CERTAIN ASPECTS OF SEMEN BIOCHEMISTRY OF BLACK BENGAL BUCK

A Thesis
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in
ANIMAL GYNAECOLOGY AND OBSTETRICS

BY

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Dedicated

to

My beloved Parents

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C E R T I F I C A T E

This is to certify that the thesis entitled "CERTAIN ASPECTS OF SEMEN BIOCHEMISTRY OF BLACK BENGAL BUCK" embodies the original work, authentically carried out by Shri Abhijit Mandal, in partial fulfilment of the requirements for the Degree of Master of Veterinary Science in Animal Gynaecology and Obstetrics of the Bidhan Chandra Krishi Viswavidyalaya.

He has carried out work under my personal supervision and guidance. The research findings presented in the thesis have not so far been submitted for any degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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(ABHIJIT MANDAL)

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LIST OF ABBREVIATIONS USED

BU	=	Bodansky Unit
^{o}C	=	Degree Centigrade
^{o}F	=	Degree Fahrenheit
d.f.	=	Degree of freedom
F	=	"F" - test value
gm	=	Gram
mEq	=	Milliequivalent
mg	=	Milligram
ml	=	Millilitre
μg	=	Microgram
mμ	=	Milimicron
M.S.	=	Mean squares
No.	=	Number
%	=	Percentage
S.S.	=	Sum of squares

Chapter : I

INTRODUCTION

I N T R O D U C T I O N

The goat (*Capra hircus*) is probably the first ruminant whose domestication took place in South Asia about 9000 years ago. The goat is a versatile animal. It is often called as "Poor man's cow" in India and as "Wet nurse" of infants in Europe. Goat is the only domestic animal which is kept for objects with little expense. It provides meat, milk, skin, fibre, manure and by-products. Goats can easily thrive well in very low quality pasture, forage, tree leaves and kitchen wastes which are generally refused by other livestock.

The vast cattle population of India cannot be used as a source of meat due to religious sentiment. Alternatively, the people of India prefer goat meat (Chevon) than any other type of meat.

The importance of goat as a subsidiary source of income is being increasingly recognised by the small, marginal and landless farmers of India. This species has

established its potential as a moderate source of employment generation for the poor people with a minimum investment. Furthermore, goat is especially privileged to be able to withstand both high and low environmental stresses with equal efficacy i.e. well adapted to the local environmental conditions.

People of West Bengal belonging to the poorest section of the community, are mainly attracted in rearing of Black Bengal goats due to its small size and prolificacy, adaptability, temperament, behaviour and high resistance to diseases. The meat production of goat and sheep is about 200 percent higher than that of cattle (Wilson, 1982). Besides, less requirement of spaces, low cost of housing and maintenance and readily available market for the disposal of milk, meat and hide are also some of the significant factors for the promotion of goat rearing.

In recent years, goats are being increasingly recognised as one of the important livestock in many parts of the tropics and sub-tropics. India ranks first among the countries of the world in goat population. There are about 105 millions of goats in India as against 501.8 millions in the world (F.A.O. 1988). This population in India is about 19 percent of that of the world.

National projects and schemes in the shape of District Rural Development Agency (DRDA), Integrated Rural Development Programme (IRDP), Draught Prone Area Programme (DRAP), Tribal Development Agency (TDA) etc. have been launched with the object of providing livestock development programme for economic upliftment of rural poor. In Integrated Rural Development Programme of West Bengal, Black Bengal bucks are distributed to the agricultural labourers and small farmers with the object of genetical upgradation of nor-descript goat of the state thereby increasing their productivity in terms of meat, milk, skin, fibre etc. But the population of Black Bengal bucks having good genetic make up is not sufficient enough to breed the vast number of non descript goat of the State of West Bengal.

A.I. in goat is, therefore, gaining momentum day by day because of its profound success in cattle breeding and for non-availability of the adequate number of male goats of well defined breed heving good genetic make up for breeding purpose. It has been claimed that high incidences of sterility in male goats has further accelerated the acceptance of A.I. in the countries like Germany, Netherland, France and India.

Large scale application of A.I. in goat has not only necessitated the evaluation of semen quality of well defined breeds of buck suitable for breeding purpose but also the

assessment of the norms of biochemical constituents of semen/ seminal plasma and the enzymatic constituents of seminal plasma of buck semen.

Seminal fructose as energy yielding agent is necessary for the survival of spermatozoa in the ejaculated semen. It has also been claimed that fructose content of semen has some relationship with the fertilising ability of spermatozoa.

There are several factors essential for spermatozoa to maintain their viability in the seminal plasma. Of the various factors, optimum pH, osmotic pressure and various metabolic activities of spermatozoa relating to generation of energy for them are most important. The osmotic pressure and pH of semen are largely dependent on electrolyte balances. The cations and anions play specific role in this regard. The most plentiful cations found in bull semen are sodium and potassium. They are most important cations in the maintenance of osmotic pressure (Cragle et al., 1958). The most important anions found in bull semen are chloride, citrate, bicarbonate and phosphate. Furthermore, these anions have distinguished role in metabolism of spermatozoa by acting as co-factor, being an integral part of different enzymes or being the important constituents of various reactions. Thus, potassium acts as co-factor in TCA cycle and inorganic phosphate plays vital role in respiration and aerobic glycolysis of spermatozoa.

Bostedt et al. (1976) opined that seminal plasma from bulls with abnormal spermatozoa had higher GOT and inorganic phosphorus levels.

Abdou et al. (1978) concluded that phosphatase activity may serve as a valuable criterion of semen quality and metabolism of spermatozoa.

The protein content of mammalian semen varies from about 3% to 7% depending on the species. In addition, the plasma may contain polypetides, probably arising from the breakdown of protein after ejaculation and free amino-acids which originate chiefly in the testes (Hafez, 1980).

It appears that the information in respect of the norms of the above biochemical constituents of semen/seminal plasma and enzymatic constituents of seminal plasma of fertile Black Bengal bucks is lacking as revealed from the available references of work in this respect.

Hence, the present study was undertaken to meet the above deficiency in knowledge with the following objectives.

1. To determine the normal levels of certain seminal biochemical and enzymatic constituents in Black Bengal bucks and these are :-

a) Initial fructose content (mg/100 ml) of semen.

b) Sodium content (mEq/litre) of seminal plasma.

c) Potassium content (mEq/litre) of seminal plasma.

d) Inorganic phosphorus content (mg/100 ml) of seminal plasma.

e) Total protein content (g/100 ml) of seminal plasma.

f) Glutamic oxaloacetic transaminase (unit/ml) of seminal plasma.

g) Glutamic pyruvic transaminase (unit/ml) of seminal plasma.

h) Acid phosphatase (BU/100 ml) of seminal plasma.

i) Alkaline phosphatase (BU/100 ml) of seminal plasma.

2. To determine whether there is any variation in respect of level of above parameters of semen between different Black Bengal bucks and to find out relationship between various biochemical and enzymatic constituents in semen under study.

Chapter: II

REVIEW OF LITERATURE

Very little work in respect of biochemistry of buck semen was undertaken so far, as revealed from the available references of work in this respect compared to the same of bull semen. Therefore, related references are too scanty. However, as the goat represents the ruminant, the references in respect of the semen biochemistry of other ruminant species along with the same of buck semen are included in the review of literature to have a comparative idea in this respect.

Initial fructose

Mann (1945) stated that the major carbohydrate component of seminal plasma was fructose and not glucose as assumed before.

Erb et al. (1956) and Hopwood et al. (1956) reported highly significant correlation between fertility and fructolysis.

Mann (1964) found that initial fructose content ranged from 300 to 900 mg% in buck semen.

Lunca et al. (1968) reported that seminal fructose in 16 A.I. bulls varied from 107 to 1046 mg%.

Patil (1970) found mean initial fructose level of 611.94 mg per 100 ml semen in Malbari bucks.

Barakat et al. (1972) reported that the mean fructose content of buck semen was 820 mg%.

Rao and Singh (1975) found the amount of 764.17 ± 25.54 and 597.00 ± 41.50 mg/100 ml of semen in Corriedale and Nali rams respectively.

Abdou et al. (1977) studied the fructose content in buffaloes and Friesian bulls and the values were 643 and 424 mg/100 ml of semen respectively.

Nafornita et al. (1977) estimated the fructose concentration of bull as 1408, 1082 and 1122 mg/100 ml of semen in the months of Dec, Jan, and Feb. respectively.

Varshney et al. (1977) reported the initial fructose content 1294.50 mg/100 ml of semen of Barbari buck.

Reddy and Raja (1979a) analysed the fructose content in buffalo semen and it was 426.64 ± 0.32 mg %.

Strzezek et al. (1979) studied the fructose concentration as 43.34 mg/100 ml of semen in Polish Large White boars.

Oramus-Kasprzyk et al. (1980) estimated the fructose concentration in 3 species. In bull, ram and boar semen, the amounts were 394, 472 and 14.2 mg/100 ml of semen respectively.

Mittal and Ghosh (1980) estimated fructose level as 795.92 ± 10.72 mg % in Beetsar bucks

Roy Choudhury and Sadhu (1981) found that the initial fructose content varied significantly between breeds of Holsteins, Jersey and Sahiwal bulls and also between seasons.

Bhela et al. (1982) studied the initial fructose content as 41.7 ± 4.80 µ moles/ml in buffalo semen.

Berader et al. (1982) determined the concentration of fructose which was 518.0 ± 149.8 mg/100 ml of semen in Nili-Ravi buffalo.

