STUDIES ON SEASONAL VARIATION IN PHYSICO-CHEMICAL CHARACTERISTICS OF NATIVE BUCK SEMEN (Capra hircus)

BY

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Sri M.SRINIVAS has satisfactorily prosecuted the course of research and that the thesis entitled "STUDIES ON SEASONAL VARIATION IN PHYSICO-CHEMICAL CHARACTERISTICS OF NATIVE BUCK SEMEN (*Capra hircus*)" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any University.

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C E R T I F I C A T E

This is to certify that the thesis entitled "STUDIES ON SEASONAL VARIATION IN PHYSICO-CHEMICAL CHARACTERISTICS OF NATIVE BUCK SEMEN (*Capra hircus*)" submitted in partial fulfilment of the requirements for the degree of MASTER OF VETERINARY SCIENCE in the major field of Animal Reproduction and Gynaecology of Andhra Pradesh Agricultural University, Hyderabad is a record of the bonafide research work carried out by Sri.M. SRINIVAS under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of the investigation have been duly acknowledged by him.

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CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

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ABSTRACT

A total of 120 ejacultes, 24 from each of 5 native bucks in each season were collected and analysed from the bucks available in the department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati.

The mean values for normal libido and semen characteristics recorded in this study were reaction time (seconds) 15.96 $\pm$ 0.28 and 21.30 $\pm$ 0.31, volume (ml)

0.70 ± 0.10 and 0.60 ± 0.02, mass motility 4.46 ± 0.06 and 4.36 ± 0.08, individual motility (per cent) 81.78 ± 0.53 and 78.06 ± 0.68, sperm concentration (x 10^9/ml) 2.45 ± 0.31 and 2.35 ± 0.02, live sperm count (per cent) 87.05 ± 0.62 and 81.11 ± 0.51, abnormal sperms (per cent), 8.98 ± 0.23 and 9.28 ± 0.20 and intact acrosomes (per cent) 73.68 ± 0.97 and 77.76 ± 0.76 in winter and summer seasons respectively.

In the present study, the reaction time was brief in winter and volume, per cent of individual motility, concentration of spermatozoa and per cent of abnormal sperms were significantly higher values were found in winter than in summer season. The difference in the value of mass motility was not significant between seasons while the per cent of intact acrosomes were higher in summer season.

The average value for different biochemical tests recorded in this investigation were pH 6.63 ± 0.01 and 6.70 ± 0.01, MBRT (minutes) 6.8 ± 0.18 and 8.3 ± 0.17 RRT (I) (seconds) 18.1 ± 0.34 and 19.9 ± 0.26, RRT (II) (seconds) 62.80 ± 0.88 and 66.90 ± 0.92 in winter and summer seasons respectively. The quality of semen was found to be good during winter than in summer season because of of lower values of MBRT, RRT(I) and RRT (II).

The mean values for various tests resistant to environment observed in this study were high temperature viability test (HTVT) (per cent) 35.0 ± 0.66 and 32.1 ± 0.36, cold shock resistant test (CSRT) (per cent) 6.16 ± 0.19 and 4.16 ± 0.16 and resistance to sodium chloride (R test value) 1425.0 ± 45.75 and 1029.2 ± 42.50 during winter and summer respectively. The quality of semen was found to be better during winter season because of lower values of HTVT and CSRT with more per cent of motile sperms resistant to sodium chloride.

Evaluation of various semen characteristics viz.,physical, biochemical and resistance to environment were good indicators to judge the semen quality and metabolism during different seasons in native bucks in artificial insemination work.

INTRODUCTION

CHAPTER I

1. INTRODUCTION

Goat is a versatile animal, it is known as "poor man's cow " in India. Goats can be kept with little expense. Marginal or undulating lands, unsuitable for other types of livestock, may be used and any inexpensive shelter will suffice.

Goat milk is cheap, wholesome, easily digestable and nutritious. About 75 per cent of total World production of goat meat is produced in tropical and subtropical countries. India contributes about 50 per cent of the total production in this region.

India's goat population is about 107 millions (FAO, 1990) out of the total World population of 446 million goats. The goat population has increased by about 19% from 1961 to 1979. Africa and India have largest goat populations. In Andhra Pradesh as on 1983 the goat population is 55.34 crores. The goat population contributes nearly 400 crores of rupees to the national income in the form of milk, meat and other products. They adapt poorly to cold climates compared to hot climates due to limited subcutaneous fat. Indigenous goats

are also resistant to many of the diseases which the other livestock are prone. Due to some reason or other the goats are neglected till now but in future goats may play a major role in the economics of the country. India has too little pasture land but has ample quantity of unconventional fodders on which goats only can survive because of its ability to digest highly lignified vegetation which neither cattle nor sheep can do. Goats seem to breed throughout the year in tropical climate. As per the literature available it reveals not much work was done on this species, and the available literature also pertains to certain countries and to certain exotic breeds only. Still much research has to be contributed for the development of native goats.

Objectives: The main objectives of this study are:

1) To study the seasonal variation (winter and summer) of semen characteristics in native bucks.
2) To study certain Biochemical characteristics of native buck semen.
3) To evaluate resistance of the sperm to its environment.

REVIEW OF LITERATURE

CHAPTER II

2. REVIEW OF LITERATURE

2.1 REACTION TIME AND PHYSICAL CHARACTERISTICS OF SEMEN:

The available literature on the service behaviour and semen characteristics in native and exotic breeds of bucks is reviewed mostly hereunder.

2.1.1 Reaction time:

Patil and Raja (1978) recorded a reaction time of 49.37 $\pm$ 2.5 seconds in Malabari bucks. The difference between seasons was not significant.

Ashmawy (1979) observed significant difference in the number of ejaculations and exhaustion time between seasons but not between months. The longest reaction time was observed in March. Mann (1980) observed uniformly good libido all through the year in African dwarf goats. Sinha *et al.* (1981) recorded a longer reaction time (25.40 to 35.75 seconds) in bucks aged over two years than in bucks below two years of age (20.85 to 35.17 seconds). However, the difference was not significant.

In Black Bengal and Saanen bucks Sinha and Singh (1982) recorded the average reaction time as 60.53 and 64.46 seconds respectively.

The average reaction time was reported to be 12.43 seconds for 1st ejaculate and 32.79 seconds for 2nd ejaculate in Baladi bucks (El-Sayed et al.,1983).

Reddy et al. (1989) observed an average reaction time of 15.75 and 19.84 seconds in local bucks below 2 years and above 2 years groups respectively during winter season and the same during hot weather period were 20.34 and 29.02 seconds respectively.

Pattnaik et al. (1991) observed a mean reaction time of 9.62 $\pm$ 0.97 seconds in Ganjam bucks and opined that it was comparitively less than that of Malabari, Black Bengal and Saanen bucks, indicating that Ganjam bucks were quick at service.

Patil and Raja (1978) observed that reaction time is significantly negatively correlated with volume (-0.267), initial motility (-0.322), sperm count(-0.051) and positively correlated with pH (+0.273) while El-Sayed et al. (1983) reported that reaction time was significantly correlated with proportion of live sper-

matozoa in 1st ejaculates (0.32) and with the proportion of motile spermatozoa (0.38) and sperm concentration (-0.20) in 2nd ejaculates.

2.1.2 Physical characteristics of semen:

2.1.2.1 Volume:

Tiwari et al. (1968) recorded a mean semen volume of 0.657 and 0.595 ml respectively in Barbari and Sannen bucks when semen was collected once daily.

Mittal and Pandey (1972) studied the ejaculate volume at weekly intervals for 2 Barbari and 2 Jamnapari bucks and concluded that the semen quality was highly significant between bucks but the differences between weeks were not significant.

In native Zambian and Boer bucks the average ejaculate volume was 0.67 ± 0.03 and 1.34 ± 0.05 ml respectively. The volume did not differ between 1st and 2nd weekly collections (Igboeli, 1974). Vinha and Megale (1974) recorded the mean ejaculate volume to be 1.48, 0.88 and 0.88 ml in Anglo-Nubian, Maroto and Maxoto bucks respectively. The average ejaculate in Katjang X Jamnapari bucks aged over 2-4 years was reported to be 0.85 ± 0.04 ml (Koh, 1975).

Mann (1980) reported the mean ejaculate volume was 0.77 ± 0.26 ml in West African Dwarf bucks born in Germany and aged 9 months to 5 years. Mohan et al. (1980) recorded a mean ejaculate volume of 0.62 ± 0.02 ml in Pashmina goats. Where as Saxena and Tripathi (1980) recorded a mean ejaculate volume of 0.37 ± 0.03 ml in Jamnapari bucks. Singh et al. (1982) recorded a mean ejaculate volume of 0.86 ± 0.09 and 1.01 ± 0.04 ml in Jamnapari and Barbari bucks respectively.

Dundar et al. (1983) recorded the mean ejaculate volume as 0.94 ± 0.11 ml in Angora goats. El-Sayed et al. (1983) recorded the volume of semen as 0.92 and 0.84 ml for 1st and 2nd ejaculates respectively in Baladi bucks.

Chaudry and Mahmood-Ul-Hassan (1984) reported the average ejaculate volume to be 0.5 ml (0.2-1.0 ml) during July-September in Barbari bucks. Further they opined that higher ejaculate viscosity was related to smaller volume and higher sperm concentration and also that greater volume was related to higher pH.

Singh et al. (1985) recorded a average ejaculate volume of 0.46, 0.55 and 0.60 ml in Black Bengal, Jamna-

pari and Jamnapari X Black Bengal bucks respectively, which were aged about 18 months.

In Nubian goats Ali and Mustafa (1986) recorded the mean ejaculate volume as 1.5 ml in Sudan while Charaborty et al. (1989) reported the same to be 0.92 ± 0.07 ml at puberty in Anglo-Nubian goats. Mendoza et al. (1989) recorded the mean ejaculate volume between April and July in 2 successive years as 0.8 ± 0.30 and 0.98 ± 0.52 ml in Angora goats.

In Ganjam bucks Pattnaik et al. (1991) recorded the mean ejaculate volume to be 0.94 ± 0.24 ml.

Sahni and Roy (1969) recorded the range in sperm volume in the 5 seasons for the Barbari and Jamnapari bucks as 0.52 ± 0.03 to 0.76 ± 0.03 and 0.72 ± 0.06 to 1.18 ± 0.01 ml respectively.

The semen volume was greatest in autumn (1.68 ml) and smallest in summer (1.30 ml) in Anglo-Nubian bucks aged 3 years as reported by Vinha (1975).

Kang and Chung (1976) recorded a mean ejaculate volume was 0.95 and 0.50 ml respectively in November-January and July-September months in native Korean goats.

They opined that ejaculate volume increased with decreasing day length and vice versa. Correlations between ejaculate volume and sperm concentration were negative while ejaculate volume was positively correlated with percentage of sperm abnormalities, pH and sperm motility.

The maximum volume of 0.63 ml was obtained during post monsoon and minimum of 0.38 ml in winter. The variation in volume of semen during different seasons was found to be highly significant (Patil and Raja, 1978). Mittal (1982) found the average ejaculate volume as 0.69 ml in Barbari bucks. Maximum ejaculate volume was noticed during summer (0.85 $\pm$ 0.25 ml) and minimum during winter (0.55 $\pm$ 0.18 ml). Greyling and Grobbelaar (1983) observed the semen quality in 2 Boer and 2 Angora bucks, over a period of one year and opined that there was no significant seasonal variation. There was no significant differences between seasons in the ejaculate volume in association with some semen characteristics (Rahman and Kandil, 1984).

Mittal (1985) studied 2 male goats during summer and autumn and reported that season had no significant effect on the ejaculate volume. Li *et al.* (1988) reported that the ejaculate volume was higher in September-

March than in April-August in Guanzhang male dairy goats. Reddy et al. (1989) studied 10 local bucks 5 below 2 years of age (group 1) and 5 over 2 years of age (group 2) and opined that season of collection affected semen volume ($P < 0.01$). They reported that semen volume was highest in winter in group 2 and in the North-east monsoon in group 1 (0.89 and 0.83 ml respectively).

Patil and Raja (1978) observed that volume of semen was non significantly correlated with initial motility, concentration of sperm, live sperm count, abnormal sperm count and pH. While El-Sayed et al. (1983) reported that ejaculate volume was significantly correlated with proportion of sperm abnormalities(-0.53) in 1st ejaculate.

2.1.2.2 Mass motility:

The semen quality of Barbari and Jamnapari bucks was evaluated by Mittal and Pandey (1972) and opined that there was highly significant difference between bucks in sperm motility.

Mohan et al. (1980) reported that the motility score (scale 1-5) averaged 4.19 $\pm$ 0.04 in 5 Pashmina

goats and there was significant difference between bucks. The physico-chemical and the morphological attributes of semen of Jamnapari bucks had the forward motility (scale 0-5) as 3.78 ± 0.07 (Saxena and Tripathi, 1980). Sinha and Singh (1982) recorded the mass motility score (0-5) as 4.44 and 4.51 in Black Bengal and Saanen bucks respectively.

El-Sayed et al. (1983) reported that the mass activity score averaged 3.06 and 3.16 for first and second ejaculates from 6 Baladi male goats.

Daudu (1984) studied the semen characters of 15 Red Sokoto male goats and reported the mass motility as 3.80 ± 0.33. Singh et al. (1985) comparatively studied the seminal quality of 2 Black Bengal, 2 Jamnapari and 2 Jamnapari X Black Bengal bucks and reported the mass motility scores as 4.75, 4.58 and 4.25 respectively in the 3 groups.

Singh et al. (1976) and Gunzel et al. (1980) concluded that the mass motility in rams obtained in winter had significantly better than those obtained in summer. However, Silva and Nunes (1984) found that season had no significant difference on mass motility in rams.

2.1.2.3 Individual motility:

A comparative study was made on seminal characteristics of 8 native Zambian and 12 Boer bucks and reported that the motility averaged 52.3 ± 1.3 and 53.2 ± 1.2% in the 2 breeds respectively (Igboeli, 1974). Vinha and Megale (1974) studied the physical and morphological aspects of 14 Anglo-Nubian, 5 Maroto and 5 Maxoto goats and reported that sperm motility averaged 76.22, 68.33 and 62.75% respectively. Koh (1975) recorded sperm motility as 85.0 ± 0.76% in Katjang X Jamnapari bucks aged 2-4 years.

Cetinkaya et al. (1980) reported that the sperm motility averaged 86% (70-90) in 5 Angora goats and when the semen was diluted and frozen and after thawing 48 hours later the sperm motility was 40-55%.

The forward motility was found to be 77.28 ± 7.75% in 6 West African Dwarf bucks as reported by Mann (1980).

Mohan et al. (1980) reported that there was significant difference in the initial motility between bucks and between collections and he recorded initial motility in 5 Pashmina goats as 60.62 ± 0.04%. Saxena and Tripathi (1980) reported that the initial sperm motility averaged

72.62 ± 1.06% in Jamnapari bucks where 10 ejaculates were collected at weekly intervals.

Singh et al. (1982) studied the semen characteristics of 5 Jamnapari and 3 Barbari bucks and reported that the initial motility was 74.01 ± 0.40 and 78.30 ± 2.48 respectively.

Chaudry and Mahmood-Ul-Hassan (1984) examined the semen from 2 Barbari bucks collected during July-September and reported that the sperm motility ranged from 65 to 80%. He opined that higher ambient temperature and humidity in September was related to lower sperm motility. Pattnaik (1991) recorded the sperm motility as 75 to 80% in five Ganjam bucks of 2-4 years age at goat breeding farm, Bhubaneswar.

The initial motility ranged in the 5 seasons for the Barbari and Jamnapari bucks respectively was 3.20 ± 0.04 - 3.98 ± 0.04 and 2.94 ± 0.02 - 4.03 ± 0.01 (Sahni and Roy, 1969). Vinha (1975) analysed the semen samples from 3 Anglo-Nubian goats aged 3 years and reported that the motility was highest in spring (86.87%) and lowest in summer (67.76%) and the difference being significant.

Djanur (1965) reported a fall in the motility of the spermatozoa in rams from 75 to 0-5% when the ambient temperature was raised from 50°F to 100°F.

Kang and Chung (1976) studied the seasonal changes in the semen characters of Korean native goats in November-January and July-September and reported that the sperm motility index in both the seasons were 83.3 and 55.1% respectively ($P < 0.05$). They further opined that motility was positively correlated with pH. Patil and Raja (1978) reported that the initial motility was significantly affected by season (summer 62.33, winter 59.33) and the average initial motility in 61 semen samples collected from 7 Malabari bucks was 66.16%. The motile spermatozoa averaged 78% (summer 82.2 $\pm$ 0.34, winter 74.5 $\pm$ 0.72) in the semen collected twice weekly from 4 Barbari bucks (Mittal, 1982).

Rahman and Kandil (1984) studied the seasonal variations in mating behaviour of male goats in association with some semen characteristics and reported that sperm motility was significantly higher in autumn than in spring or summer. Mittal (1985) studied the semen quality of 2 Jamnapari bucks under arid conditions during summer and autumn and concluded that season had no sig-

nificant effect on the sperm motility. Li et al.(1988) reported that sperm motility was higher in September-March than in April-August.

Reddy et al. (1989) studied 10 local bucks 5 below 2 years of age (group 1) and 5 over 2 years of age (group 2) and opined that the season had significant effect on individual motility. The sperm motility values in group 1 and 2 were 78.83 - 82.60% and 79.01 - 84.59% respectively. Higher motility was observed in the winter and North-eastern monsoon in both the groups and the difference was highly significantly ($P < 0.01$) in group 2 bucks.

Kang and Chung (1976) observed a positive correlation between motility and pH.

Patil and Raja (1978) reported that there were significant positive correlations between initial motility and sperm concentration (0.335), percentage of live sperm (0.836) and pH of semen (0.509).

2.1.2.4 Sperm concentration:

Mittal and Pandey (1972) evaluated the sperm quality of 2 Barbari and 2 Jamnapari bucks over a period

of 5 weeks and reported that there was significant difference between the bucks in sperm concentration and the Barbari bucks. Igboeli (1974) comparatively studied the seminal characteristics of 11 native Zambian and 12 Boer bucks and reported that the sperm concentration (x 10^9) averaged 1.65 $\pm$ 0.02 and 2.70 $\pm$ 0.03/ml respectively in the 2 breeds. The physical and morphological aspects of semen in 14 Anglo-Nubian, 5 Maxoto and 5 Maroto goats were studied and the sperm concentration in the 3 breeds was 1559154, 1107222 and 803 448/mm^3 respectively (Vinha and Megale, 1974).

Koh (1975) examined 100 ejaculates from 10 Katjang X Jamnapari bucks aged 2-4 years and reported that the sperm concentration averaged 3974.8 $\pm$ 130.8 x 10^6/ml. The sperm concentration (x 10^6) in Angora goat semen as 3674/ml (1660-5200) (Cetinkaya et al.,1980). Mohan et al. (1980) recorded the mean sperm concentration (x 10^8/ml) of 35.21 $\pm$ 1.18 in Pashmina goats. Saxena and Tripathi (1980) reported that the average sperm concentration (x 10^6/ml) was 4795.00 $\pm$ 292.9 in Jamnapari bucks.

