"INFLUENCE OF SEMINAL IONIC CONCENTRATION ON PRESERVABILITY OF LARGE WHITE YORKSHIRE AND TAMWORTH X DESI (T & D) BOARS SEMEN"

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)

RUSA (SAMASTIPUR), BIHAR

In Partial fulfillment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(Animal Reproduction Gynaecology & Obstetrics)

By

Rajesh Kumar Ranjan

Registration No. - M/VOG/19/2005-2006.

DEPARTMENT OF ANIMAL REPRODUCTION GYNAECOLOGY & OBSTETRICS

BIHAR VETERINARY COLLEGE

PATNA - 800 014

2007

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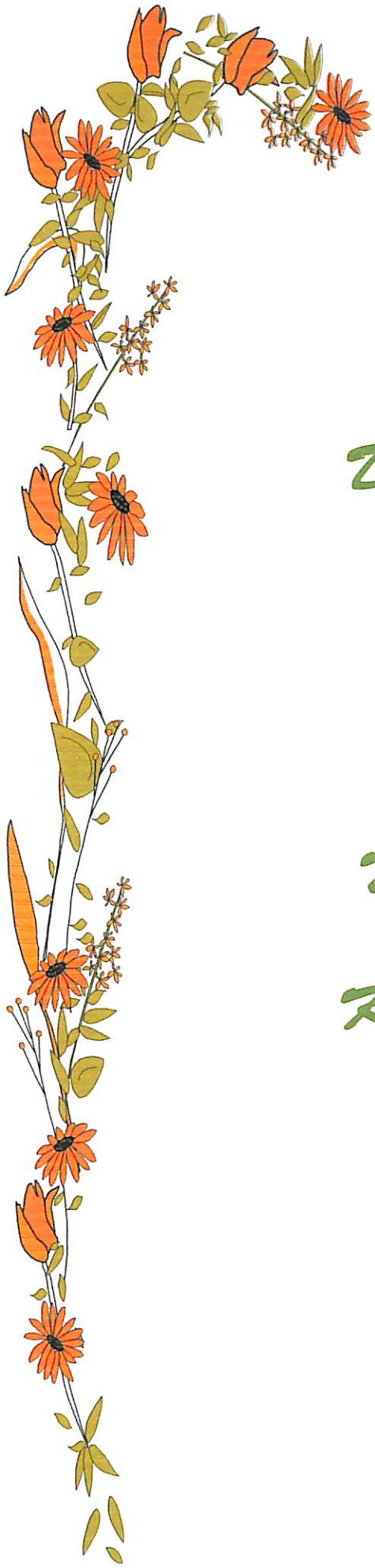

Dedicated to

My

Respected

Mother Jay

Kumari Devi

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CERTIFICATE-I

This is to certify that the thesis entitled ***"Influence of Seminal ionic concentration on preservability of large white yorkshire and Tamworth x Desi (T & D) boars semen".*** submitted in partial fulfillment of the requirements for the Degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** of the faculty of post-graduate studies, Rajendra Agricultural University, PUSA, Samastipur, Bihar is the record of bonafide research work carried out by **Dr. Rajesh Kumar Ranjan, Registration No.- M/VOG/19/2005-06,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

29/5/07

(R.P. Pandey)
Major Advisor

Endorsed :

29/5/07

(Chairman/Head of Department)

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BIHAR VETERINARY COLLEGE, PATNA- 14

RAJENDRA AGRICULTURAL UNIVERSITY, PUSA (SAMASTIPUR), BIHAR.

CERTIFICATE –II

We the undersigned members of the Advisory Committee of **Dr. Rajesh Kumar Ranjan, Registration No.- M/VOG/19/2005-06,** a candidate for the Degree of **Master of Veterinary Science** with Major in **Animal Reproduction, Gynaecology and Obstetrics,** have gone through the manuscript of the thesis and agree that the thesis entitled ***"Influence of Seminal ionic concentration on preservability of large white yorkshire and Tamworth x Desi (T & D) boars semen"*** may be submitted by **Dr. Rajesh Kumar Ranjan** in partial fulfillment of the requirements for the degree.