Kapoor (1982) estimated the average initial fructose concentration in Murrah buffalo bull as 497 ± 4 mg/100 ml of semen.

Nema et al. (1983) analysed the initial fructose content level in semen and seminal plasma of Surti buffalo bulls. The values were 647.43 ± 28.21 and 797.14 ± 32.25 mg % in semen and seminal plasma respectively.

El-sayed et al. (1983) reported fructose level in whole semen of Baladi bucks as 806.11 ± 233.97 mg%.

Dunder et al. (1983) found that the initial fructose content in Angora buck was 677.86 ± 63.26 mg/100 ml of semen.

Wildeus et al. (1984) viewed that the seminal fructose concentration in crossbred bulls decreased with advancing age.

Kapoor (1985) estimated the semen fructose concentration which averaged 491 ± 5.0 mg/100 ml of semen in Murrah buffalo bull semen and ranged from 472 ± 5.0 in Dec. and June to 508 ± 5.0 in Sept., differences being significant.

Patel et al. (1989) opined that season significantly affected fructose concentration of different breeds of bulls.

Markandeya and Pargaonkar (1989) estimated the initial fructose content as 466.511 ± 32.84 mg/100 ml semen and 404.70 ± 23.55 mg/100 ml seminal plasma in osmanabadi bucks.

Sodium and potassium :

According to the work of Nesmejanova (1938) the average concentration of sodium and potassium (mg/100 ml) in bull seminal plasma was 270 and 220 respectively.

Replacement of electrolytes by non-electrolytes was found to be beneficial in improving the semen quality (Kampschmidt et al., 1953).

White (1953) stated that high concentration of potassium depressed the viability of ram and bull spermatozoa.

Hawk et al. (1954) were of the opinion that normal ionic equilibrium and osmotic pressure were maintained in semen by means of variation in the sodium and potassium on one hand and chloride, bicarbonate and protein concentration on the other hand.

Cragle et al. (1958) found highly significant negative correlation between sodium and potassium content of bull seminal plasma.

Yassen et al. (1967) reported that the sodium and potassium concentration varied greatly between bulls. The concentrations of sodium and potassium in seminal plasma were 1768 and 1095 mcg/ml respectively.

Singh et al. (1970) estimated the Na and K concentration in Murrah buffalo bull seminal plasma as 186.89 ± 10.90 and 101.60 ± 4.45 mg% respectively.

Ratten et al. (1972) viewed that biochemical reaction at sperm cells in the seminal plasma had bearing on its livability and keeping quality which in turn have bearing on fertility.

Roy Choudhury and Sadhu (1976) stated that sodium content in the bull's seminal plasma differed significantly between seasons but not between breeds, while the potassium content in the bull's seminal plasma differed significantly between seasons and breeds of Holstein, Jersey and Sahiwal.

Thakur and Pandey (1976) studied the concentration of sodium and potassium in seminal plasma of 6 corriedale and 6 Kali rams. They reported that the sodium concentration in seminal plasma was significantly and positively correlated with motility percentage of live spermatozoa and sperm concentration. On the contrary, potassium concentration in seminal plasma was significantly and negatively correlated with these semen characters.

Semakov and Ryzhkov (1976) studied sodium and potassium concentration with undiluted semen in bulls and rams. The concentration of sodium (mç%) were 689 and 847 and the concentration of potassium (mç%) were 593 and 370 respectively.

Varshney et al. (1977) estimated the values of Na and K in the seminal plasma of buck semen as 178.05 ± 4.93 and 184.26 ± 1.98 mç% respectively

Mean semen concentrations of sodium and potassium in Surti buffaloes were 248.98 and 202.28 mç% respectively. There were no significant seasonal differences (Reddy and Raja, 1979b).

Deshmiam et al. (1981) measured the concentration of sodium and potassium (mç%) in seminal plasma of six bulls. Mean concentrations (mmol/l) of sodium and potassium were 76.3 ± 25 (range 49.6 - 102.4) and 49.9 ± 30.9 (range 14.7 - 82.4) respectively.

Nair and Nandakumaran (1982) concluded that the semen from the Jersey bull had significantly higher levels of sodium and potassium than that from the crossbreds.

Pandey et al. (1982) estimated the level of sodium and potassium in seminal plasma from Barberi and Saanen bucks. There was significant difference between the two breeds in the level of potassium but not in the level of sodium.

Nair et al. (1982) reported that the differences between bucks in sodium and potassium levels were highly significant.

Gupta et al. (1983) concluded that Surti buffalo bull showed average concentration of sodium and potassium (mg/100 ml) as 326.9 ± 57.29 and 175.52 ± 20.54 respectively.

Kanakaraj et al. (1984) studied the mean concentrations of sodium and potassium (mg/100 ml) of semen in Murrah buffalo on an average 243 ± 4.09 and 98.04 ± 2.7 respectively.

Gonzalez et al. (1984) collected semen from polwarth rams and stored at 37°C for upto 90 minutes. On semen collection and 30, 60 and 90 minutes after collection, semen sodium concentration averaged 31.7, 29.3, 32.0 and 29.0 mg/100 ml respectively and semen potassium concentration averaged 58.0, 52.8 54.9 and 58.1 mg/100 ml respectively.

Petzoldt et al. (1985) reported that seminal plasma concentration of sodium and potassium in young bulls averaged 62.75 ± 18.58 and 42.80 ± 25.72 mmol respectively and the sodium and potassium ratio was 2.72 ± 2.95. Na and K concentration varied considerally among bulls.

Petzoldt and Nehring (1986) studied that in boar semen, immediately after collection, mean sodium concentrations were 96.6 ± 12.08 n mol/litre in seminal plasma and 75.4 ± 23.12 n mol/litre in spermatozoa. Potassium concentrations were 13.1 ± 2.12 and 28.8 ± 6.54 n mol/litre respectively. Corresponding

values for ram semen were 36.0 ± 15.01, 37.8 ± 12.29, 10.8 ± 4.12 and 33.7 ± 8.63 respectively.

Markandeya and Parçaonkar (1990) reported the mean sodium and potassium concentration (mEq/litre) in Osmanabadi bucks as 77.47 and 74.58 respectively.

Inorganic phosphorus :

Pal (1957) estimated the inorganic phosphorus content in Buffalo and Bull semen as 17 mç/100 ml and 9 mç/100 ml of semen respectively.

The inorganic phosphorus concentration (mç/100 ml) in cattle whole semen was 5.9 and that in seminal plasma was 5.6 (Roy et al., 1960).

Varshney et al. (1977) estimated the inorganic phosphorus concent in seminal plasma of Barbari buck as 10.59 mç/100 ml.

Pandey et al. (1982) reported that the averege value of inorganic phosphorus in the semen samples of Saanen and Barbari bucks were 12.502 ± 0.922 and 12.278 ± 0.621 mç/100 ml seminal plasma respectively.

Nema et al. (1983) analysed the whole semen and seminal plasma of Surti buffalo bull for inorganic phosphorus. The values were 19.58 ± 0.94 and 15.51 ± 0.64 mg% respectively.

Dabas et al. (1984) reported the concentration of inorganic phosphorus in Danish Red bull and Murrah buffalo bull semen. The findings were 8.0 ± 0.5 mg/100 ml and 12.4 ± 0.4 mg/100 ml respectively.

Markandeya and Parasonkar (1990) estimated the inorganic phosphorous in Osmanabadi buck semen and this was 13.07 mg/100 ml of seminal plasma.

Total protein:

Buruiene et al. (1975) concluded that sperm protein content ranged from 0.31 to 0.49 gm % in bull semen.

Kaker and Arora (1976) reported significant positive correlations between total proteinand GOT, GPT, AKP and ACP in Young crossbred bulls.

Nafornita et al. (1977) viewed that protein content of seminal plasma of bull varied with the winter months.

Saxena and Tripathi (1981) estimated the total protein content which was 8.50 ± 0.42 gm/100 ml of semen in crossbred bulls.

Dabas et al. (1982) analysed the concentration of total protein in Red Dane and Murrah buffaloes.

Dundar et al. (1983) studied on seminal plasma of Angora bucks. They found that the concentration of protein was 2.99 ± 0.28 gm/100 ml.

Yaqub et al. (1983) observed 2.8 ± 0.13 gm/100 ml of protein in seminal plasma of Nili-Ravi buffalo semen.

Nema et al. (1983) analysed the seminal plasma of Surti buffalo-bull for total protein content and the value was 7.31 ± 0.3 g%.

Dhami and Kodagali (1987) estimated the protein content in Surti buffalo bull seminal plasma as 4.42 ± 0.07 g% and viewed that the total protein content was significantly and positively correlated with GOT (0.589).

Patel et al. (1969) concluded that season significantly affected total protein concentration of semen in crossbred bulls.

Markandeya and Pargaonkar (1989) reported that biochemical analysis of Osmanabadi buck semen revealed the total protein as 3.54 ± 0.25 gm/100 ml of seminal plasma.

Dhami et al. (1990) viewed that the seminal plasma of buffalo bulls had significantly lower levels of total protein than in the Jersey and Crossbred bulls.

Glutamic oxaloacetic transaminase (GOT) and Glutamic pyruvic transaminase (GPT) (GOT and GPT)

Elipse (1960) found that there were significant correlations of 1.849 for GOT and 0.409 for GPT between seminal plasma transaminase activity and numbers of spermatozoa per millilitre of semen of bull.