Singh et al. (1982) recorded the mean sperm concentration (x 10^6/ml) in Jamnapari and Barbari bucks as 2293 $\pm$ 728 and 1920 $\pm$ 720 while Sinha and Singh (1982)

recorded the same as 2440.15 and 2780.30 in Black Bengal and Saanen bucks. El-Sayed et al. (1983) recorded the same (x 10^6/ml) as 2723.92 and 2351.08 for 1st and 2nd ejaculates respectively in Baladi bucks.

Chaudry and Mahmood-Ul-Hassan (1984) reported that sperm concentration (x 10^9/ml) averaged 6.53 (range 5.0-8.0) during July-September in Barbari bucks. While Daudu (1984) recorded the mean sperm concentration (x 10^9 per ml) as 0.61 $\pm$ 0.05 in Red Sokoto goats aged 2- 2.5 years.

Singh et al. (1985) comparatively studied the seminal quality of pure and crossbred bucks and recorded the mean sperm concentration (x 10^6/ml) as 2619.58, 2910.33 and 2851.67 in Black Bengal, Jamnapari and Black Bengal X Jamnapari bucks respectively.

Ali and Mustafa (1986) recorded the average sperm concentration as 1.77 x 10^9/ml in Nubian goats in Sudan, while Charaborty et al. (1989) reported the same to be 1.25 $\pm$ 0.37 x 10^9/ml at puberty in Anglo-Nubian goats.

Sahni and Roy (1969) recorded the range in sperm concentration (x10^8/ml) in the 5 seasons for Barbari and

Jamnapari bucks respectively as 18.10 ± 0.10 - 24.10 ± 0.04 and 18.05 ± 1.41 - 33.09 ± 2.64. Vinha (1975) revealed that sperm concentration was highest in summer 1752 380 (spermatozoa x mm^3) and lowest in autumn (1348 636) the differences were not significant in Anglo-Nubian goats aged 3 years.

Kang and Chung (1976) recorded the mean sperm concentration of 9.86 x 10^8 and 16.02 x 10^8/ml respectively in November-January and July-September months in native Korean goats. The sperm concentration was significantly affected by season and the sperm concentration averaged 3534 x 10^6/ml in Malabari bucks (Patil and Raja, 1978). Mittal (1982) recorded the average sperm concentration (x 10^6/ml) in Barbari bucks as 2472. In summer the concentration was 2610 ± 221.24 x 10^6/ml and in winter the same was 2320 ± 94.23 x 10^6/ml .

Rahman and Kandil (1984) reported that the sperm concentration was significantly higher in autumn than in spring or summer. While Mittal (1985) reported that season had no significant effect on the sperm concentration.

Patil and Raja (1978) observed that sperm concentration had non significant positive correlations with

percentage of live sperm (+ 0.214) and pH (+ 0.235) and had significant negative correlations with abnormal sperm count (-0.286).

2.1.2.5 Live sperm count:

Igboeli (1974) comparatively studied the seminal characteristics of 11 native Zambian and 12 Boer bucks and recorded the percentage of live spermatozoa as 87.2 ± 1.0 and 87.7 ± 1.0 in the two breeds respectively. There was significant difference between bucks for the proportion of live spermatozoa which averaged 80.63 ± 0.29% in 5 Pashmina goats as reported by Mohan et al. (1980). Saxena and Tripathi (1980) reported that the proportion of live spermatozoa averaged 77.65 ± 1.04% in Jamnapari bucks.

Singh et al. (1982) recorded the mean percentage of live spermatozoa as 80.90 ± 2.32 and 83.80 ± 0.26 in Jamnapari and Barbari bucks respectively while Sinha and Singh (1982) recorded the same as 85.45 and 85.21% in Black Bengal and Saanen bucks.

Singh et al. (1985) recorded the percentage of live spermatozoa as 91.07, 90.33 and 86.37 ($P < 0.01$) in 2 Black Bengal, 2 Jamnapari and 2 Jamnapari x Black

Bengal bucks respectively. The mean per cent of live spermatozoa in 30 ram ejaculates averaged 53 (Aguirre et al.,1988).

Pattnaik et al. (1991) recorded the mean percentage of live spermatozoa as 84.83 ± 1.02 in 5 Ganjam bucks of 2-4 years age at Goat Breeding Farm, OUAT, Bhubaneswar.

Sahni and Roy (1969) reported that the percentage of live spermatozoa ranged from 65.29 ± 2.23 - 71.62 ± 1.41 and 55.75 ± 3.87 - 71.17 ± 2.82 in Barbari and Jamnapari bucks in 5 seasons and the difference in between seasons was significant only in Jamnapari bucks. Patil and Raja (1978) reported that live sperm count varied from 22% to 88% with a mean of 63.38 ± 2.58% in Malabari bucks and the percentage of live sperm was posisively correlated with pH (+0.659) and negatively correlated with the percentage of abnormal sperms (-0.069). They further reported that the variations in the percentage of live sperm at different seasons were found to be non significant.

Saxena et al. (1978) reported that there was a significant ram x month interaction for the proportion of live spermatozoa which ranged from 74.23 - 90.21%

during June - October in Muzaffarnagri rams. Chahal et al. (1979) recorded the proportion of live spermatozoa as 85.46, 86.15, 69.19 and 76.88% in winter, spring, summer and the rainy seasons respectively in Corriedale rams and the difference between seasons was highly significant.

Deka and Rao (1979) reported that the percentage of live spermatozoa averaged 84.71 $\pm$ 6.51 in 3 Dorset X Mandya, 1 Suffolk x Nellore, 1 Suffolk X Mandya and 2 Dorset X Nellore rams. They further concluded that the proportion of live spermatozoa varied significantly between rams, but not between months. Mittal and Ghosh (1979) comparatively studied the semen characteristics of Correidale, Marwari and Jaisalmeri rams maintained under hot arid conditions and recorded the mean percentage of live spermatozoa as 67.2 - 67.9, 78.6 - 80.0, 76.01 - 78.1 respectively in the 3 breeds, between June and September and found significant difference between the seasons.

Mittal (1982) studied the effect of season on the semen quality of Barbari bucks and recorded the proportion of live spermatozoa at room temperature as 72.9% and at $5^{o}c$ as 6.5%. He further reported that season had

a significant effect on the proportion of live spermatozoa at room temperature (the proportion ranged from 62.15% in winter to 82.03% in summer) and at $5^{o}c$ (ranging from 5.0% in winter to 8.9% in summer). While Greyling and Grobbelaar (1983) opined that there was no significant seasonal variation in semen quality in Boer semen and the percentage of live spermatozoa varied significantly between months. Loubser (1983) recorded the mean percentage of live spermatozoa as 50.78 in 12 Angora rams semen collected from late summer to the end of winter (February - August). They reported that the percentage of live spermatozoa did not vary significantly among seasons.

Rahman and Kandil (1984) reported that the percentage of live spermatozoa was significantly higher in autumn than in spring or summer, and Mittal (1985) reported that season had no significant effect on the percentage of live spermatozoa.

Saxena and Tripathi (1987) studied the seasonal effect on sperm morphology of Nali rams. They recorded the percentage of live spermatozoa as 49.38 to 68.04 in the first ejaculate and 45.86 to 64.00 in second ejaculate during different seasons. Further they reported that the

percentage of live spermatozoa varied significantly between the seasons in both the ejaculates. Mathur et al. (1989) comparatively studied the semen quality of Rambouillet, Soviet Merino - Dorset and Karakul rams during the summer and autumn and reported that the semen quality of all breeds was within acceptable limits (Percentage of live spermatozoa ranging from 77-87).

Patil and Raja (1978) observed that the percentage of live sperm was positively correlated with pH (+0.659) and negatively correlated with percentage of abnormal sperms (0.069).

2.1.2.6 Abnormal sperms:

Vinha and Megale (1974) recorded the percentage of abnormal spermatozoa in Anglo-Nubian, Maroto and Moxoto goats as 11.05, 11.21 and 16.35%.

The percentage of spermatozoa was recorded in Katjang X Jamnapari bucks as 3.11 $\pm$ 0.28 (Koh, 1975). Cetinkaya et al. (1980) recorded the percentage of abnormal spermatozoa in Angora goats as 2.3 while Mann(1980) recorded the same as 13.45 $\pm$ 8.77% in West African Dwarf bucks.

Saxena and Tripathi (1980) recorded the percentage of abnormal spermatozoa as 6.84 ± 0.60% in Jamnapari bucks.

Bardoloi and Sharma (1982) recorded the mean percentage of abnormal spermatozoa as 8.82 ± 2.08 (range 2.97-15.84) in goats. The percentage of abnormal spermatozoa was found to be 7.87 and 6.20 ($P < 0.01$) in Black Bengal and Sannen bucks (Sinha and Singh, 1982). Bardoloi and Sharma (1983) studied the variation in the percentage of sperm abnormalities in Beetal, Saanen and Assam goats and reported that there were no significant differences in the incidence of head, tail or total abnormalities.

Borgohain et al. (1983) tabulated the monthly incidence of various sperm abnormalities in Assam and Beetal goats and reported that the differences between months and between bucks within breeds were non significant.

The percentage of spermatozoa with normal morphology was reported in Red Sokoto goats as 80.00 ± 10.50 (Daudu, 1984). Singh et al. (1985) recorded the percentage of abnormal spermatozoa in Black Bengal, Jamnapari and Jamnapari X Black Bengal bucks as 2.12, 2.00 and 2.11 respectively.

Ali and Mustafa (1986) reported that the percentage of primary and secondary sperm abnormalities in Nubian goats were 6.7% and 15.3%. Pattnaik et al.(1991) recorded the mean percentage of total sperm abnormalities as 5.42 ± 0.31 in Ganjam bucks.

El-Wisky et al. (1971) recorded the percentage of sperm abnormalities in male Damascus goats as 12.3, 12.5, 9.0 and 11.5% during winter, spring, summer and autumn respectively.

The incidence of abnormal spermatozoa was recorded in Jamnapari, Barbari and Jamnapari X Saanen goats to be 0.5 - 4.0% and showed no seasonal variation (Sahni and Roy, 1972 a). Vinha (1975) analysed the semen samples of 3 Anglo-Nubian goats and revealed that the percentage of sperm abnormalities was highest in spring (13.72%) and lowest in autumn and winter (9.92 and 9.61%) respectively, and the difference was significant.

Patil and Raja (1978) recorded the mean abnormal sperm percentage in the semen of Malabari bucks as 4.34 ± 0.48% (Range 2.41 - 6.11). They further reported that there was significant difference (5% level) in the percentage of abnormal sperms at different seasons. The

minimum abnormality is recorded during post-monsoon and maximum during summer.

Mittal and Ghosh (1979) reported that there was significant seasonal difference between breeds for the abnormal sperm percentage and recorded the abnormal sperm percentage in Corriedale, Marwari and Jaisalmeri rams as 8.09 - 8.29, 1.21- 2.33 and 2.01 - 2.16 ($P < 0.05$).

The percentage of normal spermatozoa in Angora rams was 80.16% and it varied highly significantly between months and was highest in winter as reported by Loubser *et al.* (1983).

Carmenate and Gamcik (1984) studied the physical and morphological characters of Peliguey and Corriedale rams semen during different seasons and reported that the difference between seasons being significant for sperm head abnormalities and the total percentage of sperm abnormalities in both breeds and also for the incidence of sperm neck and tail abnormalities in Corriedales. Chaudry and Mahmood-Ul-Hassan (1984) recorded the incidence of abnormal sperm heads as 1.79% in 2 Barbari bucks during July - September. Mattos *et al.* (1984) studied the seasonal effects on the semen of German Mutton

Merino rams and reported that the percentage of abnormal spermatozoa ranged from 10 in December to 70 in August.

Mittal (1985) reported that season had no significant effect on the percentage of abnormal spermatozoa while, Gamcik and Mesaros (1986) recorded the incidence of sperm head abnormalities in Slovakian Merino rams as 14.1% and tail abnormalities as 3.3% and they further reported that there were no seasonal changes in the incidence of abnormalities.

Saxena and Tripathi (1987) studied the effect of season on the sperm morphology of Nali rams and reported that the total abnormalities in spermatozoa were 13.12 to 15.72% in both the ejaculates in all the seasons. The head abnormalities were significantly low during summer (2.96 $\pm$ 0.36 and 3.88 $\pm$ 0.39) than winter (4.36 $\pm$ 0.51 and 4.88 $\pm$ 0.62). Primary abnormalities were significantly less than the secondary abnormalities in all the seasons. The abnormalities were not affected by seasons in both the ejaculates.

Li *et al.* (1988) reported that the percentage of abnormal spermatozoa was significantly higher in September - January than in February - August in Guanzhang male dairy goats and seasonal difference was significant.

Reddy et al. (1989) studied the effect of season and age on seminal altributes of local bucks aged less than 2 years (group 1) and aged more than 2 years (group 2) and reported the incidence of abnormal spermatozoa as 6.73 - 11.00% and it was higher in group 1 than group 2 ($P < 0.05$) and lower in South west and North east monsoons than during winter and periods of hot weather.

Patil and Raja (1978) reported a non significant positive correlation between percentage of abnormal sperm and pH of semen (+ 0.125).

2.1.2.7 Acrosomal evaluation:

Aguirre et al. (1988) reported 56 per cent of intact acrosomes in 30 ram ejaculates. Mattos et al. (1984) reported that the percentage of spermatozoa with acrosomes changes from 50.0 in December to 38.0 in August in German Mutton Merino rams.

Roca et al. (1992) studied the sperm abnormalities in the semen of Murciano-Granadina goats and recorded the mean percentage of spermatozoa with damaged acrosome ranged from 3.53 $\pm$ 0.22 to 11.15 $\pm$ 0.58 . The incidence of spermatozoa with acrosome damage was highest ($P<0.05$)

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during winter and spring seasons. The monthly peak occured between February and May ($P < 0.05$). A high percentage of ($P < 0.05$) spermatozoa with damaged acrosome was also found in December.

2.2 BIOCHEMICAL TESTS:

2.2.1 Hydrogen ion concentration (pH) of semen:

Hydrogen ion concentration or pH of semen at the time of collection seems to have little practical value in the normal for predicting fertility (Swanson and Herman, 1944).

In Malabari bucks the pH of semen varied from 6.3 to 6.7 (Kurian and Raja, 1965; Patil and Raja, 1978). Koh (1975) reported the pH of semen as 6.8 in crossbred bucks. In African Dwarf bucks the pH of the semen was reported to be 6.93 (Mann, 1980). Mohan *et al.* (1980) recorded a mean pH of 6.84 $\pm$ 0.02 in the semen of Pashmina goats.

Dundar *et al.* (1983) reported the mean pH of semen as 6.685 $\pm$ 0.05 in Angora bucks. In Barbari bucks the pH ranged from 6.25 to 6.8 with an average of 6.5, they opined that greater volume was related to higher pH (Chaudry and Mahmood-Ul-Hassan, 1984). Joseph and Nair

(1989) stratified the crossbred bucks into 3 groups depending on the number of ejaculates collected per day. They recorded a mean pH of 6.87 ± 0.09 in group 1 (one ejaculate per day), 6.96 ± 0.11 in group 2 (two ejaculates per day) and 7.09 ± 0.12 in group 3 (three ejaculates) per day. Mendoza et al. (1989) recorded a mean pH of semen as 7.01 ± 0.34 in Angora bucks.

Kang and Chung (1976) recorded the annual pH range of semen as 6.8 to 7.2 and they observed that there was no marked seasonal variation in the pH in native Korean bucks. Patil and Raja (1978) opined that pH was not significantly affected by season.

The mean pH of native buck semen was 6.78 ± 0.009 in bucks aged below 2 years and 6.76 ± 0.006 in bucks aged above two years (Reddy et al.,1989).

2.2.2 Methylene Blue reduction test:

Radman and Kopijar (1960) reported and concluded that the methylene blue reduction test of 136 semen samples from 28 rams was 2-3 minutes when the semen was of good quality and 1.5 - 2 minutes when it was very good.

Gaivanovich (1974) reported that in Carpathian mountain, Tsigai, Tsigai X Carpathian mountain semicoarse

wool and Tsigai X Carpathian mountain semi fine wooled rams the methylene blue reduction of semen averaged 8.6, 3.0, 9.9 and 10.9 minutes at 9 months and 5.4, 8.7 and 6.3 minutes at 18 months of age, respectively.

El-Sherming et al. (1982) in their study Ossimi Fleisch Merino and their crossbred rams observed that Methylene blue reduction time of semen was not significantly affected by breed but was significantly affected by genotype x season interaction.

Joseph and Nair (1991) reported the mean MBRT in seconds for successive ejaculates in crossbred bucks as 259.08, ±151.60 for 1st ejaculate, 293.85 ± 152.35 for 2nd ejaculate and 404.15 ± 197.39 for 3rd ejaculates.

El-Wisky et al. (1971) reported the mean Methylene blue reduction time (min) in Damascus bucks for winter, spring, summer and autumn seasons as 6.75, 3.30, 2.74 and 9.36 respectively. The reduction time was lowest in June and July, but highest during October, El-Fouly et al. (1980) in their study on Ossimi and Rahmani rams reported the methylene blue reduction time of semen as 2.48 ± 0.21 minutes in summer and 6.19 ± 0.24 minutes in winter. The difference between seasons was significant.

Tripathi and Saxena (1983) recorded the methylene blue reduction time varying from 271.25 seconds in winter to 521.46 seconds in spring in Murrah bull semen.

Al-Wahab and Abid (1987) studied 170 ejaculates from Finnish Landrance X Awassi and Awassi rams during July -August and September and concluded that the methylene blue reduction time decreased from July and the lowest value (74 seconds) was recorded during September.

2.2.3 Resazurin reduction test:

The resazurin reduction test has been reported as one of the sensitive metabolic tests for semen quality (Erb and Ehlers, 1950) in bulls. However, very little work has appeared in the literature on its applicability, particularly no work could be found in buck semen.

Erb et al. (1952) reported that good quality bull semen samples reduced resazurin to pink colour in about one minute, and it required about four minutes to reduce it to colourless. While Flerchinger et al. (1956) reported that the average resazurin reduction time for 261 seman samples from bulls was 5.39 minutes.

Pathak et al. (1989) reported that in crossbred bulls and resazurin reduction time was higher than that reported for pure breeds by other researchers. The mean time required for second colour change (pink to colourless) in the crossbreds was 609.61 ± 69.32 seconds.

2.3 RESISTANCE TO ENVIRONMENTAL CHANGES:

Regarding these tests very little work was reported on their applicability and particularly no work could be found pertaining to buck semen.

2.3.1 High temperature viability test (HTVT):

Ludwick et al. (1948) reported high correlation between time of incubation at 100°F required for all spermatozoa to loose their motility and conception rate. Similar results were reported by Buckner et al. (1954) who estimated progressive motility after incubation for 16 and 28 hours in egg yolk citrate at 38°c.

Patel et al. (1988) observed that hot shock resistant sperm percentage in normal bulls and problem bulls were 79.31 ± 1.64 and 27.84 ± 2.38 respectively.

Joseph and Nair (1989) estimated sperm viability at 46.5°c in crossbred bucks which were grouped into 3

groups according to the number ejaculates collected per day. They recorded the mean percentage viability of spermatozoa in group 1 (one ejaculate) as 60.89 ± 17.20 53.70 ± 23.18 and 36.82 ± 22.92 at 10, 20 and 30 minutes incubation respectively. The corresponding values for group 2 (two ejaculates) were 66.07 ± 20.90, 53.62 ± 26.24 and 42.25 ± 26.57 and in group 3 (three ejaculates)were 76.53 ± 11.71, 56.14 ± 19.59 and 35.53 ± 23.99 respectively. They opined that there was no significant difference between the groups.