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DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS
BIHAR VETERINARY COLLEGE, PATNA- 14
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CERTIFICATE-III

This is to certify that the thesis entitled ***"Influence of Seminal ionic concentration on preservability of large white yorkshire and Tamworth x Desi (T & D) boars semen"*** submitted by **Dr. Rajesh Kumar Ranjan, Registration No.- M/VOG/19/2005-06,** in partial fulfillment of the requirements for the Degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, PUSA, Samastipur, Bihar was examined and approved on 29../...4../2008.

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CHAPTER - I

INTRODUCTION

INTRODUCTION

Pig industry is an emerging enterprise now a day. The problem of malnutrition resulting from shortage of quality food is likely to increase with the rapid increase in human population. The increased requirement could be achieved by increasing production of cereals and vegetables as well as production of large quantity of animal protein. The animal protein requirement can not be fulfilled by meat produced from cattle, sheep and goat only because of their lower prolificacy and prolonged generation gap. Pigs, being prolific breeder, can be a substantial alternative to meet demand of animal protein on account of its short generation interval, high prolificacy, high efficiency of food conversion, faster growth, high dressing percentage and low maintenance cost. Besides, that Pigs serve the best as scavengers of by-products and refuses, produced during production, processing and consumption of different human foods.

India posses about 14.14 million of Pigs which comes to about 1.5% of total world pig population with an annual growth rate of 0.91% (Souvenir, B.V. C., Patna, 2005). Bihar itself posses 0.67 millions pigs with an annual growth rate of 3.95% (17th Livestock census, 2003). However, about 90% of the total population are indigenous having slow growth rate,

lower feed conversion efficiency and small litter size and thus limits the magnitude of profitability.

Though, the practice of pig husbandry exist in India for centuries, its contribution to the national economy is very very less. Pig industry has remained undeveloped mainly due to religious taboo and prejudices. Pig rearing in our country is mainly in the hands of socially and economically weaker people of scheduled caste and scheduled tribes. Their unscientific breeding and unhygienic management are some contributing factors, which kept this industry in the primitive stage. Besides that, rearing of non descript type of pigs and that too without or little concentrate feed have lowered the profitability. Hence, up-gradation of local stock introducing superior germ plasm is the possible alternative to convert this enterprise profitable. During last two decades a great emphasis has been laid on the improvement of the productivity of pigs by implementing crossbreeding programme with exotic breeds of pig, having potentiality of large litter size, efficient feed conversion and high dressing percentage.

Artificial insemination programme in pigs has the primary objective of genetic improvement with maximum health control at least cost (Waltere *et al.* , 1984). Artificial insemination in pigs has shown encouraging result in protecting individual herd health by controlling infectious disease, particularly Brucellosis (Winterhalter, 1975). Besides disseminating the superior germplasm, artificial insemination

seems to be more economical in Batch farrowing. Following artificial insemination, early weaning and multiple farrowing have become common practice in some commercial swine herd of Europe.

The short storage life of spermatozoa and difficulties in detecting fertile period in Sows, are two main limitations in adopting artificial insemination at field level. The important factors which influence the preservability of semen for longer periods are Osmotic pressure, PH, buffering capacity and temperature. Among these factors, both organic and inorganic ions play a significant role.

Undoubtedly, the morphological evaluation of the semen (Phillips & Schoot, 1943, Orson & Victor, 1952, Eaton & Simmons, 1952) is helpful in assessing its quality to some extent, but mainly it is dependent upon chemical environment. The preservability and fertility is influenced directly or indirectly by various chemicals constituents present in the semen. Besides other chemical constituents, the importance of ionic concentration (Blackshaw, 1953, Salisbury & Cragle, 1956, Iritani & Nishikawa, 1964, Rattan *et al.*, 1972, Mc Grady *et al.*, 1974, Karagiannides 1976) has been found to influence the motility, preservability and fertilizing capacity of Bull, Ram, Buck and Boar Spermatozoa.