As reported by Greçoire et al. (1961), the averace number of GOT units per ml of seminal plasma was 1751 (1200-2900) in the bull.

Roussel and Stallcup (1965) studied the activity of GOT and GPT associated with spermatozoa free of seminal plasma in 149 samples of semen from 12 Holstein bulls and their average values respectively were 244.8 and 345.5 SF units/ml for GOT and 25.0 and 18.1 SF units/ml for GPT.

Chauhan and Srivastava (1973) estimated GOT and GPT in seminal plasma of buffalo bulls and the values were 166.72 ± 14.08 and 34.56 ± 4.57 units/ml respectively.

Gonzalez-Rubiera et al. (1974) reported that there were no significant differences between the breeds (Holstein-Friesian and Zebu bulls) in GOT values but Holstein-Friesian bulls had significantly higher GPT values than Zebu bulls.

Mohan et al. (1977) found that there were significant seasonal variations in transaminase activities in Murrah buffalo bull semen.

Varshney et al. (1978) working with Barbari bucks obtained the values of GOT and GPT (unit/ml) of seminal plasma as 176.23 ± 5.16 and 16.67 ± 0.67 respectively and the GOT, GPT ratio was found to be 11:1.

Ibrahim (1982) reported that the level of GOT and GPT activity in seminal plasma were significantly lower in the Hungarian simmental bulls which had excellent semen quality and freezability than the bulls which had poor quality semen.

Akusu et al. (1984) analysed the seminal plasma of West African Dwarf buck. He found that GOT activity of the seminal plasma was significantly lower for electroejaculated semen than the semen collected by artificial vagina.

Gupta and Srivastava (1985) studied the GOT activity in bovine. They found that there were no statistically significant differences between the Murrah buffalo, Danish Red and Holstein-Friesian X Tharparkar bulls in activity of the enzyme in the seminal plasma.

Karagiannidis et al. (1985) reported about the GOT activity in Chios and Friesian rams seminal plasma which were 496 ± 27.78 and 412 ± 12.97 units/ml respectively.

Khokhar et al. (1987) estimated the avereçe amount of GOT and GPT activity as 807 ± 31 and 121 SF units in seminal plasma of bull and 635 ± 28 and 94 ± 11 SF units in buffalo respectively.

Dhami and Kodagali (1987) analysed the Surti buffalo bull seminal plasma for GOT and GPT activity which were 51.50 ± 1.60 and 14.51 ± 0.97 μ mole/litre respectively.

Acid and alkaline phosphatase (ACP and AKP)

Bell and Lake (1962) measured ACP and AKP in different species of animals and man. He measured the same highest in man, comparatively low in bull, boar, rabbit and intermediate in cock and turkey.

Paul et al. (1966) viewed that the ACP and AKP level varied significantly under different environmental conditions, breeds and species of animals.

Rousse1 and Stallcup (1966) studied the seminal plasma of Holstein-Friesian bulls. He found that AKP and ACP activity was found to be 4 times higher in seminal plasma than in spermatozoa.

Kurilo (1970) measured the activity of AKP and ACP in the seminal plasma of Russian Large white boars. The amount of AKP was almost double that of ACP.

Chauhan and Srivastava (1973) estimated the ACP and AKP in buffalo seminal plasma. The amount was 315.31 ± 22.66 and 312.50 ± 24.04 (BU/100 ml) respectively.

Gasteuer (1974) analysed the activities of ACP and AKP in seminal plasma in simmental bulls. ACP value was higher in summer than in winter. Bulls with abnormal sperm had higher AKP values in the seminal plasma independent of their state of health. Bone muscle diseases were accompanied by high ACP values. Bull with chronic diarrhoea had slightly increased ACP activities.

Mohan et al. (1977) reported that there was significant seasonal variation in phosphatase activity in Murrah buffalo bull semen.

Chaudhury and Gangwar (1977) estimated the mean values of AKP and ACP in seminal plasma of Murrah buffalo bulls which were 1239 ± 47.4 and 1244 ± 54.5 KAU/100 ml of seminal plasma.

Georgiev et al. (1977) assessed the enzyme activities of seminal plasma of bulls by a modification of Fodansky's method. The alkaline/acid phosphatase ratio was 1.0 : 0.9.

Varshney et al. (1978) estimated the enzymic constituents in Barbari bucks. For ACP it was 166.11 ± 7.0

and for AKP it was 504 ± 25.82 EU/100 ml. These were estimated in whole semen.

Asghar et al. (1983) viewed that the acid and alkaline phosphatase activity in cattle seminal plasma showed no significant variation among bulls or ejaculates.

Mahmoud et al. (1986) estimated the AKP and ACP activity in the seminal plasma of bulls. The values were for AKP 37.34 ± 0.44 units/100 ml and 35.29 ± 0.46 units/100 ml in first and second ejaculates respectively. ACP activity in the seminal plasma showed the same pattern.

Dhami and Kodagali (1987) reported about AKP and ACP level in the seminal plasma of Surti buffalo bull semen which were 590.34 ± 8.70 and 252.32 ± 5.06 KAU/100 ml respectively.

The alkaline phosphatase activity observed in buffalo bulls was double the value of acid phosphatase and nearly three times higher than the values of AKP and ACP in low bulls. However, ACP values did not differ significantly between the species. The ratio of AKP : ACP observed was much higher (2.37 : 1) in buffalo than in cow bulls (0.84 : 1) (Dhami et al., 1990).

Chapter: III

MATERIALS AND METHODS

The experiment was conducted for the period of six months from June, 1990 to November, 1990.

Experimental animals

Ten Black Bengal bucks (Capra hircus) reared under the same standard of housing, feeding and management were considered in the present study. These bucks were maintained at the goat farm under the control of the Department of Animal Production and Management, Faculty of Veterinary and Animal Sciences, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal.

The details of the bucks in respect of their ages and weights are tabulated in Table-1. The ages of the bucks under study ranged from 3 years.to 4 years while their weights varied from 16 kg to 22 kg.

Table - 1. Details of ten Black Bengal bucks (*Capra hircus*) showing their ages and weights

Identification number	Age	Body weight
23	3 years	16 kg
26	3 years 3 months	18 kg
29	3 years 2 months	18.5 kg
32	3 years 8 months	19.8 kg
56	4 years	22 kg
73	3 years 1 month	16.6 kg
80	3 years	17.4 kg
84	3 years 6 months	18.4 kg
96	3 years 3 months	17 kg
99	3 years 4 months	20 kg

The experimental bucks had optimum conception rate as revealed from the buckwise conception register maintained

in the goat farm. These bucks exhibited optimum sex libido and the same was observed for three weeks before collection of semen from them.

Housing of bucks

All the bucks were housed in a shed with concrete floor. The shed had well ventilation and light with provision of fresh drinking water and attached grazing land under sunlight.

Feeding and management

All bucks were fed a ration composed of the following concentrate mixture.

Crushed gram	...	30%
Crushed wheat	...	20%
Wheat bran	...	25%
Mustard oil cake	...	25%

On an average, the animals were fed 200 gms of the above concentrate mixture per day. In addition, 5 gms of common salt, 1 gm of vitablend (Glaxo-vety) containing Vitamin A_2D_3 and 2 gms of mineral mixture were given mixed with the concentrate per day per animal.

The animals were allowed grazing for a limited period during day time and were also fed certain amount of jackfruit leaves.

Collection of semen

Semen was collected from the experimental bucks by electroejaculator which was adopted because of the fact that these animals were accustomed to ejaculate semen previously by this method. On the other hand, training of the buck would have been needed to collect semen by artificial vagina method. Hence, it was considered that the animals would respond readily to electro-ejaculator than to artificial vagina.

Description and working of electro-ejaculator

The electro-ejaculator used in this method was designed and manufactured by Micro Digisens corporation of Calcutta. The said manufacturer had made the electro-ejaculator exclusively for collection of semen from buck and the same is depicted in Fig. 1. This has a single rectal electrode about 18.7 cm long and about 1.87 cm diameter and having brass rings each of 1.25 cm broad and serially numbered 1, 2, 3, 4, 5 and 6 and between rings there is a gap of 1.25 cm. The last ring at the terminal end of the electrode is negative and the other remaining rings are positive (site selectors). These rings are connected through lead wires with a voltmeter. A rotatory

Fig - 1 - Shewing electro ejaculator of buck.

Fig - 2 - Shewing collection of semen by electro ejaculation technique in buck.

switch of the voltmeter can be worked clockwise or anticlockwise on fingers 1 to 6 corresponding to the brass rings on the rectal electrode. The power output pulses direct current (DC) at 12 volts and 1.5 amp/sec controllable in between 0-12 volts. Normally, the input power is Ac/220-250 volts/single phase/50 cycles. The local control system at the base provides easy regulation of output flow with a finger tip. With this unique safety device, this type of electro-ejaculator prevents danger of electric shock to both the breeding buck and the operator. The electric stimuli through electro-ejaculator are delivered by electrodes placed in the rectum to sympathetic and parasympathetic nerves responsible for erection of penis, emission and ejaculation of semen.

Technique of semen collection by the above model of electro-ejaculator

Prior to semen collection, the buck was placed in a lateral position on a table. Prepucial hairs were removed and the area near the prepucial orifice was cleansed by normal saline and dried.

The rectal probe properly lubricated with sterile vaseline was inserted into the rectum. At the begining of the operation of electro-ejaculator, power steps were set at '0' (zero) and the site selector was set first to its lowest setting position when there was no current in the rectal probe.

