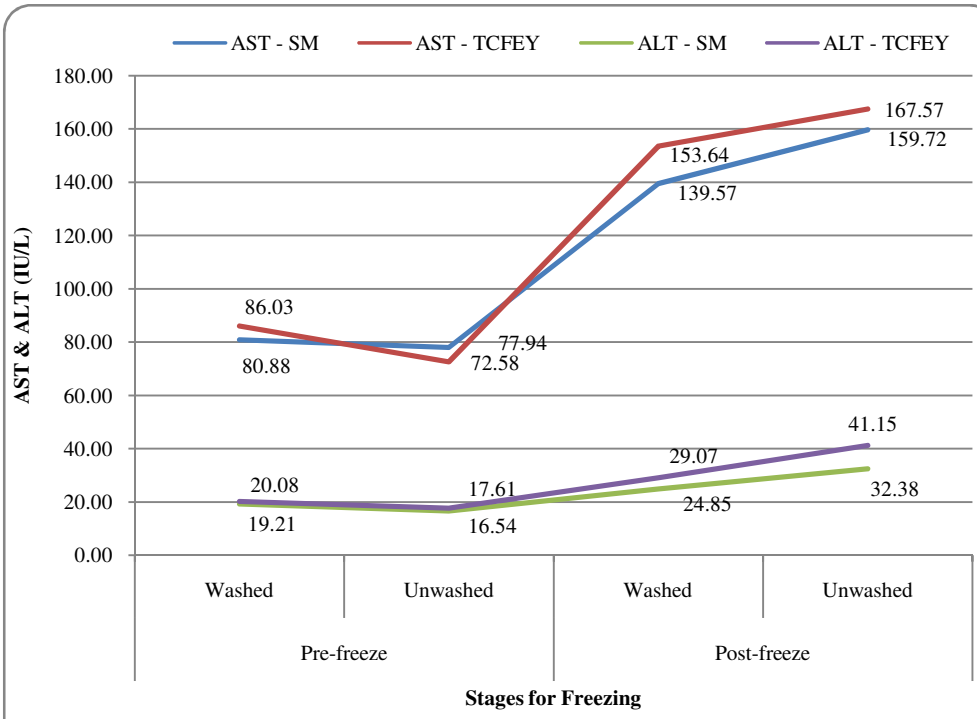

Fig. 4.6. AST & ALT (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at pre-freeze and post-freeze stages in Refrigeration group

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly higher HOST reacted sperm percent (72.50 ± 0.41 and 55.17 ± 1.02 at pre freeze and post freeze stages in washed semen) as compared to corresponding values of unwashed spermatozoa (69.54 ± 0.79 and 53.53 ± 0.94 in SM dilutor). A significantly higher HOST percent was observed (70.21 ± 0.35) and 54.17 ± 0.87 at pre freeze and post freeze stages) in washed semen as compared to corresponding values of unwashed spermatozoa (64.93 ± 0.60 and 52.25 ± 0.44 in TCFEY dilutor). The values of percent HOST reacted spermatozoa were significantly ($P<0.05$) higher in SM dilutor in washed semen at pre-freeze stage of preservation.

Analysis of variance for the washed and unwashed refrigerated Berari buck spermatozoa revealed that there was significant ($P<0.05$) difference between washed and unwashed spermatozoa at both the stages of preservation for percentage of HOST reacted spermatozoa. (Table 4.27A).

The plasma membrane integrity of washed and unwashed spermatozoa was compared by investigating the Hypo-osmotic swelling test of sperms in the seminal plasma during different stages of preservation. The results of the present study revealed that the washing of semen enhanced the maintenance of plasma membrane integrity of spermatozoa during preservation process.

iii. Leakage of enzyme (AST)

The mean of AST of washed and unwashed buck spermatozoa during pre-freeze and post-freeze stages of preservation in different dilutors are compared and depicted in Table 4.28 and results have been graphically presented in Fig. 4.6.

Table 4.28. AST (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	AST (IU/L) at Pre-freeze stage		AST (IU/L) at Post-freeze stage		Mean ± SE for Dilutors	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed		
SM	80.88^{f} ± 1.26	77.94^{g} ± 1.33	139.57^{d} ± 1.78	159.72^{b} ± 1.37	**SM** 114.53^{I} ± 20.70	**Washed** 115.03^{i} ± 18.49
TCFEY	86.03^{e} ± 1.36	72.58^{h} ± 1.44	153.64^{c} ± 1.16	167.57^{a} ± 0.93	**TCFEY** 119.96^{II} ± 23.80	**Unwashed** 119.92^{ii} ± 3.92
Mean ± SE for Stages	73.39^{A} ± 2.81		155.13^{B} ± 5.92			

Mean bearing different superscript differ significantly

Table 4.28A. ANOVA for AST of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	63.27		
Stage	1	826659.79	51384.33**	0.66
Condition	1	2822.27	175.43**	0.66
Dilutor	1	4241.27	263.63**	0.66
Stage X Condition	1	22914.39	1424.33**	0.93
Stage X Dilutor	1	4405.64	273.85**	0.93
Condition X Dilutor	1	2520.88	156.69**	0.93
Stage X Condition X Dilutor	1	165.77	10.30**	1.31
Error	497	16.09	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly higher AST values (80.88 ± 1.26 and 139.57 ± 1.78 at pre freeze and post freeze stages in washed semen) as compared to corresponding values of unwashed spermatozoa (77.94 ± 1.33 and 159.72 ± 1.37 in SM dilutor). A significantly higher AST value was observed (86.03 ± 1.36) and 153.64 ± 1.16 at pre freeze and post freeze stages) in washed semen as compared to corresponding values of unwashed spermatozoa (72.58 ± 1.44 and 167.57 ± 0.93 in TCFEY dilutor).

Analysis of variance for the AST value of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stage of preservation, washing condition, dilutors, stage X condition, stage X dilutor, condition X dilutor, stage X condition X dilutor interactions (Table 4.28A).

iv. Leakage of Enzyme (ALT)

The mean of ALT of washed and unwashed buck spermatozoa during pre-freeze and post-freeze stages of preservation in different dilutors are compared and depicted in Table 4.29 and results have been graphically presented in Fig. 4.6.

Table 4.29. ALT (Mean ± SE) of Washed and Unwashed Spermatozoa at pre-freeze and post-freeze stages in Refrigeration Group of Berari buck

Stages for Freezing	ALT (IU/L) at Pre-freeze stage		ALT (IU/L) at Post-freeze stage		Mean ± SE for Dilutors	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed		
SM	19.21^{e} ± 0.27	16.54^{g} ± 0.91	24.85^{d} ± 0.70	32.38^{b} ± 0.84	**SM** 23.24^{I} ± 3.50	**Washed** 23.30^{i} ± 2.29
TCFEY	20.08^{e} ± 0.20	17.61^{f} ± 0.89	29.07^{c} ± 1.05	41.15^{a} ± 0.46	**TCFEY** 26.98^{II} ± 5.33	**Unwashed** 26.92^{ii} ± 4.39
Mean ± SE for Stages	18.36^{A} ± 0.79		31.86^{B} ± 3.46			

Mean bearing different superscript differ significantly

Table 4.29A. ANOVA for ALT of Washed and Unwashed Semen of Berari Buck during Refrigeration

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	29.24		
Stage	1	26244.00	2760.84**	0.50
Condition	1	1885.01	198.30**	0.50
Dilutor	1	2010.03	211.45**	0.50
Stage X Condition	1	5513.06	579.97**	0.71
Stage X Dilutor	1	1100.03	115.72**	0.71
Condition X Dilutor	1	203.06	21.36**	0.71
Stage X Condition X Dilutor	1	171.17	18.01**	1.01
Error	497	9.51	-	
Total	575	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly higher ALT values (19.21 ± 0.27 and 24.85 ± 0.70 at pre freeze and post freeze stages in washed semen) as compared to corresponding values of unwashed spermatozoa (16.54 ± 0.91 and 32.38 ±0.84 in SM dilutor). A significantly higher ALT value was observed (20.08 ± 0.20) and 29.07 ± 1.05 at pre freeze and post freeze stages) in washed semen as compared to corresponding values of unwashed spermatozoa (17.61 ± 0.89 and 41.15 ± 0.46 in TCFEY dilutor).

Analysis of variance for the ALT value of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stage of preservation, washing condition, dilutors, stage X condition, stage X dilutor, condition X dilutor, stage X condition X dilutor interactions. (Table 4.29A).

4.4 Cryopreservation of Berari Buck Semen

The ejaculation of the six Berari bucks were pooled and a total of 12 pooled semen samples were utilized for during freezing in Tris – egg yolk-Glycerol (TYG) dilutor and Tris –egg yolk- Dimethyl Sulphoxide (TYD) dilutor. Freezing was carried out on the unwashed and washed semen samples.

During the process of semen cryopreservation, spermatozoa are exposed to a variable range of temperatures that promotes structural (De Leeuw *et al.,* 1990) or functional alterations (Hammerstedt *et al.,* 1990). The changes are reflected through mitochondrial and membrane dysfunction. This affects the permeability of the sperm surface to water, ions and cryoprotectant (Hagiwara *et al.,* 2009; Oldenhof *et al.,* 2010), reducing the number of viable and progressive motile sperm with uncapacitated and intact acrosomal cap prior to artificial insemination (Cormier and Bailey, 2003). Egg yolk is an integral component of semen extender that acts as a non-penetrating cryoprotectant and prevents membrane damage. The action of egg yolk may be attributed to phospholipids (Lanz *et al.,* 1965), cholesterol (Moce *et al.,* 2010) and low-density lipoprotein (LDL) content (Bergeron and Manjunath, 2006), which afford successful protection to the sperm plasma membrane against cold shock and cryoinjuries (Moussa *et al.,* 2002). The exposure of sperm to low and ultra-low temperature results in ultrastructural, biochemical and functional damages to sperm through movement of cholesterol and membrane protein (Purdy, 2006), thus altering plasma membrane integrity, leading to damaged acrosome and cryocapacitation accompanied by reduced progressive motion and viability. The egg yolk and antioxidative enzymes in extended semen prevent the loss of selective permeability and integrity of the plasma membrane (Ortman and Rodriguez, 1994), a release of intracellular enzymes (Harrison and White,1972) and lipids (Darin-bennett *et al.,* 1973), a redistribution of ions (Quinn and White, 1968), a change in the membranes of the acrosome (Jones and Martin, 1973) and mitochondria, (Watson, 1995) and imparts protection against temperature related injuries during the freeze–thawing process.

Cryopreservation as a technique for storage of buck semen has advantages but the freezing and thawing processes induce detrimental effects on the sperm ultra-structure, and the biochemical and functional integrity (Watson, 2000). There was a general decrease trend in values of pre-freeze and post thawed semen parameters such as progressive motility, live sperm, intact acrosome and HOS positive sperm, but mainly, increase in percentage of abnormal sperm in the semen diluted in extenders (Watson ,1995 and Singh *et al.*, 2016).

4.4.1 Cryopreservation of washed buck spermatozoa

The cryopreservation of washed buck spermatozoa refers to the freezing of semen samples after removing the seminal plasma by centrifugation and washing spermatozoa with the washing solution. Although egg yolk imparts cryoprotection, it simultaneously reacts with seminal plasma Glycoprotein-60 and Phospholipase A2 to form lethal compounds that lead to sperm death (Leboeuf *et al.,* 2000). Impedance to sperm respiration by egg yolk increases with concentration and may lead to decreased motility and viability (Najafi *et al.,* 2013). Furthermore, bulbourethral secretions contain Glycoprotein-60 (BUSgp60) with triacylglycerol hydrolase activity that decrease sperm motility and movement quality (Pellicer-Rubio and Combarnous, 1998). Sperm washing is considered to be beneficial in goats as it eliminates the reactive proteins but the process simultaneously removes the protective proteins and antioxidative enzymes.

a) Physical characteristics of washed cryopreserved buck spermatozoa

i. Individual motility

The mean percent individual motility of washed buck spermatozoa during different dilutors and stages of freezing is shown in Table 4.30.

Table 4.30. Individual Motility percent (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	75.00^{a} ± 0.65	63.06^{b} ± 0.93	47.08^{d} ± 0.80	**TYG** 61.71^{I} ± 8.09
TYD	74.03^{a} ± 0.59	58.06^{c} ± 1.05	39.03^{e} ± 0.62	**TYD** 57.04^{II} ±10.12
Mean ± SE for Stages	74.51^{A} ± 0.49	60.56^{B} ± 2.50	43.06^{C} ± 4.03	

Mean bearing different superscript differ significantly

Table 4.30A. ANOVA for Individual Motility of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	66.87	2.02	
Stage	2	35777.08	1080.09**	1.33
Dilutor	1	2361.34	71.29**	1.08
Stage × Dilutor	2	454.40	13.72**	1.88
Error	355	33.12	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of individual sperm motility at post diluted, post equilibrated and post thaw stages were 75.00 ± 0.65, 63.06 ± 0.93 and 47.08 ± 0.80 in TYG dilutor and 74.03 ± 0.59, 58.06 ± 1.05 and 39.03 ± 0.62 in TYD dilutor, respectively. The values of percent individual motility decreased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) differences between stages of freezing, dilutors and stage X dilutor interactions. (Table 4.30A).

Sperm motility in TYG and TYD dilutor at post diluted was found as 75.00 % and 74.03 % for washed semen. Similarly Ustuner *et al.* (2009) reported 73.0 % individual motility in TYG in Saanen goat.

In present group dilution of semen was carried out after washing of semen and sperm motility in TYG and TYD dilutor at post equilibrated stage was found as 63.06 % and 58.06 %. Similarly, Singh *et al.* (1995) reported 66.94 % in TYG and 53.61% in TYD, Naing *et al.* (2011) reported 74.58 % in TYG, Ustuner *et al.* (2009) reported 60.0 % in TYG in Saanen goat. Lower value 38.0 % was reported by Bezjian *et al.* (2013) in TYG washed semen samples at post equilibrated stage of cryopreservation in Markhor bucks.

In present group dilution of semen was carried out after washing of semen and sperm motility in TYG and TYD dilutor at post thawed stage was found as 47.08 % and 39.03 %. Similarly, Singh *et al.*(1995) reported 45.49 % in TYG and 15.33% in TYD, Dorado *et al.* (2010) 40.86 % in TYG in florida goat. Naing *et al.* (2011) 52.08 % in TYG.

Lower value 34.5% was reported by Daskin *et al.* (2011) in TYG in Angora buck, 30.1 % by Bezjian *et al.* (2013) in TYG washed semen samples at post thawed stage of cryopreservation in Markhor bucks. Higher value was reported by Ustuner *et al.* (2009) as 51.7 % in TYG in Saanen buck.

These observation are in accordance with reports of earlier workers (Snedeker and Gaunya, 1970) who reported that glycerol gave sperm motility than DMSO at any concentration. The highest mean post thaw motility percentage was reported in egg yolk tris extender containing 6% glycerol and 1 % DMSO (Snedeker and Gaunya, 1970).

Leboeuf *et al.* (2000) reported that removal of seminal plasma by centrifugation of buck semen increased motility of live spermatozoa during storage in egg yolk diluents. In addition, Islam *et al.* (2006) and Daramola and Adekunle (2017) reported beneficial effect of the removal of seminal plasma on the quality of goat semen during preservation in tris egg yolk extender.

Leboeuf *et al.* (2000) opined that removal of seminal plasma by centrifugation of buck semen increased the percentage of live spermatozoa during storage in egg yolk diluents.

The decline in sperm motility after thawing may be due to formation of peroxides from free radicals. This oxidative stress causes damage to bio-molecules and cellular components (Halliwell, 1991). The sperm membrane contains a high number of unsaturated fatty acids, which are predisposed to damage due to peroxidation, which destroys the structural integrity of plasma membrane leading to loss in motility (Aitken *et al.,* 1989; Salamon and Maxwell, 2000). The toxicity of cryoprotective agent glycerol in semen diluents causes a reduction in sperm motility and to alter the acrosome integrity by interfering with the permeability of the sperm membrane (Maxwell and Salamon, 1993; Maxwell and Watson, 1996).

ii. Live sperm percent

The mean percent of live sperms of washed buck spermatozoa for different dilutors and stages of freezing are shown in Table 4.31. The percentage of live sperm at post diluted, post equilibrated and post thaw stages were 84.64 ± 0.61, 70.88 ± 0.68 and 55.00 ± 1.18 in TYG dilutor while 80.60 ± 0.68, 66.86 ± 1.04 and 45.61 ± 0.85 in TYD dilutor, respectively. The values of live sperm percent decreased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.31A)

Table 4.31. Live Sperm percent (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	84.64^{a} ± 0.61	70.88^{c} ± 0.68	55.00^{e} ± 1.18	**TYG** 70.17^{I} ± 8.56
TYD	80.60^{b} ± 0.68	66.86^{d} ± 1.04	45.61^{f} ± 0.85	**TYD** 64.36^{II} ± 10.18
Mean ± SE for Stages	82.62^{A} ± 2.02	68.87^{B} ± 2.01	50.31^{C} ± 4.69	

Mean bearing different superscript differ significantly

Table 4.31A. ANOVA for Live Sperm of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	66.40	2.36	
Stage	2	37865.42	1347.28**	1.23
Dilutor	1	3651.70	129.93**	1.00
Stage × Dilutor	2	344.91	12.27**	1.73
Error	355	28.11	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Live sperm percent of washed semen in TYG and TYD dilutor at post diluted stage was found as 84.64 % and 80.60 %, respectively. Similarly, Ustuner *et al.* (2009) noted 15.2 % dead spermatozoa in TYG in Saanen goat. Lower value 21.6 % was reported by Bezjian *et al.* (2013) in TYG in Markhor bucks while 76.58 % live sperm was reported by Naing *et al.*(2011) in TYG in Boer bucks.

Live sperm percent of washed semen in TYG and TYD dilutor at post equilibrated stage was found as 70.88 % and 66.86 %. Similar trend was reported by Singh *et al.* (1995) as 71.77 % in TYG and 58.66 % in TYD diluors. Ustuner *et al.* (2009) reported 24.8 % dead spermatozoa in TYG dilutor in Saanen goat.

Live sperm percent of washed semen in TYG and TYD dilutor at post Thaw stage was found as 55.00 % and 45.61. Singh *et al.* (1995) reported 66.94 % in TYG and 53.61% in TYD dilutors. Ustuner *et al.* (2009) reported 24.8 % dead spermatozoa in TYG dilutor in Saanen goat and Daskin *et al.* (2011) reported 42.9 % dead sperm TYG dilutor in Angora buck. The higher value 61.08 % was observed by Naing *et al.* (2011) in TYG dilutor in Boer bucks.

In present study live sperm percent is more in TYG than TYD dilutor at post diluted, post equilibrated and post thaw stages of cryopreservation was in accordance with reports of earlier workers (Snedeker and Gaunya *et al.*, 1970) who reported that glycerol gave more live spermatozoa than DMSO at any concentration.

iii. Abnormal sperm percent

The mean percent of abnormal sperm of washed buck spermatozoa for different dilutors and stages of freezing are shown in Table 4.32.The percentage of abnormal sperm at post diluted, post equilibrated and post thaw stages were 6.90 ± 0.50, 9.93 ± 0.25 and 18.26 ± 0.19 in TYG dilutor while 8.78 ± 0.06, 10.39 ± 0.20 and 26.11 ± 0.35 in TYD dilutor, respectively. The values of abnormal sperm percent increased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.32A)**.**

Abnormal sperm percent of washed semen in TYG and TYD dilutor at post diluted stage was found as 6.90% and 8.78%. Similarly, Naing *et al.* (2011) reported 95.50% (Normal sperm) in TYG in Boer bucks. Higher value reported by Dorado *et al.* (2010) reported abnormal sperm percent which is higher 17.23% in TYG dilutor however lower value 2.2% was reported by Ustuner *et al.* (2009) in TYG dilutor in Saanen buck.

Table 4.32. Abnormal Sperms percent (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	6.90^{e} ± 6.50	9.93^{c} ± 0.25	18.26^{b} ± 0.19	**TYG** 11.70^{II} ± 3.40
TYD	8.78^{d} ± 0.06	10.39^{c} ± 0.20	26.11^{a} ± 0.35	**TYD** 15.09^{I} ± 5.53
Mean ± SE for Stages	7.84^{C} ± 0.94	10.16^{B} ± 0.23	22.19^{A} ± 3.92	

Mean bearing different superscript differ significantly

Table 4.32A. ANOVA for Abnormal Sperm of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	4.09	1.50	
Stage	2	8541.36	3134.11**	0.38
Dilutor	1	1243.73	456.36**	0.31
Stage × Dilutor	2	553.62	203.14**	0.54
Error	355	2.73	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Abnormal sperm percent in TYG and TYD dilutor at post equilibrated stage was found as 9.93 % and 10.39 % in washed semen. However, lower value of 2.6 % was noted by Ustuner *et al.* (2009) in TYG dilutor in Saanen buck.

Abnormal sperm percent in TYG and TYD dilutor at post thaw stage was found as 18.26 % and 26.11 %. Similarly, Dorado *et al.* (2010) reported 20.51 % in TYG dilutor, Naing *et al.*(2011) reported 88.42 % normal sperm in TYG dilutor in Boer bucks. However higher value 43.8 % was reported by Bezjian *et al.* (2013) in TYG dilutor in Markhor bucks, 55.0 % by Daskin *et al.* (2010) in TYG dilutor in Angora buck. The lower value 3.7 % was noted by Ustuner *et al.* (2009) in TYG dilutor in Saanen goat. Similarly, Singh *et al.*(1995) reported acrosomal and tail abnormalities tended to increase between post equilibration and post thawing stage, and were higher in extenders containing the higher levels of DMSO. Significantly lower percentages of abnormalities were recorded in the glycerol + lactose extenders.

b) Sperm functional tests of washed cryopreserved buck spermatozoa

i. Damaged acrosome percent

The mean percent of damaged acrosome of washed buck spermatozoa for different dilutors and stages of freezing are shown in Table 4.33.