Phosphorus is one of the important mineral that take part in the sperm glycolysis and there by making energy available for sperms. High level of phosphorus is stimulatory to

fructolysis (Salisbury and Nakabayshi, 1957). High concentration of magnesium improves glycolysis and motility in the presence of Potassium. However, high level of calcium depress the motility, respiration and glycolysis of Boar spermatozoa during preservation. Zinc is an integral part of carbonic anhydrase and lactic dehydrogenase enzyme in the semen. Zinc appears to be involved in the nucleic acid metabolism, protein synthesis, carbohydrate metabolism etc. Copper helps to synthesize the phospholipids that form the spermatozoan membrane. Manganese has a role in the oxidation reaction and sperm motility.

The studies on minerals profile in Boar Seminal plasma in ecological condition of Bihar is limited. So, the present investigation has been designed with the following objectives :-

1. To study the effect of genetic variation in seminal characteristics of Large White Yorkshire and Tamworth x Desi (T & D) boars semen and its preservability under agro ecological condition of Patna.
2. To assess the Macro minerals (Ca, P, Mg) and Micro minerals (Zn, Cu, Co, Mn) concentration in seminal ejaculates as well as preserved semen of different durations of both Large White Yorkshire and T & D breeds of Boars.

****

REVIEW OF LITERATURE

(1) Semen characteristics :

Murthy (1977) studied physical characteristics of boar semen preservation and artificial insemination in swine. He recorded the mean values for semen characters were as follows : total volume of ejaculate, 243.9 ml; strained volume, 189.3 ml; Gel volume, 54.6 ml; initial sperm motility, 80.9%; % of live spermatozoa, 84.5; pH, 7.8; sperm concentration, 297.3 x 10^6/ml : % of head abnormality, 2.52 : % of mid piece abnormality, 0.47; and % of total tail abnormality, 1.90.

Gamcik (1981) recorded the highest sperm concentration in Winter (203.961 million/ml.) and lowest during summer (173.584) million/ml.).

Tamuli (1982) studied the seminal characteristics and artificial insemination in pigs. He recorded mean value of different semen characters as follows : Total volume of semen 219.74 ± 4.07ml; volume of gel mass 48.38 ± 2.07 ml; 0.02; volume of strained potion 171.31 ± 3.41 ml; pH 7.75 ± 0.02; concentration of spermatozoa 226.33 ± 6.06 ($x10^6$) per ml. of semen ; progressively motile spermatozoa 85.71 ± 0.57 percent and live spermatozoa 86.90 ± 0.49 percent.

Antonyuk *et al.* (1982) recorded the sperm motility as 76, 76, 75 and 74 percent during spring, summer, autumn and winter respectively. He also reported sperm concentration as

CHAPTER - II

REVIEW OF LITERATURE

151, 163, 155 and 154 million per ml. during spring, summer, autumn and winter respectively.

Michalski and Polanska (1983) reported that the age had significant effect on semen volume. Ejaculate volume was found increased from 98 ml. at 6 months to 203 ml. at 8 month age in Hampshire x polish large white boars. They further observed an increase in sperm motility from 65 and 67 percent at 6 month of age to 72 and 79 percent at 8 month of age, in polish large white and Hampshire x polish large white boars respectively.

Kuroman and Stachowicz (1984) recorded the ejaculate volume as 300, 303, 266 and 274 ml. in winter, spring, summer and autumn respectively in Yorkshire breed of boars.

Louda and Pavlik (1984) observed 70.4, 72.7 and 70.2 percent progressively motile sperm in Duroc, Yorkshire and Belgium Landrace boars respectively.

Dubiel *et al.* (1985) reported the sperm motility as 70, 70 and 70 percent in polish large white, Duroc and crossbred boars.