2.3.2 Cold shock resistance test:

The utility of resistance to cold shock as a test was suggested by Lasley et al. (1942). This test may be useful to grade semen for varying proportion of cold shock resistant spermatozoa. It may be likely that semen with higher concentration of cold shock resistant spermatozoa may keep longer under preservation (Bishop et al.,1954; Singh et al.,1968) and may predict higher fertility rates (Lasley and Bogart, 1943; Bishop et al., 1954; Tomar and Singh, 1970).

Mittal and Pandey (1972) evaluated the semen characters in 2 Jamnapari bucks and 2 Barbari for 5 weeks at weekly intervals and reported the average per cent of live

sperm after cold shock as 6.46 ± 0.337 and 6.18 ± 0.296 for the 2 Jamnapari bucks and 5.93 ± 0.316 and 6.00 ± 0.341 for the 2 Barbari bucks.

Sahini and Roy (1972 b) recorded the average percentage of live spermatozoa subjected to cold shock at $0^{o}c$ and $5^{o}c$ were 3.1 ± 1.7 and 3.5 ± 1.4 in ram semen and 0.7 ± 0.0 and 6.9 ± 2.0 in goat semen respectively. They concluded that goat spermatozoa are quite susceptable to cold shock.

Deka and Rao (1979) reported the mean cold shock resistance index as 2.50 ± 1.81 per cent in crossbred ram semen. They concluded that cold shock resistance did not vary significantly between months or between rams.

The live sperm percentage after cold shock for buffaloe bulls in breeding (February - April and August) and non breeding (May - July) seasons was reported as 67.94 ± 4.10 and 58.92 ± 3.03 respectively. The corresponding values before cold shock were 88.68 ± 2.21 and 87.76 ± 2.78 respectively (Pangawkar, 1982).

Mohanty et al. (1985) observed the mean per cent of live sperm before and after cold shock in normal bulls

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mean per cent

bulls

as 80.49 ± 1.28 and 30.12 ± 1.75 respectively. The same for poor freezability bulls were 76.45 ± 1.07 and 27.59 ± 3.94 respectively. While Patel et al. (1988) reported the average cold shock resistant sperm percentage in normal bulls as 65.47 ± 1.32 and in problem bulls it was 23.32 ± 2.07.

2.3.3 Resistance to sodium chloride and dilution effect:

The measurement of the deleterious effect of 1% solution of sodium chloride on spermatozoal survival, as reported by Milovanov (1934), was described in detail by Anderson (1945). Subsequently Cheng et al. (1949) and Emmens and Swyer (1948) using rabbit spermatozoa showed that a similar effect could be obtained with chloride free diluents.

Izbasarov (1973) observed the mean sperm resistance (x 10^3 ml 1% Nacl) in Karakul rams aged 7-8 months and 2.5 years age groups as 36.0 - 38.3 and 40.0 - 44.0 respectively.

Patel et al. (1988) reported the mean R-test value in normal and problem crossbred bulls as 1164.81 ± 100.81 and 425.00 ± 17.68 respectively. While Tripathi and Saxena (1983) observed the mean of resistance to 1% sodium chloride

varying from 4000.00 ± 277.35 in spring to 6437.50 ± 778.45 in rainy season in the ejaculates of Murrah buffaloe bulls.

MATERIALS AND METHODS

CHAPTER III

3. MATERIALS AND METHODS

The present study was undertaken at the College of Veterinary Science, Tirupati, situated at an altitude of 182.9 meters above mean sea level on 79° longitude and 13° latitude. The maximum temperature recorded was 36.8°c in April while the minimum was 16.3°c in January. Relative humidity varied from 28% in April to 82% in January. Rainfall during the present study varied from 3.2 mm in February to 73.0 mm in May as shown in Table No.1.

The native buck is relatively large and has a graceful appearance. The average measurements are:height 89.90 ± 4.02 cm; length 80.55 ± 4.80 cm and girth 86.54 ± 3.14 cm. The various colours of the coat and black,brown, and grey. The head is roughly triangular with well set alert eyes. In the males the horns are thick and directed backwards and slightly outwards. Ears are of medium size and droopy. The barrel is proportionate to the size of animal (Fig. 1).

Experimental animals:

A total of five native bucks aged between 2 to 2½ years and weighing about 24.25 ± 2.50 kgs, available

Table No. 1: Climatological data.

Month year	Temperature celcius		Humidity		Rainfall mm
	Maximum	Minimum	Maximum	Minimum	
December'92	28.1	16.7	80	52	-
January'93	30.0	16.3	82	42	-
February'93	32.3	18.3	78	32.9	3.2
March'93	35.7	22.0	70	33	7.4
April'93	36.8	24.7	64	28	2.7
May'93	33.6	26.7	63	33.8	73.0

Fig 1: Photograph of native buck.

in the department of Gynaecology, College of Veterinary Science, Tirupati were used for this study. These were housed in a well ventilated shed and fed concentrate mixture at 500 g per animal per day besides grazing for 6 to 8 hours.

All the bucks were trained to give semen using a doe in heat initially which was substituted with a dummy later.

3.1 REACTION TIME AND PHYSICAL CHARACTERS OF SEMEN:

3.1.1 Reaction time:

Reaction time was observed during semen collection. The time that elapsed from the time the buck was introduced till it mounted the dummy with a penile erection was taken as reaction time (seconds).

Collection of semen:

Semen was collected from 5 clinically normal bucks twice a week during winter (December'92 to February'93) and summar (March'93 to May'93) as per procedure described by Hafez (1980).

The Collection was made using a soft rubber A.V. of 20 cm long and 5 cm wide in diameter. The temperature of the AV was maintained at $45^{o}c$. A smooth rubber liner

with a latex cone and with a graduated collection tube or graduated semen collection cup was used for collection of semen (Fig. 2). A total of 120 ejaculates, twenty four from each of five native bucks in two seasons (60 ejaculates in each season) were collected and various semen characters were analysed. Weekly two collections were made from each buck in two seasons. The first collection in a week was utilizes for physical characters of semen and second collection in a week was utilized for Biochemical and resistance tests for want of sufficient volume of semen.

3.1.2 Physical characteristics of semen:

3.1.2.1 Volume:

The volume of the ejaculate is measured in ml directly by graduated collection tube or semen collection cup.

3.1.2.2 Mass motility:

Mass motility was observed by placing a drop of neat semen on glass slide without cover slip under low magnification (100 x), keeping the slide warm at 37^{o}c. It was graded as per the procedure of Herman and Madden (1953) as follows:

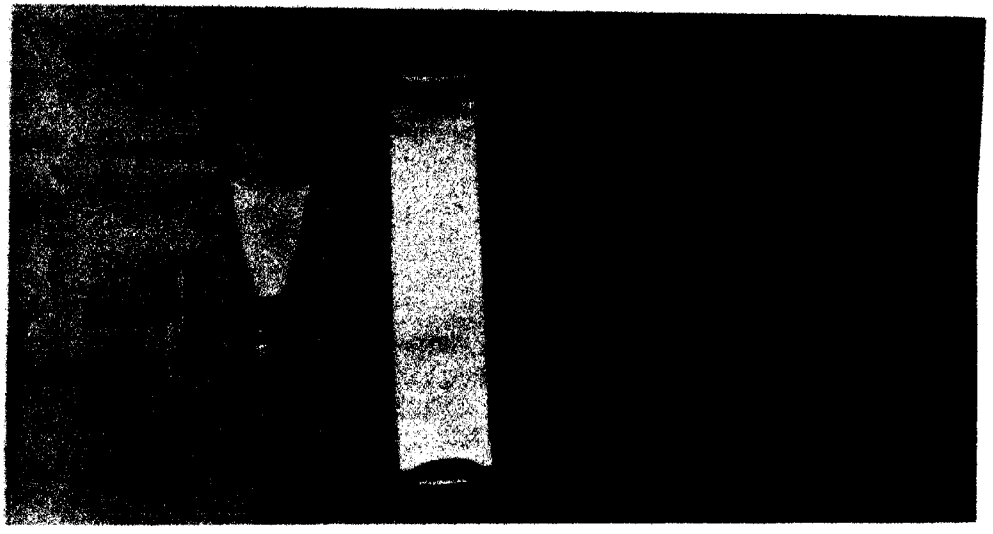


Fig 2: Photograph of semen collection equipment

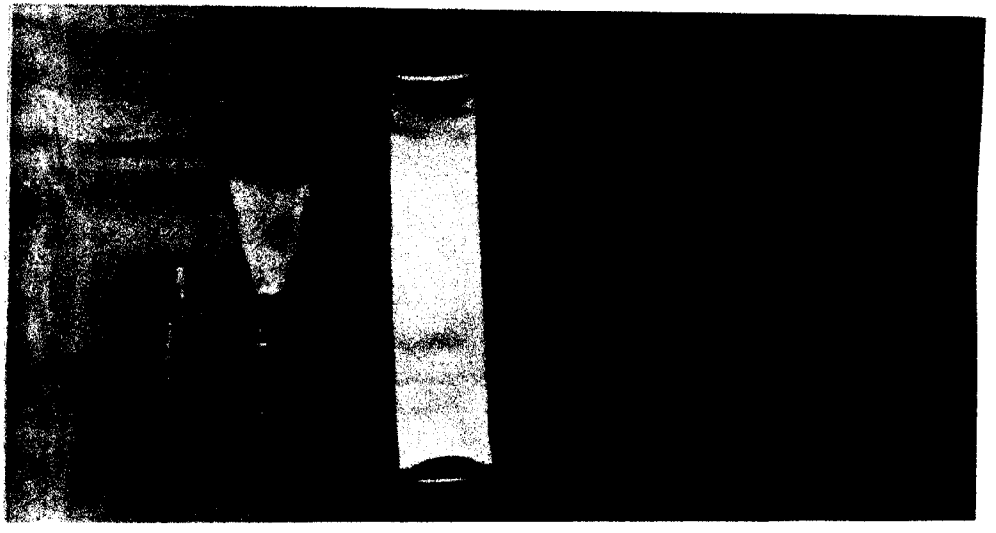


Fig 2: Photograph of semen collection equipment

5 : Excellent motility, extremely rapid swirls and eddies.

4 : Very good motility waves and eddies observed.

3 : Good motility slow formation of waves and eddies.

2 : Fair motility, vigourous movements but no waves or eddies

1 : Poor motility, weak and oscilatory movement of spermatozoa.

0 : No motility.

3.1.2.3 Individual motility:

Soon after collection, the initial sperm motility was assessed by placing a drop of diluted semen (a drop of whole semen was diluted with 2-3 drops of normal saline) on a clean glass slide maintained at $37^{o}c$ on a biotherm with a cover slip under microscope. The motility was observed under high power at 450 x magnification and expressed in terms of percentage of progressively (0-100) motile sperms as detailed below:

Criteria	Grade
0 - 20% of motile sperms	1
21 - 40% of motile sperms	2

Criteria	Grade
41 - 60% of motile sperms	3
61 - 80% of motile sperms	4
81 - 100% of motile sperms	5

3.1.2.4 Sperm concentration:

Sperm concentration was assessed by using a haemocytometer improved (Neubauer counting chamber) after diluting the semen with the dilution fluid. The dilution fluid was prepared by mixing 0.05 gm of water soluble eosin and 1.0 gm of sodium chloride in 100 ml of distilled water. The sperm concentration was expressed as millions per ml.

3.1.2.5 Live sperm count:

The live sperm count was determined from the undiluted st ained semen. Immediately after collection, a drop of st ained semen was taken from the test tube and mixed with two drops of 5 per cent eosin solution. After gentle and through mixing for a few seconds with a round end of glass rod, four drops of 10 per cent nigrosin solution was added to the mixture and once again mixed throughly.

The differential stain contained 0.67 gms. of eosin and 10 g of nigrosin in 100 ml of buffered normal saline (Dott and Foster, 1972).

A thin smear was made out of the mixture and air dried quickly. A total of 200 sperms were counted under oil immersion. The sperms that were stained with eosin were considered to be dead. The number of live sperms were expressed in percentage.

3.1.2.6 Abnormal sperms:

Immediately after collection 0.1 ml of semen was suspended in 9.9 ml of buffered saline.

Composition of buffered formal saline (Hancock, 1957).

Stock solution (Buffer)	- 100 ml
Commercial formaline	- 62.5 ml
Stock sodium chloride solution	- 150 ml
Glass distilled water	- 500 ml

Stock buffer solution:

Disodium hydrogen phosphate	21.629 g	200 ml
Glass distilled water	500 ml	
Potassium dihydrogen phosphate	22.25 g	80 ml
Glass distilled water	500 ml	

Stock sodium chloride solution:

Sodium chloride	9.01 g
Glass distilled water	500 ml

A drop of above mixture has used to prepare a wet smear for examination of sperms under high power. A total of 200 sperms were counted in each smear made during each collection from each buck, for the presence of cytoplasmic droplets, head abnormalities and abnormalities were classified as micro head, macro head, elongated or narrow head, pear shaped head, round head detached heads and double heads. The mid piece abnormalities were swollen neck, kinked neck, abaxial attachment, swollen mid piece, coiled mid piece and cork screw mid piece. Cytoplasmic droplets are proximal and distal. Tail abnormalities include coiled tail, kinked tail, brokeen tails and double tails.

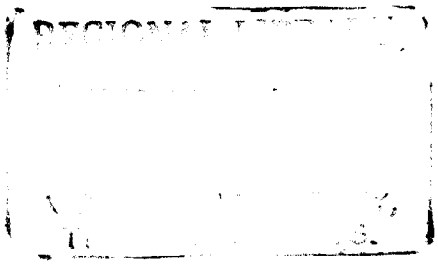
3.1.2.7 Acrosomal evaluation:

The acrosome of spermatozoa plays an important role in fertilization of the ovum. Disintegration or damage to the acrosome leads to reduced fertility. Therefore, an estimate of intact acrosomes can be used as an index of fertilising capacity of the bull. It has been established that the acrosomal integrity of spermatozoa is an important aspect of the fertilizing capacity of spermatozoa (Hafez, 1980).

Stepwise procedure:

1. Make thin smears of neat semen on a clean glass slide. If the semen is highly concentrated dilute, the semen with 2.9% sodium citrate solution and make smears.
2. Dry them in air.
3. Fix the smears in 5% formal saline for 15 minutes.
4. Wash the smears in running tap water for 20 minutes.
5. Treat the smears with chloramine T (0.5% solution) for 2-3 mts to remove mucus from the smears.
6. Wash the slides with distilled water.
7. Stain the smears with Giemsa strain for 4-5 hrs. in a coupling jar.
8. Rinse the smears with distilled water.
9. Air dry and examine under oil immersion.
10. The acrosome takes a deep purple stain and nuclear portion takes a light stain. Presence of intact healthy acrosome. Count the intact healthy acrosomes and damaged (Swollen, ruffled, fractioned, seperated, and seperating) acrosomes, a total of 200 spermatozoa should be counted for critical evaluation.

Stain preparation:



Stock solution of Giemsa:

Giemsa powder	3.8 gms
Absolute methanol (A.R.Grade)	367 ml
Glycerol	12.5 ml

Weigh Giemsa powder and grind well in a pestle and mortar. Add slowly absolute methanol and triburate till the Giemsa powder is completely dissolved. Add glycerol and store at 37°c for one week, during this period shake the bottle once a day for few minutes.

Sorenson's phosphate buffer:

Stock buffer: Solution A:

Disodium hydrogen phosphate	21.682 gms
Distilled water	500 ml
Solution B:	
Potassium Dihydrogen phosphate	22.254 gms
Distilled water	500 ml

Take 200 ml solution of A and mix with 80 m of solution B. Then from the resultant 280 ml take 100 ml as stock buffer solution.

Working solution:

Stock Giemsa solution	3 ml
Sorenson's phosphate buffer	2 ml
Double distilled water	35 ml

Formal saline solution:

Sodium chloride	9.01 gm
Formaldehyde	25 ml
Distilled water	500 ml

Composition of Hancock fixative:

Sodium chloride	10 gm
Sodium hydrogen corbonate	0.5 gm
Formaline	125 ml
Distilled water	1000 ml

3.2 BIOCHEMICAL TESTS:

Very little work was appeared in literature on its applicability, particularly on buck semen.

3.2.1 Hydrogen Ion concentration (pH) of semen:

The pH of the semen at the time of collection seems to have little practical value in the normal male

for predicting fertility (Swanson and Herman, 1944). Hydrogen Ion concentration was measured by using the narrow range indicator papers. Tear off a paper strip from the indicator book, and touch it only at the free end. Dip the tern end of indicator paper in a drop of semen. Look for the first change in colour and find a matching colour on the cover of book corresponding to the pH noted against it.

3.2.2 Methylene blue reduction test (MBRT):

The methylene blue reduction test gives an indication of the quality of semen samples for artificial insemination work in field practice (Chieffi et al.,1958). Salisbury and Van Demark (1961) included that lower the value of MBRT, better would be the semen quality.

The methylene blue reduction test is carried out according to method described by Lardy and Phillips (1941) The procedure in brief is as follows:

A volume of 0.2 ml of semen was diluted in 0.8 ml of egg yolk citrate dilutor and mixed throughly. 0.1 ml of methylene blue solution (Methylene blue solution was prepared by dissolving. 50 mg of methylene blue in 100 ml of 2.9 per cent sodium citrate buffer was added and mixed.

The mixture was then sealed with 1 cm layer of liquid paraffin and incubated in a water bath at 45°c. The time required for disappearance of colour was recorded.

3.2.3 Resazurin reduction test (RRT):

The test is carried out according to the method of Erb and Ehlers (1950) the procedure in brief is as follows:

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A volume of 0.2 ml of freshly collected undiluted semen is added with 0.1 ml of Resazurin solution (11 mg of resazurin in 200 ml distilled water) and the contents were mixed. The mixture is covered with 1 cm layer of liquid paraffin. The contents are incubated at 45°c. The time required for the change in colour from violet to pink (RRT-I) and then to colourless (RRT-II) was recorded.

3.3 RESISTANCE TO ENVIRONMENTAL CHANGES:

3.3.1 High temperature viability test(HTVT):

The longevity and viability of spermatozoa in storage is a good index of the fertilizing capacity of the spermatozoa. This test serves as a means of determining the storage potential of spermatozoa. This test may give an indication of quality of semen which reflects an conception rate (Ludwick et al.,1948).

This test involves incubation of the diluted semen with egg yolk citrate at 38°c and observing the progressive motility after 30 minutes of incubation as there was complete cessation of motility after 16 hours of incubation.

3.3.2 Cold shock resistance test:

The semen with higher concentration of cold shock resistant spermatozoa may keep longer under preservation and may produce higher fertility rates. Different methods are employed to estimate the percentage of cold shock resistant spermatozoa but the method adopted in the present study is as follows:

Diluted semen in small vials is suddenly chilled from 37°c to 0°c. After 10 minutes exposure at 0°c semen is thawed at 30°c. It is then mixed with eosinoigrosin stain (1 drop of semen with 8 to 10 drops of stain) used for live and dead count. The percentage of live spermatozoa surviving in chilled semen is determined with similar technique as used for live and dead count. This method has been used by Tomar et al. (1966).