Table 4.33. Damaged Acrosome percent (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	6.96^{f} ± 0.45	10.94^{d} ± 0.34	15.28^{b} ± 0.23	**TYG** 11.06^{II} ± 2.40
TYD	9.00^{e} ± 0.08	12.18^{c} ± 0.24	17.32^{a} ± 0.29	**TYD** 12.83^{I} ±2.42
Mean ± SE for Stages	7.98^{C} ± 1.02	11.56^{B} ± 0.62	16.30^{A} ± 1.02	

Mean bearing different superscript differ significantly

Table 4.33A. ANOVA for Damaged Acrosome of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	2.75	2.14	
Stage	2	2507.62	1951.66**	0.26
Dilutor	1	339.56	264.28**	0.21
Stage × Dilutor	2	7.79	6.06**	0.37
Error	355	1.29	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of damaged acrosome at post diluted, post equilibrated and post thaw stages were 6.96 ± 0.45, 10.94 ± 0.34 and 15.28 ± 0.23 in TYG dilutor while 9.00 ± 0.08, 12.18 ± 0.24 and 17.32 ± 0.29 in TYD dilutor, respectively. The values of damaged acrosome percent increased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.33A).

Damage acrosome percent of washed semen in TYG and TYD dilutor at post diluted stage was found as 6.96 % and 9.00 %. Similarly, Ustuner *et al.* (2009) found 7.9 % damaged acrosome percent in TYG dilutor in Saanen buck. Lower value 81.17 % of intact acrosome in TYG dilutor in Boer bucks was reported by Naing *et al.*(2011). Dorado *et al.* (2010) reported 48.10 % in TYG dilutor.

Damage acrosome percent in TYG and TYD dilutor at post equilibrated stage was found as 10.94 % and 12.18 % of washed spermatozoa however, Ustuner *et al.* (2009) recorded higher value of 14.8 % in TYG dilutor in Saanen goat.

Damage acrosome percent of washed semen in TYG and TYD dilutor at post thaw stage was found as 15.28 % and 17.32 %. Higher values of damaged acrosome percent in TYG dilutor recorded by Ustuner *et al.* (2009) 30.1% in Sanen goat, Bezjian *et al.* (2013) 70.5 % in Markhor bucks, Dorado *et al.* (2010) 51.42 %, Daskin *et al.* (2011) 36.0% in Angora buck. and Naing *et al.* (2011) reported 33.33 % damage acrosome in Boer bucks. In present study less percent of damage acrosome was noted in TYG than TYD dilutor at post diluted, post equilibrated and post thaw stages of cryopreservation was in agreement with Singh *et al.* (1995) who reported acrosomal and tail abnormalities tended to increase between post equilibration and post thawing stage, and were higher in extenders containing the higher levels of DMSO.

The cryocapacitation and acrosomal cap damage are cellular exocytic like events that influence the fertilizing ability of sperm, a fundamental prerequisite for successful conception after insemination (Lindsay *et al.,* 2005). Perusal of data on cryopreservation of washed buck semen revealed that acrosomal damaged spermatozoa increased significantly as the stages of freezing progressed.

Washing of sperm are known to cause damage to the plasma membrane and the acrosome (Lenz *et al.*, 1977). Mathur (2003) observed that irrespective of thaw temperature, percentage of intact acrosome was not affected by extenders. The beneficial effect of removal of seminal plasma on the quality of goat semen during preservation observed in the present study was in agreement with the earlier reports (Memon *et al.*, 1985; Deka and Rao, 1986; Misra *et al.*, 1993).

ii. Hypo-osmatic swelling test (HOST)

The mean percent of HOST reacted washed buck spermatozoa during post-dilution, post equilibration and post thawed stages of freezing is shown in Table 4.34.

Table 4.34. HOST percent (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	73.32^{a} ± 0.62	64.19^{c} ± 0.56	48.18^{e} ± 0.84	**TYG** 61.90^{I} ± 7.35
TYD	68.13^{b} ± 0.49	57.96^{d} ± 0.56	37.08^{f} ± 0.53	**TYD** 54.39^{II} ± 9.14
Mean ± SE for Stages	70.72^{A} ± 2.60	61.08^{B} ± 3.12	42.63^{C} ± 5.55	

Mean bearing different superscript differ significantly

Table 4.34A. ANOVA for HOST of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	1.94	0.57	
Stage	2	29335.28	8575.46**	0.43
Dilutor	1	6090.01	1780.27**	0.35
Stage × Dilutor	2	357.35	104.46**	0.60
Error	355	3.42	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of HOST positive spermatozoa at post dilution, post equilibration stage and post thawed stage was 73.32 ± 0.62, 64.19 ± 0.56 and 48.18 ± 0.84 in TYG dilutor and 68.13 ± 0.49,57.96 ± 0.56 and 37.08 ± 0.53 in TYD dilutor respectively. The values of percent HOST reacted spermatozoa decreased significantly at each stage of freezing.

The analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.34A).

An estimation of critical difference between the overall means of membrane integrity (HOST) between the two semen dilutors under study revealed that the best membrane integrity (HOST) was for TYG dilutor whereas; lower motility was in TYD dilutor at each stage of evaluation. A significant difference was reported between two dilutors.

HOST percent of washed semen in TYG and TYD dilutor at post diluted stage was found as 73.32 % and 68.13 %. Similarly, Baghel *et al.* (2014) reported 70.1 % in Barbari buck, in TYG dilutor unwashed semen samples at post diluted stage of cryopreservation.

Abnormal sperm percent of washed semen in TYG and TYD dilutor at post equilibrated stage was found as 64.19 % and 57.96 %. Similarly, 64.5 % HOST in TYG dilutor was reported by Baghel *et al.* (2014) in unwashed semen samples of Barbari bucks.

HOST percent of washed semen in TYG and TYD dilutor at post thaw stage was found as 48.18% and 37.08 %. HOST 38.0% was reported by Daskin *et al.*, (2011) in TYG dilutor in Angora buck and Baghel *et al.* (2014) reported 47.20% in Barbari buck. Similar observations for HOST after removal of seminal plasma in buck semen were in accordance with Tuli and Holtz (1994), Azeredo *et al.* (2001) and Peterson *et al.* (2007).

The membrane integrity (HOST) was higher initially and reduced gradually during preservation in both the dilutors. These changes in the seminal attributes may be the result of altered spermatozoa metabolism at low temperature during cryopreservation . The process involves a rapid reduction in temperature i.e cooling especially from 25^0c to 5^0c (White,1993). This produces cold shock, a membrane transition phase behavior is exhibited by biological membranes (Morris and Clark, 1987). Cold shock results in a loss of selective permeability and integrity of the plasma membrane (Ortman

et al., 1994), release of intracellular enzymes (Harrison and White, 1972) and lipids (Darin-bennett *et al.*, 1973; Pickett and Komarek, 1964), change in acrosome membranes (Jones and Martin, 1973), redistribution of ions (Quinn and White,1968) and mitochondria (Watson,1995) diminished metabolism.

iii. Leakage of enzymes

The data pertaining to the effect of freezing washed Berari buck semen on seminal plasma activity of AST and ALT enzymes at the post diluted, post equilibrated and post thawed stages of freezing have been presented in Table 4.35 and 4.36. The overall mean activity of AST enzyme observed at the post diluted, post equilibrated and post thawed stages were 73.06 ± 0.63, 108.03 ± 1.18 and 149.83 ± 1.44 IU/L in TYG dilutor while 74.01 ± 0.97, 111.18 ± 0.96 and 153.15 ± 0.28 in TYD dilutor respectively (Table 4.35).

On scanning the available literature, the references regarding AST and ALT in prefreez and post freezing in washed semen of Berari bucks in TYG and TYD dilutors at different time interval and temperatures were not available. Hence not compared.

Table 4.35. AST (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	AST (IU/L) at Post Diluted stage	AST (IU/L) at Post Equilibrated stage	AST (IU/L) at Post Thaw stage	Mean ± SE for Dilutors
TYG	73.06^{f} ± 0.63	108.03^{d} ± 1.18	149.83^{b} ± 1.44	**TYG** 110.31^{II} ± 22.19
TYD	74.01^{e} ± 0.97	111.18^{c} ± 0.96	153.15^{a} ± 0.28	**TYD** 112.78^{I} ± 22.86
Mean ± SE for Stages	73.54^{C} ± 0.48	109.60^{B} ± 1.58	151.49^{A} ± 1.66	

Mean bearing different superscript differ significantly

Table 4.35A. ANOVA for AST of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	19.54	2.12	
Stage	2	219196.45	23726.30**	0.70
Dilutor	1	662.56	71.72**	0.57
Stage × Dilutor	2	62.51	6.77**	0.99
Error	355	9.24	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Table 4.36. ALT (Mean ± SE) of Washed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	ALT (IU/L) at Post Diluted stage	ALT (IU/L) at Post Equilibrated stage	ALT (IU/L) at Post Thaw stage	Mean ± SE for Dilutors
TYG	15.83 ± 0.41	18.56 ± 0.61	26.25 ± 0.60	**TYG** 20.21^{II} ± 3.12
TYD	17.14 ± 0.49	19.32 ± 0.61	27.94 ± 0.21	**TYD** 21.47^{I} ± 3.30
Mean ± SE for Stages	16.49^{C} ± 0.65	18.94^{B} ± 0.38	27.10^{A} ± 0.85	

Mean bearing different superscript differ significantly

Table 4.36A. ANOVA for ALT of Washed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replication	71	3.80	1.39	
Stage	2	4444.47	1614.09**	0.38
Dilutor	1	170.00	61.74**	0.31
Stage × Dilutor	2	7.86	2.86^{NS}	0.54
Error	355	2.75	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The overall mean activity of ALT enzyme observed at the post diluted, post equilibrated and post thawed stages were 15.83 ± 0.41, 18.56 ± 0.61 and 26.25 ± 0.60 IU/L in TYG dilutor while 17.14 ± 0.49, 19.32 ± 0.61 and 27.94 ± 0.21 IU/L in TYD dilutor respectively (Table 4.36).

The analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction of AST and ALT enzyme leakage of washed buck spermatozoa. (Table 4.35A and 4.36 A).

The AST and ALT activity was lower initially and increased gradually during preservation. Leakage of AST and ALT in TYG dilutor was significantly lower than TYD dilutor at every stage of preservation. The TYG dilutor showed the lower mean value of AST and ALT whereas, TYD dilutor showed the higher mean value of AST.

The extra cellular activity of transaminases is due to their leakage into seminal plasma caused by damage inflicted upon spermatozoa (Salisbury *et al.*,1985 and Kapila, 1992).Seminal plasma transaminases are evaluated as an index of measurement of injury to spermatozoa incurred during the process of cryopreservation (Ingale *et al.*, 2000). The enzyme release from spermatozoa has generally been viewed as cellular injury (Sidhu *et al.*, 1996 and Ingale *et al.*, 2000), whereby membrane become inactive with altered permeability or destroyed resulting into loss of material therein (De-Rauck and Knight, 1964). The process of cryopreservation causes diminished intracellular enzyme activity that results from leakage of enzyme into the extracellular surrounding medium. Species release differences (Roychoudhury *et al.*, 1974) have been attributed to intrinsic differences in the cells between the species (White and Wales, 1960) as well as differences in susceptibility to membrane damage between the species (Hammerstedt *et al.*, 1990).

4.4.2 Cryopreservation of unwashed buck spermatozoa

The cryopreservation of unwashed buck spermatozoa refers to the freezing of semen samples without removing the seminal plasma.

a) Physical characteristics of unwashed cryopreserved buck spermatozoa

i. Individual motility

The mean percent individual motility of unwashed buck spermatozoa during different dilutors and stages of freezing is shown in Table 37. The percentage of individual sperm motility at post diluted, post equilibrated and post thaw stages were 72.08 ± 0.56, 60.14 ± 1.13 and 43.06 ± 0.90 in TYG dilutor while 71.11 ± 0.28, 57.08 ± 1.15 and 38.06 ± 0.51 in TYD dilutor respectively. The values of percent individual motility decreased significantly at each stage of freezing.

Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interactions. (Table 4.37A).

After unwashing of semen, sperm motility in TYG and TYD dilutor at post diluted stage was found as 72.08 % and 71.11 % , respectively. Anand *et al.* (2017) reported 75 % individual motility in TYG dilutor which is at par with the result of present investigation.

The results of the percentage of post thawed motility were comparable with the findings of Sinha *et al.* (1992), Shirbhate and Honmode (2005), Bucak and Uysal (2008). The higher values of post thawed motility have also been reported (Deka and Rao, 1986; Deka and Rao, 1987; Das and Rajkonwar, 1995; Antonie and Pattabiraman, 1999; Baruah *et al.*, 2003; Kulaksiz and Daskin, 2010). While follower workers reported lower values of post thawed motile sperms (Dutta,1996; Biswas *et al.*, 2002; Sundararaman and Edwin, 2003)

Table 4.37. Individual Motility percent (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	$72.08^{a} \pm 0.56$	$60.14^{b} \pm 1.13$	$43.06^{d} \pm 0.90$	**TYG** $58.43^{I} \pm 8.42$
TYD	$71.11^{a} \pm 0.28$	$57.08^{c} \pm 1.15$	$38.06^{e} \pm 0.51$	**TYD** $55.42^{II} \pm 9.58$
Mean ± SE for stages	$71.60^{A} \pm 0.49$	$58.61^{B} \pm 1.53$	$40.56^{C} \pm 2.50$	

Mean bearing different superscript differ significantly

Table 4.37A. ANOVA for Individual Motility of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	48.197	1.61	
Stage	2	34997.45	1181.19**	1.26
Dilutor	1	978.01	33.01**	1.03
Stage × Dilutor	2	146.07	4.93**	1.78
Error	355	29.63	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

After unwashing of semen, sperm motility in TYG and TYD dilutor at post thaw stage was found as 43.06 % and 38.06 %, respectively. While Sinha *et al.*, (1996) reported much higher values of percent post thawed motile sperms, such as 50.22 ± 0.83 in Beetal, 45.22 ± 0.71 in Black Bengal and 47.88 ± 0.57 in Crossbred bucks. Agarwal (2001) reported 64.85 %, Naing *et al.* (2010) reported 60 % in Boer bucks.and Jha *et al.* (2018) found 49 % in Nepalese Indigenous Khari buck.

The lower values of post thawed motile sperms i.e 23.1% in Glycerol dilutor and 17 % in DMSO dilutor were observed in Markhoz goat (Farshad *et al.*, 2009a) and 30.63 in TYG dilutor (Bane, 2004).

ii. Live sperm percent

The mean percent of live sperms of unwashed buck spermatozoa for different dilutors and stages of freezing are shown in Table 4.38.The percentage of live sperm at post diluted, post equilibrated and post thaw stages were 79.78 ± 0.41, 68.18 ± 1.23 and 50.97 ± 0.85 in TYG dilutor while 78.99 ± 0.33, 65.96 ± 1.13 and 44.39 ± 0.64 in TYD dilutor, respectively. The values of live sperm percent decreased significantly at each stage of freezing.

Table 4.38. Live Sperm percent (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	79.78^{a} ± 0.41	68.18^{b} ±1.23	50.97^{d} ± 0.85	**TYG** 66.31^{I} ± 8.37
TYD	78.99^{a} ± 0.33	65.96^{c} ± 1.13	44.39^{e} ± 0.64	**TYD** 63.11^{II} ± 10.09
Mean ± SE for stages	79.38^{A} ± 0.40	67.07^{B} ± 1.11	47.68^{C} ± 3.29	

Mean bearing different superscript differ significantly

Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.38A).

Table 4.38A. ANOVA for Live Sperm of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	42.91	1.63	
Stage	2	36780.11	1395.02**	1.19
Dilutor	1	1105.28	41.92**	0.97
Stage × Dilutor	2	327.66	12.43**	1.68
Error	355	26.37	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

After unwashing of semen and live sperm percent in TYG and TYD dilutor at post diluted stage was found as 79.78 % and 78.99 %. Similarly, live sperm percent in TYG dilutor was recorded by Naing *et al.*(2011) as 71.83 % in Boer bucks, Singh *et al.* (2016) 70.27 % in unwashed semen samples at post diluted stage of cryopreservation. Higher value of 89.3 % was reported by Ahmed *et al.* (2014) in TYG dilutor in Beetal bucks and 99.5 % by Saraswat (2011).

Live sperm percent of unwashed semen in TYG and TYD dilutor at post equilibrated stage was found as 68.18 % and 65.96 %. Similarly, Ustuner *et al.* (2009) recorded 31.4 % dead sperms in TYG dilutor in Saanen goat.

Higher value for live sperm count i.e. 78.55% and 78.77% in TYG and TYD dilutor was reported by Dagli (2011) in Osmanabadi bucks and 78.77% and 79.66% in Sirohi bucks. Ahmed *et al.* (2014) reported 89.3 % live sperm in TYG in Beetal bucks while 80.37 % was reported by Ramachandran *et al.* (2015) in Jamunapari in TYG unwashed semen samples at post equilibrated stage of cryopreservation which is higher than result of present study. This could be attributed to cryoprotectant, dilutor used, breed, age etc..

Live sperm percent of unwashed semen in TYG and TYD dilutor at post thaw stage was found as 50.97 % and 44.39 %. Live sperm percent of unwashed semen in TYG dilutor in post thaw cryopreserved semen reported as 39 % by Daskin *et al.* (2011) in Angora buck, 57.08 % by Naing *et al.* (2011) in Boer bucks, 57.97 % by Beltran *et al.* (2013), 41.01 % by Ramachandran *et al.* (2015), 49.2 % by Ranjan *et al.* (2014) in Jamunapari, 50.48 % by Singh *et al.* (2016) in Black Bangal buck, 50.8 % by Saraswat (2011) and 49 % by Jha *et al.* (2018).

Higher value 78.55% and 78.77% in TYG and TYD dilutor was reported by Dagli (2011) in Osmanabadi and 78.77% and 79.66% in Sirohi bucks, 56.0 % was reported by Mushtaq *et al.* (2014) in TYG in Beetal bucks, 77.92 % in 6% glycerol and 60.52 % in 6% DMSO by Sikarwar *et al.* (2015), 77.70 % in Sirohi buck by Sikarwar *et al.* (2016), 84.05 % by Gangwar *et al.*, (2015) in Barbari buck, at 6% glycerol level. Lower value was reported by Ustuner *et al.* (2009) as 71.1 % dead sperm in TYG in Saanen goat in unwashed semen samples at post thaw stage of cryopreservation.

iii. Abnormal sperm percent

The mean percent of abnormal sperms of unwashed buck spermatozoa for different dilutors and stages of freezing are shown in Table 4.39.

Table 4.39. Abnormal Sperms percent (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	$7.93^{f} \pm 0.15$	$12.06^{d} \pm 0.22$	$23.79^{b} \pm 0.71$	**TYG** $14.59^{II} \pm 4.75$
TYD	$10.32^{e} \pm 0.30$	$13.94^{c} \pm 0.40$	$26.92^{a} \pm 0.47$	**TYD** $17.06^{I} \pm 5.04$
Mean ± SE for stages	$9.13^{C} \pm 1.19$	$13.00^{B} \pm 0.94$	$25.35^{A} \pm 1.56$	

Mean bearing different superscript differ significantly

Table 4.39A. ANOVA for Abnormal Sperms of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	3.38	1.82	
Stage	2	10344.65	5585.97**	0.31
Dilutor	1	657.61	355.10**	0.26
Stage × Dilutor	2	13.91	7.52**	0.45
Error	355	1.85	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of abnormal sperm at post diluted, post equilibrated and post thaw stages were 7.93 ± 0.15, 12.06 ± 0.22 and 23.79 ± 0.71 in TYG dilutor while 10.32 ± 0.30,13.94 ± 0.40 and 26.92 ± 0.47 in TYD dilutor, respectively. The values of abnormal sperm percent increased significantly at each stage of freezing.

Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.39A).

Abnormal sperm percent of unwashed semen in TYG and TYD dilutor at post diluted stage was found as 7.93 % and 10.32 %. Similarly, 9.58 % abnormal sperm was reported by Singh *et al.* (2016) in TYG unwashed semen samples at post dilution stage of cryopreservation.

Lower value of abnormal sperm percent in TYG dilutor was reported as 2.6 % by Ustuner *et al.* (2009) in Saanen goat, 3.67 % by Naing *et al.* (2011) in Boer bucks and 3.6 % by Mushtaq *et al.* (2014) in Beetal bucks. This type of variations was due to species difference.

In present group dilution of semen was carried out after unwashing of semen and abnormal sperm percent in TYG and TYD dilutor at post equilibrated stage was found as 12.06 % and 13.94 %. Lower values of abnormal sperm percent in TYG reported as 3.1 % (Ustuner *et al.* 2009) in Saanen buck, 1.74 % (Ramachandran *et al.* ,2015) in Jamunapari in unwashed semen samples at post equilibrated stage of cryopreservation. Similarly,12.8 abnormal sperrm % was reported by Ahmed *et al.* (2014) in TYG dilutor in Beetal bucks. Higher value, 16.60% and 16.11% in TYG and TYD dilutor was reported by Dagli (2011) in Osmanabadi and 14.33% and 15.72% in Sirohi bucks. However, compared with observation of Ari and Daskin (2010) in Tushin ram semen (49.50 to 65.7 % sperm abnormality) the present findings show much encouraging results. This could be attributed to cryoprotectant efficacy in preserving the sperm morphology.

After unwashing of semen abnormal sperm percent in TYG and TYD dilutor at post thaw stage was found as 23.79 % and 26.92 %. Abnormal sperm 18.61% and 17.50% in TYG and TYD dilutor was reported by Dagli (2011) in Osmanabadi and 17.55% and 18.05% in Sirohi bucks which are slightly lower. Also lower value of abnormal sperm percent in TYG dilutor was 4.4 % by Ustuner *et al.* (2009) in Saanen goat, 16.81 % by Singh *et al.* (2016), 12.91% by Sundararaman *et al.* (2016), 9.50 % by Naing *et al.*,(2011) in Boer bucks, 18.8 % by Mushtaq *et al.* (2014) in Beetal bucks, 2.84 % by Ramachandran *et al.* (2015) in Jamunapari buck and 12.9 % in 6% Glycerol and 20.3 % in 6% DMSO by Buyukleblebici *et al.* (2014) in Angora buck in unwashed semen samples at post thaw stage of cryopreservation were reported.