After turning on power and probe switches, site selector was advanced until buck reacted slightly. This stimulus was continued upto 2 to 3 seconds. The stimulus was applied immediately advancing the site selector ring further than the previous one. This sequence was repeated with setting of site selector to its upper position than the previous one. Successful emission of electro-ejaculated semen from buck was obtained when the selector was set at 1 to 4 ring and when a maximum of five stimuli within time limit of 3 to 5 seconds were given. By operating the electro-ejaculator in the above way semen was collected in a collecting tube through funnel held by hand under the penis of the buck (Fig. 2).

Frequency and number of ejaculates

A total of 120 ejaculates were collected from ten experimental bucks at the rate of ejaculation twice in a week for six weeks from each buck.

Biochemical analysis of semen/seminal plasma

Number of ejaculates considered for study of different biochemical parameters

Out of 12 ejaculates of each buck, 4 ejaculates were utilised for the estimation of initial fructose content of semen and sodium and potassium content of seminal plasma,

4 ejaculates were utilised for the estimation of inorganic phosphorus and protein content of seminal plasma and the remaining 4 ejaculates were utilised for studying Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT) and Acid and Alkaline phosphatase (ACP and AKP activities in seminal plasma.

Separation of seminal plasma from neat semen

A certain volume of semen was taken in a centrifuge tube and the tube was centrifuged at 3500 r.p.m. for half an hour. This facilitated the separation of seminal fluid from neat semen. The separated seminal plasma was then removed completely for estimation of different biochemical parameters under study and was kept in the refrigerator for use.

Initial fructose content of semen

The initial fructose content of semen was estimated according to the conventional method followed by Mann (1948) as modified by Bishop et al. (1954). Soon after the collection of semen, 0.1 ml of semen was dropped in a tube containing 0.9 ml of distilled water. For deproteinisation of semen, 2 ml of each of 2% zinc sulphate and N/10 sodium hydroxide were added The sample was heated in a boiling water bath. It was then cooled and filtered. 2 ml of this filtrate was taken in a

15 ml tube. In another tube, 2 ml of fructose solution containing 0.2 mg of fructose (0.1 mg/ml) was taken. 2 ml of distilled water was taken in a third tube labelled as "blank". 2 ml of 0.1% alcoholic resorcinol and 6 ml of 30% HCl were added to each tube. All the three tubes were kept in the water bath at 80-85°C for 10 minutes and then cooled. The samples were compared against blank to find out the optical density of solution in the other two test tubes with the help of colorimeter.

Calculation

$$\frac{\text{Optical density (O.D) of unknown}}{\text{Optical density of standard}} \times 0.2 \times \frac{5}{0.1 \times 2} \times 100$$

= mg of fructose/100 ml of semen.

Estimation of Sodium and Potassium content of seminal plasma

These two biochemical parameters of seminal plasma were estimated according to the method adopted by Oser (1979). The seminal plasma diluted one in hundred with distilled water was introduced in the form of continuous fine spray into the non-luminous gas flame of a flame photometer. Air under pressure was used to maintain high burning temperature

and thereby keeping the luminosity of flame at a minimum. By using proper filter of different actions granting the emitted light, wave length characteristics of sodium or potassium as the case may be, was isolated. The ion concentration was determined by serial dilution of standard solution and standard curve was prepared accordingly. The standard graph was referred to determine sodium or potassium content and expressed as mEq/litre of seminal plasma.

Inorganic phosphorus content of seminal plasma

The method followed by Fiske and Subbarow (1925) was adopted to determine the level of this parameter.

Reagents

(1) Trichloracetic acid (10%)
(TCA)

10 gms of reagent grade TCA was dissolved in water and diluted to 100 ml.

(2) 10 N sulphuric acid

Carefully 450 ml of conc. sulphuric acid was added to 1300 ml of water. To check, 10 ml of the solution was diluted to 100 ml in a volumetric flask, mixed and titrated a 10 ml portion with standard 1N sodium hydroxide. From the titration result, the original solution was adjusted, if necessary, to make it exactly 10N.

(3) Ammonium molybdate solution

In an one litre volumetric flask, 25 gms of ammonium molybdate was taken. The same was diluted with 200 ml distilled water. Further, 300 ml distilled water and 300 ml 10N H_2SO_4 were added to it. Finally the volume was made upto 1 litre with distilled water.

(4) Sodium bisulphite solution (15%)

15 gms of sodium bisulphite was dissolved in 100 ml distilled water.

(5) Sodium sulphite solution (20%)

20 gms of sodium sulphite was dissolved in 100 ml distilled water .

(6) Aminonapthol sulphonic acid

In a glass stoppered cylinder, 195 ml of 15% sodium sulphite solution was taken and to it added 0.5 gm of 1, 2, 4 aminonapthol sulphonic acid and 5 ml of 20% sodium sulphite solution. It was then shaken to dissolve and stored in the refrigerator for use.

(7) Stock standard phosphate solution

In a volumetric flask of one litre, 0.351 gm of pure dry monopotassium phosphate was taken and 10 ml of 10N sulphuric acid were added to it. Then distilled water was

added and mixed properly to dissolve the reagent perfectly. Finally, it was made upto 1000 ml mark with distilled water. The solution was kept in refrigerator for use.

(8) Working standard phosphate solution

5 ml of stock standard solution was added to 15 ml of distilled water. 5 ml of the solution contained 0.1 mg of phosphorus.

Preparation of standard curve

Different concentrations of working standard phosphate solution which contained 0.02 mg of phosphorus per ml of the same i.e. 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06, 0.07, 0.08 were taken in different test tubes while triple distilled water instead of standard phosphate solution was taken in a test tube which served as blank. The volume of all the test tubes including the blank was made upto 5 ml using triple distilled water. Then 1 ml of molybdate solution was added and using 3.6 ml of tripple distilled water, the volume in each tube was made upto 9.6 ml. Lastly, 0.4 ml aminonapthol sulphonic acid was mixed, waited for 5 minutes and the colour intensities were read at 660 mμ in the systronic colorimeter while the density of blank was set at [0]. The different values were plotted on the graph paper and the standard curve was drawn.

Determination of Inorganic phosphorus

0.5 ml of seminal plasma was taken in a test tube and deproteinised with 9.5 ml of 10% TCA. It was then allowed to stand for 5 minutes and filtered. To 5 ml of filtrate, 1 ml of ammonium molybdate solution and 0.4 ml of aminonapthol sulphonic acid were added. Then the solution was made upto 10 ml by distilled water. The solution was mixed and was allowed to stand for 5 minutes.

In case of blank, 5 ml of triple distilled water was taken in a test tube. To it, 1 ml of ammonium molybdate solution and 0.4 ml of aminonapthol sulphonic acid were added and mixed thoroughly. The tubes were allowed to stand for 5 minutes. The colour intensity was read in a systronic colorimeter at 660 mμ wave length. The optical density (O.D.) of each sample was compared with O.D. of standard curve. The amount of inorganic phosphorus of seminal plasma was expressed as mg%.

Total protein content of seminal plasma

To determine the total protein content (g/100 ml) of seminal plasma the method as described by Wooton (1974) was followed.

Reagents

(1) Biuret reagent

9 gm of sodium-potassium tartarate was dissolved in 500 ml of 0.2 N sodium hydroxide solution in 1000 ml volumetric flask. 3 gm of copper sulphate ($CuSO_4$, $5H_2O$) and 5 gm of potassium iodide were then added and dissolved. Finally, volume was made upto the mark with 0.2 N sodium hydroxide solution.

(2) Bovine serum albumin

200 mg of bovine serum albumin was dissolved in 10 ml of distilled water.

Preparation of standard curve

Ascending concentrations of bovine serum albumin was taken separately in different test tubes like 2, 4, 6, 8, 10, 12, 14 mg and a blank with distilled water were taken. The different reagents were added according to standard procedure and the final colour was measured colorimetrically (Systronic's colorimeter) at 540 mμ wave length. The optical density was plotted against the concentration of bovine serum albumin and standard curve was thus drawn.

Procedure of estimation

0.2 ml of seminal plasma was taken in a test tube. 2.8 ml of distilled water was then added and mixed well.

A blank tube with 3 ml of distilled water was also taken. 5 ml of biuret reagent was added to each tube and mixed well. The colour developed was measured after 30 minutes in a colorimeter (Systronic's colorimeter) at 540 mμ wave length. The optical density thus obtained from different samples was then compared with optical density of standard curve and expressed as gm/100 ml of seminal plasma.

Transaminases of seminal plasma

The levels of glutamic oxaloacetic and glutamic pyruvic transaminase (unit/ml) i.e. GOT and GPT of seminal plasma were determined following the method of Yatzidis (1960).

Reagents

(1) Aspartate transaminase

(0.2M D-L aspartic acid and 0.002M)
-Keto glutaric acid

This substrate was prepared by adding 2.66 gm D-L aspartic acid 0.03 gm keto glutaric acid and 2.00 gm of dipotassium hydrogen phosphate in distilled water and volume of 100 ml was made.

(2) Alanine transaminase

(0.2 M D-L alanine and 0.002 M -Keto glutaric acid)

D-L alanine 1.78 gm, -Keto glutaric acid 0.03 gm and dipotassium hydrogen phosphate 2.00 gm were added to distilled water and the volume was made upto 100 ml.

(3) Aniline citrate reagent -

20 gms citric acid was dissolved in 20 ml distilled water and equal volume of redistilled aniline was added for the preparation of aniline citrate reagent. Each time freshly prepared aniline citrate reagent was used. The reagent was diluted with distilled water at the ratio of 1:5.