Bhuyan (1989) recorded mean values of different semen characteristics as follows : volume of gel mass 33.75 ± 2.53 and 20.81 ± 1.04 ml; volume of filtered portion 156.71 ± 9.65 and 107.31 ± 5.46ml; total volume of semen 191.31 ± 11.84 and 129.41 ± 6.42ml., progressively motile spermatozoa 78.88 ± 0.70 and 81.63 ± 1.30 percent, sperm concentration 239.33

± 17.55 and 257.92 ± 13.32 million per ml. in Hampshire and crossbred boars respectively.

Rao *et al.* (1991) evaluated the semen characteristics of native boars aged 14-24 months. The average value of different characters were as follows ; Total ejaculate volume 138.9 ± 3.67 ml; strained volume 108.94 ± 3.23 ml; gel volume 30.0 ± 1.66 ml; initial motility 83.31 ± 1.52 percent ; pH 7.74 ± 0.05; sperm concentration 283.01 ± 12.76 million per ml. and percent of live sperm 81.70 ± 1.43.

Rao *et al.* (1992) studied the seminal characteristics of crossbred (Desi x Large white Yorkshire) boars and found as follows : Reaction time (minutes) 1.58 ± 0.11; duration of ejaculation 3.39 ± 0.10 min; Total volume 163.43 ± 8.0 ml; strained volume 133.54 ± 6.11 ml; gel volume 29.89 ± 1.89 ml; initial sperm motility 85.14 ± 0.35 percent; pH 7.24 ± 0.01; sperm concentration 274.0 ± 9.83 million per ml. and live sperms 83.47 ± 1.64 percent.

Pandey and Singh (1998) studied the influence of season on coital behaviour and seminal characters of large white Yorkshire boars. The mean value of volume (ml.), pH, initial motility (%), progressive motility (%), live sperm count (%) and sperm concentration were recorded as 224.783 ± 4.803, 7.55 ± 0.029, 85.00 ± 0.713, 71.84 ± 0.246, 83.101 ± 0.390 and 282.851 ± 4.953 million/ml. respectively during winter season.

Kansi (2007) studied the seminal attributes in different breed of boars and reported as follows : strained volume (ml.)

153.26 ± 3.14, 155.78 ± 3.37 and 135.86 ± 2.41; gel volume (ml.) 39.96 ± 0.82, 40.61 ± 0.88 and 35.69 ± 0.67; Total volume (ml.) 193.22 ± 3.96, 196.39 ± 4.25 and 171.28 ± 3.03; initial sperm motility (%) 81.72 ± 0.52, 83.06 ± 0.49 and 85.55 ± 0.51; Live sperm (%) 79.56 ± 0.45, 81.06 ± 0.41 and 83.55 ± 0.81; sperm concentration (million/ml.) 280.28 ± 2.84, 280.77 ± 2.44 and 274. 28 ± 2.78 in large white Yorkshire, Tamworth and T & D boars semen respectively.

(2) Morphological abnormalities of spermatozoa :

Cerovsky (1981) recorded head abnormality, proximal cytoplasmic droplet, tail abnormality and proximal protoplasmic droplet migrating to tail in boar semen as 0.36, 0.3, 2.06 and 3.79 percent respectively.

Tamuli (1982) recorded the mean incidence of head, mid piece and tail abnormalities as 2.01, 1.01, and 1.20 percent respectively in landrace boars.

Louda and Pavlik (1984) found an incidence of abnormal spermatozoa as 12.5, 11.2 and 14.9 percent in Duroc, Duroc x Belgian landrace and Belgian landrace boars respectively.

Dubiel *et al.* (1985) observed the percentage of sperm abnormalities in polish large white, Duroc and crossbred boars and reported as 4.05, 4.0 and 3.4 percent respectively.

Bhuyan (1989) recorded mean incidence of head, mid piece and tair abnormalities in Hampshire and crossbred boars as 2.00 ± 0.29 and 1.34 ± 0.21, 17.70 ± 1.85 and 11.15 ± 1.27, 3.08 ± 0.45 and 3.33 ± 0.51 percent respectively.