Higher value as 35.7 % in TYG and 32.1% in TYD dilutor was for abnormal sperm percent reported by Bezerra *et al.* (2009) and 65.0% in TYG dilutor was reported by Daskin *et al.*, (2011) in Angora buck. These types of variations in sperm morphology are observed due to different types of extenders used, handling of semen, species difference and season of semen collection.

b) Sperm functional tests of unwashed cryopreserved buck spermatozoa

i. Damaged acrosome percent

The mean percent of damaged acrosome of unwashed buck spermatozoa for different dilutors and stages of freezing are shown in Table 4.40.

Table 4.40. Damaged Acrosome percent (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	$6.15^{f} \pm 0.37$	$9.00^{d} \pm 0.14$	$12.81^{b} \pm 0.31$	**TYG** $9.32^{II} \pm 1.93$
TYD	$7.06^{e} \pm 0.29$	$10.07^{c} \pm 0.38$	$15.03^{a} \pm 0.30$	**TYD** $10.72^{I} \pm 2.32$
Mean ± SE for stages	$6.60^{C} \pm 0.45$	$9.54^{B} \pm 0.53$	$13.92^{A} \pm 1.11$	

Mean bearing different superscript differ significantly

Table 4.40A. ANOVA for Damaged Acrosome of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	1.85	1.71	
Stage	2	1950.29	1796.21**	0.24
Dilutor	1	211.12	194.44**	0.19
Stage × Dilutor	2	18.59	17.12**	0.34
Error	355	1.09	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The percentage of damaged acrosome percent at post diluted, post equilibrated and post thaw stages were 6.15 ± 0.37, 9.00 ± 0.14 and 12.81 ± 0.31 in TYG dilutor while 7.06 ± 0.29, 10.07 ± 0.38 and 15.03 ± 0.30 in TYD dilutor, respectively. The values of damaged acrosome percent increased significantly at each stage of freezing.

Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.40A).

Damage acrosome percent of unwashed spermatozoa in TYG and TYD dilutor at post diluted stage was found as 6.15 % and 7.06 %. Ustuner *et al.* (2009) documented 8.5 % damaged acrosome in TYG dilutor in Saanen goat. However, higher value in TYG dilutor as 22.17 % by Naing *et al.*,(2011)in Boer bucks and 87.16 % by Singh *et al.* (2016) in unwashed semen samples at post thaw stage of cryopreservation were reported.

Damage acrosome percent of unwashed semen in TYG and TYD dilutor at post equilibrated stage was found as 9.00 % and 10.07 %. However, higher value for damage acrosome percent was recorded by Ustuner *et al.* (2009) as 13.7 % in TYG dilutor in Saanen goat. Dagali (2001) noted damage acrosome percent value in TYG and TYD dilutor as 13.11 % and 11.50 % in Osmanabadi and 14.78 % and 12.62 % in Sirohi bucks, respectively which is slightly higher.

After unwashing of semen, damage acrosome percent in TYG and TYD dilutor at Post Thaw stage was found as 12.81 % and 15.03 %. Bezerra *et al.* (2009) reported damaged acrosome 13.1% in TYG and 19.4 % in TYD dilutor. Dagli (2011) reported post thaw damage acrosome percent in TYG and TYD dilutor as 15 % and 13.06 % in Osmanabadi and 17.11% and 13.56% in Sirohi bucks. Sundararaman *et al.* (2016) reported 13.96 %, Sikarwar *et al.* (2016) 15.65 % in TYG dilutor and Sikarwar *et al.* (2015) 20.74 % in 6% glycerol and 35.82 % in 6% DMSO. However, higher values as 39.7 % by Ustuner *et al.* (2009) in TYG dilutor in Saanen goat, 36.08 % by

Naing *et al.*(2011) in TYG in Boer bucks, 41 % by Daskin *et al.* (2011) in TYG dilutor in Angora buck, 50.01 % by Ranjan *et al.* (2014), in Jamunapari, 51.90 % by Ustuner *et al*., (2015) and 38 % by Singh *et al.* (2016) were recorded in TYG unwashed semen samples at post thaw stages of cryopreservation.

Lower value as 1.27 % by Beltran *et al.* (2013) in TYG dilutor, 3.1 % in 6% Glycerol and 6.0 % in 6% DMSO was documented by Buyukleblebici *et al.* (2014) in Angora buck.

In Barbari buck semen, a slightly higher value of the percentage acrosomal integrity of spermatozoa was recorded as 91.57±0.91 at 6% glycerol level (Gangwar *et al.,*2015). In Black Bengal buck semen, the percentage acrosomal integrity of spermatozoa was 93.38 ± 0.25 at 7% glycerol level (Ray *et al.*, 2015). On the other hand, Shakeel (1999) reported non significant difference in acrosomal status between the diluents. However, some of the authors (Ahmed *et al.*, 2014; Ustuner *et al.*, 2009; Dorado *et al.*, 2009; Ustuner *et al.*, 2015) have recorded the intact acrosome in the range of 45.20 to 60.8%. This could be attributed to the difference in breeds, species, dilutors, age of bucks etc.

The significance of an acrosomal cap is known to be a prerequisite for successful fertilisation. Researchers suggested that cryopreservation induces reduction in acrosome integrity of frozen semen samples of goats (Aboagla and Terada, 2004). Acrosome membrane-intact sperm ranged between 40% and 70% in Canary (Cabrera *et al.,* 2005) and Florida bucks (Hidalgo *et al.,* 2007). It is presumed that loss of the acrosomal cap during freezing and thawing of buck sperm is similar to that demonstrated in bull sperm (Bamba and Cran 1988). Greater release of the acrosomal enzyme hyaluronidase was noticed after freezing and thawing in the sperm of buffalo bulls (Akhtar and Chaudhry 1989). Therefore, it would be meaningful to study the relationship between fertility and acrosome damage due to freezing and thawing in goat semen, also.

On the contrary Khan and Ijaz (2007) found that the integrity of acrosome membrane decreased numerically in diluted and frozen thawed semen as compared to diluted semen but no significant difference was observed among undiluted, diluted and frozen-thawed semen.

ii. Hypo-osmatic swelling test (HOST)

The mean percent of HOST reacted unwashed buck spermatozoa during post-dilution, post equilibration and post thawed stages of freezing is depicted in Table 4.41. The percentage of HOST positive spermatozoa at post dilution, post equilibration stage and post thawed stage was 73.32 ± 0.62, 63.14 ± 0.31 and 45.38 ± 0.65 in TYG dilutor while 66.99 ± 0.39,56.03 ± 0.70 and 36.03 ± 0.88 in TYD dilutor respectively. The values of percent HOST reacted spermatozoa decreased significantly at each stage of freezing.

The analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction. (Table 4.41A).

HOST percent of unwashed semen in TYG and TYD dilutor at Post Diluted stage was found as 73.32 % and 63.14 %.Similar to present findings, Baghel *et al.* (2014) reported 72.2 % in Barbari buck, Singh *et al.* (2016) found 68.58 % in TYG unwashed semen samples at post diluted stage of cryopreservation.

Host positive sperm percent of unwashed semen in TYG and TYD dilutor at post equilibrated stage was found as 63.14% and 56.03 %. Similarly, Baghel *et al.* (2014) reported 66.5 % in Barbari in TYG dilutor which is in relation with result of study. Dagli (2011) reported HOST values in TYG and TYD dilutor 72.50% and 74.22% in Osmanabadi and 71.00% and 72.11% in Sirohi bucks respectively which are higher than present observation. This may be due to difference in the species and source of spermatozoa.

Table 4.41. HOST percent (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	Post Diluted	Post Equilibrated	Post Thaw	Mean ± SE for Dilutors
TYG	$73.32^{a} \pm 0.62$	$63.14^{c} \pm 0.31$	$45.38^{e} \pm 0.65$	**TYG** $58.24^{I} \pm 8.16$
TYD	$66.99^{b} \pm 0.39$	$56.03^{d} \pm 0.70$	$36.03^{f} \pm 0.88$	**TYD** $55.38^{II} \pm 9.06$
Mean ± SE for stages	$68.23^{A} \pm 3.17$	$56.18^{B} \pm 3.56$	$46.03^{C} \pm 4.68$	

Mean bearing different superscript differ significantly

Table 4.41A. ANOVA for HOST of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	4.98	1.22^{NS}	
Stage	2	17787.59	4364.82**	0.47
Dilutor	1	881.23	216.24**	0.38
Stage × Dilutor	2	17031.70	4179.34**	0.66
Error	355	4.08	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

HOST percent in TYG and TYD dilutor at Post Thaw stage was found as 45.38 % and 36.03 %.

Baghel *et al.* (2014) reported HOST as 51.87% in Barbari buck, Singh *et al.* (2016) 47.02 % ,Sundararaman *et al.*(2016) 43.73 % in TYG dilutor unwashed semen at post thaw stage of cryopreservation. Higher value 49.9 % in 6% glycerol and 41.9 % in 6% DMSO in Angora goat by

Buyukleblebici *et al.* (2014) has been documented. In TYG and TYD dilutor 68.83% and 72.66 % in Osmanabadi while 68.83% and 70.11% in Sirohi bucks was reported by Dagli (2011).

Lower value 11.8 % in TYG dilutor and 11.1% in TYD dilutor by Bezerra *et al.* (2009) and 30.0% by Daskin *et al.*, (2011) in TYG dilutor in Angora buck was reported.

In present study, the mean value for hypo osmotic reacted spermatozoa in unwashed samples was higher than (Khalili *et al.*, 2009) but lower (Ahmed *et al.*, 2014) than the previous reports. Plasma membrane integrity is of prime importance for the freezing and fertility of the sperm cells. The hypo-osmotic swelling assay has been described as a useful test for assessing functional integrity of the plasma membrane in humans (Jeyendran *et al.,* 1984). In the existing study, the plasma membrane integrity of the sperm was lowered by equilibration and declined further after freezing and thawing, as has previously been reported in goat semen (Azerêdo, 2001). A similar decrease in the membrane integrity of sperm after freezing and thawing has been reported in bulls (Correa and Zavos, 1994), boars (Vazquez *et al.,* 1997) and stallions (Neild *et al.,* 1999). These finding are supported by the fact that the lipid components of the plasma membrane of buck semen are significantly reduced after freezing (Holt and North, 1984).

Analysis of variance (ANOVA) showed that there was significant ($P<0.01$) decline of post-thaw plasma membrane integrity in the presence of DMSO. This is in agreement with Rasul *et al.* (2007) in buffalo bull. Type of cryoprotectant used exert significant effect ($P<0.01$) as post-thawed semen's motility, defected acrosomes, abnormal sperm, along with the sperm's plasma membrane integrity (Soylu *et al.,* 2007).

iii. Leakage of enzymes

The data pertaining to the effect of freezing unwashed Berari buck semen on seminal plasma activity of AST and ALT enzymes at the post diluted, post equilibrated and post thawed stages of freezing have been presented in Table 4.42 and Table 4.43, respectively.

Table 4.42. AST (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	AST (IU/L) at Post Diluted stage	AST (IU/L) at Post Equilibrated stage	AST (IU/L) at Post Thaw stage	Mean ± SE for Dilutors
TYG	$68.14^{f} \pm 0.95$	$110.01^{d} \pm 0.62$	$151.99^{b} \pm 1.03$	**TYG** $110.05^{II} \pm 24.20$
TYD	$69.36^{e} \pm 1.74$	$113.28^{c} \pm 0.90$	$154.86^{a} \pm 0.15$	**TYD** $112.50^{I} \pm 24.68$
Mean ± SE for stages	$68.75^{C} \pm 0.61$	$111.65^{B} \pm 1.63$	$153.42^{A} \pm 1.44$	

Mean bearing different superscript differ significantly

Table 4.42A. ANOVA for AST of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	11.29	0.99	
Stage	2	258121.34	22791.57**	0.78
Dilutor	1	650.23	57.41**	0.64
Stage × Dilutor	2	42.31	3.74*	1.10
Error	355	11.33	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Table 4.43. ALTpercent (Mean ± SE) of Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stage of Freezing / Dilutors	ALT (IU/L) at Post Diluted stage	ALT (IU/L) at Post Equilibrated stage	ALT (IU/L) at Post Thaw stage	Mean ± SE for Dilutors
TYG	$13.46^{f} \pm 0.59$	$20.10^{d} \pm 0.19$	$28.31^{b} \pm 0.46$	**TYG** $20.62^{II} \pm 4.29$
TYD	$14.26^{e} \pm 0.14$	$21.92^{c} \pm 0.48$	$28.93^{a} \pm 0.49$	**TYD** $21.70^{I} \pm 4.24$
Mean ± SE for stages	$13.86^{C} \pm 0.40$	$21.01^{B} \pm 0.91$	$28.62^{A} \pm 0.31$	

Mean bearing different superscript differ significantly

Table 4.43A. ANOVA for ALT of Unwashed Semen of Berari buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	4.92	1.31	
Stage	2	7842.23	2085.98**	0.45
Dilutor	1	126.75	33.72**	0.37
Stage × Dilutor	2	14.92	3.98*	0.63
Error	355	3.76	-	
Total	431	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

The overall mean activity of AST enzyme observed at the post diluted, post equilibrated and post thawed stages were 68.14 ± 0.95, 110.01 ± 0.62 and 151.99 ± 1.03 IU/L in TYG dilutor while 69.36 ± 1.74, 113.28 ± 0.90 and 154.86 ± 0.15 in TYD dilutor, respectively (Table 4.42). The analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing and dilutors. A significant (P<0.05) difference was observed in stage

X dilutor interaction of AST and ALT enzyme leakage of unwashed buck spermatozoa. (Table 4.42A and 4.43 A).

Leakage of AST was significantly increased in both the dilutors as period of preservation increase. Similar to the present findings Dagli *et al.* (2011) also found highly significant (P<0.01) rise in the seminal plasma GOT enzyme levels in frozen thawed semen as compared to that in fresh diluted, cooled and equilibrated semen. difference between dilutors as well as stages of preservation.

A significant variation was observed among the two semen dilutors for AST activity, being apparently lower in TYG dilutor followed by TYD dilutor. In present investigation values of AST activity were recorded as 110.01 IU/ L and 113.28 IU/L at post equilibrated stage in TYG and TYD dilutor, respectively. Similarly, Dagli (2011) reported AST value at post equilibrated stage in TYG and TYD dilutor as 110.30 IU/L and 101.33IU/L in Osmanabadi and 87.55 IU/L and 86.16 IU/L in Sirohi bucks, respectively.

In present investigation values of AST activity were recorded as 151.99 IU/ L and 154.86 IU/L at post thawed stage in TYG and TYD dilutor, respectively. Similarly, Dagli (2011) reported AST value at post thaw stage in TYG and TYD dilutor as 116.33 IU/L and 108.11 IU/L in Osmanabadi and 98.44 IU/L and 91.94 IU/L in Sirohi bucks, respectively.

The variations in leakage of GOT have been attributed to differences in breeds (Sinha *et al.*, 2000), individual variations between the bucks of same breed (Tuli *et al.*, 1991), age of bucks (Tiwari, 2000), season of semen collection (Baruah *et al.*, 1992), sperm wash or sperms with intact plasma (Tuli *et al.*, 1991), rate of dilution as well as composition of diluent (Singh *et al.*, 1996), glycerol levels (Bonadonna *et al.*, 1974), equilibration periods (Joshi *et al.*, 1990), cooling rates (Bhosrekar, 1975) and freezing rates.

The overall mean activity of ALT enzyme observed at the post diluted, post equilibrated and post thawed stages were 13.46 ± 0.59, 20.10 ± 0.19 and 28.31 ± 0.46 IU/L in TYG dilutor while 14.26 ± 0.14, 21.92 ± 0.48 and 28.93 ± 0.49 in TYD dilutor, respectively (Table 4.43).

A significant variation was observed among the two semen dilutors for ALT activity, being apparently lower in TYG dilutor followed by TYD dilutor. In present investigation values of ALT activity were recorded as 20.10 IU/ L and 21.92 IU/L at Post Equilibrated stage in TYG and TYD dilutor, respectively. Similarly, Dagli (2011) reported ALT value at post equiliberated stage in TYG and TYD dilutor as 20.06 IU/L and 19.49 IU/L in Osmanabadi and 20.11 IU/L and 20.38 IU/L in Sirohi bucks, respectively. In present study there was higher AST release than ALT in Berari bucks semen at post diluted and post equilibrated stage. Chauhan *et al.* (1994) in Jamnapari bucks also observed similar type of trend.

A significant variation was observed among the two semen dilutors for ALT activity, being apparently lower in TYG dilutor followed by TYD dilutor. In present investigation values of ALT activity were recorded as 28.31 IU/ L and 28.93 IU/L at post thawed stage in TYG and TYD dilutor, respectively. Similarly, Dagli (2011) reported ALT value at post thaw stage in TYG and TYD dilutor as 22.66 IU/L and 21.72 IU/L in Osmanabadi and 24.16 IU/L and 24.77 IU/L in Sirohi bucks, respectively.

In present study there was higher AST release than ALT in Berari bucks semen at post thaw stage. Similar report has been observed by Chauhan *et al.* (1994) in Jamnapari bucks.

Sinha *et al.* (1996) reported that extracellular activities of transaminases (AST and ALT) were significantly higher in extenders containing DMSO than lactose. Leakage of these enzymes was increased found to increase from the pre freeze to the post freeze stage.

From the result it can be opined that glycerol was better cryoprotectant than DMSO for Berari buck semen. Glycerol 6 % significantly ($P<0.05$) improved sperm post-thaw progressive motility, live sperm count

and acrosomal integrity and effectively protects the sperm damages compared to ethylene glycol during cryopreservation.(Sikarwar *et al.*,2016). Swelum *et al.* (2011) also reported that 7% glycerol in tris improved postthaw semen characteristics compared to skim milk based extenders in bull semen cryopreservation. Kulaksiz *et al.* (2013) indicated on the interaction between goat breed and glycerol concentration and reported that 7 % glycerol concentration in Saanen semen cryopreservation provided optimum freezability. Angora goat semen needed 5 % glycerol to provide optimum freezability. Moreover, Kilis goat semen could be successfully cryopreserved using 5, 7 and 9 % glycerol concentrations. Zeidan *et al.* (2002) reported that combined use of glycerol and DMSO causes less damage in the acrosome structure after freezing thawing in a rabbit sperm while Singh *et al.* (1995) reported that both motility and percentage of live spermatozoa were improved with a combination of glycerol and DMSO. Kulaksiz *et al.* (2010) reported variation in glycerol concentration in different goat breeds and reported that 7% glycerol provided optimum freezability in Saanen and 5 % provided optimum freezability in Kilis goat breed. Biswas *et al.* (2002) used 5%, 7% and 10 % of glycerol for freezing of buck semen and documented that motility and viability of thawed sperm frozen in 7% glycerol concentration were superior to those of sperm frozen and thawed in 5% and 10% glycerol concentration. Furthermore, Sonmez and Demira (2004) tested different concentration of glycerol and reported that 5% glycerol provided successful cryopreservation of ram semen and higher concentration of glycerol (7%) negatively affected post thaw semen quality. However, Awad (2011) compared 3% and 6 % glycerol concentration in cryopreservation of ram semen and did not found any differences in post thaw semen parameters between the two concentrations of glycerol.

Sikarwar *et al.* (2015) reported that the highest subjective sperm motility rate obtained was at 6% glycerol (41.7500 ± 0.085) and at 6% DMSO (21.7857 ± 1.17). Live sperm count was also higher at 6% Glycerol (77.9220 ± 0.22 %) than 6% DMSO (60.5221±0.25 %). Similarly intact acrosome percent was higher in 6% glycerol (79.2687±0.20 %) than 6%

DMSO (64.1869 ± 0.19c %). The cryoprotectants are added to extenders to protect the sperm from damage during freezing process (Singh *et al.* 1995). The level and type of cryoprotectants in semen diluent influence these events and their effects on the sperm cells during freezing (Harairy *et al.*, 2011).Glycerol can cause great osmotic damage to spermatozoa because Glycerol passes through the sperm membrane much slower than other cryoprotectants (Guthrie *et al.*, 2002). However, cryoprotectants, such as DMSO has been shown to protect spermatozoa against cryodamage as well as glycerol (Lovelock and Dishop, 1959). Researchers added glycerol and DMSO as cryoprotectants to the extender for freezing of Red deer epididymal sperm and DMSO showed the highest toxicity and glycerol showed the lowest (Fernandez *et al.*, 2005).

Similar with our result that researchers (Kundu *et al.*, 2000) determined the highest sperm motility (35%) in the extender which 6% glycerol was added. Singh *et al.* (1995) observed glycerol and DMSO combined use have given better results for viability and motility in a goat sperm freezing study. Glycerol (2%) and DMSO (4%) combined use causes less damage in the acrosome structure after freezing thawing in a rabbit sperm (Zeidan *et al.*, 2002). Glycerol has osmotic and toxic effects on the plasma membrane and metabolism of cryopreserved cells. It is responsible for the disorganization of sperm plasma membrane and reducing motility and fertilizing ability. Higher concentrations of glycerol lead to cell death (Swelum *et al.*, 2011). Glycerol enhances preservation of the acrosome and plasma membrane of ram spermatozoa, despite being toxic when used in high concentrations. Similar to the findings of the literature, plasma membrane integrity and acrosomal defects were less deteriorated at the groups at 6% Glycerol group.

The variations in ALT release have been attributed to differences in breeds (Sinha *et al.*, 2000), individual variations between the bucks (Tuli *et al.*, 1991), age of bucks (Tiwari, 2000), season of semen collection (Baruah *et al*., 1992), washing or non washing of sperms (Tuli *et al.*, 1991), rates of dilution and composition of diluent (Singh *et al.*, 1993 and

Singh *et al.*, 1996), glycerol levels (Bonadonna *et al.*, 1974), equilibration periods (Joshi *et al.*, 1990), cooling rates (Bhosrekar, 1975) and freezing rates.