(4) Dinitrophenyl hydrazine reagent (0.002M)

2,4, Dinitrophenyl hydrazine 0.04 gm and concentrated hydrochloric acid 5 ml were added to 100 ml distilled water.

The reagent was diluted in the HCl with 15-20 ml distilled water with vigorous shaking and then made upto 100 ml with distilled water.

(5) Aqueous solution of sodium hydroxide (0.75M)

35 gm of sodium hydroxide was dissolved in 1000 ml of distilled water.

(6) Stock pyruvic acid solution (1%)

This solution was prepared by dissolving 0.625 gm of sodium pyruvate in 50 ml distilled water.

(7) Working standard of pyruvic acid solution

The stock standard of pyruvic acid solution was diluted with distilled water at the ratio of 1:99 so that 1 ml of working standard solution contained 100 µg of pyruvic acid.

Preparation of standard curve

Ascending concentrations of working standard were taken separately in different test tubes like 10 µg, 20 µg,

40 µg, 60 µg, 100 µg and a blank with distilled water was taken. The different reagents were added to each tube according to standard procedure and the final colour was measured by means of systronic double celled colorimeter at 500 mµ wave length. The optical density was plotted against the concentrations of pyruvic acid and a standard curve was thus drawn.

Procedure of estimation

To 0.8 ml of saline in a test tube, 0.2 ml of seminal plasma was added with a pipette and the contents were mixed. Then 0.5 ml of substrate for GOT or GPT was added and placed in water bath for one hour at 37°C. Immediately after incubation, 0.5 ml of the diluted aniline citrate was pipetted into it to stop the enzymatic reaction. 0.5 ml dinitrophenyl hydrazine was then added and shaken vigorously for 5 minutes. Exactly after 5 minutes, 3.0 ml of 0.75 M sodium hydroxide was added and mixed. The colour developed was measured after 30 minutes in systronic double celled colorimeter at 500 mµ. The blank was a mixture of reagents prepared in the same way as that of sample, except that the enzymatic preparation was replaced with saline. Each test sample was followed by a control sample but the enzyme activity was not allowed and that was achieved by adding aniline citrate before mixing

enzyme preparation. The optical density thus obtained from different samples was then plotted on the previously prepared standard curve and the concentration of pyruvic acid thus obtained was expressed as unit/ml of seminal plasma.

Acid and alkaline phosphatase (BU/100 ml) of seminal plasma

This enzymatic parameter of seminal plasma was estimated as per the method followed by Hawk et al. (1965).

Reagents

(1) Alkaline phosphatase substrate

In a 100 ml volumetric flask, successively 3 ml of petroleum ether, about 80 ml of distilled water, 0.5 gm of sodium glycerophosphate, 0.29 gm of sidium diethyl barbiturate and water were introduced and it was made upto 100 ml with distilled water and stored in the refrigerator for use.

(2) Acid phosphatase substrate

This was prepared like that of the alkaline phosphatase substrate except that 5 ml of 1N acetic acid was added. pH was adjusted to 5.0.

(3) 30% Trichloracetic acid (TCA)

30 gm analytical reagent grade TCA was taken in a 100 ml volumetric flask and dissolved into 100 ml of distilled water.

(4) Ammonium molybdate solution

In an one litre volumetric flask, 25 gm of ammonium molybdate was taken. It was diluted with 200 ml distilled water and again 300 ml of 10N H_2SO_4 was added to it. Finally, the volume was made upto one litre with distilled water.

(5) Sodium bisulphite solution (15%)

15 gms of sodium bisulphite was dissolved in 100 ml distilled water.

(6) Sodium sulphite solution (20%)

20 gm of sodium sulphite was dissolved in 100 ml of distilled water.

(7) Aminonapthol sulphonic acid

In a glass stoppered cylinder, 195 ml of 15% sodium sulphite solution was taken and to it added 0.5 gm of 1, 2, 4 aminonapthol sulphonic acid and 5 ml of 20% sodium sulphite solution. It was then shaken to dissolve and stored in the refrigerator.

Standard curve for inorganic phosphorus was utilised for the estimation.

Procedure of estimation

9 ml of alkaline or acid phosphatase substrate was taken separately in test tubes and placed in a water bath at 37°C until the fluid reached incubation temperature. 1 ml of seminal plasma was mixed with it and incubated for one hour. After incubation, 2 ml of 30% TCA was mixed with it and allowed to stand for 2 minutes. Then, it was filtered through a low ash filter paper. Each test sample was followed by a control sample. Control sample contained all the reagents of test sample but the enzyme activity was not allowed and this was achieved by adding 30% T.C.A. before mixing enzyme preparation.

Now, 5 ml of each filtrate of both control and test were taken into 2 separate test tubes.

Inorganic phosphorus, thus liberated from the enzymatic activity of seminal plasma was measured by systronic colorimeter at 660 mμ wave length. The calibrated standard curve for inorganic phosphorus was utilized for measurement of inorganic phosphorus liberated during enzymatic activity and expressed as BU/100 ml of seminal plasma.

Statistical analysis

Statistical analysis of data obtained in the experiment were analysed with the help of 'Micro-system' analysis following the method of Snedecor and Cochran (1968).

Chapter: IV

RESULTS AND DISCUSSION

Initial fructose

The mean value of initial fructose content (mg/100 ml) of semen in Black Bengal buck was found to be 568.64 ± 107.34 (Table-2) while Fig. 3 indicated buckwise variation of this seminal attribute.

Analysis of variance as revealed from Table-3 clearly indicated that there was a highly significant variation of initial fructose concentration between different bucks.

The present finding of mean value was in agreement with that of Mann (1964) who reported that in his work initial fructose content ranged from 300 to 900 mg% in buck semen. The levels of initial fructose concentration as worked out by different workers in different breeds of buck other than Black Bengal buck were as follows.

Patil (1970) found mean initial fructose level of 611.94 mg/100 ml of semen in Malbari bucks. Varshney et al.

Table - 2. Mean values (± SE) of seminal biochemical and enzymatic constituents in Black Bengal buck

Constituents	Mean values ± SE	Number of observations
Initial fructose content (mg/100 ml) of semen	569.64 ± 107.34 (464.28 - 714.28)	40
Sodium content (mEq/litre) of seminal plasma	75.18 ± 2.41 (60.24 - 83.47)	40
Potassium content (mEq/litre) of seminal plasma	63.65 ± 1.85 (54.15 ± 81.04)	40
Inorganic phosphorus content (mg/100 ml) of seminal plasma	11.76 ± 0.36 (10.11 - 13.54)	40
Total protein content (g/100 ml) of seminal plasma	5.21 ± 0.16 (4.05 - 6.36)	40
GOT (unit/ml) of seminal plasma	130.07 ± 2.22 (112.17 - 143.30)	40
GPT (unit/ml) of seminal plasma	15.38 ± 1.16 (10.30 - 22.31)	40
Acid phosphatase (BU/100 ml) of seminal plasma	81.97 ± 2.14 (70.41 - 102.29)	40
Alkaline phosphatase (BU/100 ml) of seminal plasma	228.79 ± 6.93 (187.92 - 263.52)	40

Figures in parentheses indicate range

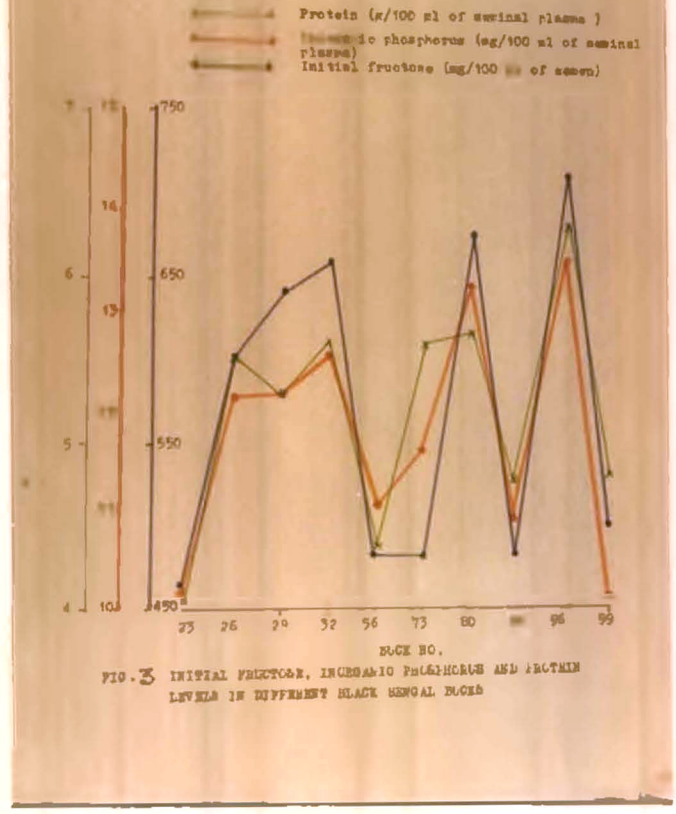

FIG. 3 INITIAL FRUCTOSE, INORGANIC PHOSPHORUS AND PROTEIN LEVELS IN DIFFERENT BLACK BENGAL BUCKS

(1977) reported the initial fructose content 1294.50 mg/100 ml of semen of Barbari buck. Mittal and Ghosh (1980) estimated fructose level as 795.92 ± 10.72 mg% in parbatsar bucks. El-Sayed et al. (1983) reported fructose level in whole semen of Baladi bucks as 806.11 ± 337.97 mg%. Markandeya and Pargaonkar (1989) estimated the initial fructose content as 496.511 ± 32.84 mg/100 ml of semen in Osmanabadi bucks.