Pandey and Singh (1997) studied abnormalities of large white Yorkshire boars spermatozoa in different season. They recorded the average percent of head abnormalities, fractured neck, mid piece defect, tail abnormality, proximal cytoplasmic droplet and distal cytoplasmic droplet as 2.575 ± 0.055, 0.095 ± 0.001, 0.494 ± 0.046, 2.172 ± 0.178, 1.147 ± 0.078 and 0.942 ± 0.105 respectively.

Kansi (2007) observed head abnormality, fractured neck, mid piece defect, Tail abnormality, proximal cytoplasmic droplet, distal cytoplasmic droplet as 2.54 ± 0.148, 2.61 ± 0.128 and 2.72 ± 0.148, 0.208 ± 0.065, 0.28 ± 0.075 and 0.24 ± 0.071, 0.472 ± 0.066, 0.50 ± 0.063 and 0.58 ± 0.080, 2.152 ± 0.138, 2.31 ± 0.163 and 2.31 ± 0.155, 1.236 ± 0.109, 1.47 ± 0.122 and 1.49 ± 0.161, 0.944 ± 0.095, 1.50 ± 0.088 and 1.19 ± 0.122 in large white Yorkshire, Tamworth and T & D boars semen respectively.

(3) Acrosomal abnormalities :

Cerovsky (1981) reported the incidence of acrosomal defect in boars for the year 1976 and 1980 as 1.88 and 0.76 percent respectively.

Tamuli (1982) reported the percentage of normal acrosome in landrace boar as 3.11 with a range of 0 to 7 percent.

Strzezek and Slaweta (1984) observed 90.1 percent spermatozoa with normal acrosomal ridge in boars.

(4) Preservation of semen :

Cerovsky and Vinter (1986) studied sperm survival in Modena, Beltsville, Kiev and NG diluent. They observed sperm survival as 39.0, 47.1, 45.5 and 50.1% after 24 h and 31.9, 38.4, 37.1 and 45.9 after 48h, 30.5, 30.4, 29.9 and 40.8% after 72 h and 25.8, 23.6, 28.0 and 32.4% after 96 h respectively in preserved semen at 37^{0}C.

Joshi and Kharche (1990) studied the effect of preservation of diluted semen at varying interval on semen quality in crossbred bulls. They observed that in fresh semen the percent live spermatozoa in group I, II and III were 89.45 ± 0.613%, 88.35 ± 0.711% and 91.45 ± 0.881 respectively. The corresponding value were 85.80 ± 0.489, 85.20 ± 0.694 and 87.35 ± 0.818% 80.70 ± 0.747, 80.15 ± 0.952 and 83.70 ± 0.992% and 75.30 ± 0.926, 76.35 ± 1.008 and 79.05 ± 1.122% at 24, 48 and 72 hour of preservation respectively.

Bhuyan *et al.* (1992) studied the effect of Kiev, GPSE-1 and BL-1 extenders on semen quality of crossbred boars and recorded that there was no significant difference in mean percent of progressive motile sperm, live sperm and intact acrosome at 0 hour of preservation among the extenders but progressively motile sperm, live sperm and intact acrosome dropped significantly in BL-1 extender at 24 and 48 hour of preservation than in Kiev and GPSE-1 extenders.