Kim *et al.* (2011) documented that in boar sperm cryopreservation, membrane intactness were higher in 5% and 7% DMSO than 3% DMSO (P<0.05). Sperm motility of 5% DMSO was lower than that of 3% glycerol (P<0.005) and membrane intactness were lower in 5% DMA and DMSO than 3% glycerol (P<0.05). DMA and DMSO didn't improve sperm quality and glycerol remains the most useful for boar sperm cryopreservation.

Silva (2006) reported that in goats, it was previously demonstrated that the addition of 7% glycerol or 5% DMF to a skim milk-based extender promoted numerically higher results for post-thawing subjective motility and vigor with the use of glycerol in spite of the absence of significant difference. Farshad *et al.* (2009a) determined that extender containing 7 % glycerol added at 37^0C was significantly (P<0.05) shared better than 7% glycerol added at 5^0C and extender containing 1.75 % DMSO added at both temperatures. In conclusion the results of indicated that glycerol was still the cryoprotectant of choice for freezing of Markhoz goat sperm.

4.4.3 Comparison between washed and unwashed cryopreserved buck spermatozoa

Comparison between washed and unwashed cryopreserved buck spermatozoa were done to judge the effect of washing on post diluted and post freeze quality of Berari buck spermatozoa during cryopreservation.

a) Physical characteristics of washed and unwashed cryopreserved buck spermatozoa

i. Individual sperm motility

The mean percent individual sperm motility of washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in TYG and TYD dilutors are shown in Table 4.44 and results have been graphically presented in Fig. 4.7.

Table 4.44. Individual Motility percent (Mean ± SE) of Washed and Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	Post Diluted		Post Equilibrated		Post Thaw		Mean ± SE for Diluters	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	75.00 ± 0.65	72.08 ± 0.56	63.06 ± 0.93	60.14 ± 1.13	47.08 ± 0.80	43.06 ± 0.90	**TYG** 60.07^{I} ± 5.27	**Washed** 59.38^{i} ± 5.89
TYD	74.03 ± 0.59	71.11 ± 0.28	58.06 ± 1.05	57.08 ± 1.15	39.03 ± 0.62	38.06 ± 0.51	**TYD** 56.23^{II} ± 6.24	**Unwashed** 56.92^{ii} ± 5.74
Mean ± SE for Stages	73.06^{A} ± 0.89		59.58^{B} ± 1.32		41.81^{C} ± 2.07			

Mean bearing different superscript differ significantly

Table 4.44A. ANOVA for Individual Motility of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	74.94		
Stage	2	70757.40	2199.30**	0.93
Condition	1	1300.46	40.42**	0.76
Dilutor	1	3189.35	99.13**	0.76
Stage X Condition	2	17.13	0.53^{NS}	1.31
Stage X Dilutor	2	557.41	17.33**	1.31
Condition X Dilutor	1	150.00	4.66*	1.07
Stage X Condition X Dilutor	2	43.06	1.34^{NS}	1.85
Error	781	32.17	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Data pertaining to freezability of washed and unwashed buck spermatozoa showed non significantly higher individual sperm motility (75.00 ± 0.65, 63.06 ± 0.93 and 47.08 ± 0.80) at post diluted, post equilibrated and post thawed stage in washed semen, as compared to the corresponding values of unwashed spermatozoa (72.08 ± 0.56, 60.14 ± 1.13 and 43.06 ± 0.90) in TYG dilutor. Higher sperm motility (74.03 ± 0.59, 58.06 ± 1.05 and 39.03 ± 0.62) at post diluted, post equilibrated and post thawed stage in washed semen as compared to corresponding values of unwashed spermatozoa (71.11 ± 0.28, 57.08 ± 1.15 and 38.06 ± 0.51) in TYD dilutor but the difference was non-significant.

The analysis of variance for the individual sperm motility percentage of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between washed and unwashed spermatozoa at each stage of freezing, washing condition, dilutors and stage X dilutor interaction. A non significant difference was observed in stage X condition X dilutor interaction. (Table 4.44A).

According to Choe *et al.* (2006), the presence of seminal plasma influences sperm motility and percentage of dead spermatozoa of equilibrated goat semen. In agreement with these studies, we found that the presence of seminal plasma affected the motility and spermatozoa at 5^0C in equilibrated and post-thaw semen are observed in present study.

After thawing, acrosomes were affected by seminal plasma more than those at 5^0C, in diluted and equilibrated semen

Kozdrowski *et al.*, (2007) studied the semen was centrifuged in order to remove its plasma and diluted in Tris buffer extender containing glucose, citric acid and glycerol with 20% addition of egg yolk. the removal of semen plasma through centrifugation improved motility properties of goat semen following the freezing/thawing procedure. It may be the result of sperm washing that removes the reactive seminal proteins preventing cell membrane disruption lethal to sperm. (Anand *et al.*, 2017)

Removal of seminal plasma improved the percentage of motile spermatozoa in frozen-thawed samples ($P<0.05$). These findings about with separation of seminal plasma are in disagreement with those of Ritar and Salmon (1983), Chauhan and Anand (1990), Tuli and Holtz (1994) and Azerado *et al.* (2001) verified a higher percentage of motility when the seminal plasma was present than when it was removed. Ritar and Salamon (1983) who obtained higher rates of live spermatozoa after thawing when the seminal plasma had been removed. Also, Ustuner *et al.* (2009) obtained similar findings with current study, and pointed out that the presence of buck seminal plasma had a detrimeantal effect on post-thaw motility. Hence, the solution used for seminal plasma separation may have been important. The seminal plasma during the non-breeding season under temperate climate conditions is capable of stimulating motility of ejaculated and epidiymal sperm (Corteel,1992)

Likewise, Tuli *et al.* (1991) obtained similar results estimating spermatozoa motility with subjective and computer methods in fresh goat and boar semen, and freezing/thawing bull, goat and boar semen. It is noted that the results of estimates of the percentage of motile spermatozoa following freezing/thawing obtained by different authors are not uniform, which indicates that an array of factors may affect post-freezing properties of goat semen. According to Ritar and Salamon (1982) the removal of semen plasma has a favorable effect on its freezing/thawing properties which was confirmed as per present studies. Similar results with regard to the percentage of motile spermatozoa were presented by Ritar *et al.* (1990) and Ritar and Salamon (1982).

According to Ritar *et al.* (1990) freezing/thawing properties of goat semen are affected, among others, by the temperature at which straws are filled with semen, degree of dilution, methods of cooling and freezing, and the way of semen storage. They point to the fact that goat semen freezes better in pellets than in straws. Ritar and Salamon (1982) think that the motility of goat semen after freezing/thawing is influenced by an array of interacting factors, such as individual features of the male, month of semen collection, quantity of

added yolk and the removal of semen plasma. Contradictory to this (Anand *et al.*, 2017) observed more percent live sperm concentration and percent Intact Acrosome at after equilibration and after thawing in unwashed semen. This, however, contradicts with the findings of Tuli and Holtz (1994), Gil *et al.* (2000), Azeredo *et al.* (2001) and Peterson *et al.* (2007) who reported that the removal of seminal plasma decreased motility in frozen-thawed spermatozoa. Furthermore, Angora buck sperm frozen, with or without centrifugation/ washing in the Bioxcell extender, demonstrated higher percentages of subjective motility (58.1 ± 3.0% and 53.5 ± 3.8%, respectively) compared to that of the groups Tris extender (40.9 ± 1.8% with centrifugation and 45.0 ± 3.1% without centrifugation) (Sariozkan *et al.*, 2010). Where as, Bezjian *et al.*, 2013, observed statistically non significant difference between progressive motility, percent live sperm abnormality and percent Intact Acrosome at after equilibration and after thawing in unwashed semen.

The results obtained in this study indicated that high concentration (18%) of egg yolk did not properly increase the post-thaw motility of non-washed semen regardless of the season. Memon *et al.* (1985) reported a drastic deterioration in post-thaw cell survival when non-washed spermatozoa were diluted in an extender with 11% egg yolk. In contrast with present study results of, Daskın and Tekin (1996) reported that the non-washed post-thaw sperm motility extended with 20% egg yolk was better than that without egg yolk extending during the breeding season. Cabrera *et al.* (2005) found that washing negatively affected the cryosurvival of semen diluted with extender contained 6% or 12% egg yolk when out of season. Differences in the response of egg yolk concentrations could be related to the toxicity of EYCE in seminal plasma (Leboeuf *et al.*,2000), season of semen production, breed, and buck (Purdy,2006 and Tuli and Holtz, 1994). Leboeuf *et al.* (2000) reported that removal of seminal plasma by centrifugation of buck semen increased motility and the percentage of live spermatozoa during storage in egg yolk diluents. In addition, Islam *et al.* (2006) and Daramola and Adekunle (2017) obtained beneficial effect of the removal of seminal plasma on the quality of goat semen during preservation in tris egg yolk extender.

Some enzymes in seminal plasma, originating from bulborethral gland secretion, catalyse the hydrolysation of egg yolk lecithin and milk triglycerides of the extender, releasing sperm toxic substances (lysolecithin and fatty acids) that lead to sperm damage (Pellicer Rubio and Combamous, 1998). Removal of seminal plasma is usually carried out in artificial insemination programme in order to reduce its adverse effect on spermatozoa and improve sperm quality (Marti *et al.,* 2006). The improvement in sperm qualityin the present study indicated the beneficial effects of separating sperm by centrifugation from deleterious effects of coagulating enzyme such as phospholipase A in bulbourethral gland before dilution and freezing. Leboeuf *et al.* (2000) reported that phospholipase A coagulates egg yolk extender and reduces the viability of sperm cells. In contrast, some findings indicated positive results without centrifugation (Cabrera *et al.*, 2005; Ari and Daşkin, 2010). Seminal plasma protects the sperm from damage by oxidative stress (Saleh *et al.*, 2002), notwithstanding, ejaculated semen contains aging spermatozoa, defects, leukocytes and particle debris that can reduce sperm survival. Therefore, the separation of mammalian spermatozoa from seminal plasma by centrifugation is preferred and supported by several studies in order to increase motility and fertility after freeze thaw procedure (Kozdrowski *et al.*, 2007).In addition, different findings indicated that removal of the seminal fluid of chilled semen increases the sperm survival and motility (Kozdrowski *et al.*, 2007).

Goat semen differs from other mammalian species due to the presence of enzymes in the seminal plasma that react with egg yolk and milk, resulting in damaged spermatozoa following production of toxic compounds (Pellicer Rubio and Combarnous, 1998). Furthermore, the lifespan of stored sperm is increased by centrifugation (Pellicer Rubio *et al.*, 1997). The results of study are further supported by the work of Marti *et al.* (2006) who showed that centrifugation yielded higher percentages of capacitated spermatozoa.

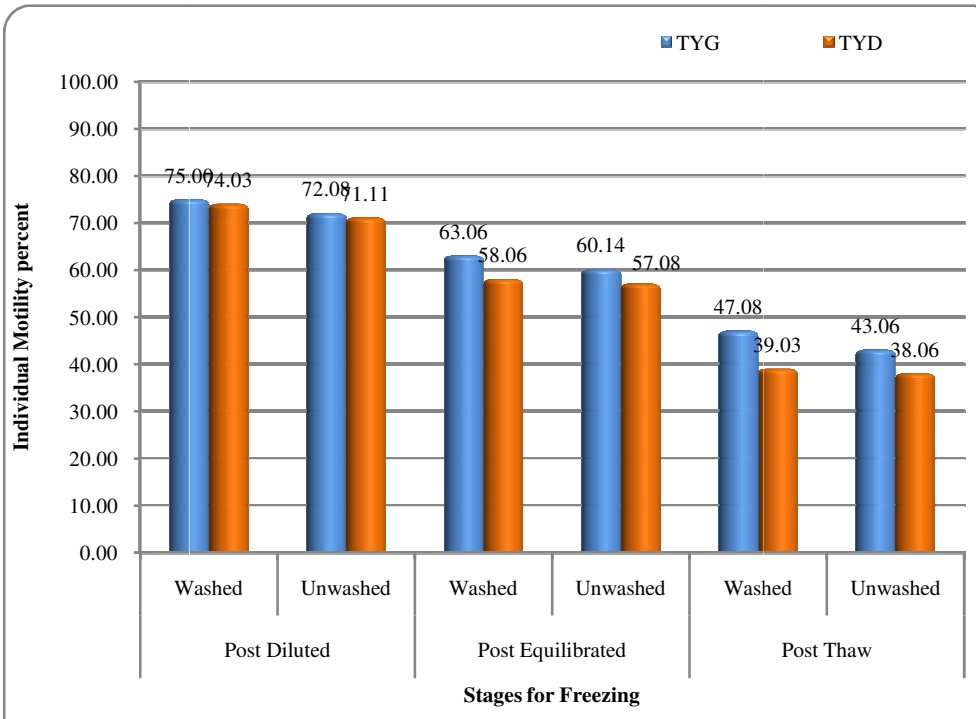

Fig. 4.7. Individual Motility percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at different freezing stages in Cryopreservation group

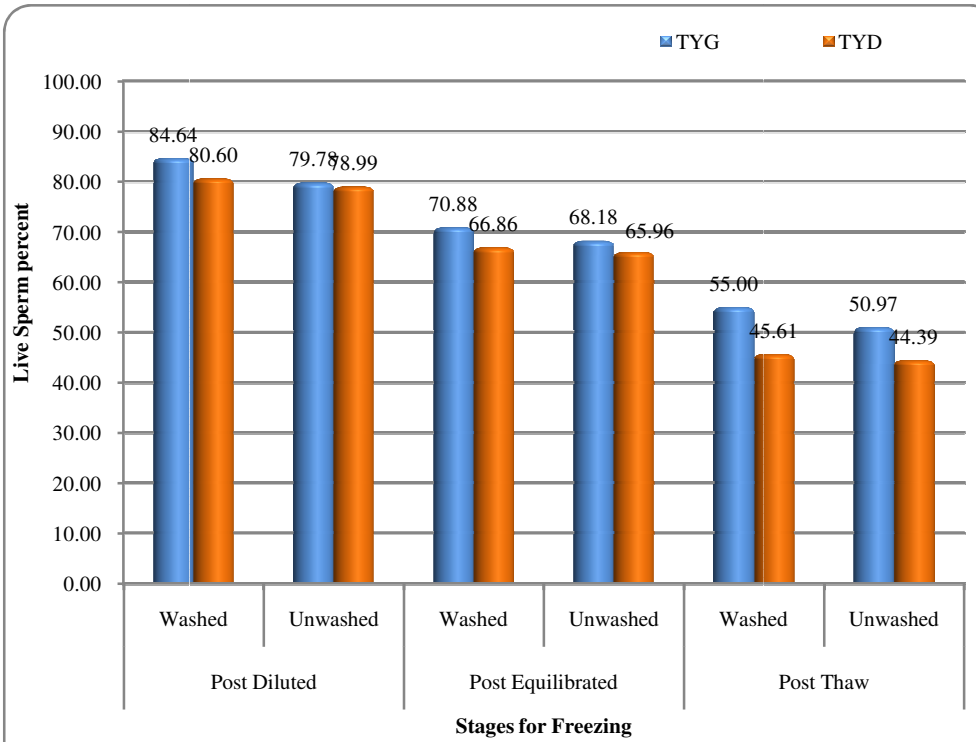

Fig. 4.8. Live Sperm percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at different freezing stages in Cryopreservation group

ii. Live sperm percent

The mean percent of live sperm of washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in TYG and TYD dilutors are shown in Table 4.45 and results have been graphically presented in Fig. 4.8.

Data pertaining to freezability of washed and unwashed buck spermatozoa showed non significantly higher live sperm percentage (84.64 ± 0.6, 70.88 ± 0.68 and 55.00 ±1.18) at post diluted, post equilibrated and post thawed stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (79.78 ± 0.41, 68.18 ± 1.23 and 50.97 ± 0.85) in TYG dilutor. A non significantly higher live sperm percent was observed (80.60 ± 0.68, 66.86 ± 1.04 and 45.61 ± 0.85) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (78.99 ± 0.33, 65.96 ± 1.13 and 44.39 ± 0.64) in TYD dilutor.

The analysis of variance for the live sperm percentage of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between washed and unwashed spermatozoa at each stage of freezing, washing condition, dilutors, stage dilutor interaction and washing condition X dilutor interaction while a non-significant difference observed between stage X condition X dilutor interaction. (Table 4.45A).

Table 4.45. Live Sperm percent (Mean ± SE) of Washed and Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	Post Diluted		Post Equilibrated		Post Thaw		Mean ± SE for Diluters	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	84.64 ± 0.61	79.78 ± 0.41	70.88 ± 0.68	68.18 ± 1.23	55.00 ± 1.18	50.97 ± 0.85	**TYG** $68.24^{I} \pm 5.42$	**Washed** $67.26^{i} \pm 6.09$
TYD	80.60 ± 0.68	78.99 ± 0.33	66.86 ± 1.04	65.96 ±1.13	45.61 ± 0.85	44.39 ± 0.64	**TYD** $63.73^{II} \pm 6.41$	**Unwashed** $64.71^{ii} \pm 5.91$
Mean ± SE for Stages	$81.00^{A} \pm 1.26$		$67.97^{B} \pm 1.07$		$48.99^{C} \pm 2.46$			

Mean bearing different superscript differ significantly

Table 4.45A. ANOVA for Live Sperm of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	72.27		
Stage	2	74608.08	2652.63**	0.87
Condition	1	1408.11	50.06**	0.71
Dilutor	1	4387.51	156.00**	0.71
Stage X Condition	2	37.47	1.33^{NS}	1.23
Stage X Dilutor	2	662.50	23.56**	1.23
Condition X Dilutor	1	369.47	13.14**	1.00
Stage X Condition X Dilutor	2	10.06	0.34^{NS}	1.73
Error	781	28.13	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Washing of buck spermatozoa was clearly beneficial for their survival during incubation shortly after resuspension (fresh sperm) or after freeze-thawing (Drobnis *et al.,* 1980).

iii. Abnormal sperm percent

The mean abnormal sperm percent of washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in TYG and TYD dilutors are shown in Table 46 and results have been graphically presented in Fig. 4.9.

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly lower abnormal sperm percentage (6.90 ± 6.50, 9.93 ± 0.25 and 18.26 ± 0.19) at post diluted, post equilibrated and post thawed stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (7.93 ± 0.1, 12.06 ± 0.22 and 23.79 ± 0.71) in TYG dilutor. A significantly lower abnormal sperm percentage was observed (8.78 ± 0.26, 10.39 ± 0.20 and 26.11 ± 0.35) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (10.32 ± 0.30, 13.94 ± 0.40 and 26.92 ± 0.47) in TYD dilutor.

The analysis of variance for the abnormal sperm percentage of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between washed and unwashed spermatozoa at each stage of freezing, washing condition, dilutors, stage X condition interaction, stage X dilutor interaction, condition X dilutor interaction and stage X condition X dilutor interaction. (Table 4.46A).

Percentage of abnormal spermatozoa was higher in unwashed and washed groups reported by Daskin *et al.* (2010)

Azeredo *et al.* (2001) reported that, removal of seminal plasma decreased minor defects (18.40%) increased major defect (8.20%) in frozen samples.