On comparision between the present finding and those of above workers in breeds other than Black Bengal bucks, it will be revealed that the initial fructose concentration was higher in the other breeds of buck except in Osmanabadi buck where the same was lower compared to the Black Bengal buck under study. This variation between Black Bengal bucks and other breeds of bucks might be due to breed characteristics.

Table - 3 Analysis of variance of initial fructose

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	342981.70	38109.08	3.31**
Error	30	345660.66	11522.02	

** Significant at 1% level i.e. P < 0.01

Markandeya and Pargaonkar (loc.cit) working with Osmanabadi bucks found significant variation of initial fructose concentration within individuals of the same breed and similar result was obtained with Black Bengal bucks.

Sodium and Potassium

From Table-2, it will be evident that the mean level of sodium was 75.18 ± 2.41 mEq/litre of seminal plasma in Black Bengal buck while the same of potassium was 63.65 ± 1.85 mEq/litre of seminal plasma (Table-2). The buckwise variations of these two seminal attributes were represented graphically in Fig.4.

By analysis of variance (Table-4 and Table-5) it was observed that these two seminal characteristics had highly significant variations between different Black Bengal bucks under study.

There are few reports on the bulk cations namely sodium and potassium in the semen of bucks. Bulk cations play a major role in the sperm cell metabolism. Cations in semen reflects the functional status of acessory sex glands and metabolic activity of spermatozoa (Mann, 1964). Cations play role in biochemical reactions of sperm cells in the natural medium viz. seminal plasma and this has bearing on its

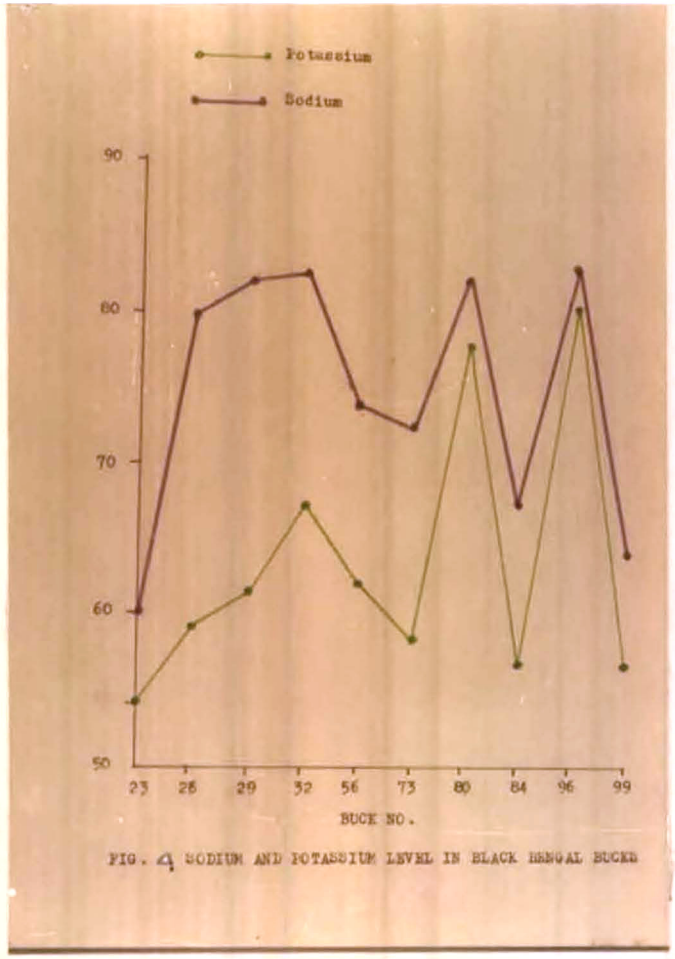

FIG. 4 SODIUM AND POTASSIUM LEVEL IN BLACK BENGAL BUCKS

Table - 4 Analysis of variance of Sodium

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	2486.10	276.23	47.50**
Error	30	174.45	5.81	

** Significant at 1% level i.e. P < 0.01

Table - 5. Analysis of variance of Potassium

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	3068.96	341.00	99.24**
Error	30	103.08	3.44	

** Significant at 1% level i.e. P<0.01

livability and keeping quality which in turn have bearing on fertility (Rattan et al., 1972).

Varshney et al. (1977) estimated the values of sodium and potassium in the seminal plasma of buck semen as 178.05 ± 4.93 and 184.26 ± 1.98 mg% respectively. These values were not in agreement with the present finding which were lower. On the contrary, the levels of sodium and potassium as found in the Black Bengal buck were in close proximity with the findings of Markendeya and Parçaonkar (1990) who reported the semen sodium and potassium concentration in Osmanabadi buck seminal plasma as 77.47 and 74.58(mç%) respectively.

While working with bull semen, different workers reported higher values of sodium and potassium concentrations in seminal plasma and the following were the findings of different workers with bull seminal plasma. Nesmejanova (1938) reported the averaçe concentration of sodium and potassium (mg/100 ml) 278 and 228 respectively. The concentrations of sodium and potassium in seminal plasma as reported by Yaasen et al. (1967) were 1768 and 1095 mcg/ml respectively. Singh et al. (1970) estimated the sodium and potassium concentration as 186.89 ± 10.90 and 101.60 ± 4.45 mg% respectively. The higher values obtained in the bull seminal plasma and also in the other breeds of buck compared to the values

in the seminal plasma of Black Bengal buck might be due to species and breed variation.

Yassen et al. (1967) and Roy choudhury and Sadhu (1976) reported that the sodium and potassium concentrations varied significantly between bulls. Nair and Nandakumaran (1982) reported that the differences between bucks in sodium and potassium levels were highly significant. The present result in this respect with Black Bengal buck corroborated with the findings of above workers.

Inorganic phosphorus

Table-2 indicates that the mean level of inorganic phosphorus in the seminal plasma of Black Bengal buck was 11.76 ± 0.36 mg/100 ml.

By analysis of variance (Table-6) it was found that the differences in the level of inorganic phosphorus among Black Bengal bucks under study were highly significant. Fig-3 indicated buckwise variation of this seminal characteristic.

Varshney et al. (1977) found the level of inorganic phosphorus in seminal plasma of Barbari buck to be 10.59 mg/ 100 ml. Pandey et al. (1982) while working with Saanen and Barbari bucks reported level of 12.503 and 12.278 mg/100 ml

Table - 6 Analysis of variance of Inorganic phosphorus

Source of Variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	52.82	5.8	41.43**
Error	30	4.19	0.14	

** Significant at 1% level i.e. P < 0.01.

of seminal plasma respectively. Markandeya and Parçaonkar (1990) estimated the inorganic phosphorus in Osmanabadi buck semen and this was 13.07 mg/100 ml of seminal plasma. The mean level of inorganic phosphorus obtained in the Black Bengal buck was more or less the same as obtained by the above workers in Barbari and Osmanabadi buck but higher level was obtained in Saanen buck as reported by Pandey et al. (1982).

Pandey et al. (1982) working with Barbari and Sannen bucks reported that differences in the levels of inorganic phosphorus in buck semen of different breeds were found to be highly significant (P < 0.01) while the differences among bucks of the same breed were found to be non-significant.

On the contrary, in the present finding, highly significant differences in the level of this seminal characteristic were obtained between different bucks in Black Bengal breed. Possibly, this might be due to variation of breed, managemental practices and environmental factors.

Total protein

The mean value of total protein in seminal plasma of Black Bengal buck was 5.21 ± 0.16 g/100 ml (Table-2) and the buckwise variation was depicted in Fig.3. Analysis of variance (Table-7) showed highly significant variation of this parameter between different bucks under study.

Table - 7 Analysis of variance of total protein

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	17.25	1.92	64**
Error	30	0.81	0.03	

** Significant at 1% level i.e. P < 0.01

Very limited works were undertaken to determine the level of this parameter in the semen picture of different species of animals including bucks on studying the available report of works in this respect in bucks it was observed that Dunder et al. (1983) studied this parameter in the seminal plasma of Angora bucks and found that the concentration of protein was 2.99 ± 0.28 g/100 ml while Markandya and Parroonkar (1989) reported level of protein as 3.54 ± 0.25 g/100 ml of seminal plasma in Osmanabadi buck. The findings of the above workers were of lower values than that obtained in the present study with Black Bengal buck.

The protein content of seminal plasma was found to be variable in bull and buffalo bull seminal plasma. Baruiana et al. (1975) reported that sperm protein ranged from 0.31 to 0.49 g% in bull semen. Saxena and Tripathi (1981) estimated the total protein content which was 8.50 ± 0.42 g/100 ml of semen. Yakub et al. (1983) found 2.8 ± 0.13 g/100 ml of protein in seminal plasma of Nili-Ravi buffalo semen while in Surti buffalo bull the value was found by Nema et al. (1983) as 7.31 ± 0.3 g%. Dhami and Kodagali (1987) estimated the same in surti buffalo bull seminal plasma as 4.42 ± 0.07 g%. It will be evident that the present finding with Black Bengal buck was higher than the same obtained by Baruiana et al. (1975) and Yakub et al. (1983) while the level as worked out

in the present study was lower compared to the same reported by Saxena and Tripathi (1981) and Nema et al. (1983). This difference in the levels might be due to species and breed variation. References in respect of significant variations of this parameter of seminal plasma within individuals of the same breed of buck were not available but Yakub et al. (1983) found significant variations in the protein content of seminal plasma between bulls. The present finding with Black Bengal buck was in conformity with the finding of above workers.