Lalrintuanga (1994) studied preservation of Boar semen in liquid state at 5^{0}C and 15^{0}C. He observed sperm motility,

live sperm count and intact acrosome at 0 hour (i.e. just after dilution) as 81.79 ± 0.63, 91.32 ± 0.50 and 94.36 ± 0.36 percent respectively. The sperm motility in Kiev, Modena, Zornobsa and LEY extenders at 48 hours of preservation was 6.57 ± 0.89, 6.32 ± 1.07, 5.18 ± 0.98 and 51.43 ± 1.99 percent respectively at 5°C and 54.11 ± 1.92, 53.04 ± 1.90, 50.00 ± 1.54 and 58.57 ± 1.73 percent respectively at 15°C. The corresponding values for live sperm count were 16.23 ± 1.56, 14.48 ± 2.33, 13.29 ± 2.22 and 65.82 ± 1.45 percent at 5°C and 69.25 ± 1.68, 68.21 ± 1.56, 65.70 ± 1.59 and 73.84 ± 1.23 percent at 15°C the percent of intact acrosome were 20.23 ± 1.70, 18.57 ± 3.68, 17.41 ± 3.56 and 69.80 ± 1.70 at 5°C and 72.37 ± 1.41, 71.48 ± 1.67, 69.00 ± 1.55 and 77.89 ± 1.33 at 15°C.

Kutty *et al.* (1996) recorded average counts of live with infact acrosome (LI), live with damaged acrosome (LD), Dead with intact acrosome (DI) and dead with damaged acrosome (DD) sperms in the semen sample as 81.45, nil, 12.00, 6.55 and 39.57, 0.18, 49.45, 10.80 and 22.74, 0.36, 50.55, 26.35 at 0, 24 and 48 h respectively.

Pandey and Singh (1997) studied acrosomal changes during liquid preservation of boar semen with BTS, Kiev and BL-1 dilutors. The incidence of Damaged apical ridge (DAR), missing apical ridge (MAR) and loose acrosomal cap (LAC) were recorded as 0.750 ± 0.217, 2.500 ± 0.230, 10.416 ± 0.254 and 14.833 ± 0.726; 0.0, 0.0, 0.416 ± 0.261 and 1.083 ± 0.288;

1.66 ± 0.207, 1.750 ± 0.278, 3.750 ± 0.271 and 4.166 ± 0.297 at 0, 24, 48 and 72 h preservation in Kiev dilutor respectively.

Pandey and Singh (1998) reported mean percentage of sperm motility, live sperm, head abnormality and Tail abnormality in Kiev dilutor as 80.291 ± 0.266, 84.258 ± 0.238, 2.650 ± 0.133 and 1.837 ± 0.229, 70.583 ± 0.248, 80.900 ± 0.194, 2.560 ± 0.101 and 3.425 ± 0.192, 66.250 ± 0.129, 74.983 ± 0.294, 2.430 ± 0.130 and 5.858 ± 0.341, 61.525 ± 0.520, 70.433 ± 0.503, 2.660 ± 0.005 and 11.533 ± 0.479 at 0, 24, 48 and 72 hours of preservation respectively.

Naidu and Rao (1999) studied preservation of native boar semen. They reported that sperm motility was higher in all diluents at 15°C and was found more that 50% after 48 hours storage. Motility was highest in samples diluted using GPSE at all times.

Lalrintuanga *et al.* (2002) studied preservation of boar semen at 5°C and 15°C. They reported that the semen quality was significantly ($P<0.05$) better in LEY extender followed by Kiev, modena and zornobsa extenders. The difference between Kiev and Modena and between modena and zornobsa was not significant. The sperm motility (50% or more) and live sperm count (more than 65%) were maintained upto 48 h of preservation in all the extenders at 15°C and only in LEY extender at 5°C.

Das *et al.* (2006) studied morphological characteristics of spermatozoa in Hampshire and crossbred boars preserved with

BTS diluent. The motility of spermatozoa were 82.33 ± 0.29 and 80.28 ± 0.27, 7.38 ± 0.29 and 70.50 ± 0.27, 62.61 ± 0.29 and 57.28 ± 0.27 and 58.56 ± 0.29 and 54.19 ± 0.27 at 0, 24, 48 and 72h respectively. The live percent of spermatozoa were 89.67 ± 0.30 and 86.89 ± 0.33, 80.03 ± 0.30 and 76.56 ± 0.33, 64.72 ± 0.30 and 61.11 ± 0.33 and 59.81 ± 0.30 and 54.58 ± 0.33 at 0, 24, 48 and 72 h respectively. The normal acrosomal integrity of Hampshire and crossbred spermatozoa were 86.69 ± 0.38 and 85.03 ± 0.37, 77.22 ± 0.38 and 75.14 ± 0.37, 61.47 ± 0.38 and 56.97 ± 0.37 and 56.97 ± 0.37 and 51.64 ± 0.37 at 0, 24, 48 and 72 h respectively.