Table 4.46. Abnormal Sperms percent (Mean ± SE) of Washed and Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	Post Diluted		Post Equilibrated		Post Thaw		Mean ± SE for Dilutors	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	6.90^{j} ± 0.50	7.93^{i} ± 0.15	9.93^{g} ± 0.25	12.06^{f} ± 0.22	18.26^{d} ± 0.19	23.79^{c} ± 0.71	**TYG** 13.15^{II} ± 2.69	**Washed** 13.40^{ii} ± 3.00
TYD	8.78^{h} ± 0.06	10.32^{g} ± 0.30	10.39^{g} ± 0.20	13.94^{e} ± 0.40	26.11^{b} ± 0.35	26.92^{a} ± 0.47	**TYD** 16.08^{I} ± 3.37	**Unwashed** 15.83^{i}±3.15
Mean ± SE for Stages	8.48^{C} ± 0.72		11.58^{B} ± 0.91		23.77^{A} ± 1.95			

Mean bearing different superscript differ significantly

Table 4.46A. ANOVA for Abnormal Sperm of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	5.40		
Stage	2	18813.19	8295.59**	0.25
Condition	1	1276.04	562.67**	0.20
Dilutor	1	1855.04	817.97**	0.20
Stage X Condition	2	72.82	32.11**	0.35
Stage X Dilutor	2	369.19	162.80**	0.35
Condition X Dilutor	1	46.29	20.41**	0.28
Stage X Condition X Dilutor	2	198.34	87.46**	0.50
Error	781	2.27	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

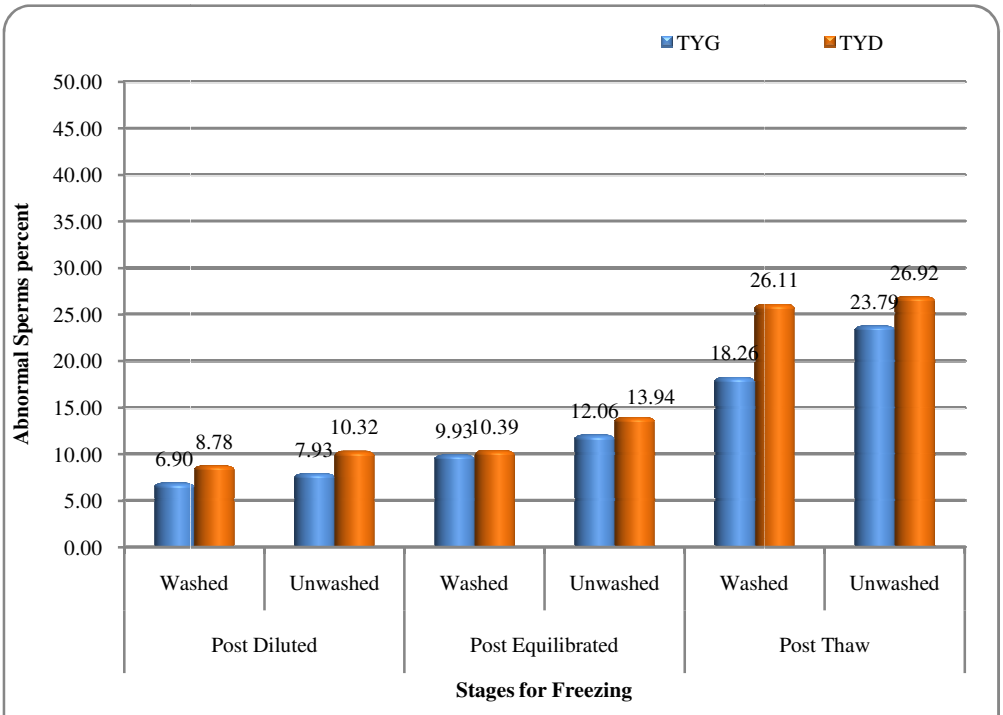

Fig. 4.9. Abnormal Sperms percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at different freezing stages in Cryopreservation group

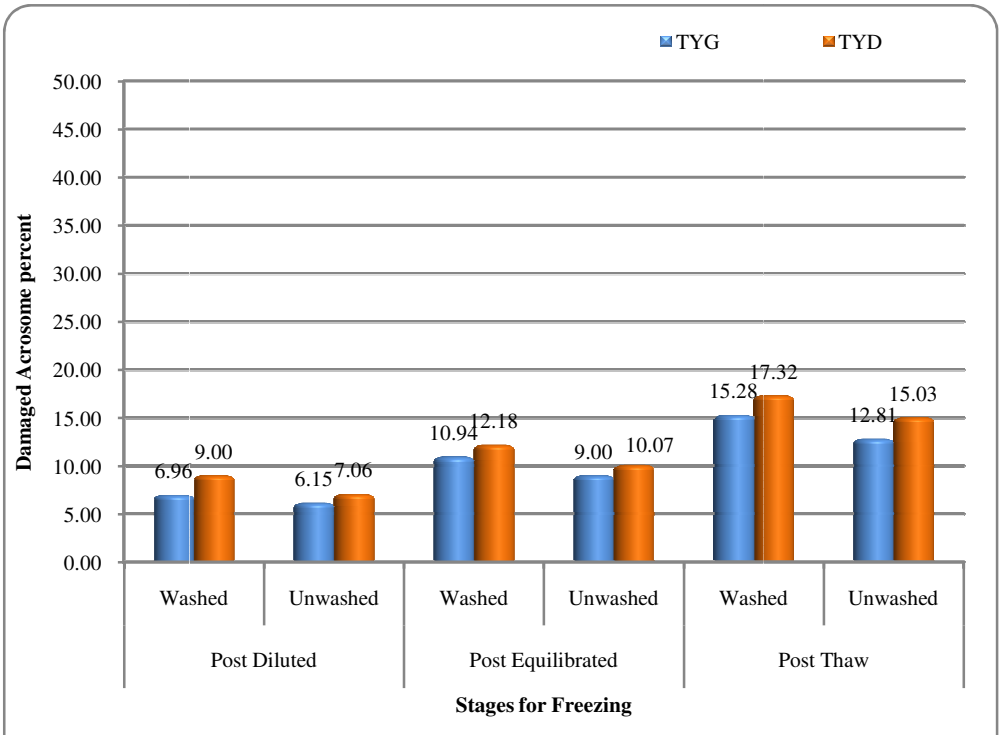

Fig. 4.10. Damaged Acrosome percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at different freezing stages in Cryopreservation group

b) Sperm function tests washed and unwashed cryopreserved buck spermatozoa

i. Damaged acrosome percent

The mean percent of damaged acrosome of washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in TYG and TYD dilutors are shown in Table 4.47 and results have been graphically presented in Fig. 4.10.

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly higher damaged acrosome percent (6.96 ± 0.45, 10.94 ± 0.34 and 15.28 ± 0.23) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (6.15 ± 0.37, 9.00 ± 0.14 and 12.81 ± 0.31) in TYG dilutor. A significantly higher damaged acrosome percent was observed (9.00 ± 0.08, 12.18 ± 0.24 and 17.32 ± 0.29) at post diluted, post equilibrated and post thawed stages) in washed semen as compared to corresponding values of unwashed spermatozoa (7.06 ± 0.29,10.07 ± 0.38 and 15.03 ± 0.30) in TYD dilutor respectively. The values of percent damaged acrosome were significantly ($P<0.01$) higher in TYD dilutor in washed semen at post thawed stage of freezing.

Analysis of variance for the washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was significant ($P<0.01$) difference between washed and unwashed spermatozoa at both the stages of freezing, washing condition, dilutors, stage X condition interaction, stage X dilutor interaction and stage X condition X dilutor interaction for percentage of damaged acrosome. (Table 4.47A).

Acrosome abnormality after thawing was higher in unwashed Angora goat semen compared with washed Angora goat semen. According to Pellicer-Rubio and Combarnous (1998), the physiological function of BUSgp60 in goat seminal plasma could be related to the acrosome reaction because it has been showed a high degree of similarity with lipases of the PL-RP2 subfamily, which present lipase and phospholipase activities, for participation in the energy metabolism of spermatozoa.

Table 4.47. Damaged Acrosome percent (Mean ± SE) of Washed and Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	Post Diluted		Post Equilibrated		Post Thaw		Mean ± SE for Diluters	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	6.96^{h} ± 0.45	6.15^{i} ± 0.37	10.94^{e} ± 0.34	9.00^{g} ±0.14	15.28^{b} ± 0.23	12.81^{c} ± 0.31	**TYG** 10.19^{II} ± 1.41	**Washed** 11.95^{i} ± 1.57
TYD	9.00^{g} ± 0.08	7.06^{h} ± 0.29	12.18^{d} ± 0.24	10.07^{f} ± 0.38	17.32^{a} ± 0.29	15.03^{b} ± 0.30	**TYD** 11.78^{I} ± 1.57	**Unwashed** 10.02^{ii} ±1.39
Mean ± SE for Stages	7.29^{C} ± 0.60		10.55^{B} ± 0.67		15.11^{A} ± 0.92			

Mean bearing different superscript differ significantly

Table 4.47A. ANOVA for Damaged Acrosome of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	3.76		
Stage	2	4439.13	3845.55**	0.17
Condition	1	803.11	695.72**	0.14
Dilutor	1	543.08	470.47**	0.14
Stage X Condition	2	18.79	16.27**	0.25
Stage X Dilutor	2	17.96	15.55**	0.25
Condition X Dilutor	1	7.60	6.58*	0.20
Stage X Condition X Dilutor	2	8.42	7.29**	0.35
Error	781	1.15	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

On the other hand, Manjuah and Therien (2002) observed that some proteins called bovine seminal plasma proteins (BSP 1,2,3,4) had important roles stabilization of acrosome membrane. So removal of goat seminal plasma containing BUSgp60 (called EYCE) and addition of ovine or bovine seminal plasma containing BSP 1,2,3,4 or BSP like proteins may have decreased acrosome abnormality in washed Angora goat semen. Similarly, Azeredo *et al.* (2001) reported that, more or increased damaged acrosome (83.36 %) in washed frozen samples and (83.15 %) in unwashed semen.

ii. Hypo-osmatic swelling test

The mean percent of actual HOST reacted washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in different dilutors are compared and depicted in Table 48 and results have been graphically presented in Fig. 4.11.

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly higher HOST reacted sperm percent (73.32 ± 0.62, 64.19 ± 0.56 and 48.18 ± 0.84) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (73.32 ± 0.62, 63.14 ± 0.31 and 45.38 ± 0.65) in TYG dilutor. A significantly higher HOST percent was observed (68.13 ± 0.49, 57.96 ± 0.56 and 37.08 ± 0.53) at post diluted, post equilibrated and post thawed stages) in washed semen as compared to corresponding values of unwashed spermatozoa (66.99 ± 0.39, 56.03 ± 0.70 and 36.03 ± 0.88) in TYD dilutor respectively. The values of percent HOST reacted spermatozoa were significantly ($P<0.05$) higher in TYG dilutor in washed semen at post diluted and post equilibrated stages of freezing.

Analysis of variance for the washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was significant ($P<0.05$) difference between washed and unwashed spermatozoa at both the stages of freezing, washing condition, dilutors, stage X condition interaction, stage X dilutor interaction and stage X condition X dilutor interaction for percentage of HOST reacted spermatozoa (Table 4.48A).

Table 4.48. HOST percent (Mean ± SE) of Washed and Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	Post Diluted		Post Equilibrated		Post Thaw		Mean ± SE for Diluters	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	73.32^{a} ± 0.62	73.32^{a} ± 0.62	64.19^{d} ± 0.56	63.14^{e} ± 0.31	48.18^{h} ± 0.84	45.38^{j} ± 0.65	**TYG** 59.59^{I} ± 4.92	**Washed** 57.47^{ii} ±5.51
TYD	68.13^{b} ± 0.49	66.99^{c} ± 0.39	57.96^{f} ± 0.56	56.03^{g} ± 0.70	37.08^{i} ± 0.53	36.03^{k} ± 0.88	**TYD** 55.92^{II} ± 5.76	**Unwashed** 57.94^{i}± 5.71
Mean ± SE for Stages	62.89^{A} ± 1.68		57.39^{B} ±1.98		52.83^{C} ± 3.01			

Mean bearing different superscript differ significantly

Table 4.48A. ANOVA for HOST of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	3.09		
Stage	2	7301.63	2043.07**	0.31
Condition	1	48.64	13.61**	0.25
Dilutor	1	2741.34	767.06**	0.25
Stage X Condition	2	26744.53	7483.38**	0.44
Stage X Dilutor	2	6949.68	1944.59**	0.44
Condition X Dilutor	1	4.89	1.37^{NS}	0.36
Stage X Condition X Dilutor	2	23648.76	6617.16**	0.62
Error	781	3.57	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

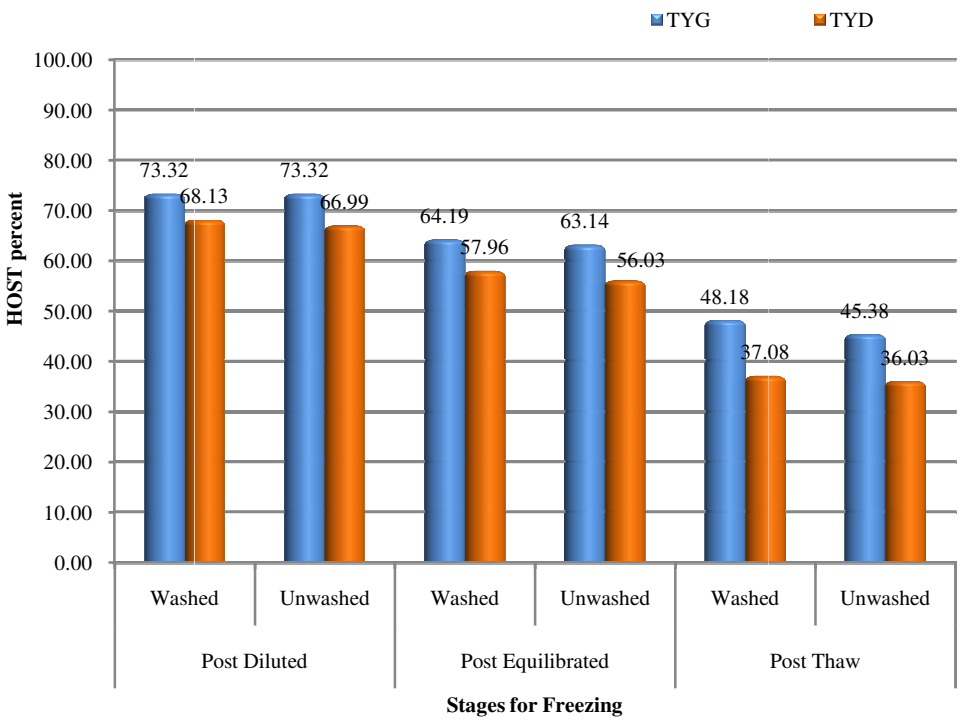

Fig. 4.11. HOST percent (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at different freezing stages in Cryopreservation group

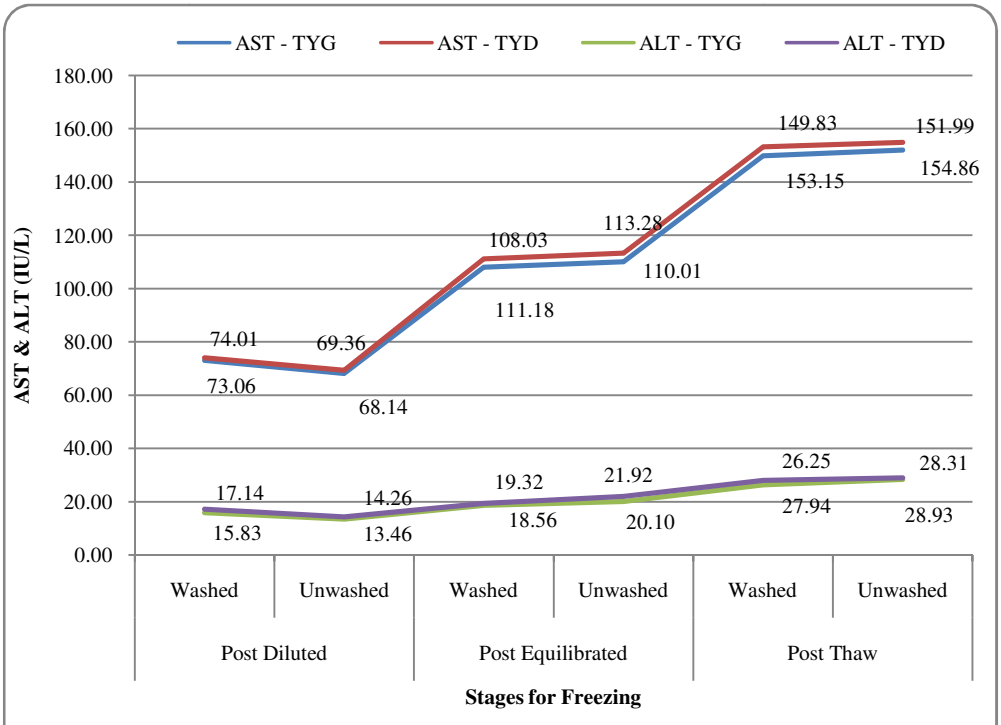

Fig. 4.12. AST & ALT (Mean ± SE) of Washed and Unwashed Spermatozoa in different dilutor at different freezing stages in Cryopreservation group

Plasma membrane plays an important role in maintaining the structural and biochemical function integrity of spermatozoa within the limits of osmotic stress. When exposed to hypo osmotic conditions, the biochemically active sperm cell membrane absorbs fluid from the extracellular medium to maintain equilibrium between fluid compartments, intracellular and extracellular. Absorption of fluid results in swelling of the cell due to expansion of the cell membrane which in turn culminates in curling of the tail (Jayendran *et al.*, 1984). Therefore, the proportion of hypo osmotic reacted spermatozoa in semen indicates the presence of spermatozoa with normal structural and functional integrity.

The plasma membrane integrity of washed and unwashed spermatozoa was compared by investigating the hypo-osmotic swelling test of sperms in the seminal plasma during different stages of preservation. The results of the present study revealed that the washing of semen enhanced the maintenance of plasma membrane integrity of spermatozoa during freezing process.

It may be pointed out that washing and addition of ovine or bovine seminal plasma positively affected membrane integrity. Nevertheless Azerado *et al.* (2001) did not find any difference between washing and not washing goat semen after thawing for membrane integrity. Different results may have rooted from different washing solutions. Memon *et al.* (1985), Ritar and Salamon (1982) indicated that removal of the seminal plasma is necessary for maximizing post-thaw motility and acrosomal integrity in goat semen. But according to Azeredo *et al.* (2001). Chauhan and Anand (1990) and Tuli and Holtz (1994) plasma is beneficial also when the spermatozoa are frozen with the egg yolk containing diluents.

iii. Leakage of Enzyme (AST)

The mean of AST of washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in different dilutors are compared and depicted in Table 4.49 and results have been graphically presented in Fig. 4.12.

Table 4.49. AST (Mean ± SE) of Washed and UnwashedSpermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	AST (IU/L) at Post Diluted stage		AST (IU/L) at Post Equilibrated stage		AST (IU/L) at Post Thaw stage		Mean ± SE for Diluters	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	73.06 ± 0.63	68.14 ± 0.95	108.03 ± 1.18	110.01 ± 0.62	149.83 ± 1.44	151.99 ± 1.03	**TYG** 110.18^{II} ± 14.70	**Washed** 111.54 ±14.27
TYD	74.01 ± 0.97	69.36 ± 1.74	111.18 ± 0.96	113.28 ± 0.90	153.15 ± 0.28	154.86 ± 0.15	**TYD** 112.64^{I} ± 15.05	**Unwashed** 111.57 ± 15.47
Mean ± SE for Stages	71.14^{C} ± 1.42		110.63^{B} ± 1.10		152.46^{A} ± 1.02			

Mean bearing different superscript differ significantly

Table 4.49A. ANOVA for AST of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	17.30		
Stage	2	476217.31	45022.40**	0.53
Condition	1	15.84	1.50^{NS}	0.43
Dilutor	1	1312.76	124.11**	0.43
Stage X Condition	2	1100.48	104.04**	0.75
Stage X Dilutor	2	102.37	9.67**	0.75
Condition X Dilutor	1	0.30	0.00^{NS}	0.61
Stage X Condition X Dilutor	2	2.50	0.24^{NS}	1.06
Error	781	10.58	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Data pertaining to freezability of washed and unwashed buck spermatozoa showed non-significantly higher AST values (73.06 ± 0.63, 108.03 ± 1.18 and 149.83 ± 1.44) at post diluted, post equilibrated and post thawed stages in washed semen) as compared to corresponding values of unwashed spermatozoa (68.14 ± 0.95, 110.01 ± 0.62 and 151.99 ± 1.03 in TYG dilutor). A non-significantly higher AST value (74.01 ± 0.97, 111.18 ± 0.96 and 153.15 ± 0.28) was observed at post diluted, post equilibrated and post thawed stages) in washed semen as compared to corresponding values of unwashed spermatozoa (69.36 ± 1.74, 113.28 ± 0.90 and 154.86 ± 0.15) in TYD dilutor.

Analysis of variance for the AST value of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing , dilutors, stage X condition interaction and stage X dilutor interaction. A non significant difference was observed in stage X condition X dilutor interaction. (Table 4.49A)

iv. Leakage of Enzyme (ALT)

The mean of ALT of washed and unwashed buck spermatozoa during post diluted, post equilibrated and post thawed stages of freezing in different dilutors are compared and depicted in Table 4.50 and results have been graphically presented in Fig.4.12.

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly higher ALT values (15.83 ± 0.41, 18.56 ± 0.61 and 26.25 ± 0.60) at post diluted, post equilibrated and post thawed stages in washed semen) as compared to corresponding values of unwashed spermatozoa (13.46 ± 0.59, 20.10 ± 0.19 and 28.31 ± 0.46) in TYG dilutor. A significantly higher ALT value was observed (17.14 ± 0.49, 19.32 ± 0.61 and 27.94 ± 0.21) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (14.26 ± 0.14, 21.92 ± 0.48 and 28.93, ± 0.49 in TYD dilutor).

Table 4.50. ALT (Mean ± SE) of Washed and Unwashed Spermatozoa at different freezing stages in Cryopreservation Group of Berari buck

Stages for Freezing	ALT (IU/L) at Post Diluted stage		ALT (IU/L) at Post Equilibrated stage		ALT (IU/L) at Post Thaw stage		Mean ± SE for Diluters	Mean ± SE for Conditions
Condition / Dilutors	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed		
TYG	15.83i ± 0.41	13.46^{k} ± 0.59	18.56^{g} ± 0.61	20.10^{e} ± 0.19	26.25^{c} ± 0.60	28.31^{b} ± 0.46	**TYG** 20.42^{II} ± 2.38	**Washed** 20.84^{ii}±2.05
TYD	17.14^{h} ± 0.49	14.26^{j} ± 0.14	19.32^{f} ± 0.61	21.92^{d} ± 0.48	27.94^{b} ± 0.21	28.93^{a} ± 0.49	**TYD** 21.59^{I} ± 2.40	**Unwashed** 21.16^{i} ± 2.71
Mean ± SE for Stages	15.17^{C} ± 0.82		19.97^{B} ± 0.72		27.86^{A} ± 0.57			

Mean bearing different superscript differ significantly

Table 4.50A. ANOVA for ALT of Washed and Unwashed Semen of Berari Buck during cryopreservation

Source of variation	Degrees of freedom	Mean sum of squares	F cal	Critical Difference Values (5%)
Replications	71	5.07		
Stage	2	11812.37	3587.79**	0.30
Condition	1	22.37	6.79**	0.24
Dilutor	1	295.17	89.65**	0.24
Stage X Condition	2	474.32	144.07**	0.42
Stage X Dilutor	2	1.01	0.31^{NS}	0.42
Condition X Dilutor	1	1.58	0.48^{NS}	0.34
Stage X Condition X Dilutor	2	21.78	6.62**	0.60
Error	781	3.29	-	
Total	863	-	-	

* : Significant (P<0.05) **: Significant (P<0.01) NS : Non-significant

Analysis of variance for the ALT value of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing , washing condition, dilutors, stage X condition interaction and stage X condition X dilutor interactions. (Table 4.50A).

The investigators (Corteel *et al*, 1974; Drobnis *et al.* 1980, Ritar and salamon,1982) reported that washing of buck spermatozoa was clearly beneficial for their survival during incubation shortly after resuspension (fresh sperm) or after freeze-thawing which is concurrence with observation of present study.