3.3.3 Resistance to sodium chloride and dilution effect:

R test is assessed by the ability of spermatozoa to with stand the action of 1% sodium chloride solution. The resistance is donated as the millilitres of 1% sodium chloride solution required to stop the progressive motility of all spermatozoa in 0.02 ml of semen.

The procedure is as follows:

0.02 ml of semen is pipetted into a 200 ml capacity flask from a burette containing 1% sodium chloride, solution. Volumes each of 10 ml are added to semen. After each addition of 10 ml of solution a drop of semen is examined under the microscope for motility. Sodium chloride solution is added at intervals till progressive motility of spermatozoa is ceased.

Calculation of R value:

$$= \frac{\text{Ml of sodium chloride solution required}}{0.02}$$

The data obtained was subjected to statistical analysis as per the methods described by Snedecor and Cochran (1968).

RESULTS

CHAPTER IV

4. RESULTS

The present investigations on "Seasonal variation in physical, biochemical (pH, MBRT and RRT) and resistance to environmental changes (HTVT, CSRT and resistance to sodium chloride) were carried out in semen of native bucks during winter and summer seasons.

A total of 120 ejacultes, twenty four from each of five native bucks in two seasons (60 ejaculates in each season) were collected and the various semen characteristics analysed.

4.1 REACTION TIME AND PHYSICAL CHARACTERISTICS OF SEMEN:

The data on the reaction time and the various semen characteristics of native bucks obtained in this study is shown in Table 2.

4.1.1 Reaction time:

The normal reaction time in the native bucks varied from 14.5 $\pm$ 0.38 to 17.7 $\pm$ 0.43 seconds with an average of 15.96 $\pm$ 0.28 seconds during winter season,

Table 2: Mean for reaction time and physical characteristics of semen in two seasons.

Season	Month	Reaction time seconds	Volume ml	Mass motility per cent	Individual motility per cent	Sperm conc. x 10^9	Live sperms per cent	Abnormal sperms per cent	Intact acro-somes percent
	Dec	14.50 ±0.38	0.66 ± 0.01	4.60 ±0.11	84.25 ±0.86	2.56 ±0.08	88.65 ±1.27	8.60 ±0.29	70.65 ±1.17
Winter	Jan	15.70 ±0.35	0.70 ±0.02	4.40 ±0.13	80.10 ± 1.52	2.69 ±0.10	87.00 ±0.84	8.85 ±0.25	73.40 ±1.52
	Feb	17.70 ±0.43	0.76 ±0.03	4.35 ±0.10	81.00 ±0.96	2.44 ±0.06	84.50 ±0.57	9.50 ±0.25	76.70 ±2.00
	Mean	15.96 ±0.28	0.70 ±0.10	4.46 ±0.06	81.78 ±0.53	2.45 ±0.31	87.05 ±0.62	8.98 ±0.23	73.68 ±0.97
	Mar	19.35 ±0.39	0.73 ±0.02	4.65 ±0.10	81.30 ±0.98	2.37 ±0.03	84.50 ±0.57	8.15 ±0.26	76.45 ±1.62
Summer	Apr	21.30 ±0.34	0.59 ±0.03	4.45 ±0.13	77.84 ±0.87	2.34 ±0.04	81.40 ±0.73	8.90 ±0.22	77.55 ±1.23
	May	22.95 ±0.37	0.49 ±0.03	4.00 ±0.15	74.50 ±1.05	2.35 ±0.06	77.65 ±0.59	10.80 ±0.21	79.30 ±0.91
	Mean	21.30 ±0.31	0.60 ±0.02	4.36 ±0.08	78.06 ±0.68	2.35 ±0.02	81.11 ±0.51	9.28 ±0.20	77.76 ±0.76

while in summer season it ranged from 19.35 ± 0.39 to 22.95 ± 0.37 seconds, with a mean of 21.30 ± 0.31 seconds (Table 2, Fig. 3).

There was a significant difference ($P < 0.01$) in all interactions viz.,between weeks, between bucks, between seasons and season x bucks (Table 3).

4.1.2 Physical characteristics of semen:

4.1.2.1 Volume:

In native buck the ejaculate volume yielded 0.66 ± 0.01 to 0.76 ± 0.03 ml with a mean of 0.70 ± 0.10 ml in winter and 0.73 ± 0.02 to 0.49 ± 0.03 ml with an average of 0.60 ± 0.02 ml during summer season (Table 2 and Fig.3).

The difference in the volume was found to be significant ($P < 0.01$) between bucks and between seasons. The interaction between weeks and season x bucks was found to be non significant (Table 4).

4.1.2.2 Mass motility:

The mass motility during winter season ranged between 4.60 ± 0.11 to 4.35 ± 0.10 with a mean of 4.46 ±

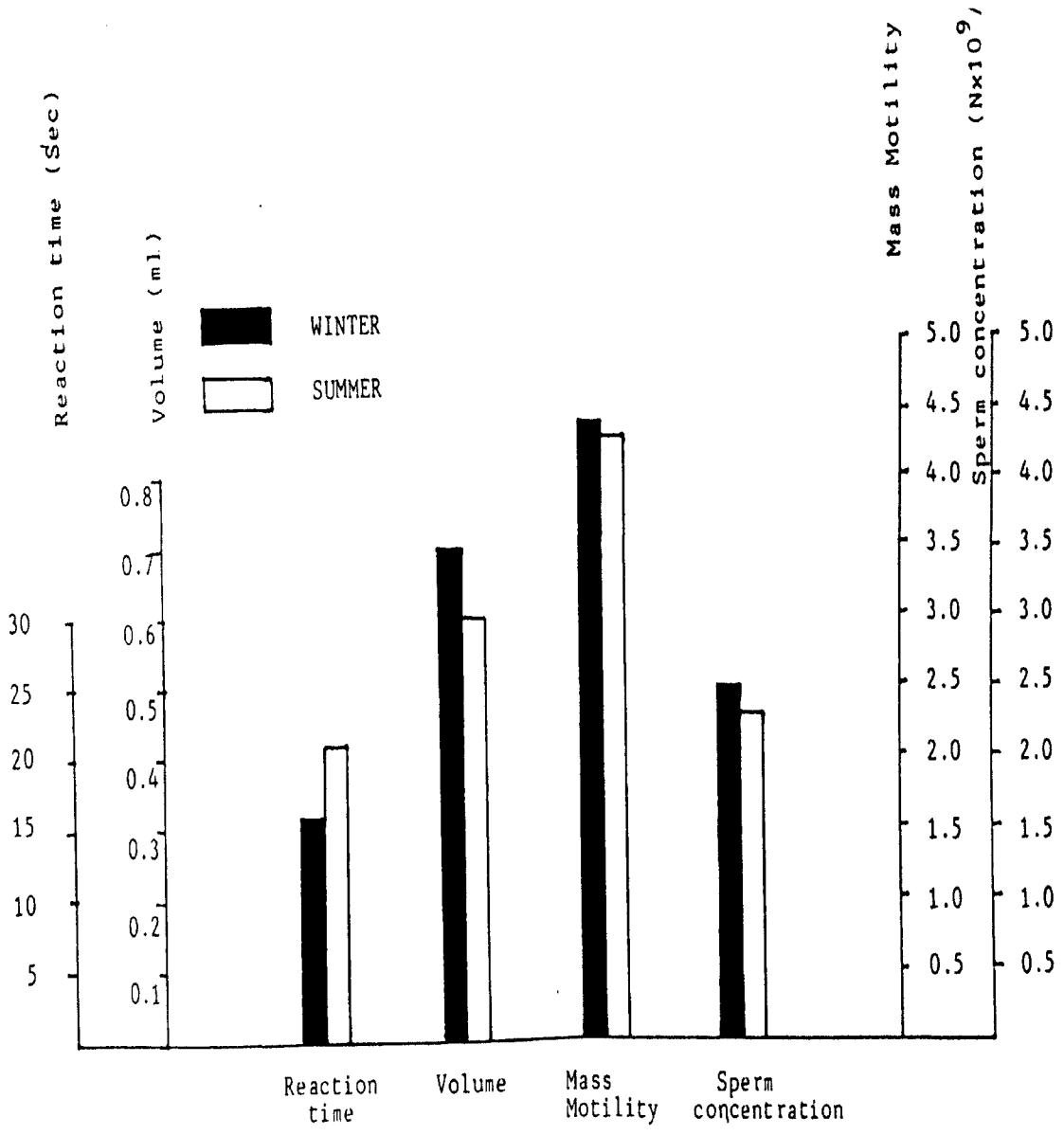


Figure 3 : Physical characteristics of semen during winter and summer seasons.

Table 3: Anova for reaction time:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	262.5664	23.8697	14.2557**	1.80	2.38
2.	Between seasons	1	821.6328	821.6328	490.7028**	3.95	6.92
3.	Between bucks	4	60.5000	15.1250	9.0331**	2.48	3.56
4.	Season x Buck	4	94.7031	23.6758	14.1399**	2.48	3.56
5.	Error	99	165.7656	1.6744			
6.	Total	119	1405.1680				

** ($P < 0.01$)

Table 4: Anova for volume:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	0.1807	0.0164	1.0748^{NS}	1.80	2.38
2.	Between seasons	1	0.3203	0.3203	20.9637**	3.95	6.92
3.	Between bucks	4	0.8963	0.2241	14.6653**	2.48	3.56
4.	Season x Buck	4	0.0847	0.0212	1.3853^{NS}	2.48	3.56
5.	Error	99	1.5127	0.0153			
6.	Total	119	2.9947				

** ($P < 0.01$)

NS = Non significant

0.06 per cent. The average per centof mass motility of semen samples was 4.36 ± 0.08 varying from 4.65 ± 0.10 to 4.00 ± 0.15 in summer season (Table 2 and Fig.3)

No significant difference was observed between seasons and seasons x buck, but was significant between weeks ($P < 0.05$) and between bucks ($P < 0.01$) (Table 5, Fig. 3).

4.1.2.3 Individual motility:

The individual motility in native bucks ranged from 80.1 ± 1.52 to 84.25 ± 0.86 per cent with a mean of 81.78 ± 0.53 per cent in winter. Where as during summer season the per cent varied between 74.50 ± 1.05 to 81.30 ± 0.98 with an average of 78.06 ± 0.68 (Table 2, Fig 3).

The percentage of individual motility was found to be significant ($P < 0.01$) between weeks, between seasons and between bucks (Table 6).

4.1.2.4 Sperm concentration:

The mean concentration of spermatozoa was recorded $2.45 \pm 0.31 \times 10^9$ per ml of semen with a range of $2.44 \pm 0.06 \times 10^9$ to $2.69 \pm 0.01 \times 10^9$ per ml in winter season and $2.35 \pm 0.06 \times 10^9$ to $2.37 \pm 0.03 \times 10^9$ ml with an average $2.35 \pm 0.2 \times 10^9$ in summer season (Table, 2 Fig.3).

Table 5: Anova for mass motility:

S. No	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	5.2917	0.4811	1.8956*	1.80	2.38
2.	Between seasons	1	0.2083	0.2083	0.8206NS	3.95	6.92
3.	Between bucks	4	10.7834	2.6959	10.6225**	2.48	3.56
4.	Season x Buck	4	1.5833	0.3958	1.5596NS	2.48	3.56
5.	Error	99	25.1250	0.2538			
6.	Total	119	42.9917				

* ($P < 0.05$)

** ($P < 0.01$)

NS = Non significant

Table 6: Anova for individual motility:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	646.4375	58.7670	4.1142**	1.80	2.38
2.	Between seasons	1	414.3750	414.3750	29.0095**	3.95	6.92
3.	Between bucks	4	1271.8130	317.9531	22.2592**	2.48	3.56
4.	Season x Buck	4	59.5625	14.8906	1.0425NS	2.48	3.56
5.	Error	99	1414.1250	14.2841			
6.	Total	119	3806.3130				

** ($P < 0.01$)

NS = Non significant.

The influence of season was significant ($P < 0.05$) where as it was highly significant between bucks and season x bucks ($P < 0.01$) while no difference was noticed between weeks (Table 7).

4.1.2.5 Live sperm count:

It was observed in this study that the percentage of live spermatozoa ranged from 84.5 ± 0.57 to 88.65 ± 1.27 with a mean value of 87.05 ± 0.62 in winter season. The mean value of percentage of live sperms during summer was found to be 81.10 ± 0.51 and varied between 77.65 ± 0.59 to 84.50 ± 0.57 (Table 2, Fig. 4).

The live sperm count was found to be significant ($P < 0.01$) in all interactions viz.,between weeks, between seasons, between bucks and seasons x bucks (Table 8).

4.1.2.6 Abnormal sperms:

The mean per cent of total abnormal spermatozoa in native bucks varied from 8.60 ± 0.29 to 9.50 ± 0.25 with a mean value of 8.98 ± 0.23 during winter season. The mean per cent of abnormal sperms during summer collections was observed as 9.28 ± 0.20 ranging between 8.15 ± 0.26 to 10.80 ± 0.21 (Table 2, Fig. 4).

Table 7: Anova for sperm concentration:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	0.6993	0.0636	1.0571^{NS}	1.80	2.38
2.	Between seasons	1	0.2560	0.2560	4.2578*	3.95	6.92
3.	Between bucks	4	3.0134	0.7533	12.5276**	2.48	3.56
4.	Season x Buck	4	2.0471	0.5118	8.5106**	2.48	3.56
5.	Error	99	5.9533	0.0601			
6.	Total	119	11.9691				

* ($p < 0.05$)

** ($p < 0.01$)

NS = Non significant

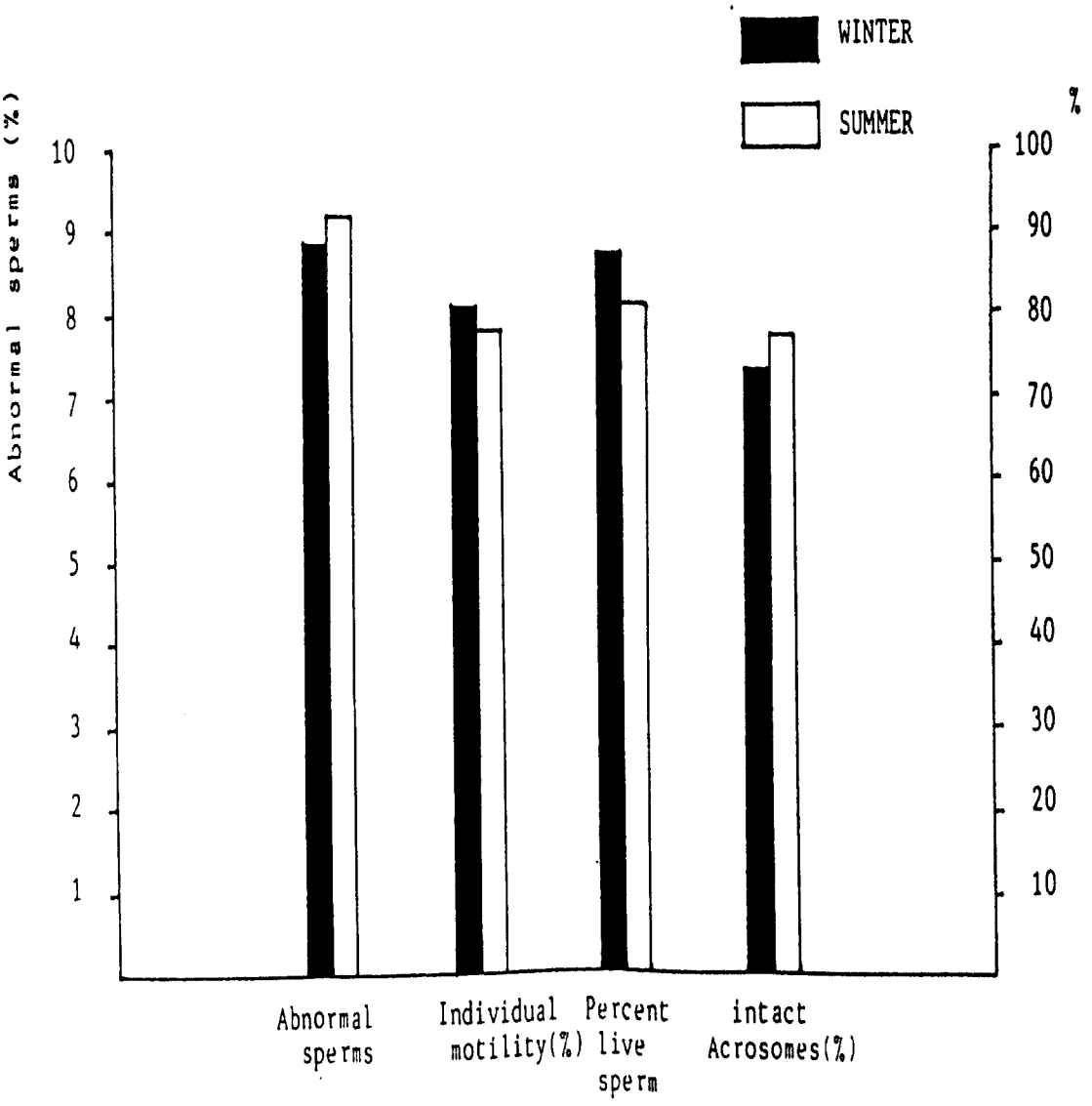


Figure 4 : Physical characteristics of semen during winter and summer seasons.

Table 8: Anova for live sperm count:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	626,6875	56.9716	7.8911**	1.80	2.38
2.	Between seasons	1	1050.1880	1050.1880	145.4614**	3.95	6.92
3.	Between bucks	4	872.0000	218.0000	30.1952**	2.48	3.56
4.	Season x Buck	4	136.3750	34.0938	4.7223**	2.48	3.56
5.	Error	99	714.750	7.2197			
6.	Total	119	3400.000				

** ($P < 0.01$)

The difference in the per cent of abnormal spermatozoa was found to be highly significant ($P < 0.01$) between weeks and season x bucks. However, higher per cent of abnormal sperms were observed in summer when compared to winter, though it was not significant(Table 9, Fig. 5 to 22).

Among the various sperm abnormalities viz.,head, midpiece and tail were observed in the present study.

Of the various head abnormalities studied were micro head (Fig. 5), Gaint head (Fig.6), Pear shaped head (Fig. 7), Detached acrosome (Fig. 8), Incomplete acrosome (Fig. 9), Swollen acrosome (Fig. 10). Out of midpiece abnormalities observed were distal protoplasmic droplet showing live and dead sperms (Fig.11), Loose heads(Fig.12), double midpiece (Fig.13), thickened midpiece (Fig. 14), Kinked midpiece (Fig.15), abaxial attachment (Fig.16), bent midpiece (Fig.17), bent midpiece and coiled tail (Fig.18), dag defect (Fig.19) and distal protoplasmic droplet (Fig.20). The tail abnormalities noticed were bent tail (Fig.21) and simple coiled tail (Fig.22).