Kansi (2007) observed the motility percentage in large white Yorkshire and T & D boar semen diluted with Kiev dilutor and found as 81.22 ± 0.38 and 82.11 ± 0.42, 67.28 ± 0.39 and 70.00 ± 0.42, 57.28 ± 0.39 and 62.06 ± 0.45, 50.33 ± 0.37 and 54.94 ± 0.51, 44.28 ± 0.35 and 49.77 ± 0.68 at 0, 24, 48, 72 and 96 hours of preservation respectively. The corresponding mean values of live sperm percentage were 83.28 ± 0.34 and 86.39 ± 0.41, 72.11 ± 0.36 and 76.22 ± 0.46, 63.22 ± 0.36 and 68.17 ± 0.49, 57.16 ± 0.35 and 61.11 ± 0.52, 52.44 ± 0.35 and 54.22 ± 0.46 at 0, 24, 48, 72, 96 hour of preservation respectively. The incidence of head abnormality percentage were found as 2.76 ± 0.19 and 2.82 ± 0.22, 2.56 ± 0.19 and 2.96 ± 0.26, 2.49 ± 0.18 and 2.58 ± 0.24, 2.63 ± 0.18 and 2.78 ± 0.27, 2.50 ± 0.14 and 2.96 ± 0.24 at 0, 24, 48, 72 and 96 hours of preservation respectively. The mean tail

abnormality percentage was 1.82 ± 0.18 and 2.13 ± 0.21, 3.11 ± 0.21 and 3.26 ± 0.27, 5.83 ± 0.25 and 5.99 ± 0.30, 11.04 ± 0.43 and 11.11 ± 0.49, 16.09 ± 0.37 and 16.43 ± 0.40 at 0, 24, 48, 72 and 96 hours of preservation respectively.

(5) Seminal minerals :

Mann (1964) recorded average value of calcium, magnesium and inorganic phosphorus in boar semen were 5mg/100ml., 11mg/100ml. and 17 mg/100ml. respectively.

Blom and Wolstrup (1976) assessed zinc content in semen samples from four young Danish Jersey bull showing the typical "Dag defect". The zinc content in normal spermatozoa and seminal plasma were 190 ± 15.8 M µg/g d.w. and 81 ± 31.2 M µg/g d.w. They further reported an elevated values of zinc content 137 ± 75.2 m µg/g d.w. were found in the seminal plasma of abnormal bulls.

Gottardi and Brunel (1978) studies mineral content and enzyme activity of Boar semen collected at different interval. They observed that difference in calcium, magnesium, potassium and sodium levels due to collection interval were not significant, but inorganic phosphorus was found significantly higher at the longest collection interval (1.80 mg/100ml.) than at the shortest interval of collection (1.46mg/100ml).

Saha and Sidhu (1982) studied biochemical attributes for 2 semen samples from each of 2 Dorest, 2 suffolk and 2 Muzaffarnagri rams. They recorded the average chloride

content as 98.44, 94.82 and 106.00 mg/100ml. of seminal plasma respectively. The calcium and magnesium content were found as content 9.83, 8.00 and 9.33 and 2.07, 2.50 and 2.53 respectively.