The removal of seminal plasma increased Boer buck semen quality in cryopreservation process. This is in agreement with the findings of Ritar and Salamon (1982); Machado and Simplicio (1992) ; Love *et al.* (2005) and Kozdrowski *et al.* (2007) who reported a beneficial effect of removing seminal plasma on the freezability of semen. This, however, contradicts with the findings of Tuli and Holtz (1994), Gil *et al.* (2000), Azeredo *et al.* (2001) and Peterson *et al.* (2007) who reported that the removal of seminal plasma decreased motility in frozen-thawed spermatozoa. Furthermore, Angora buck sperm frozen, with or without centrifugation/washing in the Bioxcell extender, demonstrated higher percentages of subjective motility (58.1 ± 3.0% and 53.5 ± 3.8%, respectively) compared to that of the groups Tris extender (40.9 ± 1.8% with centrifugation and 45.0 ± 3.1% without centrifugation) (Sariozkan *et al.*, 2010). Some of these contraindications may be attributed to several factors, such as season, different processing procedures (namely, washing solution, centrifugation regimes, cooling and freezing rate), and the concentration of seminal plasma remaining after centrifugation. Moreover, species, breeds and individual variation are also critical factors because the compositions of seminal plasma and sperm membrane vary greatly between species and individuals. Among other, seasonal variation is one of the most important considerations (Leboeuf *et al.*, 2000).

Contradictory to this Ferreira *et al.* (2014) also reported that presence of seminal plasma and higher concentration of egg yolk in extender provides a higher viability in cryopreserved goat semen.

4.5 Conception rate of preserved semen

4.5.1 Conception rate in refrigerated semen in Berari goats

The conception rate in washed and unwashed refrigerated semen of Berari buck is depicted in Table 4.51 and Fig. 4.13. The conception rate of Berari buck semen preserved in washed refrigerated and unwashed refrigerated semen in estrus synchronized does was 62.50, and 37.50 percent, respectively. The overall conception rate for Berai buck refrigerated semen was 50.00 percent. Higher conception rate was observed for semen preserved in washed refrigerated semen as compared with unwashed refrigerated semen. It indicates that washed refrigerated semen was found to be more suitable for refrigeration preservation of Berari buck semen as compared to unwashed refrigerated semen.

Table 4.51. Conception rate in washed and unwashed groups inseminated with refrigerated semen in Berari Goats

Sr. No.	Particulars	No of Goats synchronized	No of Goats Inseminated	No of Goats detected conceived	Conception Rate (%)
1	Washed refrigerated	8	8	5	62.50%
2	Unwashed refrigerated	8	8	3	37.50%
Overall		16	16	8	50.00%

Mara *et al.* (2007) recoded that at day 50 from AI the percentages of pregnant goats were 71.4 % (30/42) with skim milk, 61.4 % (27/44) with TEMPOL and 48.8 % (22/45) with TEMPOL+HA, with significant differences between skim milk and TEMPOL + HA dilutor. Percentage kidding with unwashed semen with milk extender was 59.1%

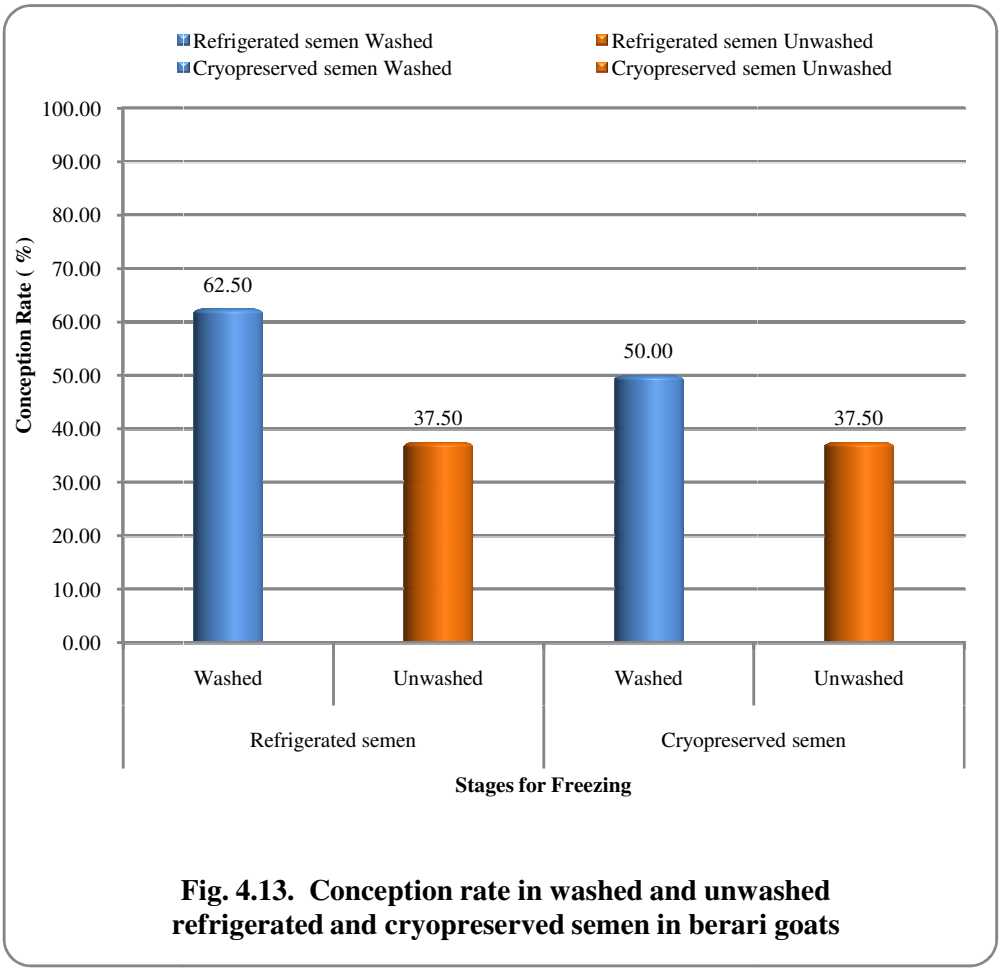

Fig. 4.13. Conception rate in washed and unwashed refrigerated and cryopreserved semen in berari goats

reported by Leboeuf *et al.* (2004) while Arrebola *et al.* (2013) reported 60% fertility in refrigerated semen.

Yotov (2015) evaluated the effect of semen extender with soybean lecithin and/or low concentration of glycerol on the quality of goat chilled-stored semen. Group I (n=6) was inseminated with 0.5 ml (400×106 sperm/ml) chilled-stored semen diluted with Ext. 1 and group II (n=6) received the same dose, but diluted with Ext.2. On day 20 after artificial insemination, the pregnancy rate in the first and the second groups was 83.3%s and 50%, respectively.

The conception rate in goats after AI depends on several factors, such as farm, age of the goat, size of the group to be inseminated, deep deposition of the semen in the cervix, number of previous kiddings of the goat and milk production. (Arrebola *et al.*, 2013).

Conception rates with fresh or chilled semen are satisfactory (65 to 75%) but miserably low (10 to 30%) if frozen-thawed semen is used (Olesen, 1993). Paulenz *et al.* (2002) reported a 51.5% lambing rate using semen stored at 5^0C diluted in a commercial tris-based extender.

Successful artificial insemination program involve multidimensional management including the detection of heat, proper time of insemination, semen storage method, handling and quality of semen, the efficiency of AI technician, ewes health, breed, feeding and others (Pervage *et al*.,2009).

4.5.2 Conception rate with cryopreserved semen in Berari goats

The conception rate in washed and unwashed cryopreserved semen of Berari buck is presented in Table 4.52 and graph Fig. 4.13. The conception rate of Berari buck semen preserved in washed cryopreserved semen and unwashed cryopreserved semen in estrus synchronized does was 50.00 and 37.50 percent, respectively. The overall conception rate for Berai buck cryopreserved semen was 43.75 percent. Higher conception rate was observed for semen preserved in washed cryopreserved semen as compared with unwashed cryopreserved semen. It indicates that washed cryopreserved

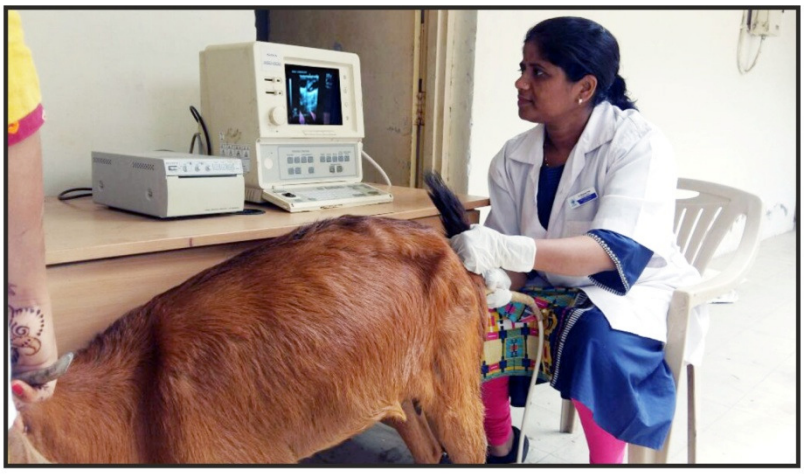

Plate 4.5. Pregnancy diagnosis by tramsrectal Ultrasonography method in inseminated goat

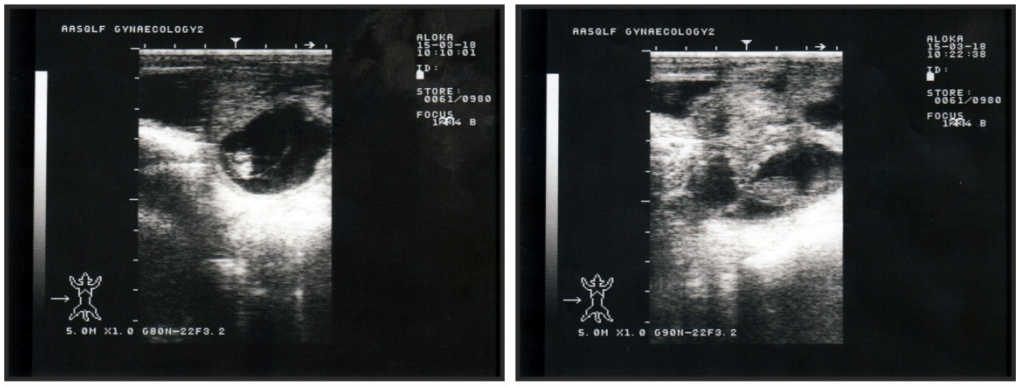

Plate 4.6. Single embryo along with embryonic vesicles

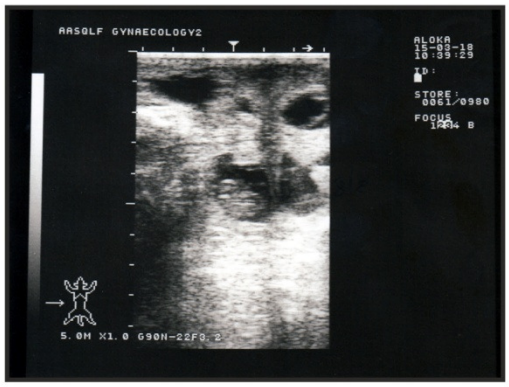

Plate 4.7. Twin embryo along with embryonic vesicles

semen was found to be more suitable for cryopreservation of Berari buck semen as compared to washed cryopreserved semen.

Table 4.52. Conception rate in washed and unwashed groups inseminated with cryopreserved semen in Berari Goats

Sr. No.	Particulars	No of Goats synchronized	No of Goats Inseminated	No of Goats detected Conceived	Conception Rate (%)
1	Washed cryopreserved	8	8	4	50.00%
2	Unwashed cryopreserved	8	8	3	37.50%
Overall		16	16	7	43.75%

Dorado *et al.* (2010) reported pregnancy rates for washed TRIS as 35.70 %. High conception rate in the present study may be due to the fact that the numbers of spermatozoa per dose (200 X 10^6 millions) used in our experiments exceeds the number of sperm needed to obtain the highest fertility achievable for most males. Therefore, the fertilizing potential of individual sperm in most semen samples could be masked by the excess sperm inseminated, as described in bulls (Shannon and Vishwanath, 1995).

Similarly, conception rate found by Salvador *et al.* (2005) was 57%.In this work, the effect of several factors on pregnancy after AI with frozen-thawed semen in MG goat was studied. Overall pregnancy rate results obtained in this work are similar to fertility results obtained in other breeds with frozen semen; in Angora goat: 51% (Ritar and Salamon 1983), in Saanen and Alpine goats: 65% (Leboeuf *et al.* 1998) and in German breeds WDE and BDE: 62% (Fischer *et al.,* 2001; Salvador *et al.*, 2005).

Singh *et al.*, (1995) reported 45.45% conception rate in TYG. Overall pregnancy rate results obtained in this work are similar to fertility results obtained in other breeds with frozen semen; in Angora goat: 51% (Ritar and Salamon 1983), in Saanen and Alpine goats: 65% (Leboeuf *et al.,* 1998) and in German breeds WDE and BDE: 62% (Fischer *et al.,* 2001).

The conception rate of Berari buck semen preserved in unwashed cryopreserved semen in estrus synchronized does was 37.50 percent.

Conception rate using buck semen preserve at ultra low temperature for AI in the range of 50.0 to 80.0 was reported by Ritar and Salamon (1983), Chauhan and Anand (1990), Sinha *et al.* (1995), Greyling and Nest (2000) and Beltran *et al.* (2013).These findings were higher than the finding of present study. Variation in conception rate may be due to variation in dilutor, breeds, age, climatic condition and breeding management

Similarly, Restall *et al.* (1987) reported 43.2 % and Mohammed *et al.* (2012) reported 45.8 % conception rate using frozen thawed semen. Ashmawy *et al.* (2010) reported conception rate using pellet frozen semen in 50% Damascus and 50% Baladi cross bred bucks as 31.57% which is lower than that recorded in present study.

Dagli (2011) reported 33.33 and 30.55 %S conception rate using Osmanabadi and Sirohi buck frozen semen using Tris egg yolk glycerol dilutor. These findings were slightly lower than those recorded in present study. The difference in observations may be due to variation in breeds, dilutors used, fertility of female etc. According to Salvador *et al.* (2005) the factors such as depth of semen deposited, inseminator skill, farm, sire, expression of estrus etc also affect the conception rate in does.

Batisa *et al.* (2009) has reported 43.6% of conception rate in Majorera goat breed using frozen thawed semen. In India, Bhattacharyya *et al.* (2012) reported higher pregnancy rate (71.43%) in Kashmir local goats which could be due to the use of Boer pellet semen with more volume and higher post thaw motility during breeding season.

Kharche *et al.* (2013) observed fertility following AI using frozen semen of Jamunapari bucks. Out of 32 Jamunapari goats inseminated, 17 become pregnant with a pregnancy rate of 53.12%. The pregnancy rate based on actual kidding was recorded to be 44.44% in goats restrained manually for insemination (group 1) whereas it was non significantly higher (64.28%) in goats restrained using specially designed AI cra te.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present work on "Seminal attributes preservation and fertilizing ability of refrigerated and cryopreserved semen of Berari buck" was carried out at the Department of Animal Reproduction, Gynaecology and Obstetrics, PGIVAS, Akola (Maharashtra). The research was planned to record the scrotal biometry, physical characteristics, functional tests of washed neat semen as well as unwashed semen of Berari buck preserved by refrigeration and cryopreservation. The conception rate with washed and unwashed refrigerated and cryopreserved semen in Berari goats was studied.

5.1 Scrotal Biometry

5.1.1 Scrotal circumference

The overall mean of scrotal circumference was 24.62 ± 0.51 cm in Berari buck. The highest scrotal circumference was observed in buck No.2 (26.50 cm) while the lowest was observed in buck No. 6 (23 cm). It was observed that the individual variation among bucks was significant ($P<0.05$).

The scrotal circumference was found to be highly significantly ($P<0.01$) and positively correlated with testicular length (0.859), testicular width (0.812), ejaculate volume (0.581), seminal pH (0.176), mass motility (0.423), Individual motility (0.337), live sperm count (0.420) and sperm concentration (0.433).

5.1.2 Testicular length

The overall mean of testicular length was measured as 7.67 ± 0.30 cm in Berari buck. The individual variation among bucks was significant ($P<0.05$).A significant and positive correlation of testicular length with testicular width (0.861), ejaculate volume (0.433), mass motility (0.370), live sperm count (0.422) and sperm concentration (0.397) was observed.

Correlation of testicular length with scrotal pH was statistically non-significant.

5.1.3 Testicular width

The mean of testicular width of Berari buck was 5.04 ± 0.23 cm. ranged between 5.90 to 3.90 cm. The highest testicular width was measured in buck No.2 (5.90 cm) while lowest in buck No 6 (3.90 cm). The individual variation among bucks was significant (P<0.05).Testicular width was significantly (P<0.05) and positively correlated with ejaculate volume (0.440), mass motility (0.319), individual motility (0.314), live sperm count (0.319) and sperm concentration (0.282) whereas positively and non-significantly correlated with seminal pH (0.069).

5.2 Seminal Attributes of Berari Buck Neat Semen in Refrigerated and Cryopreserved Group

5.2.1 Physical characteristics of neat semen in refrigerated group and cryopreserved group

a) Colour

The colour of Berari buck semen was found creamy in all the semen samples during research work.

i. Ejaculate volume

The overall mean volume of Berari buck neat semen was observed as 1.84 ± 0.06 ml (Range :0.80 ml to 1.60 ml) and 1.22 ± 0.04 ml (Range : 0.90 to 1.60 ml) in refrigerated and cryopreserved group, respectively. The individual variation among bucks was highly significant (P<0.01) in refrigerated group and significant (P<0.05) in cryopreserved group.

In refrigerated group the ejaculate volume was found to be significantly (P<0.05) and positively correlated with mass motility (0.293), individual motility (0.36), live sperm count (0.426), sperm concentration (0.560) and HOST (0.396). The negative significant (P<0.05) correlation of ejaculate volume was found with the damaged acrosome (-0.367) and AST

(-0.410). Whereas, the correlation of ejaculate volume with abnormal sperm count (-0.228) and ALT (-0.036) were negative and statistically non-significant. In cryopreserved group the ejaculate volume was found to be significantly ($P<0.05$) and positively correlated with seminal pH (0.29), mass motility (0.28), live sperm count (0.371), sperm concentration (0.32) and HOST (0.40). The negative significant ($P<0.05$) correlation of ejaculate volume was found with abnormal sperm count (-0.24), damaged acrosome (-0.29) and AST (-0.27). The correlation of ejaculate volume with individual motility (0.23) and ALT (0.09) were positive but was statistically non-significant.

ii. Seminal pH

The mean pH value of Berari buck semen was noted as 6.78 ± 0.01 (Range: 6.60 to 7) in refrigerated group while 6.79 ± 0.01 (Range: 6.70 to 7.00) in cryopreserved group and the difference is non-significant among both groups.

In refrigeration group, the correlation of seminal pH was non significantly and positively correlated with mass motility (0.205), individual motility (0.121), live sperm count (0.189), sperm concentration (0.196) and HOST (0.095). The negative significant ($P<0.05$) correlation of ejaculate volume was found with damaged acrosome (-0.308). The correlation of seminal pH with abnormal sperm count (-0.196), AST (-0.163) and ALT (-0.029) were negative and statistically non-significant. In cryopreserved group, Seminal pH was significantly and positively correlated with sperm content (0.32). The seminal pH was significantly and positively correlated with mass motility (0.12), individual motility (0.07), live sperm count (0.21), HOST (0.21), AST (0.14) and ALT activity (0.02) and negatively correlated with abnormal sperm count (-0.16), damage acrosome (-0.10).

iii. Mass motility

Mass motility of Berari buck semen ranged between +3 to +5 with a mean value of 3.92 ± 0.14 in refrigerated and 4.06 ± 0.12 in

cryopreserved group. The individual variation among bucks was highly significant (P<0.01).

In refrigerated group mass motility was significantly (P<0.05) and positively correlated with individual sperm motility (0.383), live sperm count (0.451), sperm concentration (0.280) and HOST(0.362). Negatively significant correlation of the mass motility was observed with the abnormal sperm count (-0.319), damaged acrosome (-0.259) and AST (-0.240). However ALT (-0.046) alone was observed negatively and non-significantly correlated with mass motility. In cryopreserved group, mass motility was significantly (P<0.05) and positively correlated with live sperm count (0.33), sperm concentration (0.32), HOST (0.38) and non-significantly and negatively correlated with abnormal sperm count (-0.02), damage acrosome (-0.21), AST (-0.09). The correlation of mass motility with ALT (0.01) was positive but non-significant.

iv. Individual sperm motility

The individual sperm motility averaged 84.44 ± 0.88 percent in refrigerated group while 85.28 ± 0.95 percent in cryopreserved group in Berari buck semen with a range of 70 to 90 percent. The individual variation among bucks was non-significant.

In the refrigerated group, the correlation between individual sperm motility and live sperm count (0.949) and HOST (0.764) was positive and significant (P<0.05).The correlation of Individual sperm motility with sperm concentration (0.182) was positive but non-significant. The values of abnormal sperm count (-0.322), damaged acrosome (-0.610) were negatively correlated with motility. The individual motility was negatively and significantly correlated with abnormal sperm count (-0.322) and damaged acrosome (-0.610). A statistically non-significant and negative correlation of individual motility with AST (-0.117) and ALT (-0.088) was observed. In the cryopreserved group, the correlation between individual sperm motility and live sperm count (0.47) was positive and significant. Individual sperm motility was positive with the sperm concentration (0.15) and HOST (0.08) but

statistically non-significant. The negative and significant correlation was recorded with the abnormal sperm count and damaged acrosome (-0.37). Individual motility was found to be negative with both the enzymes AST (-0.20) and ALT (-0.08) but they differ non-significantly.

v. Live sperm count

The percentage of live spermatozoa was 86.42 ± 0.88 (Range: 74 to 93) and 90.01 ± 0.66 (Range : 81 to 95) in refrigerated and cryopreserved groups, respectively. The differences between the bucks were found to be highly significant ($P<0.01$) in refrigerated while significant in ($P<0.05$) in cryopreserved group. In refrigerated group, the percentage of live sperm was significantly and positively correlated with sperm concentration (0.0266) and HOST (0.788) while negative and significant ($P<0.05$) correlation was found with abnormal sperm count (-0.337) and damaged acrosome (-0.661). A statistically non-significant negative correlation with AST (-0.124) and ALT (-0.085) enzymes were also observed.