4.1.2.7 Acrosomal evaluation:

In native bucks it was observed that the mean percentage of intact acrosomes ranged from 70.65 $\pm$ 1.17

Table 9: Anova for abnormal sperms:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	80.4658	7.3151	5.6636**	1.80	2.38
2.	Between seasons	1	2.6992	2.6992	2.0898NS	3.95	6.92
3.	Between bucks	4	5.2832	1.3208	1.0226NS	2.48	3.56
4.	Season x buck	4	25.5508	6.3877	4.9456**	2.48	3.56
5.	Error	99	127.8672	1.2916			
6.	Total	119	241.8662				

** ($P < 0.01$)

NS = Non significant

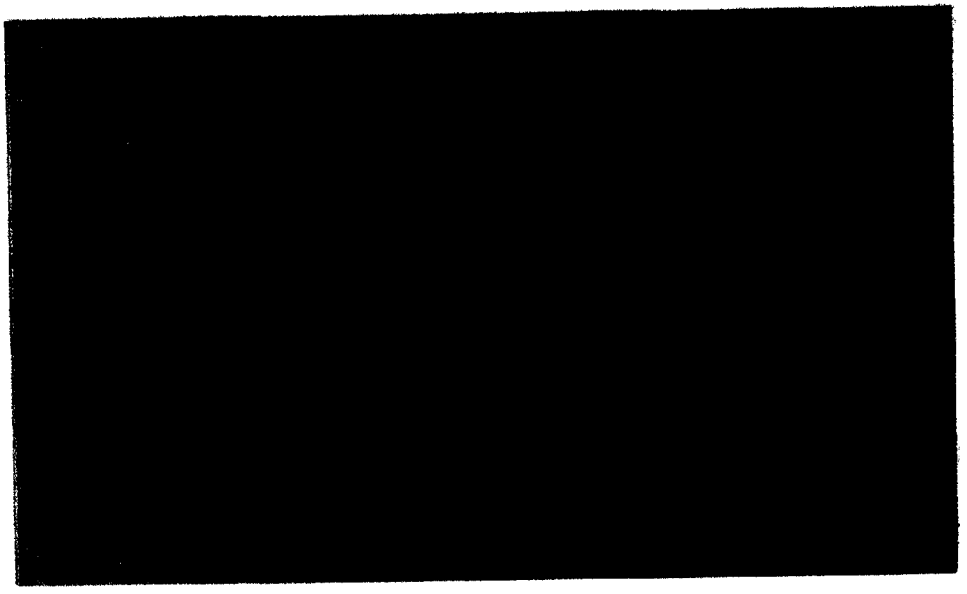


Fig.5: Photimicrograph of the spermatozoa of native buck. Abnormalities of head: "Micro head".(Williams stain X 1000 x)

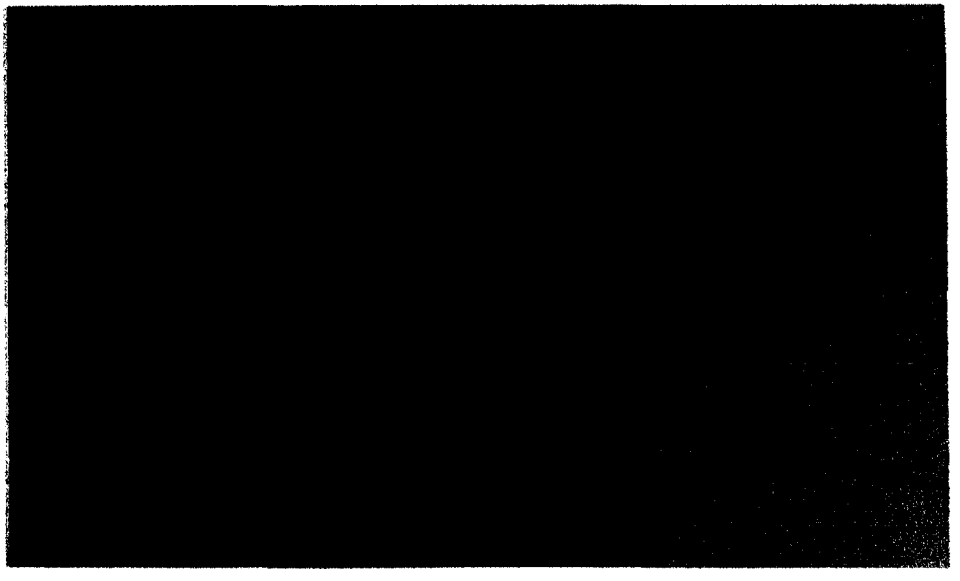


Fig.6: Photomicrograph of the spermatozoa of native buck. Abnormalities of head: "Gaint head". (Williams stain X 1000 x).

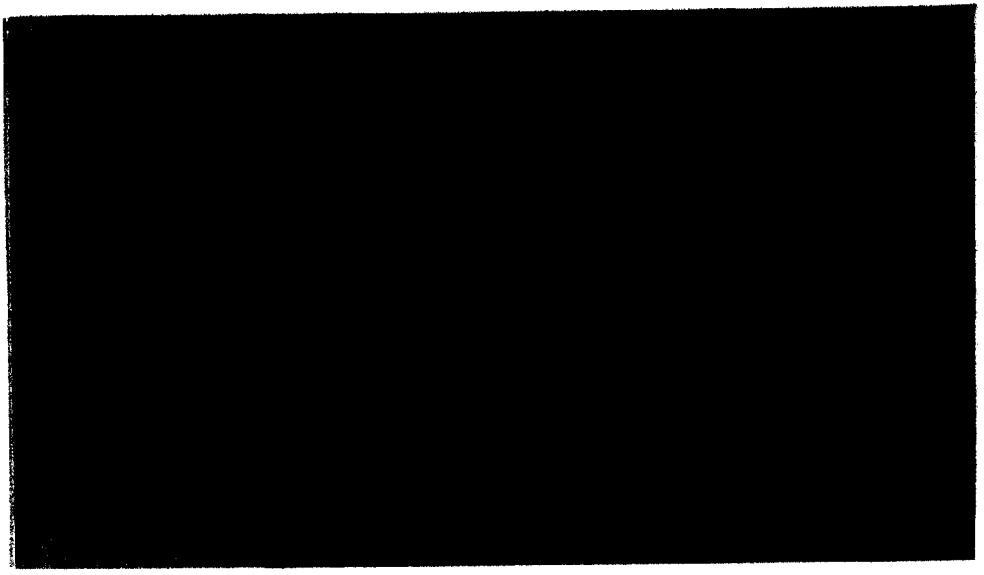


Fig:7: Photomicrograph of the spermatozoa of native buck. Abnormalities of head: "Pear shaped head".(Williams stain X 1000 x).

Fig.8: Photomicrograph of the spermatozoa of native buck. Abnormalities of acrosome: "Detached acrosome". (Giemas stain X 1000 x)

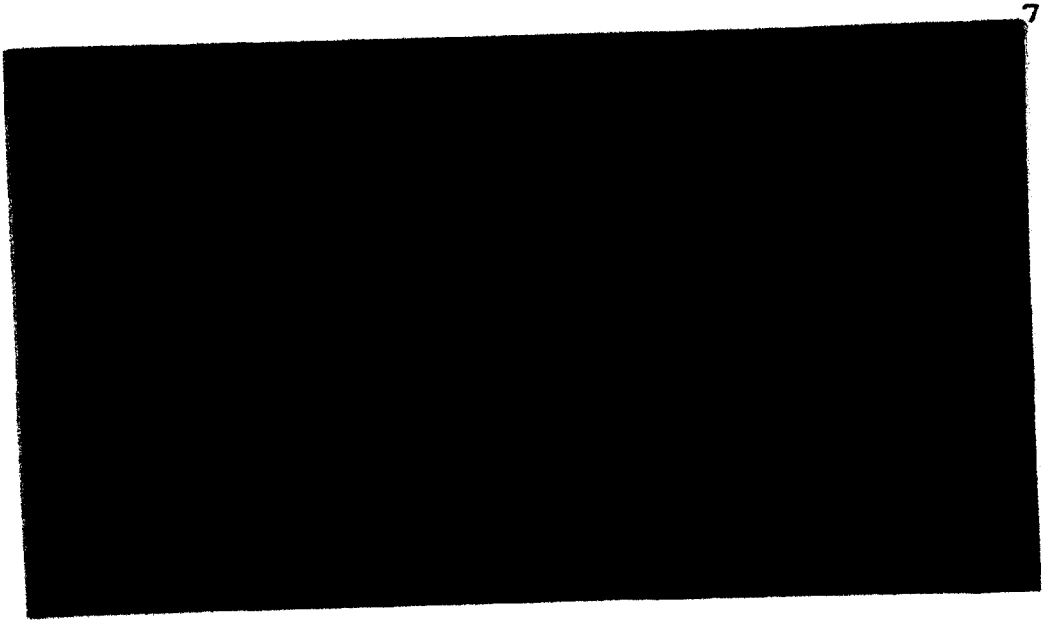


Fig.9: Photomicrograph ofthe spermatozoa of native buck. Abnormalities of acrosome: "Incomplete acrosome". (Williams stain x 1000 x)

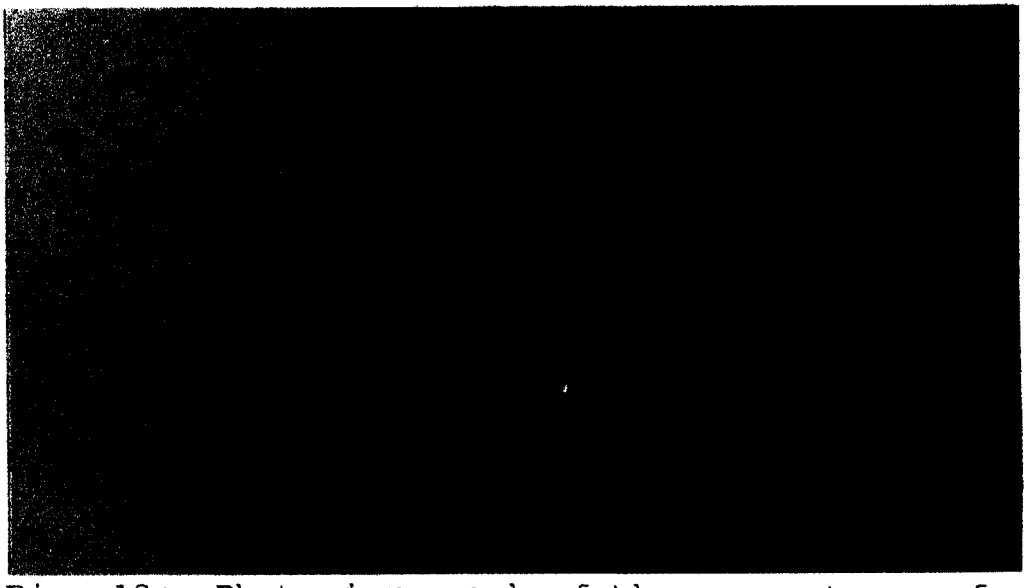


Fig. 10: Photomicrograph of the spermatozoa of native buck. Abnormalities of acrosome: "Swollen Acrosome" (Williams stain x 1000 x)

Fig.11: Photomicrograph of the spermatozoa of native buck: Live and dead spermatozoa "Distal protoplasmic droplet". (Eosin & Nigrosine X 1000 x).

Fig. 12: Photomicrograph of the spermatozoa of native buck. "Loose heads". (Giemsa stain x 1000 x).

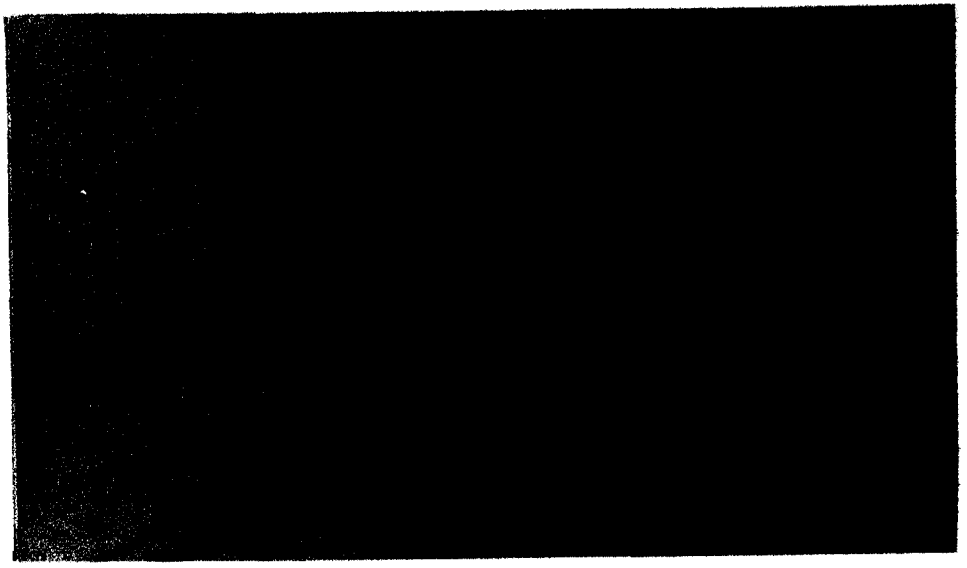


Fig 13: Photomicrograph of the spermatozoa of native buck. Abnormalities of midpiece: "Double midpiece" (Williams stain x 1000 x)

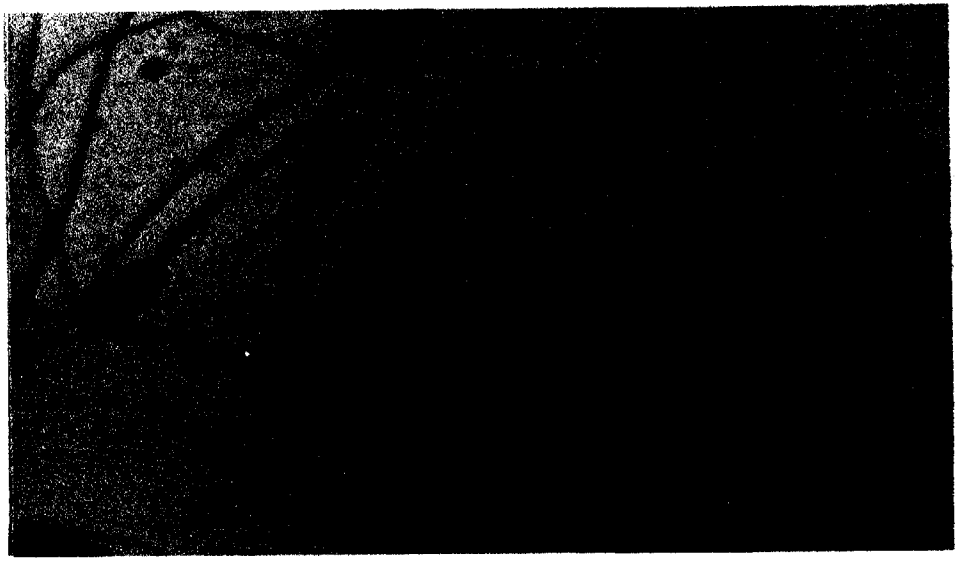


Fig.14: Photomicrograph of the spermatozoa of native buck. Abnormalities of midpiece: "Thickened midpiece" (Williams stain x 1000x)

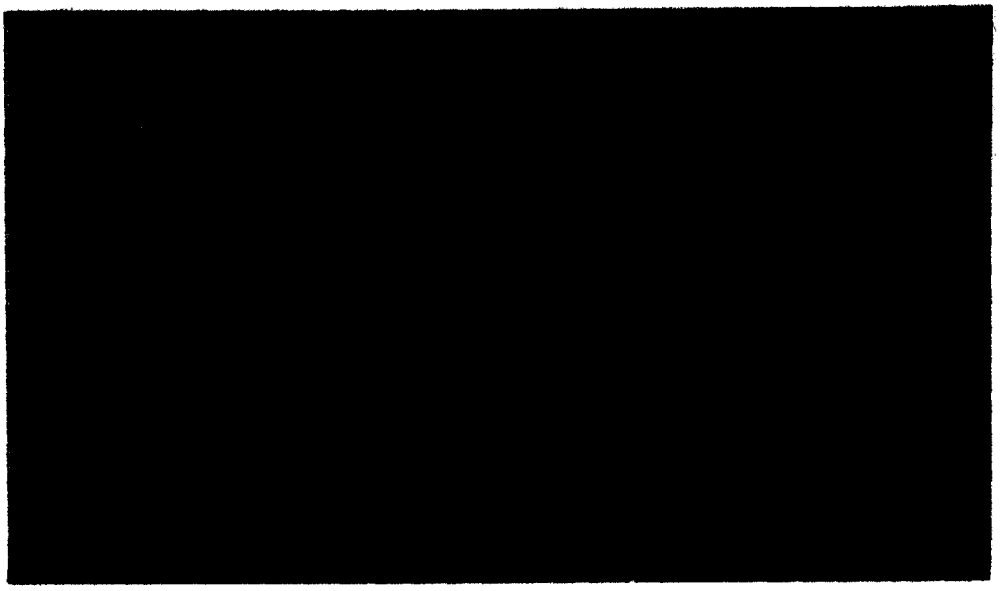


Fig.15: Photomicrograph of the spermatozoa of native buck. Abnormalities of midpiece: "Kinked mid piece" (Williams stain x 1000x)

Fig.16: Photomicrograph of the spermatozoa of native buck. "Abaxial attachment" (Williams stain x 1000 x).

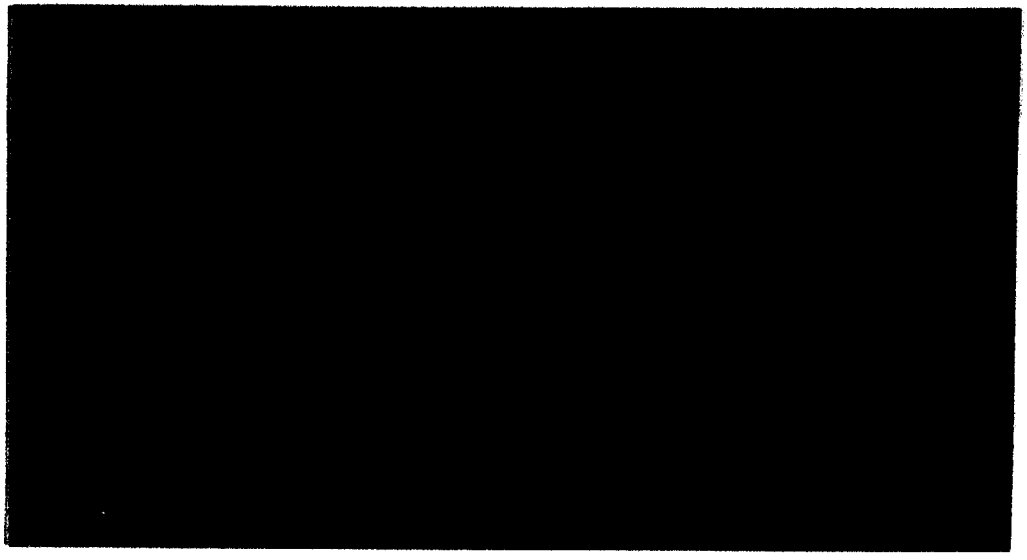


Fig.17: Photomicrograph of the spermatozoa of native buck. Abnormalities of midpiece: "Bent mid piece" (Eosin & Nigrosine x 1000 x).