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)

The mean concentrations of GOT and GPT activities (unit/ml) of seminal plasma in Black Bengal buck were 130.07 ± 2.22 and 15.38 ± 1.16 respectively (Table-2). Buckwise variations of these two seminal attributes were graphically presented in Fig. 5.

Analysis of variance (Table-8 and Table-9) clearly indicated highly significant variations of these two seminal enzymatic constituents between different Black Bengal bucks.

It has been claimed by many workers that GOT activity in seminal plasma is significantly correlated with the various semen characteristics. Levels of GPT is significantly

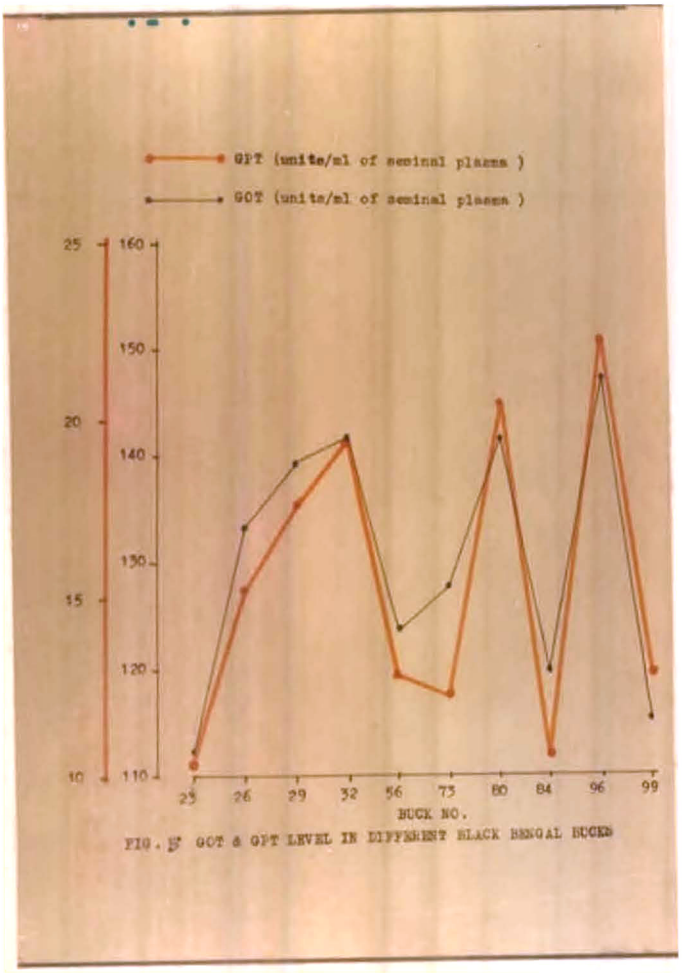

FIG. 5 GOT & GPT LEVEL IN DIFFERENT BLACK BENGAL BUCKS

Table - 8 Analysis of Variance of GOT

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	5533.54	614.84	124.74**
Error	30	147.87	4.93	

** Significant at 1% level i.e. P/ 0.01

Table - 9 Analysis of variance of GPT

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	681.21	75.69	55.65**
Error	30	40.98	1.36	

**Significant at 1% level i.e. P / 0.01

correlated with concentration, percent live and percent abnormal cells. Transaminases are mostly intra-cellular and have an important role in the catabolism of glutamate by bovine spermatozoa.

The available literatures in respect of studies of these two parameters of seminal plasma indicated that very little work was done in the sphere of buck and no work was undertaken in Black Bengal buck. Under Indian condition Varshney et al. (1978) studied the GOT and GPT activities in the seminal plasma of adult Barbari bucks. They found the levels of GOT and GPT to be 176.23 ± 5.16 and 16.67 ± 0.67 units/ml in the seminal plasma. Their result indicated that GOT value was much higher than GPT value and the GOT, GPT ratio was found to be 11:1 in buck semen. Present study showed the GOT, GPT ratio in Black Bengal buck seminal plasma to be 9:1 which was in close accordance with the finding of Varshney et al. (loc.cit.) and the finding of Flipse (1960) in case of bull seminal plasma, but differs slightly from a ratio of 19:1 reported in bull seminal plasma by Roussel and Stallcup (1965). Chauhan and Srivastava (1973) estimated GOT and GPT in seminal plasma of buffalo bulls and the values were 166.72 ± 14.08 and 34.56 ± 4.57 units/ml respectively which did not conform with the present finding obtained in the Black Bengal buck under study. Gregoire et al. (1961)

reported higher value of GOT in the bull. Akusu et al. (1984) found that GOT activity of the seminal plasma was significantly lower for electro-ejaculated semen than the semen collected by artificial vagina. The low value obtained in the present study might be due to the fact that semen from Black Bengal buck under study was collected by electro-ejaculation method.

Gonzalez-Rubiera et al. (1974) reported that there were no significant variation between the breeds (Holstein-Friesian and Zebu bulls) in GOT values but Holstein-Friesian bulls had significantly higher GPT values than Zebu bulls. But no work was done to find out whether GOT and GPT values differed significantly between individuals within breed. Therefore, finding of significant variation between different Black Bengal bucks could not be compared.

Acid phosphatase (ACP) and Alkaline phosphatase (AKP)

The mean level of ACP and AKP (BU/100 ml) in the seminal plasma of Black Bengal buck was tabulated in Table-2 and these were found to be 81.97 ± 2.14 and 228.79 ± 6.93 while buckwise variation of these two seminal enzymatic constituents were graphically represented in Fig.6.

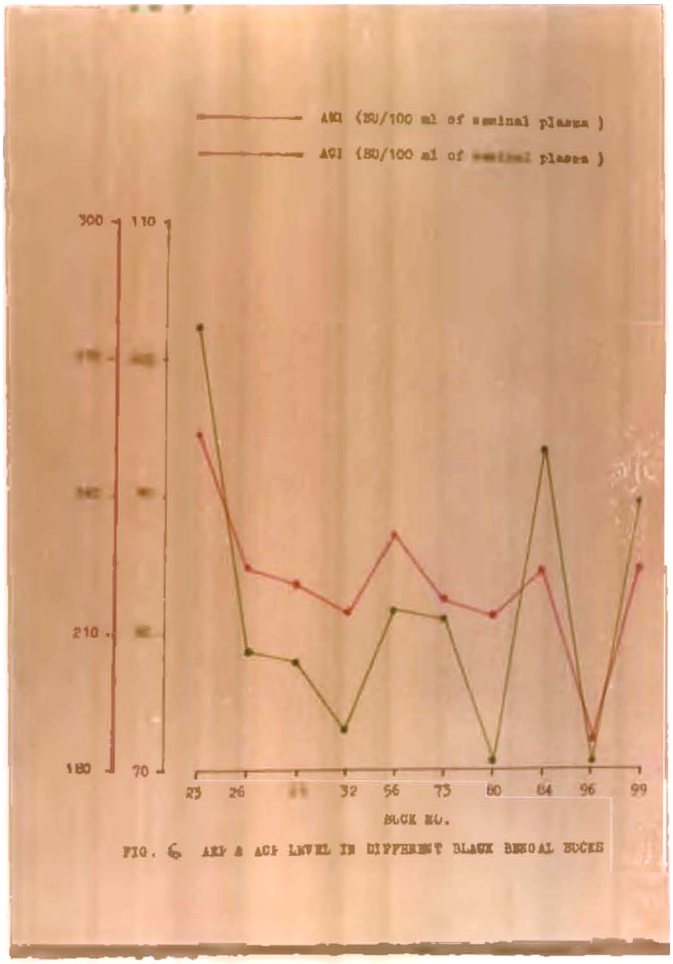

FIG. 6 AKI & ACI LEVEL IN DIFFERENT BLACK BENGAL BUCKS

Analysis of variance (Table-10 and Table-11) revealed a highly significant variation of ACP and AKP in the seminal plasma between Black Bengal bucks under study.

ACP and AKP like other seminal enzymatic constituents are concerned with oxidative metabolic pathway and provide direct energy for sperm cell metabolism (White, 1958). Semen phosphatases play important role in sperm metabolism. It has been claimed by many workers that these enzyme activities are subjected to breed difference (Roy et al., 1960), species (Bell and Lake, 1962).

Available references in respect of the studies of the level of these two seminal parameters indicated that the only work in buck semen was conducted by Varshney et al. (1978). They, working with semen of Barbari buck, found ACP and AKP levels as 166.11 ± 70 and 504.45 ± 25.82 (BU/100 ml) which were more or less double the levels of ACP and AKP obtained in the present study with the seminal plasma of Black Bengal buck. This difference in level might be due to the fact that the present study in Black Bengal buck was with seminal plasma while Varshney et al. (loc.cit.) considered whole semen.

The goat semen was characterised by a three times higher alkaline phosphatase activity as compared to acid phosphatase. The significance of so conspicuously high alkaline phosphatase is not clear.