In cryopreserved group, the percentage of live sperm was highly significantly and positively correlated with HOST (0.27) while positive and non-significant correlation was found with abnormal sperm concentration (0.22) and ALT (0.12). Negative and non-significant correlation of the live sperm count with abnormal sperm count (-0.53) and damaged acrosome (-0.55) was observed. A statistically non - significant negative AST(-0.23) enzyme correlation of live sperm was also observed.

vi. Abnormal sperm count

In refrigerated group, the abnormal sperm count in Berari buck semen ranged between 3 to 8 percent with an overall mean value of 5.08 ± 0.26 percent. The individual variation in abnormal sperm count was non-significant. While in cryopreserved group, the abnormal sperm count in Berari buck semen ranged between 2 to 5 percent with an overall mean value of 3.72 ± 0.20 percent. The individual variation in abnormal sperm count was statistically significant ($P<0.05$).

In refrigerated group, the correlation revealed that the abnormal sperm count was significant and positively correlated with damage acrosome (0.234) and negatively with sperm concentration (-0.245) and HOST (-0.248). The correlation of abnormal sperm count with AST (0.097) was negative but was statistically non-significant. In cryopreserved group, the statistical results for the correlation revealed that the abnormal sperm count was significant ($P<0.05$) and positively correlated with damaged acrosome (0.56). However the correlation of abnormal sperm count with AST enzyme activity (0.06) was positive but statistically non-significant. The correlation of abnormal sperm count with sperm concentration (-0.09), HOST (-0.06) and ALT (-0.13) was negative and statistically non-significant.

vii. Sperm concentration

The mean sperm concentration of Berari buck semen in refrigerated group was $3417.36 \pm 35.93 \times 10^6$/ml with a range of 3000×10^6/ml to 3700×10^6/ml while in cryopreserved group was $3664.58 \pm 22.76 \times 10^6$/ml with a range of 3500×10^6/ml to 3950×10^6/ml. The mean value of sperm concentration per ml of semen showed highly significant differences between bucks ($P<0.01$).

In refrigerated group, sperm concentration showed significant and positive correlation with HOST (0.250). The correlation between sperm concentration and damage acrosome (-0.28) and AST (-0.28) enzyme activity was negative and statistically significant. The sperm concentration exhibited negative and non-significant correlation with the ALT (-0.139) enzyme activity. In cryopreserved group, sperm concentration showed significant ($P<0.05$) and positive correlation with HOST (0.38). The correlation between sperm concentration and damaged acrosome (-0.18), AST (-0.13) and ALT (-0.03) was negative and non-significant.

b) Sperm Function Tests

i. Damaged acrosome

The percentage of damaged acrosome recorded was 2.85 ± 0.17 (Range: 2.00 to 5.00 percent) and 2.72 ± 0.19 (Range:1.00 to 7.00 percent) in refrigerated and cryopreserved group, respectively. The differences between bucks were found to be highly significant (P<0.01).

In refrigerated group, the positive non-significant correlation was noted with both the enzymes i.e. AST (0.231) and ALT (0.194) while the percentage of damaged acrosome was significant and negatively correlated with HOST (-0.568). In cryopreserved group, the percentage of damaged acrosome was positively correlated with AST (0.02), ALT (0.10) enzymes and negatively correlated with HOST (-0.21) but non-significant.

ii. Hypo-osmatic swelling test (HOST)

In refrigerated group, the average percentage HOST was 80.40 ± 0.41 with a range of 74 to 83 percent. The differences between the bucks were found to be highly significant (P<0.01) while in cryopreserved group the overall mean percentage of HOST was 81.53 ± 0.18 with a range of 78 to 85 percent. The differences between the bucks were found to be non-significant.

In refrigerated group, the correlation of HOST with both the enzymes i.e AST (0.177), ALT (0.088) were negative and statistically non-significant. In cryopreserved group the percentage of HOST reacted sperm was positively correlated with ALT activity (0.01) while negatively correlated with AST (-0.06) activity but are non-significant

iii. Aspartate Aminotransferase (AST)

The overall mean AST activity (IU/L) observed in seminal plasma of Berari buck was 56.140 ± 0.86 (Range : 49 IU/L to 62 IU/L) and 41.88 ± 0.66 (Range : 35 IU/L to 46 IU/L) in refrigerated and cryopreserved groups, respectively. The individual variation among bucks was highly significant (P<0.01)

In refrigerated group the AST activity was positively correlated with ALT activity (0.017) and statistically non-significant while in cryopreserved group it was negatively (-0.06) and non-sigificantly correlated with ALT activity.

iv. Alanine Aminotransferase (ALT)

In refrigerated group the ALT activity of seminal plasma of Berari buck ranged between 9 IU/L to 17 IU/L with an overall mean value of 13.00 ± 0.70 IU/L. The individual variation among bucks was highly significant (P<0.01).

In cryopreserved group the overall mean value of ALT enzyme activity of seminal plasma of Berari buck neat semen in cryopreserved was 9.06 ± 0.14 with a range of 7 IU/L to 14 IU/L. The individual variation among bucks was insignificant.

c) Physical characteristics of washed spermatozoa of Berari buck:

i. Individual sperm motility

The effect of dilutor stages of preservation was found to be highly significant (P<0.01). The values of percent motility decreased significantly at different stages in both dilutors.

The individual sperm motility (percent) during preservation at Pre-freeze and Post-freeze stages in SM dilutor were 75.00 ± 0.81, and 69.86 ± 0.90 respectively. Whereas, the corresponding values for the TCFEY dilutor were 73.06 ± 1.90 and 68.19 ± 1.19, respectively. The sperm motility was higher initially and reduced gradually during preservation in both the dilutors.

The overall means of percent motility in the semen dilutors in present study revealed that the best motility was in skim milk dilutor whereas; the lower motility was observed in TCFEY dilutor. The interaction between buck and dilutor, buck and stage and dilutor and stage were found to be statistically non-significant (Table 4.9A).

ii. Live sperm count

The percentage of live sperm at pre-freeze and post freeze stage was 79.65 ± 0.80 and 75.33 ± 0.72 in SM dilutor and 81.15 ± 0.29 and 76.60 ± 1.00 in TCFEY dilutor, respectively. The percent live sperm count was declinined at each stage of preservation.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between dilutors and stages of preservation. The stage X dilutor interaction was non-significant..

iii. Abnormal sperm count

The percentage of abnormal sperms at pre-freeze and post freeze stages was 5.47 ± 0.16 and 6.01 ± 0.43 in SM dilutor and 5.99 ± 0.24 and 7.17 ± 0.30 in TCFEY dilutor, respectively. The values of percent live sperm count decreased significantly at subsequent stage of preservation in both dilutors.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservtion, different dilutors and stage X dilutor interaction.

d) Sperm function tests of washed spermatozoa of Berari buck

i. Damaged acrosome percentage

The percent of damaged acrosome at pre-freeze and post freeze stage was 7.07 ± 0.46 and 9.18 ± 0.27 in SM dilutor and 8.04 ± 0.40 and 10.50 ± 0.24 in TCFEY dilutor, respectively. The values of percent damaged acrosome were increasing significantly (P<0.05) at stage of preservation.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation and different dilutors. The stage X dilutor interaction was non-significant .

ii. Hypo-osmatic swelling test

The percentage of HOST positive spermatozoa at pre-freeze and post freeze stage was 72.50 ± 0.41 and 55.17 ± 1.02 in SM dilutor and 70.21 ± 0.35 and 54.17 ± 0.87 in TCFEY dilutor, respectively. The values of percent HOST reacted spermatozoa decreased significantly (P<0.01) at stage of preservation.

The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation and both dilutors. The stage X dilutor interaction was non-significant (Table 4.13A).

iii. Leakage of enzymes

The overall mean activity of AST enzyme of washed buck spermatozoa observed was 80.88 ± 1.26 and 139.57 ± 1.78 IU/L in SM dilutor. 86.03 ± 1.36 and 153.64 ± 1.16 IU/L in TCFEY dilutor in pre-freeze and post-freeze stages respectively. The analysis of variance for the washed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of preservation, dilutors, stage X dilutor interaction.

The AST activity was lower initially and increased gradually during preservation. Leakage of AST in SM dilutor was significantly lower than TCFEY dilutor at stage of preservation. The SM dilutor showed the lower mean value of AST whereas, TCFEY dilutor showed the higher mean value of AST in the present study.

The overall mean activity of ALT enzyme of washed buck spermatozoa observed was 19.21 ± 0.27 and 24.85 ± 0.70 IU/L in SM dilutor. 20.08 ± 0.20 and 29.07 ± 1.05IU/L in TCFEY dilutor in pre-freeze and post-freeze stages, respectively. The value of AST and ALT enzyme leakage increased significantly (P<0.01) at each stage of preservation, respectively.

The plasma membrane integrity of washed buck spermatozoa was detoriated significantly at different stages of preservation as detected by leakage of enzymatic constituents in seminal plasma.

e) Physical characteristics of unwashed spermatozoa of Berari buck

i. Individual Sperm motility

The percentage of individual sperm motility at pre-freeze and post freeze stage was 71.11 ± 2.44 and 67.08 ± 1.13 in SM dilutor and 70.14 ± 1.79 and 65.28 ± 0.70 in TCFEY dilutor, respectively. The values of percent individual motility decreased significantly at each stage of preservation.

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of preservation and significant ($P<0.05$) difference between dilutors. The stage X dilutor interaction was non-significant (Table 4.16A).

ii. Percentage of live spermatozoa

The percentage of live sperm at pre-freeze and post freeze stage was 76.99 ±1.06 and 71.44 ± 0.55 in SM dilutor and 75.28 ± 0.70 and 70.28 ± 0.90 in TCFEY dilutor, respectively. The values of percent individual motility decreased significantly at each stage of preservation.

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of preservation and dilutors. The stage X dilutor interaction was non-significant (Table 4.17A).

iii. Abnormal sperm percentage

The percentage of abnormal sperm at pre-freeze and post freeze stage was 6.61 ± 0.06 and 15.68 ± 0.09 in SM dilutor and 8.07 ± 0.19 and 17.07 ± 0.12 in TCFEY dilutor, respectively. The values of percent individual motility increased significantly at each stage of preservation. The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between dilutors

and stages of preservation. The stage X dilutor interaction was non-significant (Table 4.18A).

Stage of preservation had also a marked effect on the mean percent morphological abnormalities. The abnormal sperm count was lower initially and increase gradually during stages of preservation.

f) Sperm function tests of unwashed spermatozoa of Berari buck

i. Damaged acrosome percentage

The percentage of damaged acrosome at pre-freeze and post freeze stage was 4.90 ± 0.08 and 9.68 ± 0.11 in SM dilutor and 6.90 ± 0.16 and 10.83 ± 0.06 in TCFEY dilutor, respectively. The values of percent individual motility increased significantly at each stage of preservation.

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between dilutors, stages of preservation and stage X dilutor interaction.

ii. Hypo-osmatic swelling test

The percentage of HOST positive spermatozoa at pre-freeze and post freeze stage was 69.54 ± 0.79 and 53.53 ± 0.94 in SM dilutor and 64.93 ± 0.60 and 52.25 ± 0.44 in TCFEY dilutor, respectively. The values of percent HOST reacted spermatozoa decreased significantly ($P<0.01$) at each stage of preservation.

The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between dilutors, stages of preservation and stage X dilutor interaction.

iii. Leakage of enzymes

The overall mean activity of AST enzyme of unwashed buck spermatozoa observed was 77.79 ± 1.33 and 159.72 ± 1.37 IU/L in SM dilutor while 72.58 ± 1.44 and 167.57 ± 0.93 IU/L in TCFEY dilutor in pre-freeze and

post-freeze stages, respectively. The analysis of variance for the unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between both stages of preservation, dilutors and stage X dilutor interaction.

The AST activity was lower initially and increased gradually during preservation. Leakage of AST in SM dilutor was significantly lower than TCFEY dilutor at stage of preservation. The SM dilutor showed the lower mean value of AST whereas, TCFEY dilutor showed the higher mean value of AST. The plasma membrane integrity of unwashed buck spermatozoa was detoriated significantly at different stages of preservation as detected by leakage of enzymatic constituents in seminal plasma.

g) Comparison of Physical Characteristics of washed and unwashed refrigerated buck spermatozoa

i. Individual sperm motility

Data pertaining to preservation of washed and unwashed buck spermatozoa showed non significantly higher sperm motility (75.00 ± 0.81 and 69.86 ± 0.90) at pre freeze and post freeze stage in washed semen, as compared to the corresponding values of unwashed spermatozoa (71.11 ± 2.44 and 67.08 ± 1.13) in SM dilutor. Higher sperm motility (73.06 ± 1.90 and 68.19 ± 1.19) at pre-freeze and post-freeze stage in washed semen as compared to corresponding values of unwashed spermatozoa (70.14 ± 1.79 and 65.28 ± 0.70) in TCFEY dilutor but the difference was non-significant.

The analysis of variance for the individual sperm motility percentage of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between washed and unwashed spermatozoa at each stage of preservation, washing condition, and dilutors. A non-significant difference observed between stage X condition X dilutor interaction.

ii. Live sperm percent

Data pertaining to preservation of washed and unwashed buck spermatozoa showed non significantly higher live sperm percentage (79.65 ±

0.80 and 75.33 ± 0.72) at pre freeze and post freeze stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (76.99 ± 1.06 and 71.44 ± 0.55) in SM dilutor. A non significantly higher live sperm percent was observed (81.15 ± 0.29 and 76.60 ± 1.00) at pre-freeze and post-freeze stages in washed semen as compared to corresponding values of unwashed spermatozoa (75.28 ± 0.70 and 70.28 ± 0.90) in TCFEY dilutor.

The analysis of variance for the live sperm percentage of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between washed and unwashed spermatozoa at each stage of preservation, washing condition, and washing condition X dilutor interaction while a non-significant difference observed between stage X condition X dilutor interaction.

iii. Abnormal sperm percent

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly lower abnormal sperm percentage (5.47 ± 0.16 and 6.01 ± 0.43) at pre freeze and post freeze stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (6.61 ± 0.06 and 15.68 ± 0.09) in SM dilutor. A significantly lower abnormal sperm percentage was observed (5.99 ± 0.24 and 7.17 ± 0.30) at pre-freeze and post-freeze stages in washed semen as compared to corresponding values of unwashed spermatozoa (8.07 ± 0.19 and 17.07 ± 0.12) in TCFEY dilutor.

The analysis of variance for the abnormal sperm percentage of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between washed and unwashed spermatozoa at each stage of preservation, washing condition, dilutors, stage X condition interaction, condition X dilutor interaction, stage X condition X dilutor interaction while a non-significant difference observed between stage X condition X dilutor interaction.

h) Comparison of Sperm function tests of washed and unwashed refrigerated buck spermatozoa

i. Damaged acrosome percent

Data pertaining to preservation of washed and unwashed buck spermatozoa at pre freeze stage showed significantly higher damaged acrosome percentage (7.07 ± 0.46 and 8.04 ± 0.40 in SM and TCFEY dilutor, respectively) in washed semen than the unwashed semen (4.90 ± 0.08 and 6.90 ± 0.16 in SM and TCFEY dilutor, respectively). At post freeze stage the values of acrosomal damaged were having an apparent difference between washed semen (9.18 ± 0.27 and 10.50 ± 0.24 in SM and TCFEY dilutor, respectively) and unwashed semen (9.68 ± 0.11 and 10.83 ± 0.06 in SM and TCFEY dilutor respectively).

ii. Hypo-osmatic swelling test

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly higher HOST reacted sperm percent (72.50 ± 0.41 and 55.17 ± 1.02 at pre freeze and post freeze stages in washed semen) as compared to corresponding values of unwashed spermatozoa (69.54 ± 0.79 and 53.53 ± 0.94 in SM dilutor). A significantly higher HOST percent was observed (70.21 ± 0.35) and 54.17 ± 0.87 at pre freeze and post freeze stages) in washed semen as compared to corresponding values of unwashed spermatozoa (64.93 ± 0.60 and 52.25 ± 0.44 in TCFEY dilutor). The values of percent HOST reacted spermatozoa were significantly ($P<0.05$) higher in SM dilutor in washed semen at pre-freeze stage of preservation.

Analysis of variance for the washed and unwashed refrigerated Berari buck spermatozoa revealed that there was significant ($P<0.05$) difference between washed and unwashed spermatozoa at both the stages of preservation for percentage of HOST reacted spermatozoa.

The plasma membrane integrity of washed and unwashed spermatozoa was compared by investigating the Hypo-osmotic swelling test of sperms in the seminal plasma during different stages of preservation. The results of the present study revealed that the washing of semen enhanced the

maintenance of plasma membrane integrity of spermatozoa during preservation process.

iii. Leakage of enzyme (AST)

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly higher AST values (80.88 ± 1.26 and 139.57 ± 1.78 at pre freeze and post freeze stages in washed semen) as compared to corresponding values of unwashed spermatozoa (77.94 ± 1.33 and 159.72 ± 1.37 in SM dilutor). A significantly higher AST value was observed (86.03 ± 1.36) and 153.64 ± 1.16 at pre freeze and post freeze stages) in washed semen as compared to corresponding values of unwashed spermatozoa (72.58 ± 1.44 and 167.57± 0.93 in TCFEY dilutor).

Analysis of variance for the AST value of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stage of preservation , washing condition, dilutors, stage X condition, stage X dilutor, condition X dilutor, stage X condition X dilutor interactions.

iv. Leakage of Enzyme (ALT)

Data pertaining to preservation of washed and unwashed buck spermatozoa showed significantly higher ALT values (19.21 ± 0.27 and 24.85 ± 0.70 at pre freeze and post freeze stages in washed semen) as compared to corresponding values of unwashed spermatozoa (16.54 ± 0.91 and 32.38 ±0.84 in SM dilutor). A significantly higher ALT value was observed (20.08 ± 0.20) and 29.07 ± 1.05 at pre freeze and post freeze stages) in washed semen as compared to corresponding values of unwashed spermatozoa (17.61 ± 0.89 and 41.15± 0.46 in TCFEY dilutor).

Analysis of variance for the ALT value of washed and unwashed refrigerated Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stage of preservation , washing condition, dilutors, stage X condition, stage X dilutor, condition X dilutor, stage X condition X dilutor interactions.

i) Physical characteristics of washed cryopreserved buck spermatozoa

i. Individual motility

The percentage of individual sperm motility at post diluted, post equilibrated and post thaw stages were 75.00 ± 0.65, 63.06 ± 0.93 and 47.08 ± 0.80 in TYG dilutor and 74.03 ± 0.59, 58.06 ± 1.05 and 39.03 ± 0.62 in TYD dilutor, respectively. The values of percent individual motility decreased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interactions.

ii. Live sperm percent

The percentage of live sperm at post diluted, post equilibrated and post thaw stages were 84.64 ± 0.61, 70.88 ± 0.68 and 55.00 ± 1.18 in TYG dilutor while 80.60 ± 0.68, 66.86 ± 1.04 and 45.61 ± 0.85 in TYD dilutor, respectively. The values of live sperm percent decreased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction.

iii. Abnormal sperm percent

The percentage of abnormal sperm at post diluted, post equilibrated and post thaw stages were 6.90 ± 0.50, 9.93 ± 0.25 and 18.26 ± 0.19 in TYG dilutor while 8.78 ± 0.06, 10.39 ± 0.20 and 26.11 ± 0.35 in TYD dilutor, respectively. The values of live sperm percent increased significantly at each stage of freezing (Table 4.32).

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction (Table 4.32A)**.**

j) Sperm functional tests of washed cryopreserved buck spermatozoa

i. Damaged acrosome percent

The percentage of damaged acrosome percent at post diluted, post equilibrated and post thaw stages were 6.96 ± 0.45, 10.94 ± 0.34 and 15.28 ± 0.23 in TYG dilutor while 9.00 ± 0.08, 12.18 ± 0.24 and 17.32 ± 0.29 in TYD dilutor, respectively. The values of damaged acrosome percent increased significantly at each stage of freezing.

Analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction.

ii. Hypo-osmatic swelling test (HOST)

The percentage of HOST positive spermatozoa at post dilution, post equilibration stage and post thawed stage was 73.32 ± 0.62, 64.19 ± 0.56 and 48.18 ± 0.84 in TYG dilutor and 68.13 ± 0.49,57.96 + 0.56 and 37.08 ± 0.53 in TYD dilutor respectively. The values of percent HOST reacted spermatozoa decreased significantly at each stage of freezing.

The analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction.

iii. Leakage of enzymes

The overall mean activity of AST enzyme observed at the post diluted, post equilibrated and post thawed stages were 73.06 ± 0.63, 108.03 ± 1.18 and 149.83 ± 1.44 IU/L in TYG dilutor while 74.01 ± 0.97, 111.18 ± 0.96 and 153.15 ± 0.28 in TYD dilutor respectively.The overall mean activity of ALT enzyme observed at the post diluted, post equilibrated and post thawed stages were 15.83 ± 0.41, 18.56 ± 0.61 and 26.25 ± 0.60 IU/L in TYG dilutor while 17.14 ± 0.49, 19.32 ± 0.61 and 27.94 ± 0.21 in TYD dilutor respectively .The analysis of variance for the washed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01)

difference between stages of freezing, dilutors and stage X dilutor interaction of AST and ALT enzyme leakage of washed buck spermatozoa.

The AST and ALT activity was lower initially and increased gradually during preservation. Leakage of AST and ALT in TYG dilutor was significantly lower than TYD dilutor at every stage of preservation. The TYG dilutor showed the lower mean value of AST and ALT whereas, TYD dilutor showed the higher mean value of AST.