Fig. 18: Photomicrograph of the spermatozoa of native buck. Abnormalities of mid piece "Bent mid piece and coiled tail"(Giemsa stain x 1000 x).

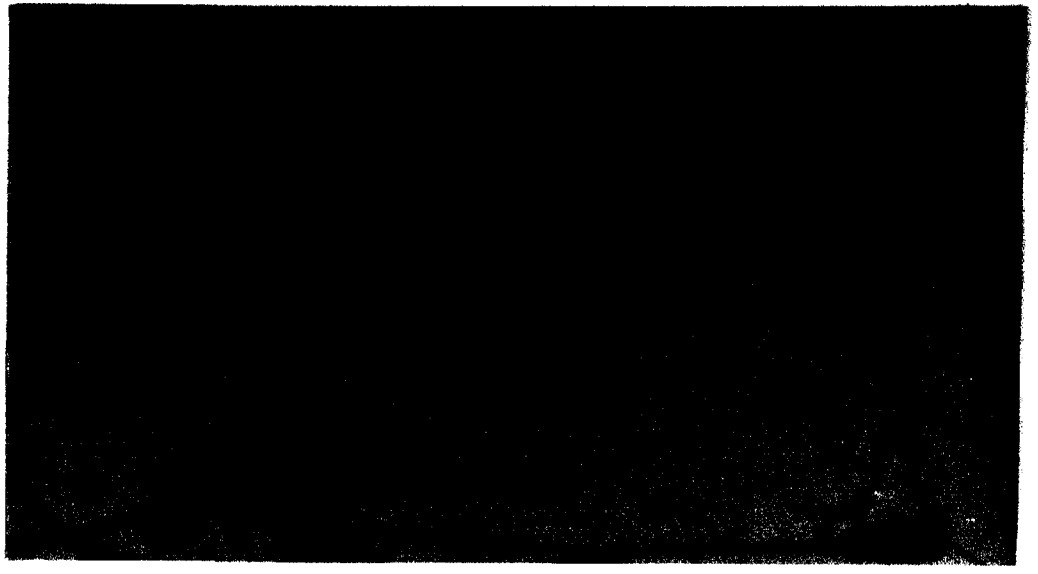


Fig.19: Photomicrograph of the spermatozoa of native buck: "Dag defect".(Williams stain x 1000 x).

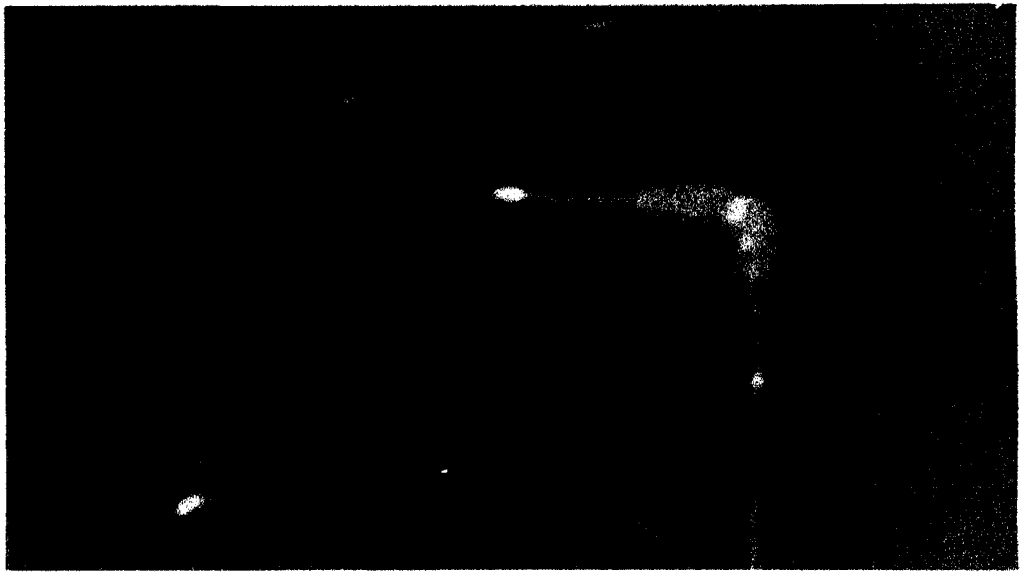


Fig.20: Photomicrograph of the spermatozoa of native buck." Distal protoplasmic droplets" (Eosin & Nigrosine x 1000 x)

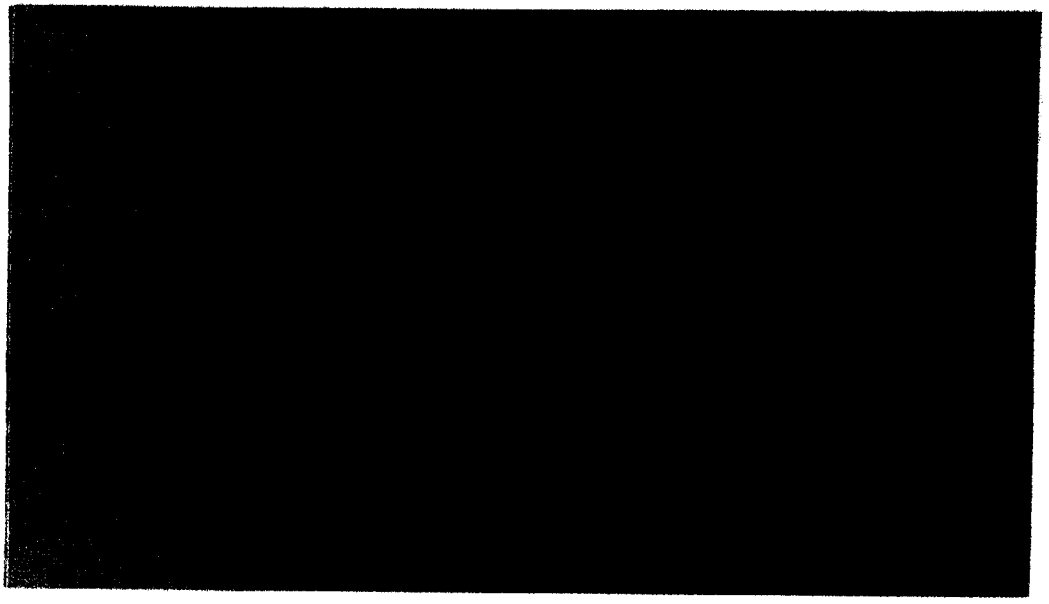


Fig.21: Photomicrograph of the spermatozoa of native buck. Abnormalities of tail: "Bent tail" (Williams stain x 1000 x).

Fig.22: Photomicrograph of the spermatozoa of native bucks. Abnormalities of tail: "Coiled tail" (Williams stain x 1000 x)

Table 10. Anova for intact acrosome.

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	423.8125	38.5284	2.8684**	1.80	2.38
2.	Between seasons	1	500.1875	500.1875	37.2390**	3.95	6.92
3.	Between bucks	4	3636.7500	909.1875	67.6891**	2.48	3.56
4.	Season x Buck	4	45.4375	11.3594	0.8457 NS	2.48	3.56
5.	Error	99	1329.7500	13.4318			
6.	Total	119	5935.9380				

** ($P < 0.01$)

NS = Non significant

Table 11: Biochemical characters of semen:

Season	Month	pH	MBRT minutes	RRT-I seconds	RRT-II seconds
	Dec	6.63 ± 0.03	6.7 ± 0.30	20.1 ± 0.61	65.60 ± 1.40
Winter	Jan	6.60 ± 0.02	6.8 ± 0.27	17.8 ± 0.36	61.95 ± 1.40
	Feb	6.67 ± 0.03	6.9 ± 0.35	16.6 ± 0.50	60.85 ± 1.57
	Seasonal mean	6.63 ± 0.01	6.8 ± 0.18	18.1 ± 0.34	62.80 ± 0.88
	Mar	6.69 ± 0.02	8.0 ± 0.27	20.1 ± 0.47	70.45 ± 1.14
Summer	Apr	6.70 ± 0.03	8.6 ± 0.34	20.1 ± 0.36	65.80 ± 1.78
	May	6.72 ± 0.02	8.4 ± 0.28	19.7 ± 0.49	64.45 ± 1.47
	Seasonal mean	6.70 ± 0.01	8.3 ± 0.17	19.9 ± 0.26	66.90 ± 0.92

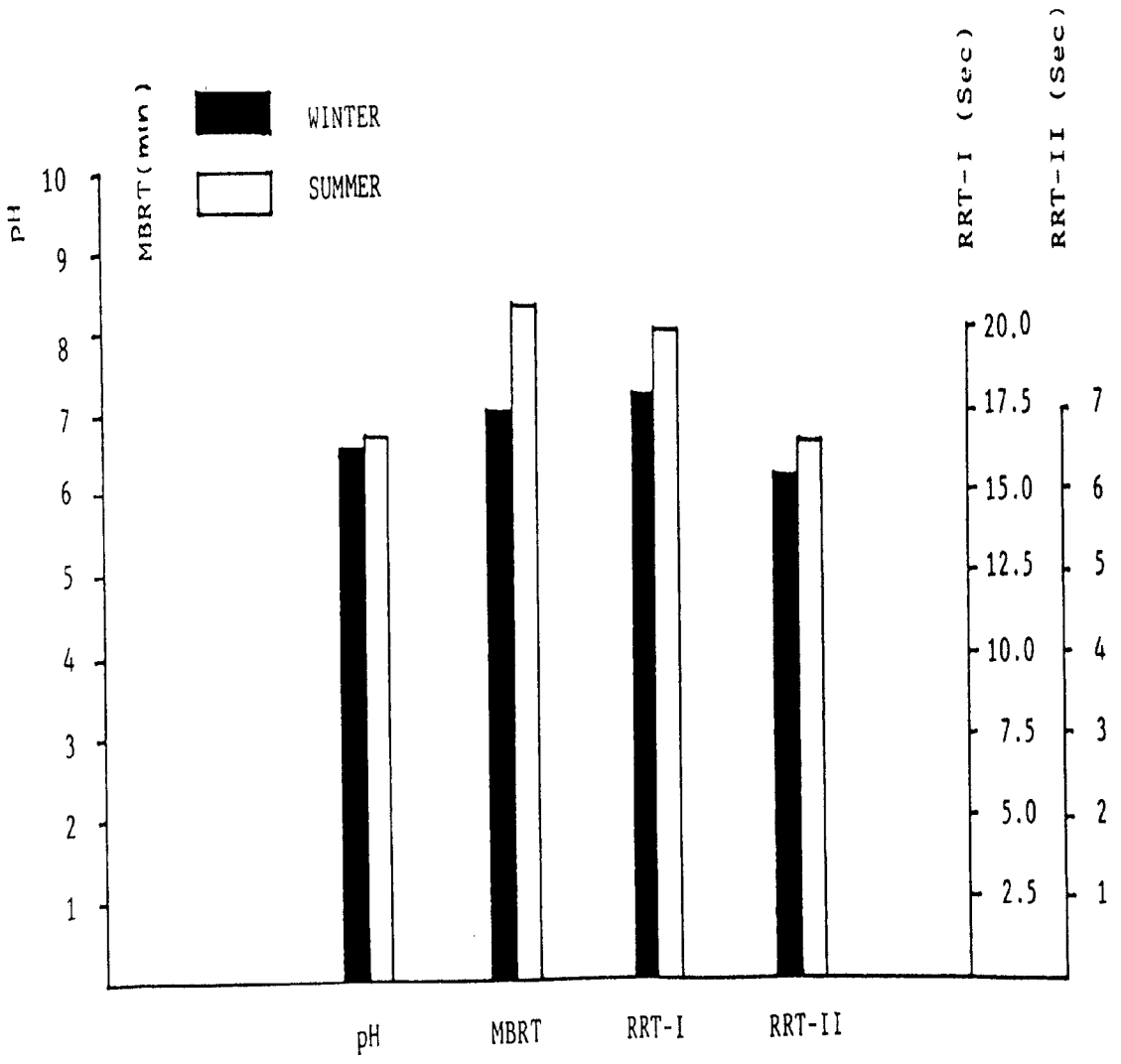


Figure 28: Biochemical characteristics of semen during winter and summer seasons.

Table 12: Anova for pH.

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	0.1108	0.0101	0.6856^{NS}	1.80	2.38
2.	Between seasons	1	0.1411	0.1411	9.6010**	3.95	6.92
3.	Between bucks	4	0.3916	0.979	6.6609**	2.48	3.56
4.	Season x Buck	4	0.2549	0.0637	4.3354**	2.48	3.56
5.	Error	99	1.4551	0.0147			
6.	Total	119	2.3535				

** ($P < 0.01$)

NS = Non significant

average of 6.8 ± 0.18 minutes for samples collected during winter. In summer season the value ranged between 8.0 ± 0.27 to 8.6 ± 0.34 minutes with a mean of 8.3 ± 0.17 minutes (Table 11, Fig. 23, 24 and 28).

A highly significant difference was noticed ($P < 0.01$) between bucks, between season and season X buck but was not observed between weeks (Table 13).

4.2.3 Resazurin reduction test:(RRT I):

The time taken to reduce violet colour to pink (RRT I) was ranged between 16.6 ± 0.50 to 20.1 ± 0.61 seconds with a mean value of 18.1 ± 0.34 seconds in winter collections. The mean value during summer collections was found to be 19.9 ± 0.26 seconds varying between 19.7 ± 0.49 to 20.1 ± 0.36 seconds (Table 11, Fig. 25, 26 and 28).

The difference in the value was highly significant ($P < 0.01$) between seasons and season x bucks but was not significant between bucks. However the value was significant ($P < 0.05$) between weeks (Table 14).

Resazurin reduction rest II: (RRT II):

The duration taken to reduce colour from pink to colourless (RRT II) varied from 60.85 ± 1.57 to 65.5 ±

Fig.23: Photograph showing the semen sample after the addition of methylene blue.

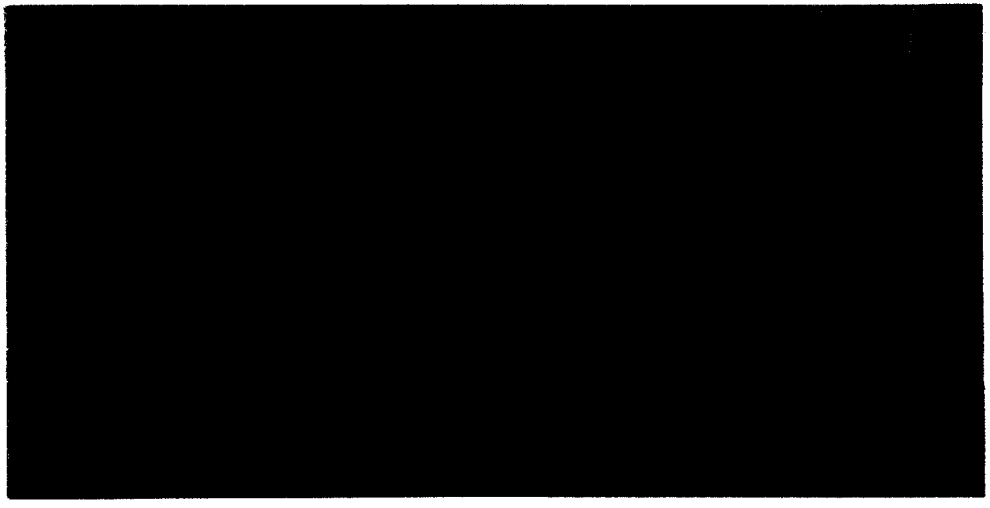


Fig.24: Photograph showing the colourless stage of methylene blue.

Table 13: Anova for MBRT.

S. No.	Source of variation	df	SS	MS	F cal	F Table value 5%	F Table value 1%
1.	Between weeks	11	20.9663	1.9060	1.2680^{NS}	1.80	2.38
2.	Between seasons	1	70.5332	70.5232	46.9588**	3.95	6.92
3.	Between bucks	4	35.0830	8.7708	5.8393**	2.48	3.56
4.	Interaction season x buck	4	29.8838	7.4709	4.9739**	2.48	3.56
5.	Error	99	148.7002	1.5020			
6.	Total	119	305.1665				

** ($P < 0.01$)

NS = Non significant

Fig.25: Photograph showing the semen sample after addition of Resazurin.

Fig.26: Photograph showing the RRT (I) stage in violet to pink.

Table 14: Anova for Resazurin reduction test-I (RRT-I):

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	99.0664	9.0060	1.8051*	1.80	2.38
2.	Between seasons	1	97.1992	97.1992	19.4818**	3.95	6.92
3.	Between bucks	4	3.5469	0.8867	0.1777NS	2.48	3.56
4.	Season x Buck	4	79.7188	19.9297	3.9945**	2.48	3.56
5.	Error	99	493.9336	4.9892			
6.	Total	119	776.4649				

* ($P < 0.05$)

** ($P < 0.01$)

NS = Non significant

1.40 seconds with a mean value of 62.80 ± 0.88 seconds during winter collection. In semen samples collected during summer season, the mean value was recorded as 66.90 ± 0.92 seconds which varied between 64.45 ± 1.47 to 70.45 ± 1.14 seconds (Table 11, Fig. 27 and 28).

A highly significant difference was observed ($P < 0.01$) between weeks, between seasons and between bucks but not so in season x bucks (Table 15).

4.3 RESISTANCE TO ENVIRONMENTAL CHANGES:

4.3.1 High temperature viability test (HTVT):

The average percentage of motility of spermatozoa after incubation varied between 34.4 ± 1.01 to 36.0 ± 1.10 with a mean of 35.0 ± 0.66 per cent in winter season. The semen samples collected summer season showed a mean value of 32.1 ± 0.36 per cent ranging between 31.0 ± 0.64 to 33.9 ± 0.44 (Table,16, Fig. 29)

The interaction between weeks was significant ($P < 0.01$) and a highly significant difference ($P < 0.01$) was noticed between seasons and between bucks but not in season x bucks (Table 17).

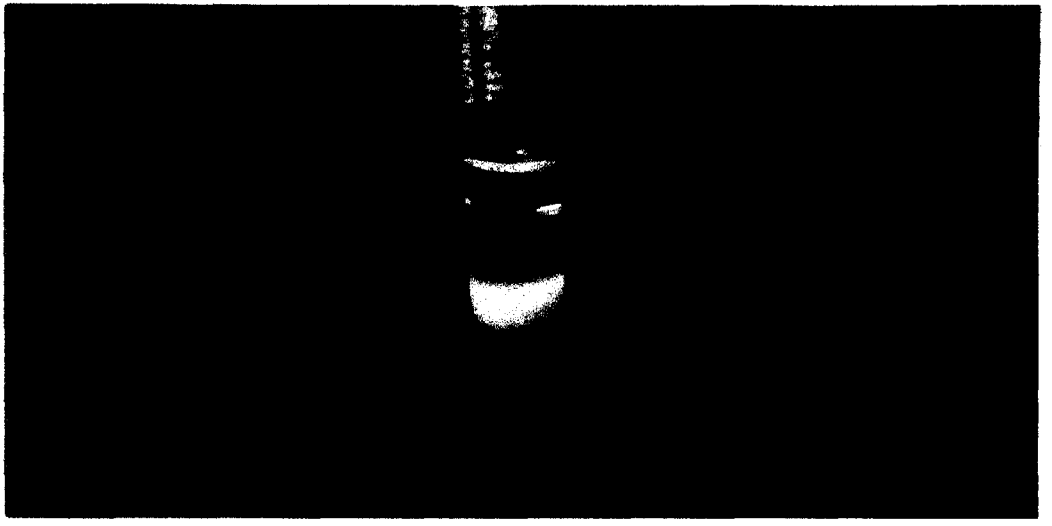


Fig. 27: Photograph showing the RRT (II) stage in pink to colourless.