Table - 10 Analysis of variance of ACP

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	3946.60	438.51	75.22**
Error	30	174.89	5.83	

** Significant at 1% level i.e. P < 0.01

Table - 11 Analysis of variance of AKP

Source of variation	d.f.	S.S.	M.S.S.	F
Between Bucks	9	13548.02	1505.34	31.29**
Error	30	1443.41	48.11	

** Significant at 1% level; i.e. P < 0.01

Asghar et al. (1983) viewed that acid and alkaline phosphatase activity in cattle seminal plasma showed no significant variation among bulls or ejaculates. On the contrary, in the present study, highly significant variations of level of acid and alkaline phosphatase between different Black Bengal bucks under study were obtained.

Correlations between various biochemical and enzymatic constituents in semen/seminal plasma

Table-12 represented values of simple correlation coefficient between biochemical and enzymatic constituents in semen/seminal plasma of Black Bengal bucks under study. Studies in respect of correlation of seminal parameters of buck semen were not undertaken so far as revealed from the study of available references.

It will be evident from the above table that the initial fructose content had highly significant and negative correlations with ACP (-0.8416) and AKP (-0.7816) and highly significant and positive correlations with total protein (0.8037), inorganic phosphorus (0.9152), Na (0.8833), K (0.8480), GOT (0.9380) and GPT (0.9830).

As no such study in the above respect was undertaken with buck semen, this finding could not be compared.

There was significant positive correlation between sodium and potassium (0.7486). Potassium content was positively and significantly correlated with initial fructose (0.8480), total protein (0.7407), GOT (0.8311) and GPT (0.9060). But the same was negatively and significantly correlated with ACP (-0.8095) and AKP (-0.8490). Nair et al. (1982) viewed

Table - 12 Correlation coefficient of seminal biochemical and enzymatic constituents of Black Bengal Bucks

Constituents	Total protein	Potassium	GOT	GPT	ACP	AKP	Initial fructose	Sodium
Inorganic phosphorus (mg/100 ml)	0.0040**	0.0014**	0.9769**	0.9199**	-0.9151**	-0.0418**	0.9152**	0.9347**
Total protein(g/100ml)		0.7407**	0.0720**	0.0108**	-0.0444**	-0.9179**	0.0037**	0.7989**
Potassium (mEq/litre)			0.0311**	0.9060**	-0.0095**	-0.0490**	0.0480**	0.7486**
GOT (Unit/ml)				0.9395**	-0.9369**	-0.0374**	0.9380**	0.9707**
GPT (Unit/ml)					-0.0078**	-0.0294**	0.9030**	0.0026**
ACP (BU/100 ml)						0.0336**	-0.0416**	-0.9535**
AKP (BU/100 ml)							-0.7016**	-0.7537**
Initial Fructose (mg/100 ml)								0.0033**
Sodium (mEq/litre)								

** Significant at 1% level i.e. P < 0.01

that there was a positive correlation ($P < 0.01$) between the levels of sodium and potassium in bull seminal plasma. The same trend was observed in the present study. The potassium cation is directly related with the maintenance of excitability of sperm, optimum seminal pH. This is one of the main electrolytes influencing sperm viability.

The correlation coefficient of different biochemical and enzymatic constituents indicated that total protein content was positively and significantly correlated with other constituents except ACP and AKP with which it was negatively and significantly correlated. Dhami and Kodagoli (1987) working with semen of Surti buffalo bulls observed that total protein was significantly correlated with GOT (0.589). Similar trend was observed in the present study with Black Bengal buck semen while Dabas et al. (1982) observed non-significant correlations of total protein with GOT and GPT in buffalo semen. Kaker and Arora (1976) reported significantly positive correlation between total protein and GOT, GPT, ACP and AKP.

From correlation studies, it was observed that the inorganic phosphorus content was significantly and positively correlated with the other seminal constituents under study except ACP and AKP with which it was significantly and negatively correlated. In absence of available references in

respect of this study with semen of buck and other species, the present findings could not be compared.

The correlation coefficient values of GOT and GPT enzymes indicated that these two enzymes had significant and positive correlations with initial fructose, total protein, sodium, potassium, inorganic phosphorus and had negative and significant correlations with ACP and AKP while they were significantly and positively correlated among themselves. On the contrary, Dhami and Kodagali (loc.cit.) found that GOT activity in seminal plasma of Surti buffalo bull had a highly significant and negative correlation with GPT (-0.830).

Kaker and Arora (1976) reported significantly positive correlations of both GOT and GPT with AKP and ACP in crossbred bulls and this finding was not in agreement with the finding of the present study where significantly negative correlations were obtained with seminal plasma of Black Bengal buck. Debas et al. (1982) reported positive correlation of seminal GPT with GOT in buffalo bulls which was in conformity with the present study.

ACP and AKP had highly significant and positive correlation between themselves (0.8336) and the other correlations with ACP and AKP were negative and significant. Dhami

and Kodaçali (loc.cit.) also found that seminal plasma AKP of Surti buffalo bull had no significant correlations with total protein, GOT, GPT, sodium and potassium. In their work ACP activity was significantly and positively correlated with sodium (0.722) and potassium (0.595) and this finding was not in conformity with the findings of the present study. Like the present finding, Kaker and Arora (loc.cit.) also reported significant inter-relationship between AKP and ACP.

Chapter : V

SUMMARY AND CONCLUSION

Semen of ten Black Bengal bucks Capra hircus aged between 3 years and 4 years having weight range between 16 kg and 22 kg maintained under identical conditions of housing, feeding and management were considered for the present study to know the normal levels of certain seminal biochemical and enzymatic constituents. The seminal characteristics considered were (a) initial fructose content (mg/100 ml) of semen, (b) sodium and potassium content (mEq/litre) of seminal plasma, (c) Inorganic phosphorus content (mg/100 ml) of seminal plasma, (e) Glutamic oxaloacetic transaminase and Glutamic pyruvic transaminase (unit/ml) of seminal plasma and Acid and alkaline phosphatase (BU/100 ml) of seminal plasma. 40 ejaculates were considered to determine the level of each of the above seminal parameters.

The mean value of initial fructose content (mg%) of semen was found to be 569.64 $\pm$ 107.34 having buckwise variation from 464.28 to 714.28. Analysis of variance indicated that there were significant ($P < 0.01$) variations between bucks.

Sodium and potassium concentration of seminal plasma (mEq/litre) showed on an average level of 75.18 ± 2.41 and 63.65 ± 1.85, the range being from 60.24 to 83.47 and from 54.15 to 81.04 respectively. It was observed by analysis of variance that the buckwise variations were highly significant ($P < 0.01$).

The average value of inorganic phosphorus content of seminal plasma (mg%) was 11.76 ± 0.36 and the range was from 10.11 to 13.54. By analysis of variance it was revealed that the level of inorganic phosphorus differed significantly ($P < 0.01$) between bucks.

The mean value of total protein content (g%) of seminal plasma was 5.21 ± 0.16, the range being from 4.05 to 6.36. Analysis of variance indicated highly significant ($P < 0.01$) variations between different bucks.

GOT and GPT (unit/ml) activities in seminal plasma showed an average value of 130.07 ± 2.22 with a range from 112.17 to 143.30 and 15.38 ± 1.16 with a range from 10.30 to 22.31 respectively. The variations in the level of these two seminal enzymatic constituents differed significantly ($P < 0.01$) between bucks as revealed by analysis of variance.

The mean level of acid and alkaline phosphatase (EU%) activities in seminal plasma was found to be 81.97 ± 2.14 and

228.79 ± 6.93, the range being from 70.41 to 102.29 and from 187.92 to 263.52 respectively. Analysis of variance clearly indicated a highly significant variation of these two seminal enzymatic constituents between different Black Bengal bucks.

Correlations between various seminal biochemical and enzymatic constituents taken into consideration were studied. It was observed that initial fructose content was significantly and positively correlated with total protein, inorganic phosphorus, sodium, potassium, GOT and GPT while the same significantly and negatively correlated with acid and alkaline phosphatase.

There was significant positive correlation between sodium and potassium content of seminal plasma. Potassium had significant and positive correlations with initial fructose, total protein, GOT and GPT but sodium and potassium were significantly and negatively correlated with ACP and AKP.

Total protein was positively and significantly correlated with other seminal constituents except with ACP and AKP where significant and negative correlations existed.

Inorganic phosphorus had significant and positive correlations with other seminal constituents under study except with ACP and AKP where it was found significant and negative correlations.

There was highly significant and positive correlation between ACP and AKP and the other correlations with ACP and AKP were negative and significant.

It may be concluded that the levels of all seminal biochemical and enzymatic constituents of Black Bengal bucks as studied in the present investigation did not follow the same trend as observed with other breeds of buck and therefore requires detailed investigation to find out the definite reasons for such variation between different breeds of buck which were taken into consideration so far by different workers in their studies.

Chapter: VI

FUTURE SCOPE OF RESEARCH

Owing to limitation of time, facilities and other handicaps, the present investigation with Black Bengal buck semen, though exhaustive, could not be completed in all respects.

Therefore, it may be appropriate to list some aspects which seem to be promising for the future scope of research as follows.

1. To study the biochemical and enzymatic constituents of semen with reference to freezability and fertility.

2. To investigate the physico-chemical constituents of semen and their interrelationship during different seasons.

3. To determine the critical levels of seminal biochemical and enzymatic constituents responsible for obtaining optimum fertility and conception.

4. To study whether the different ages and weights of animals affect the levels of different seminal biochemical and enzymatic constituents.

5. To compare the semen biochemistry of Black Bengal buck with that of other breeds of buck for assessing the extent of variations if any, in this respect.

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