5.4.2 Cryopreservation of unwashed buck spermatozoa

a) Physical characteristics of unwashed cryopreserved buck spermatozoa

i. Individual motility

The percentage of individual sperm motility at post diluted, post equilibrated and post thaw stages were 72.08 ± 0.56, 60.14 ± 1.13 and 43.06 ± 0.90 in TYG dilutor while 71.11 ± 0.28, 57.08 ± 1.15 and 38.06 ± 0.51 in TYD dilutor respectively. The values of percent individual motility decreased significantly at each stage of freezing.Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interactions.

ii. Live sperm percent

The percentage of live sperm at post diluted, post equilibrated and post thaw stages were 79.78 ± 0.41, 68.18 ± 1.23 and 50.97 ± 0.85 in TYG dilutor while 78.99 ± 0.33, 65.96 ± 1.13 and 44.39 ± 0.64 in TYD dilutor, respectively. The values of live sperm percent decreased significantly at each stage of freezing.Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing, dilutors and stage X dilutor interaction.

iii. Abnormal sperm percent

The percentage of abnormal sperm at post diluted, post equilibrated and post thaw stages were 7.93 ± 0.15, 12.06 ± 0.22 and 23.79 ±

0.71 in TYG dilutor while 10.32 ± 0.30,13.94 ± 0.40 and 26.92 ± 0.47 in TYD dilutor, respectively. The values of live sperm percent increased significantly at each stage of freezing.

Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction.

b) Sperm functional tests of unwashed cryopreserved buck spermatozoa

i. Damaged acrosome percent

The percentage of damaged acrosome percent at post diluted, post equilibrated and post thaw stages were 6.15 ± 0.37, 9.00 ± 0.14 and 12.81 ± 0.31 in TYG dilutor while 7.06 ± 0.29, 10.07 ± 0.38 and 15.03 ± 0.30 in TYD dilutor, respectively. The values of damaged acrosome percent increased significantly at each stage of freezing.Analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction.

ii. Hypo-osmatic swelling test (HOST)

The percentage of HOST positive spermatozoa at post dilution, post equilibration stage and post thawed stage was 73.32 ± 0.62, 63.14 ± 0.31 and 45.38 ± 0.65 in TYG dilutor while 66.99 ± 0.39, 56.03 ± 0.70 and 36.03 ± 0.88 in TYD dilutor respectively. The values of percent HOST reacted spermatozoa decreased significantly at each stage of freezing. The analysis of variance for the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing, dilutors and stage X dilutor interaction.

iii. Leakage of enzymes

The overall mean activity of AST enzyme observed at the post diluted, post equilibrated and post thawed stages were 68.14 ± 0.95, 110.01 ± 0.62 and 151.99 ± 1.03 IU/L in TYG dilutor while 69.36 ± 1.74, 113.28 ± 0.90 and 154.86 ± 0.15 in TYD dilutor, respectively . The analysis of variance for

the unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing and dilutors. A significant (P<0.05) difference was observed in stage X dilutor interaction of AST and ALT enzyme leakage of unwashed buck spermatozoa.

c) Comparison of Physical characteristics of washed and unwashed cryopreserved buck spermatozoa

i. Individual sperm motility

Data pertaining to freezability of washed and unwashed buck spermatozoa showed non significantly higher individual sperm motility (75.00 ± 0.65, 63.06 ± 0.93 and 47.08 ± 0.80) at post diluted, post equilibrated and post thawed stage in washed semen, as compared to the corresponding values of unwashed spermatozoa (72.08 ± 0.56, 60.14 ± 1.13 and 43.06 ± 0.90) in TYG dilutor. Higher sperm motility (74.03 ± 0.59, 58.06 ± 1.05 and 39.03 ± 0.62) at post diluted, post equilibrated and post thawed stage in washed semen as compared to corresponding values of unwashed spermatozoa (71.11 ± 0.28, 57.08 ± 1.15 and 38.06 ± 0.51) in TYD dilutor but the difference was non-significant.

The analysis of variance for the individual sperm motility percentage of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between washed and unwashed spermatozoa at each stage of freezing, washing condition, dilutors and stage X dilutor interaction. A non significant difference was observed in stage X condition X dilutor interaction.

ii. Live sperm percent

Data pertaining to freezability of washed and unwashed buck spermatozoa showed non significantly higher live sperm percentage (84.64 ± 0.6, 70.88 ± 0.68 and 55.00 ±1.18) at post diluted, post equilibrated and post thawed stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (79.78 ± 0.41, 68.18 ± 1.23 and 50.97 ± 0.85) in TYG dilutor. A non significantly higher live sperm percent was observed (80.60 ± 0.68, 66.86 ± 1.04 and 45.61 ± 0.85) at post diluted, post equilibrated and post

thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (78.99 ± 0.33, 65.96 ± 1.13 and 44.39 ± 0.64) in TYD dilutor.

The analysis of variance for the live sperm percentage of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between washed and unwashed spermatozoa at each stage of freezing, washing condition, dilutors, stage dilutor interaction and washing condition X dilutor interaction while a non-significant difference observed between stage X condition X dilutor interaction.

iii. Abnormal sperm percent

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly lower abnormal sperm percentage (6.90 ± 6.50, 9.93 ± 0.25 and 18.26 ± 0.19) at post diluted, post equilibrated and post thawed stages in washed semen, as compared to the corresponding values of unwashed spermatozoa (7.93 ± 0.1, 12.06 ± 0.22 and 23.79 ± 0.71) in TYG dilutor. A significantly lower abnormal sperm percentage was observed (8.78 ± 0.26, 10.39 ± 0.20 and 26.11 ± 0.35) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (10.32 ± 0.30, 13.94 ± 0.40 and 26.92 ± 0.47) in TYD dilutor.

The analysis of variance for the abnormal sperm percentage of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between washed and unwashed spermatozoa at each stage of freezing, washing condition, dilutors, stage X condition interaction, stage X dilutor interaction, condition X dilutor interaction and stage X condition X dilutor interaction.

d) Comparison of Sperm function tests washed and unwashed cryopreserved buck spermatozoa

i. Damaged acrosome percent

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly higher damaged acrosome percent (6.96 ± 0.45, 10.94 ± 0.34 and 15.28 ± 0.23) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (6.15 ± 0.37,9.00 ± 0.14 and 12.81 ± 0.31) in TYG dilutor. A significantly higher damaged acrosome percent was observed (9.00 ± 0.08, 12.18 ± 0.24 and 17.32 ± 0.29) at post diluted, post equilibrated and post thawed stages) in washed semen as compared to corresponding values of unwashed spermatozoa (7.06 ± 0.29,10.07 ± 0.38 and 15.03 ± 0.30) in TYD dilutor respectively. The values of percent damaged acrosome were significantly ($P<0.01$) higher in TYD dilutor in washed semen at post thawed stage of freezing.

Analysis of variance for the washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was significant ($P<0.01$) difference between washed and unwashed spermatozoa at both the stages of freezing, washing condition, dilutors, stage X condition interaction, stage X dilutor interaction and stage X condition X dilutor interaction for percentage of damaged acrosome.

ii. Hypo-osmatic swelling test

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly higher HOST reacted sperm percent (73.32 ± 0.62, 64.19 ± 0.56 and 48.18 ± 0.84) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (73.32 ± 0.62, 63.14 ± 0.31 and 45.38 ± 0.65) in TYG dilutor. A significantly higher HOST percent was observed (68.13 ± 0.49, 57.96 ± 0.56 and 37.08 ± 0.53) at post diluted, post equilibrated and post thawed stages) in washed semen as compared to corresponding values of unwashed spermatozoa (66.99 ± 0.39, 56.03 ± 0.70 and 36.03 ± 0.88) in TYD

dilutor respectively. The values of percent HOST reacted spermatozoa were significantly (P<0.05) higher in TYG dilutor in washed semen at post diluted and post equilibrated stages of freezing.

Analysis of variance for the washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was significant (P<0.05) difference between washed and unwashed spermatozoa at both the stages of freezing, washing condition, dilutors, stage X condition interaction, stage X dilutor interaction and stage X condition X dilutor interaction for percentage of HOST reacted spermatozoa.

iii. Leakage of Enzyme (AST)

Data pertaining to freezability of washed and unwashed buck spermatozoa showed non-significantly higher AST values (73.06 ± 0.63, 108.03 ± 1.18 and 149.83 ± 1.44) at post diluted, post equilibrated and post thawed stages in washed semen) as compared to corresponding values of unwashed spermatozoa (68.14 ± 0.95, 110.01 ± 0.62 and 151.99 ± 1.03 in TYG dilutor). A non-significantly higher AST value (74.01 ± 0.97, 111.18 ± 0.96 and 153.15 ± 0.28) was observed at post diluted, post equilibrated and post thawed stages) in washed semen as compared to corresponding values of unwashed spermatozoa (69.36 ± 1.74, 113.28 ± 0.90 and 154.86 ± 0.15) in TYD dilutor.

Analysis of variance for the AST value of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant (P<0.01) difference between stages of freezing , dilutors, stage X condition interaction and stage X dilutor interaction. A non significant difference was observed in stage X condition X dilutor interaction.

iv. Leakage of Enzyme (ALT)

Data pertaining to freezability of washed and unwashed buck spermatozoa showed significantly higher ALT values (15.83 ± 0.41, 18.56 ± 0.61 and 26.25 ± 0.60) at post diluted, post equilibrated and post thawed stages in washed semen) as compared to corresponding values of unwashed

spermatozoa (13.46 ± 0.59, 20.10 ± 0.19 and 28.31 ± 0.46) in TYG dilutor. A significantly higher ALT value was observed (17.14 ± 0.49, 19.32 ± 0.61 and 27.94 ± 0.21) at post diluted, post equilibrated and post thawed stages in washed semen as compared to corresponding values of unwashed spermatozoa (14.26 ± 0.14, 21.92 ± 0.48 and 28.93, ± 0.49 in TYD dilutor).

Analysis of variance for the ALT value of washed and unwashed cryopreserved Berari buck spermatozoa revealed that there was highly significant ($P<0.01$) difference between stages of freezing , washing condition, dilutors, stage X condition interaction and stage X condition X dilutor interactions.

5.5 Conception Rate of Preserved Semen

5.5.1 Conception rate in refrigerated semen in Berari goats

The conception rate of Berari buck semen preserved in washed refrigerated and unwashed refrigerated semen in estrus synchronized does was 62.50, and 37.50 percent, respectively. The overall conception rate for Berai buck refrigerated semen was 50.00 percent. Higher conception rate was observed for semen preserved in washed refrigerated semen as compared with unwashed refrigerated semen. It indicates that washed refrigerated semen was found to be more suitable for refrigeration preservation of Berari buck semen as compared to unwashed refrigerated semen.

5.5.2 Conception rate with cryopreserved semen in Berari goats

The conception rate of Berari buck semen preserved in washed cryopreserved semen and unwashed cryopreserved semen in estrus synchronized does was 50.00 and 37.50 percent, respectively. The overall conception rate for Berai buck cryopreserved semen was 43.75 percent. Higher conception rate was observed for semen preserved in washed cryopreserved semen as compared with un washed cryopreserved semen. It indicates that washed cryopreserved semen was found to be more suitable for cryopreservation of Berari buck semen as compared to unwashed cryopreserved semen.

In the light of above results following conclusions are made: -

- The seminal attributes of Berari buck neat semen was within normal physiological range for reproduction as well as preservation.
- Berari buck semen was preserved at refrigeration and ultra low temperature showed reduced postthaw quality of semen in the physical characteristics and sperm function tests which was within the permissible limit.
- The refrigeration and cryopreservation method of preservation has significant negative effect on plasma membrane integrity and leakage of AST and ALT enzymes.
- The conception rate of washed refrigerated and cryopreserved semen showed higher conception rate than unwashed semen indicating washing of sperms has beneficial better semen preservation.
- There was significant difference was observed between the dilutors at all stages i.e pre-freeze and post-freeze in refrigerated and cryopreserved semen . SM was found to be superior to TCFEY in refrigerated and TYG was found to be superior to TYD in cryopreserved semen of Berari buck.

CHAPTER VI

PROPOSED AREA FOR FUTURE RESEARCH

Based on the observation of present research, the improved fertility potential of cryopreserved goat semen is major thrust research area for popularizing this technology along with reduction in the price of the semen doses.

Following are the some of important areas in buck semen preservation

1. Combination of different cryoprotectants levels as well as dilution for buck semen cryopreservation.
2. Incorporation of antioxidant as well as stabilizer in semen dilutors.
3. Sex sorting of buck semen.

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VITA

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THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	**:**	**SEMINAL ATTRIBUTES, PRESERVATION AND FERTILIZING ABILITY OF REFRIGERATED AND CRYOPRESERVED SEMEN OF BERARI BUCKS**
b)	**Full name of student**	**:**	**Patil Manjusha Ganeshrao**
c)	**Name and address of Major advisor**	**:**	**Dr. M. V. Ingawale** Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, PGIVAS, Akola
d)	**Degree to be awarded**	**:**	Ph.D.
e)	**Year of award of degree**	**:**	2020
f)	**Major subject**	**:**	Animal Reproduction, Gynaecology and Obstetrics
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i)	**Signature of Student**	**:**	
j)	**Signature, Name and address of forwarding authority**	**:**	

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ABSTRACT

The present investigation on "Seminal attributes, preservation and fertilizing ability of refrigerated and cryopreserved semen berari bucks" was conducted for study of scrotal biometry, evaluation of seminal characteristics, assessment of effect of dilutors for the preservation of buck semen at refrigeration temperature. The research was carried out on 144 semen samples

of 6 bucks for various macroscopic, microscopic, sperm function tests and enzyme activity on neat semen and on semen diluted with in refrigerated semen Skim milk (SM) and Tris citric acid fructose egg yolk (TCFEY) dilutors while in cryopreserved semen TYG (Tris egg yolk glycerol) and TYD (Tris egg yolk di-methyl sulphoxide) diluters, at different stages of pre-freezing and post freezing. The macroscopic, microscopic, sperm function tests and enzyme activity in neat semen of Berari bucks indicated that the quality of neat semen was very good and within normal prescribed limits.

Similarly the microscopic, sperm function tests and enzyme activity in the semen of Berari bucks diluted with different diluters both in refrigerated with semen while in cryopreserved semen indicated that there was slight reduction in the quality of semen at every stage freezing of semen In refrigerated SM diluter was found to be superior than TCFEY While in cryopreserved semen TYG diluter was found to be superior than TYD at all the stages i.e. pre-freezing and post- freezing . In all diluters post thaw quality of semen was gradually reduced i.e. reduction in post thaw motility percentage and live sperm percentage, increase in sperm abnormality percentage, damage acrosome percentage and increase in enzyme leakage. This reduction in quality was within the prescribed limit. There were indications of increased leakage of enzymes into the plasma from pre-freezing to post thaw stage.

Total 32 oestrous synchronized goats were inseminated with refrigerated and cryopreserved semen of Berari bucks. Conception rate of washed (removal of seminal plasma) semen of refrigerated or cryopreserved group was higher for Berari bucks. This indicates that washing plays an important role in semen preservation.

Thus, on the basis of results obtained from the present research work, it can be concluded that the quality of diluted semen declines gradually during freezing of semen. However, this decline is gradual and within the normal permissible limits. The comparison between the results of washed and unwashed refrigerated or cryopreserved buck spermatozoa revealed that quality of semen was better in washed semen samples than in unwashed semen samples at each stages of freezing and gives better conception rate.

प्रबंध सारांश

१.	**प्रबंधाचे शिर्षक**	**: बेरारी बोकडाच्या वीर्याची थंड व अतिशीत पद्धतीने जतन व गुणधर्माची तपासणी**
२.	**विद्यार्थ्याचे पूर्ण नांव**	**: पाटील मंजुषा गणेशराव**
३.	**मुख्य मार्गदर्शकाचे नांव व पत्ता**	: डॉ. महेशकुमार वि. इंगवले सहायक प्राध्यापक, पशुप्रजनन, स्त्रीरोग व प्रसूतीशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला
४.	**प्रदान केली जाणारी पदवी**	: आचार्य पदवी
५.	**पदवी प्रदान करण्याचे वर्ष**	: २०२०
६.	**मुख्य विषय**	: पशुप्रजनन, स्त्रीरोग व प्रसूतीशास्त्र
७.	**प्रबंधामधील एकुण पाने**	: २५०
८.	**प्रबंध सारांशामधील एकुण शब्द**	: ४०५
९.	**विद्यार्थ्याची सही**	:
१०.	**प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता**	:

(चैतन्य एच. पावशे)
विभाग प्रमुख
पशुप्रजनन, स्त्रीरोग व प्रसूतीशास्त्र विभाग
स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था,
अकोला.

सारांश

"बेरारी बोकडाच्या वीर्याची थंड व अतिशीत पद्धतीने जतन व गुणधर्माची तपासणी" या संशोधन प्रकल्पात बोकडाच्या वृषणाचे आकारमान, विर्याच्या गुणधर्माची तपासणी, वीर्यातील विविध द्रावणांचा, तसेच वीर्यद्रव विरहित व वीर्यद्रव सहित, विर्याचा थंड व अतिशीत पद्धतीने विर्याच्या साठवणुकीवर होणार परिणाम ह्या बाबींचा अभ्यास करण्यात आला.

या संशोधन प्रकल्पात ६ बेरारी बोकडांच्या १४४ वीर्य नमुन्यावर संशोधन करण्यात आले. यात विर्याच्या विविध चाचण्या घेण्यात आल्या, जसे की सुक्ष्मदर्शक वापरून, सुक्ष्मदर्शक न वापरता, वीर्यातील विरकांच्या (ए.एस.टी. व ए.एल.टी.) चाचण्या, इत्यादी. त्यानंतर उत्कृष्ट वीर्याचा प्रत्येक नमुना दोन भागात म्हणजे धुतलेले (वीर्यद्रव विरहित) व न धुतलेले (वीर्यद्रव्य सहित) असा विभागला गेला. हे वीर्य साठवणुकीच्या थंड (४^{0}से.) पद्धतीने स्कीम मिल्क व ट्रीस ही दोन द्रावणे वापरून, तर अतिशीत पद्धतीने (-१९६^{0}से.) टी.वाय.जी. व टी.वाय.डी. ही दोन द्रावणे वापरून साठवण्यात आले. ह्या विर्याच्या विविध टप्प्यावर विविध चाचण्या (सूक्ष्मदर्शक यंत्र वापरून शुक्राणूंची तपासणी, शुक्राणूंच्या आवरणाच्या चाचण्या, वीर्यातील विरकांच्या चाचण्या) घेऊन प्रत ठरविण्यात आली. ह्या चाचण्यांच्या निष्कर्षावरून हे लक्षात आले की, बेरारी बोकडांच्या वीर्याची प्रत उत्तम असून विविध गुण वैशिष्ठ्ये सामान्य निर्धारीत प्रमाणात होती.

थंड (४^{0}से.) पद्धतीने तसेच अतिशीत (-१९६^{0}से.) पद्धतीने वीर्य जेव्हा विविध द्रावणात साठविले जाते तेव्हा त्याची सूक्ष्मदर्शक वापरातील प्रत, कार्य व विरकांचे प्रमाण चाचण्यांतील साठवणुकीच्या विविध टप्प्यावर थोडी थोडी कमी होते. थंड पद्धतीने स्कीम मिल्क व ट्रिस या द्रावणात, तर अतिशीत पद्धतीत टी.वाय.जी. व टी.वाय.डी. या द्रावणात साठविलेल्या वीर्याची प्रतवारीवरून थंड पद्धतीने स्कीम मिल्क हे द्रावण तर अतिशीत पद्धतीत टी.वाय.जी. हे द्रावण अधिक चांगले होते. सगळ्याच द्रावणामध्ये वीर्य साठवणूक पश्चात वीर्याची प्रत कमी झाली होती. जसे कि, शुक्राणूंची गती व जिवंत शुक्राणूंचे प्रमाण कमी झाले होते तर शुक्राणूंच्या अवगुणांचे प्रमाण, शुक्राणूंच्या डोक्यावरील कवचाला इजेचे प्रमाण व वीर्यातील विरकांच्या स्रवणाचे प्रमाण हे वाढले होते. अशा प्रकारे वीर्याची प्रत हि एका मर्यादेपर्यंतच साठवणूक पश्चात कमी झाली होती.

बत्तीस बेरारी शेळ्यांचे माजाचे एकत्रीकरण करून थंड (४^{0}से.) व अतिशीत (-१९६^{0}से.) पद्धतीने साठविलेल्या वीर्याने कृत्रिम रेतन करण्यात आले. कृत्रिम रेतनानंतर गर्भधारणेवरून असे लक्षात येते कि, धुतलेल्या (वीर्यद्रव

विरहित) विर्यामुळे गर्भधारणेचे प्रमाण हे न धुतलेल्या (वीर्यद्रव सहित) वीर्यापेक्षा जास्त आहे. हे थंड व अतिशीत वीर्यामध्ये आढळून आले. यावरून असे लक्षात आले की, वीर्यद्रव धुणे ही वीर्य साठवणुकीकरता महत्वाची बाब आहे.

सदर संशोधनातून असा निष्कर्ष काढता येतो की, बेरारी बोकडांचे वीर्य साठवितांना त्याची प्रतवारी कमी होते परंतु थंड किंवा अतिशीत पद्धतीने साठविलेल्या वीर्याची नंतरची प्रतवारी कृत्रिम रेतनास वापरण्याजोगी चांगली असते. धुतलेले (वीर्यद्रव विरहित) वीर्य वापरून केलेल्या कृत्रिम रेतनामुळे गर्भधारणा होणाऱ्या शेळ्यांचे प्रमाण हे न धुतलेल्या (वीर्यद्रव सहित) विर्याच्या वापराने होणाऱ्या गर्भधारणेच्या प्रमाणापेक्षा जास्त आढळून आले व प्रतवारी ही चांगली दिसली. त्यामुळे धुतलेले (वीर्यद्रव विरहित) वीर्य हे प्रतवारीत जास्त चांगले दिसून आले.