Table 15: Anova for Resazurin reduction test-II (RRT-II).

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	1010.7190	91.8835	3.1489**	1.80	2.61
2.	Between seasons	1	504.3125	504.3125	17.2830**	3.95	6.92
3.	Between bucks	4	1905.4060	476.3516	16.3248**	2.48	3.56
4.	Season x Bucks	4	62.0938	15.5234	0.5320NS	2.48	3.56
5.	Error	99	2888.7810	29.1796			
6.	Total	119	6371.3130				

** ($P < 0.01$)

NS = Non significant

Table 16: Mean for resistance to environmental characters:

Season	Month	HTVT %	Coldshock % of live	Sodium chloride & Dil.effect
	Dec	36.0 ± 1.10	6.60 ± 0.33	1300.0 ± 65.19
Winter	Jan	35.8 ± 1.23	6.10 ± 0.32	1525.0 ± 82.72
	Feb	34.4 ± 1.01	5.80 ± 0.31	1450.0 ± 78.26
	Seasonal mean	35.0 ± 0.66	6.16 ± 0.19	1425.0 ± 45.75
	Mar	33.9 ± 0.44	5.05 ± 0.30	1325.0 ± 79.25
Summer	Apr	31.7 ± 0.62	3.55 ± 0.21	925.0 ± 39.90
	May	31.0 ± 0.64	3.90 ± 0.18	837.5 ± 31.99
	Seasonal mean	32.1 ± 0.36	4.16 ± 0.16	1029.2 ± 42.50

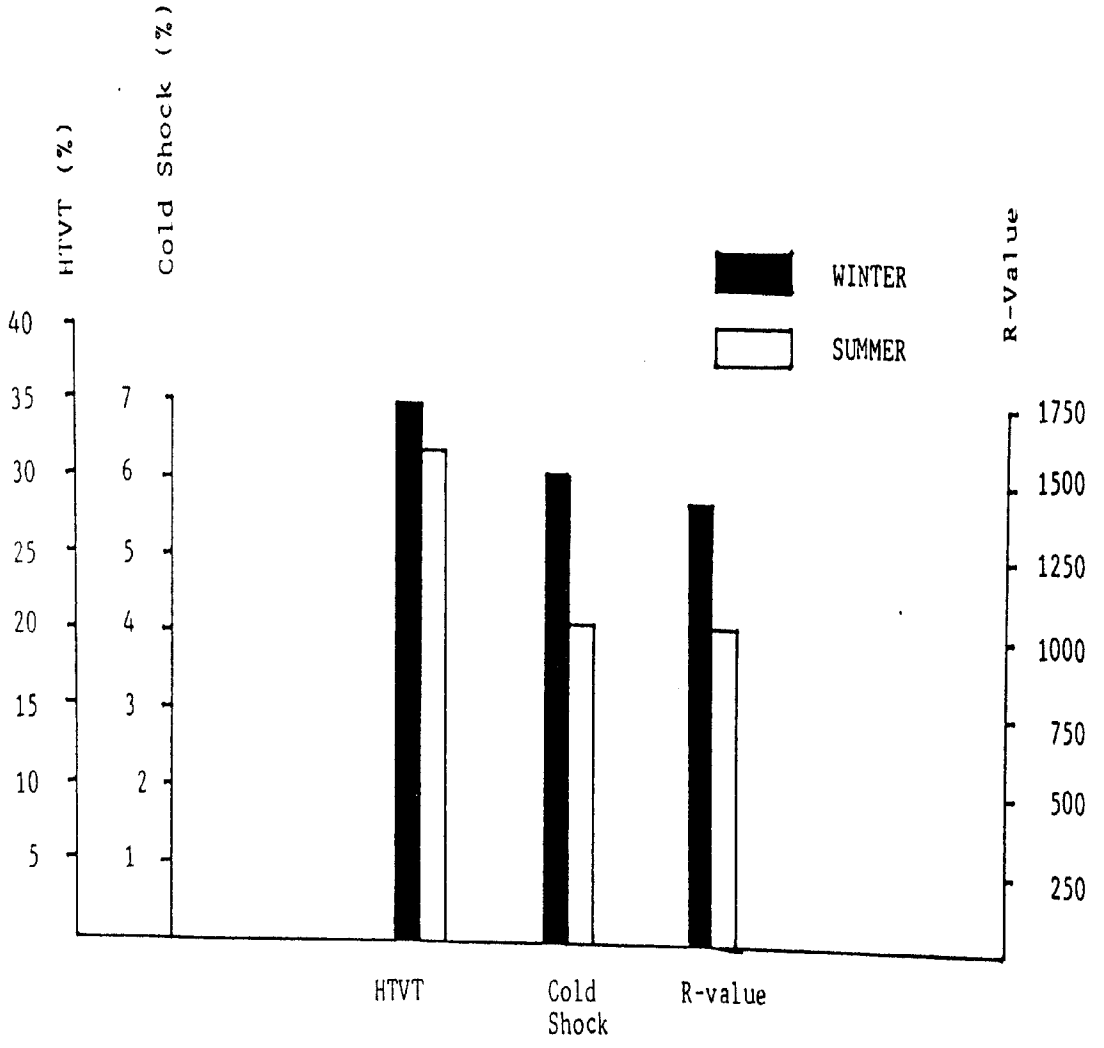


Figure 29: Resistance to environmental changes during Winter and Summer seasons.

Table 17: Anova for HTVT.

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	283.0938	25.7358	1.9083*	1.80	2.58
2.	Between seasons	1	261.0781	261.0781	19.3586**	3.95	6.92
3.	Between bucks	4	502.2188	125.5547	9.3097**	2.48	3.56
4.	Season x Buck	4	77.0469	19.2617	1.4282NS	2.48	3.56
5.	Error	99	1335.1560	13.4864			
6.	Total	119	2458.5940				

* ($P < 0.05$)

** ($P < 0.01$)

NS = Non significant

4.3.2 Cold shock resistant test (CSRT):

The mean per cent of live spermatozoa after subjecting to cold shock was found to be 6.16 ± 0.19 and varied between 5.80 ± 0.31 to 6.60 ± 0.33 in winter season. In summer season the semen samples ranged from 3.55 ± 0.21 to 5.05 ± 0.30 per cent of live sperms with a mean of 4.16 ± 0.16 (Table 16, Fig. 29). In all interactions the difference was highly significant ($P < 0.01$) (Table 18).

4.3.3 Resistant to sodium chloride and dilution effect:

The average R test value was reported to vary between 1300.0 ± 65.19 to 1525.0 ± 82.72 with a mean of 1425.0 ± 45.75 in samples evaluated during winter season. In summer season the mean value was found to be 1029.2 ± 42.50 ranging between 837.5 ± 31.99 to 1325.0 ± 79.25 (Table 16, Fig. 29).

A highly significant difference ($P < 0.01$) was noticed between seasons while it was not significant in all other interactions (Table 19).

Correlations study:

Under the present study, various correlations were observed within and between reaction time, physical,

Table 18: Anova for cold shock:

S. No.	Source of variation	df	SS	MS	F cal	F table value 5%	F table value 1%
1.	Between weeks	11	35.0667	3.1879	3.6165**	1.80	2.38
2.	Between seasons	1	120.0000	120.0000	136.1346**	3.95	6.92
3.	Between bucks	4	71.8335	17.9584	20.3750**	2.48	3.56
4.	Season x Buck	4	32.5000	8.1250	9.2174**	2.48	3.56
5.	Error	99	87.2666	0.8815			
6.	Total	119	346.6668				

** ($P<0.01$)

Table 19: Anova for Resistance to sodium chloride and dilution effect.

S. No.	Source of variation	df	SS	MS	F cal	F table value	
						5%	1%
1.	Between weeks	11	1418224.0	128929.5	1.0634^{NS}	1.80	2.38
2.	Between seasons	1	4700512.0	4700512.5	38.7678**	3.95	6.92
3.	Between bucks	4	194748.0	48696.0	0.4016^{NS}	2.48	3.56
4.	Season x Buck	4	57296.0	14324.0	0.1181^{NS}	2.48	3.56
5.	Error	99	12003540.0	121247.80			
6.	Total	119	18374350.0				

** ($P < 0.01$)

NS = Non significant

pH and resistance to environmental changes during winter and summer (Tables 20 and 21) seasons seperately.

Correlations for biochemical parameters were not undertaken due to the fact that their values were estimated from the second ejaculate of the week.

A significant positive correlations were observed between reaction time and abnormal sperms ($r=0.4706$; $P< 0.01$); and also with pH ($r=0.2610$, $P< 0.05$) during winter season and the same during summer season was positively significantly correlated with volume ($r=0.3429$, $P < 0.01$) and abnormal sperms ($r=0.4559$, $P< 0.01$).

Volume showed non significant correlations with other parameters during winter season where as in summer it showed significant positive correlations with individual motility ($r=0.3161$, $P< 0.05$), sperm concentration ($r=0.2833$, $P< 0.05$), per cent live sperm ($r=0.5899$, $P< 0.01$), HTVT ($r=0.3021$, $P< 0.05$), cold shock($r=0.3207$, $P < 0.05$) and R value ($r=0.4560$, $P< 0.01$).

Mass motility was significantly positively correlated with individual motility ($r= 0.5735$, $P< 0.01$) during winter and summer season ($r=0.7900$, $P < 0.01$). Mass

Table20: Correlation coefficients(r) among various physical characteristics, resistance to environment tests and pH of native buck semen in winter season.

	Reaction time	Volume	Mass motility	Individual motility	Sperm concentration	Percent live sperm	Abnormal sperms	Intact acrosomes	HTVT	Cold shock	R'value	pH
Reaction time	1.0000	-0.1005	-0.0297	-0.1871	-0.2161	-0.6908**	0.4706**	0.0298	-0.2111	-0.4198**	0.1488	0.2610*
Volume	-	1.000	-0.1491	-0.1625	0.0697	0.1420	-0.0814	0.1404	-0.1087	-0.0407	0.0489	0.0069
Mass motility	-	-	1.0000	0.5735**	-0.0513	-0.0351	-0.2293	-0.4435**	0.1506	0.2253	-0.0803	0.1659
Individual motility	-	-	-	1.0000	-0.0339	0.0449	-0.1500	-0.2242	0.1201	0.1456	0.1037	-0.0072
Sperm concentration	-	-	-	-	1.0000	0.1850	-0.0487	-0.0936	0.1152	0.1098	-0.1516	-0.0995
Percent live sperm	-	-	-	-	-	1.0000	-0.4004**	0.0162	0.3817**	0.4451**	0.0169	-0.1118
Abnormal sperms	-	-	-	-	-	-	1.0000	0.1660	-0.1324	-0.2206	0.1753	0.0130
Intact acrosomes	-	-	-	-	-	-	-	1.0000	-0.2561*	0.0810	-0.0114	-0.0398
HTVT	-	-	-	-	-	-	-	-	1.0000	0.3338**	-0.1414	-0.3947**
Cold shock	-	-	-	-	-	-	-	-	-	1.0000	0.0718	-0.3947**
R value	-	-	-	-	-	-	-	-	-	-	1.0000	0.1418
pH	-	-	-	-	-	-	-	-	-	-	-	1.0000

** ($p < 0.01$); * ($P < 0.05$)

Table21 : Correlation coefficients (r) among various physical characteristics, resistance to environment tests and pH of native buck semen in summer season.

	Reaction time	Volume	Mass motility	Individual motility	Sperm conc.	Percent live sperm	Abnormal sperms	Intact acrosomes	HTVT	Cold shock	'R'value	pH
Reaction time	1.0000	0.3429**	-0.0584	-0.2679*	0.1653	-0.4432**	0.4559**	-0.1265	-0.3704**	-0.2570*	-0.4777**	0.0937
Volume	-	1.0000	0.1997	0.3161*	0.2833*	0.5899**	-0.3762**	0.1238	0.3021*	0.3207*	0.4580**	-0.0371
Mass Motility	-	-	1.0000	0.7900**	0.0232	0.3433**	-0.4926**	-0.2617*	-0.0085	0.0472	0.4724**	0.2765*
Individual motility	-	-	-	1.0000	-0.0124	0.2921*	-0.6344**	-0.2518	-0.0205	-0.0670	0.5633**	0.2767*
Sperm concentration	-	-	-	-	1.0000	0.2325	0.0329	-0.1522	-0.1093	0.1705	0.2246	-0.2535
Percent live sperm	-	-	-	-	-	1.0000	-0.4888**	-0.1306	0.6875**	0.5350**	0.4814**	-0.1475
Abnormal sperms	-	-	-	-	-	-	1.0000	0.1098	-0.2966*	-0.1095	-0.4954**	-0.0728
Intact acrosome	-	-	-	-	-	-	-	1.0000	-0.2387	0.0233	-0.1313	-0.0492
HTVT	-	-	-	-	-	-	-	-	1.0000	0.5046**	0.2415	-0.0868
Cold shock	-	-	-	-	-	-	-	-	-	1.000	0.5021**	-0.3231*
R value	-	-	-	-	-	-	-	-	-	-	1.0000	-0.0719
pH	-	-	-	-	-	-	-	-	-	-	-	1.0000

** (P<0.01); *(P< 0.05)

motility was positively significantly correlated with per cent live sperm ($r=0.3433$, $P< 0.01$), R value ($r=0.4724$, $P<0.01$) and pH ($r=0.2765$, $P< 0.05$) in summer season.

Individual motility did not show significant correlation with the parameters during winter season but in summer season it showed positive significant correlations with per cent live sperm ($r=0.2921$, $P< 0.01$), R value ($r=0.5633$, $P< 0.01$) and pH ($r=2767$, $P< 0.05$).

Per cent live sperm showed significant positive correlations with HTVT ($r=0.3817$, $P< 0.01$) and cold shock ($r=0.4451$, $P< 0.01$) during winter season and with HTVT ($r =0.6875$, $P < 0.01$), cold shock ($r=0.5350$, $P< 0.01$) and R value ($r =0.4814$, $P< 0.01$) in summer season.

High temperature viability test (HTVT) showed a significant positive correlation with cold shock ($r=0.3338$, $P< 0.01$) in winter and ($r=0.5046$, $P<0.01$) summer seasons.

Cold shock showed a positive significant correlation with R value ($r=0.5021$, $P< 0.01$) in summer season, but no significant correlation was observed during winter season.

During winter season, reaction time was significantly negatively correlated with per cent live sperm ($r=-0.6908$, $P< 0.01$) and cold shock ($r= -0.4198$, $P< 0.01$) where as in summer, reaction time was significantly negatively correlated with individual motility ($r= -0.2679$, $P< 0.05$); per cent live sperm ($r= -0.4432$, $P< 0.01$), HTVT ($r = -0.3704$, $P< 0.01$); cold shock ($r= -0.2570$, $P< 0.05$) and R value ($r= -0.4777$, $P< 0.01$).

Volume showed significant negative correlation with abnormal sperms ($r= - 0.3762$, $P< 0.01$) in summer season only.

D257

In winter season mass motility had significant negative correlation with intact acrosomes ($r= -0.4435$, $P< 0.01$) whereas in summer it was significantly negatively correlated with abnormal sperms ($r= -0.4926$, $P< 0.01$) and intact acrosomes ($r= -0.2617$, $P< 0.05$).

Individual motility showed significant negative correlation with abnormal sperms ($r= -0.6344$, $P< 0.01$) during summer season only.

Percent live sperm was significantly negatively correlated with abnormal sperms in winter ($r= -0.4004$, $P< 0.01$) and summer ($r=-0.4888$, $P< 0.01$) seasons.

Abnormal sperms had significant negative correlations with HTVT ($r = -0.2966$, $P < 0.05$) and R value ($r = -0.4954$, $P < 0.01$) in summer season only.

Intact acrosomes were significantly negatively correlated with HTVT ($r = -0.2561$, $P < 0.05$) while HTVT was significantly negatively correlated with pH ($r = -0.3947$, $P < 0.01$) during winter season only.

Cold shock was significantly negatively correlated with pH inwinter ($r = -0.3947$, $P < 0.01$) and in summer ($r = -0.3231$, $P < 0.05$) seasons.

DISCUSSION

CHAPTER V

5. DISCUSSION

In the present investigation sex behaviour, semen characteristics viz., physical, biochemical and resistance to environmental changes were studied during winter and summer seasons respectively.

The mean reaction time in native bucks was found to be 15.96 ± 0.28 and 21.30 ± 0.31 seconds during winter and summer seasons respectively (Table 2).

The reaction time in the present study is in agreement in Baladi bucks (El-Sayed *et al.*, 1983) and in native bucks (Reddy *et al.*, 1989). The duration was much lower when compared to the reaction time reported earlier in Malabari bucks (49.39 ± 2.5 seconds) by Patil and Raja (1978), in Black Bengal (60.53 seconds) and Saanen bucks (64.46 seconds) by Sinha and Singh (1982). However, Pattnaik *et al.* (1991) reported very short duration in Ganjam bucks (9.62 ± 0.97 seconds).

A significant variation in the reaction time ($P < 0.01$) between weeks, between bucks, between seasons and season x bucks was observed in the present study

(Table 3). The present observations were akin to those of El-Wisky et al. (1971) and Reddy et al. (1989) who reported a decline in libido in bucks during summer, while Ashmawy (1979) witnesed in bucks the longest reaction time during March but with no significant difference between seasons. Umasankar et al. (1989) also recorded significant difference between animals.

In the present study the duration of courtship and the completion of the act of coitus was brief in winter collections than in summer. Perhaps the bucks did not tolerate much due to high temperature in summer(Reddy et al.,1989).

The average values for ejaculate volume in native bucks was 0.70 ± 0.10 in winter and 0.60 ± 0.02 ml in summer season (Table 2).

The present observations are similar to those of Barbari and Saanen bucks (Tiwari et al.,1968), in West African Dwarf bucks (Mann, 1980) in Pashmina bucks (Mohan et al.,1980) and in native bucks (Reddy et al.,1989). However, much higher values than the present observations were also reported in Jamnapari (0.86 ml) and Barbari bucks

(1.01 ml) by Singh et al. (1982) in Nubian goats (1.5 ml) by Ali and Mustafa (1986) and in Ganjam bucks (0.94 ml) by Pattnaik et al. (1991).

Variation in the volume of semen was highly significant ($P < 0.01$) between bucks and between seasons found in the present observations (Table 4). The lowest volume (0.49 $\pm$ 0.03 ml) was recorded in the month of May (summer season) in all the bucks, while the highest volume (0.76 $\pm$ 0.03 ml) of semen recorded in the month of February (winter season) (Table 2). Similar findings were observed by Vinha (1975), Kang and Chung (1976), Li et al. (1980), Sinha et al. (1981) and Reddy et al. (1989). However, contrary to the present findings Greyling and Grobbelaar (1983), Rahman and Kandil (1984) and Mittal (1985) reported that there was no significant difference between seasons in the ejaculate volume.

Similar to the present observations Krishnamurthy (1991) reported a significant difference between bucks ($P < 0.01$).

The diurnal light effect, high ambient temperature during hot months might have contributed to the reduced volume of semen.

In winter season the average mass motility was found to be 4.46 ± 0.06 and 4.36 ± 0.08 in summer season in the present study (Table 2).

Similar values were reported by Mohan et al.(1980) in Pashmina goats and Sinha and Singh (1982) in Black Bengal and Saanen bucks and Singh et al. (1985) in three breeds of goats. Where as lower values were reported in Jamnapari bucks (3.78 ± 0.07) by Saxena and Tripathi (1980), in Baladi male goats (3.06) by El-Sayed et al. (1983) and in Red Sokoto goats (3.80 ± 0.33) by Daudu (1984).

The difference in the value was found to be significant between bucks only but not between seasons(Table 5) Mohan et al. (1980) also observed a significant difference between bucks. Silva and Nunes (1984) observed similar results that season had no effect on mass motility in rams. However, Singh et al. (1976) and Gunzel et al. (1980) concluded that the mass motility in rams obtained in winter was significantly better than those obtained in summer, though significant difference between seasons was not noticed in the present investigation.

The average per cent of individual sperm motility in the present study was observed to be 81.78 ± 0.53 in winter season and 78.06 ± 0.68 in summer season(Table 2).

These results were in close confirmity with the observations made by Mann (1980) in West African Dwarf bucks, by Singh et al. (1982) in Jamnapari bucks and by Pattnaik et al. (1991) in Ganjam bucks, while (Igboeli 1974) and Mohan et al. (1980) observed low percentage of individual motility in native Zambian (52.30%), Boer (53.2%) and Pashmina bucks (60.62%) respectively.

The variation in the per cent of individual motility in this study was found to be highly significant between weeks, between seasons and between bucks ($P < 0.01$) (Table 6). Under the present investigation, significantly higher per cent of sperm motility was observed during winter over summer season. Patil and Raja (1978), Rahman and Kandil (1984), Li et al. (1988) and Reddy et al. (1989) reported significant difference between seasons in bucks. However, Mittal (1985) concluded that season had no effect in Jamnapari bucks.

Significant difference was noticed between bucks, which is in agreement with the findings of Mohan et al. (1980) in Anglo nubian bucks.

The reduced motility of spermatozoa in this study during summer months might be due to the adverse climatic

Kang and Chung (1976), Gamcik et al. (1979) and Rahman and Kandil (1984) reported that the sperm concentration was significantly higher in winter than in summer months, whereas Mittal (1985) recorded that season has no significant effect. But contrary to the present findings Vinha (1975) and Neves et al. (1980) revealed that sperm concentration was highest in summer and lowest in other seasons in bucks and rams respectively.

The lower level of concentration of the spermatozoa may be due to reduced volume of semen obtained during hot weather period as sensitivity of seminal plasma production to season was more readily appraised than that of spermatogenic process (Corteel, 1971).

Similarly Saxena and Tripathi (1980) also observed a significant difference in sperm concentration between Jamnapari bucks.

The concentration of the sperm variation might be due to breed (Mittal, 1982) seasons (Chaudry and Mahmood-Ul-Hassan, 1984) and frequency of semen collection (Tomer and Singh ,1970).

The mean live sperm count observed in this study was 87.05 ± 0.62 and 81.11 ± 0.51 during winter and summer seasons respectively (Table 2).

Similar values were reported by Igboeli (1974), Mohan et al. (1980), Singh et al. (1982), Pattnaik et al. (1991) in bucks, while Singh et al. (1985) recorded percentage of live spermatozoa as high as 91.07 and 90.33 in Black Bengal and Jamnapari bucks respectively.

Some investigators reported lower value than that was recorded in the present study (Jeelani and Nambiar, 1965), Patil and Raja, 1978; Saxena and Tripathi, 1980).

The live sperm count was found to be highly significant ($P < 0.01$) in all interactions in the present study (Table 8). The variation between season were similar to the observations made by Sahni and Raoy (1969) in Jamnapari bucks, Chahal et al. (1979) in Corriedale rams, Mittal (1982) in Barbari bucks, Rahman and Kandil (1984) in male goats and Saxena and Tripathi (1987) in rams. However, no significant difference was observed between seasons as reported by Patil and Raja (1978) in Malabari bucks, Loubser et al. (1983), Silva and Nunes (1984) in rams Mittal (1985) and Reddy et al. (1989).

Mohan et al. (1980) also found significant difference between Pashmina bucks similar to the present findings.

During the present study the low level of percentage of live spermatozoa during summer may be because of the high environmental temperature leading to disturbed spermatogenesis. Testicular hypoxia may also play a role in heat induced spermatogenic disturbences (Mc Donald, 1975)

In native bucks it was observed that the mean percentage of abnormal sperms was 8.98 ± 0.23 in winter and 9.28 ± 0.20 in summer seasons respectively (Table 2).

Bardoloi and Sharma (1982) in bucks and Sinha and Singh (1982) in Black Bengal goats and Reddy et al. (1989) in native buck reported similar observations to the present study. Some investigators reported higher per cent of abnormal spermatozoa as by Vinha and Megale (1974) in different breeds (11.05, to 16.35%), by Mann (1980) in West African Dwarf bucks (13.45 ± 8.77%) and Saxena and Tripathi (1980) in Nali rams in two ejaculations (13.12 to 15.72%).

However low percentage of abnormal spermatozoa was reported in Katjang x Jamnapari bucks as 3.11 ±

0.28 (Koh, 1975), 2.3% in Angora goats (Cetinkaya et al., 1980) and 2.12 to 2.11% in three breeds of goats (Singh et al.,1985).

The percentage of abnormal spermatozoa differed significantly ($P< 0.05$) between weeks and season x bucks (Table 9).

Mittal (1985), Gamcik and Mesaros (1986), Saxena and Tripathi (1987) reported that the abnormalities were not affected by season, while Patil and Raja (1978) Mittal and Ghosh (1979) Carmenate and Gamcik (1984), Li et al. (1988) and Reddy et al. (1989) pointed out that difference between seasons was significant. However, the increased percentage of abnormal spermatozoa 10.80 $\pm$ 0.21 was found in the month of May (summer season). This might be due to high environmental temperature which might have caused disturbed spermatogenesis (Djanur,1965 and Reddy et al.,1989).

In native bucks the mean per cent of intact acrosomes was found to be 73.68 $\pm$ 0.97 and 77.76 $\pm$ 0.76 in winter and summer seasons respectively (Table 2).

Mattos et al. (1984) and Aguirre et al. (1988) reported 56% and 50% in December and 38.0% during August

in rams respectively. Roca et al. (1992) recorded the acrosomal damage was highest during winter and spring seasons. The incidence of intact acrosomes is significantly higher ($P< 0.01$) during summer than winter season and other interactions except season x bucks in the present observations.

It has been established that the acrosomal integrity of spermatozoa is an important aspect of the fertilizing capacity of the spermatozoa (Hafez, 1980).

In the present observation the mean hydrogen ion concentration (pH) of semen was found to be 6.63 ± 0.01 during winter and 6.70 ± 0.01 in summer seasons respectively.

This was in agreement with values reported by Kurian and Raja (1965), Patil and Raja (1978) and Dundar et al. (1983) (Table 11) in bucks.

Significant ($P< 0.01$) variation was observed between season, between bucks and season x bucks only in the present study (Table 12). These findings are in accordance with the values of Tomer and Singh (1970) who reported higher pH in summer and humid seasons in cattle. While

Kang and Chung (1976) and Patil and Raja (1978) opined that pH was not significantly affected by season in goats.

The difference in the pH of semen might be due to varying proportion of several secretions involved and the time lapse in estimating the pH from the time of collection to recording (Salisbury and Van Demark, 1961).

The average value for reduction of methylene blue was noticed as 6.8 $\pm$ 0.18 minutes in winter and samples collected during summer season was 8.3 $\pm$ 0.17 minutes (Table 11).

Similar values were reported by Gaivanovich(1974) in carpathian rams at 9 months age. However much lower values were reported by Radman and Kopijar (1960) and Tripathi and Saxena (1983) in murrah bulls and Al-Wahab and Abid (1987) in rams.

In the present investigations highly significant ($P < 0.01$) difference was observed between seasons, between bucks and season x bucks, but not between weeks (Table 13). The semen quality was found to be superior in winter over summer seasons because of lower value in winter season observed in the present study. Similarly Tripathi and

Saxena (1983) and Patel et al.(1988) also recorded the M.B.R.Time was much lower in winter in murrah bulls. However, El-Fouly et al. (1980) reported that M.B.R.Time of semen is less in summer than in winter in rams. The MBRT gives an indication of the quality of semen samples for artificial insemination work in field practice (Chieffi et al., 1958), Salisbury and Van Demark (1961) concluded that lower the value of MBRT better would be the semen quality.

The time taken to reduce violet colour to pink(RRT-I) was averaged to be 18.1 ± 0.34 seconds in winter and in summer collection the mean duration was found to be 19.9 ± 0.26 seconds (Table 11).

The average time recorded in the present study was found to be higher as compared to the findings of Erb et al. (1952) and Pathak et al. (1989) in bull semen.

The difference in the values was highly significant between seasons and season x bucks, but was significant between weeks but insignificant between bucks in the present study (Table 14). RRT is one of the sensitive tests to determine the metabolic activity of semen.

The mean duration taken to reduce colour from pink to colourless was observed to be 62.80 ± 0.88 seconds in winter and 66.90 ± 0.92 seconds in summer season (Table 11). A highly significant ($P < 0.01$) difference was observed between weeks, between seasons and between bucks but not in season x bucks.

MBRT and RRT both proved to be good indicators of sperm metabolism and thus, indicating the semen quality in bucks. However, amongst the two tests, RRT appeared to be more sensitive than the MBRT as it has two end points both requiring a shorter time than MBRT (Patel, 1991).

The average percentage of motility of spermatozoa after 30 minutes of incubation was found to be 35.0 ± 0.66 and 32.1 ± 0.36 in winter and summer respectively (Table 16).

Patel *et al.* (1988) opined that percentage of motility after HTVT was significantly lower in problem bulls (27.84 ± 2.38) than in normal bulls (79.31 ± 1.64). The present findings are akin to the values (36.87 ± 22.92 reported by Joseph and Nair (1989) after 30 minutes of incubation in crossbred bucks.

The variations between seasons, between bucks and between weeks is statistically significant (Table 17). This test may give indication of quality of semen which reflects on conception (Ludwick et al.,1948).

The mean per cent of live spermatozoa after subjecting to cold shock was noticed as 6.16 ± 0.19 during winter collections. In summer season the average per cent was recorded as 4.16 ± 0.16 (Table 16).

Spermatozoa resistant after cold shock treatment may be useful to grade the semen quality (Lasley et al., 1942). High percentage of spermatozoa after the test may keep longer under preservation (Bishop et al.,1954) and Singh et al. (1968) and may predict higher fertility rates (Lasley and Bogart, 1943 and Tomar and Singh,1970).

The values in the present study support to the findings of Mittal and Pandey (1972) and Sahni and Roy (1972 b) in goat semen. They further reported lower values in rams which was endorsed by Deka and Rao (1979) in rams and concluded that goat spermatozoa may be susceptable to cold shock. Mohanthy et al. (1985) and Patel et al. (1988) reported the average per cent sperms resistant to cold shock was higher than in problem bulls.

In the present collections the values are significant between seasons and all interactions (Table 18). Similar findings were reported by Deka and Rao (1979) that cold shock resistant sperms did not vary significantly between months or between rams.

Spermatozoa resistant after cold shock treatment may be useful to grade the semen quality (Lasley et al., 1942). High percentage of spermatozoa after the test may keep longer under preservation (Bishop et al.,1954 and Singh et al.,1968) and may predict higher fertility rates (Lasley and Bogart, 1943 and Tomer and Singh, 1970).

In winter season the average R value resistant to sodium chloride was recorded as 1425.0 ± 45.75 and 1029.2 ± 42.50 during summer season (Table 16).

Tripathi and Saxena (1983) reported higher R test value 4000.00 to 6437.50 in the ejacultes of murrah buffaloe bulls. White Patel et al. (1988) observed lower R test value as 1164.81 in normal and 425.00 in problem bulls and 40.0 to 44.0 in Karakul rams (Izbasarov, 1973). It may have practical value in artificial insemination work to differentiate between normal and problematic male animals.

Highly significant difference ($P < 0.01$) was noticed between seasons only and non significant in all other interactions (Table 19).

Correlations study:

Under the present study, various correlations were seen within and between reaction time, physical, pH and resistance to environmental changes during winter and summer seasons.

These studies highlighted the fact that reaction time showed significant positive correlation with pH during winter, where as it showed negatively significant correlation with individual motility in summer. Volume showed nonsignificant correlations with abnormal sperms and pH. These findings are in accordance with that of Patil and Raja (1978). But in contrast to the result of Patil and Raja (1978), volume was positively significantly correlated with individual motility and sperm concentration. A negative significant correlation between volume and abnormal sperms in summer was also reported by El-Sayed et al.(1983).

Individual motility was positively correlated with pH in this study, similar to the results of Kang and Chung

(1976) with pH and per cent live sperm which is in lieu with the reports of Patil and Raja (1978). Non significant correlation between sperm concentration and other parameters in both seasons of this study are similar to the non significant per cent live sperm and pH and negatively significant correlation with abnormal sperm count made by Patil and Raja (1978).

Mass motility was positively correlated with per cent live sperm which is similar to the findings of Chahal *et al.* (1979) and Patel (1991) in ram semen.

Per cent live sperm was negatively correlated with abnormal sperms in both seasons which is in accordance with the findings of Patil and Raja (1978).

SUMMARY

CHAPTER VI

6. SUMMARY

A total of 5 native bucks aged between 2 to 2½ years and weighing about 24.25 ± 2.50 kgs available in the Department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati were used for this study. Semen was collected twice a week during winter and summer seasons. A total of 120 ejaculates, 24 from each of 5 bucks in each season were collected and analysed.

The mean values for normal libido and semen characteristics recorded in this study were reaction time (seconds) 15.96 ± 0.28 and 21.30 ± 0.31, volume (ml) 0.70 ± 0.10 and 0.60 ± 0.02, mass motility 4.46 ± 0.06 and 4.36 ± 0.08, individual motility (per cent) 81.78 ± 0.53 and 78.06 ± 0.68, sperm concentration (x 10^9ml) 2.45 ± 0.31 and 2.35 ± 0.02, live sperm count (per cent) 87.05 ± 0.62 and 81.11 ± 0.51, abnormal sperms (per cent) 8.98 ± 0.23 and 9.28 ± 0.20 and intact acrosomes(per cent) 73.68 ± 0.97 and 77.76 ± 0.76 in winter and summer seasons respectively.

In the present study the duration of courtship and completion of the act of coitus was brief in winter

(15.96 ± 0.28 seconds) than in summer (21.30 ± 0.31 seconds) collections. Perhaps the bucks may not tolerate due to high temperature in summer season.

The lowest volume (0.49 ± 0.32 ml) was recorded in the month of May (summer season) in all the bucks while the highest volume (0.765 ± 0.03 ml) was recorded in the month of February (winter season). The diurnal light effect, high ambient temperature during hot months might have contributed to the reduced volume of semen.

The difference in the value of mass motility was not significant between seasons but significant between bucks.

Significantly higher per cent of individual sperm motility was observed during winter (82.45 ± 0.53) than in summer (78.23 ± 0.68) season. The reduced motility of spermatozoa in this study during summer months might be due to the adverse climatic conditions such as high environmental temperature which might have placed animals under stress.

The concentration of spermatozoa was significant between season ($P < 0.05$) and highly significant between bucks, season x bucks ($P < 0.01$). Lower level of concen-

tration of the sperms may be due to reduced volume of semen obtained during hot weather period as sensitivity of seminal plasma production to season was more readily appraised than that of spermatogenic process.

The per cent of live sperm count was found to be highly significant between seasons and all other interactions ($P < 0.01$). The low level of per cent of live spermatozoa in this study during summer season may be because of the high environmental temperature leading to disturbed spermatogenesis. Testicular hypoxia may also play a role in heat induced spermatogenic disturbences.

The percentage of abnormal spermatozoa differed significantly ($P < 0.01$) between weeks and season x bucks. However the increased per cent of abnormal spermatozoa (10.80 ± 0.21) was observed in the month of May (summer season) compared to winter season (9.15 ± 0.23). This might be due to high environmental temperature which might have caused disturbed spermatogenesis.

The mean per cent of intact acrosomes were significant ($P < 0.05$) in all interaction except season X bucks. The incidence of intact acrosomes is significantly higher ($P < 0.01$) during summer than in winter season.

The average values for different biochemical tests recorded in this investigation were pH 6.63 ± 0.01 and 6.70 ± 0.01, MBRT (minutes) 6.8 ± 0.18 and 8.3 ± 0.17 RRT (I)(seconds) 18.1 ± 0.34 and 19.9 ± 0.26 and RRT (II) (seconds) 62.80 ± 0.88 and 66.90 ± 0.92 in winter and summer seasons respectively.

The value of pH was found to be significant ($P < 0.01$) between seasons and all other interactions except between weeks. The difference in the pH of semen might be due to the varying proportion of several secretions involved and the time lapse in estimation of the pH from the time of collection of recording.

The semen quality was found to be superior in winter (6.81 minutes) than in summer season (8.35 minutes) because of lower value of methylene blue reduction time in winter. This gives an indication of the quality of semen samples for artificial insemination work in field practice.

The difference in value of RRT (I) and RRT (II) was significant ($P < 0.01$) between seasons. The influence of season on RRT (I) and RRT (II) was found to be

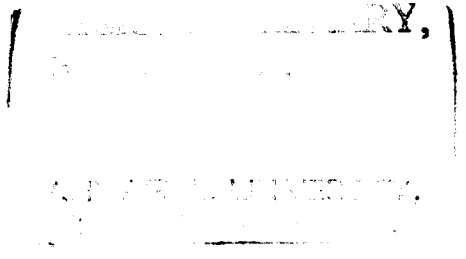
significant. Because of lower value during winter than in summer season, the quality of semen was found to be superior in winter season. This is one of the sensitive test to determine the metabolic activity of semen.

The mean values for various tests resistance to environment observed in this study were HTVT (per cent) 35.0 $\pm$ 0.66 and 32.1 $\pm$ 0.36, CSRT (per cent) 6.16 $\pm$ 0.19 and 4.16 $\pm$ 0.16 and resistance to sodium chloride (R test value) 1425.0 $\pm$ 45.75 and 1029.2 $\pm$ 42.50 during winter and summer seasons respectively.

The variation in the percentage of motility after incubation of semen was found to be significant between winter (35.08) and summer (32.13) season. It may be useful to grade the quality of semen based on th percent of live sperms after HTVT.

Spermatozoa resistant after cold shock was significant ($P < 0.01$) between seasons and other interactions. The semen collected during winter season (6.16%) was found to be superior than in summer season (4.16%),because higher percentage of spermatozoa after the test may keep longer under preservation and may predict higher fertility rates.

The average R test value between winter(1425.0) and summer (1029.16) seasons was significant ($P< 0.01$) and on significant in all other interactions. Some investigators concluded that R test value was higher in normal bulls than in problem bulls.



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