Pandey *et al.* (1982) assessed the levels of certain ions in the semen of saanen and Barbari Bucks. They reported that the semen samples of Barbari bucks had significantly higher level of Ca (14.875 ± 0.144 mg/100ml.) in comparison to the semen samples of Saanen bucks (11.433 ± 0.063 mg/100ml). The average level of Mg in the semen samples of Saanen and Barbari bucks were 11.433 and 11.033 mg/100ml. respectively. The average value of inorganic phosphorus in the semen samples of saanen and Barbari bucks were 18.502 ± 0.922 and 12.278 ± 0.621 mg/100ml respectively.

Petzoldt and Nehring (1986) studied ion levels in spermatozoa and seminal plasma of boar and ram semen. They reported that the mean sodium concentration of boar semen were 96.6 ± 12.08 nmol/litre in seminal plasma and 75.4 ± 23.12 nmol/litre in spermatozoa. Potassium concentration were 13.1 ± 2.12 and 28.8 ± 6.54 nmol/litre in seminal plasma and spermatozoa respectively.

Robertson and Watson (1986) studied calcium transport in diluted or cooled ram semen. They reported that even 2 to 4 fold dilution caused an increase in intracellular calcium content. A proportionate increase in intracellular calcium also observed with the lowering of the temperature of diluted

semen. After an initial increase in intracellular Ca content, spermatozoa appeared able to restore a low intracellular Ca content over a period of 24 h at < more intracellular Ca content over a period of 2 h at < more or = 22^0. This ability was lost at < less or = 16^0.

Pangawkar *et al.* (1988) studied electrolyte composition of seminal plasma in relation to freezability in HF bulls. They observed mean concentration of Ca and Mg as 36.7 ± 1.3 and 8.9 ± 0.1, 34.7 ± 1.5 and 9.3 ± 0.1, 36.5 ± 0.9 and 9.3 ± 0.1 mg percent in freezability group I, II and III respectively.

Pangawkar *et al.* (1988) also assessed protein, sialic acid and zinc concentration in the seminal plasma of Bulls in relation to freezability of semen. They observed mean concentration of Zn in freezability group I, II and III as 2.53 ± 0.05, 2.32 ± 0.04 and 2.39 ± 0.01 mg percent respectively.

Patel *et al.* (1989) studied seminal biochemical profiles and their interrelationships in crossbred bulls. They recorded mean value of Ca, inorganic phosphorus and magnesium in Kankrej x Jersey and Kankrej x HF bull semen as 44.29 ± 1.42, 15.44 ± 0.58, 3.77 ± 0.16 and 45.96 ± 1.34, 13.34 ± 0.53, 3.82 ± 0.17 mg percent respectively.

Bhavsar *et al.* (1989) studied seminal trace elements with reference to freezability and fertility of Mehsana buffalo bulls. The overall means of Zinc, iron, copper and manganese in seminal plasma and spermatozoa were recorded as 3.367 ± 0.801, 1.986 ± 0.022, 0.271 ± 0.010 and 0.122 ± 0.002

mg/100ml. and 0.0460 ± 0.0048, 0.0205 ± 0.0026, 0.0042 ± 0.0001 and 0.00149 ± 0.0003 mg/10^9 sperm respectively.

Markandeya *et al.* (1991) studied chemical composition of seminal plasma of Red Kandhari and crossbred Bulls. They observed that the levels of sodium, potassium, chloride, calcium, and inorganic phosphorus were 71.06 mEq per litre, 16.18 mEq per litre, 149.80 mg%, 14.97 mg% and 4.03 mg% in seminal plasma of Red Kandhari bulls whereas the level of these ions in crossbred dulls seminal plasma were 67.23 mEq per litre, 27.60 mEq per litre, 117.65 mg%, 20.93 mg% and 3.27 mg% respectively.

Dhami and Sahni (1993) assessed certain biochemical and mineral constituents of seminal plasma of bull and buffalo bull. They observed acid soluble phosphate 65.57 ± 3.47 & 91.00 ± 3.32 mg%, ester phosphate 55.37 ± 3.21 & 79.79 ± 3.07 mg%, inorganic phosphate 10.31 ± 0.48 & 12.28 ± 0.37 mg%, sodium 337.37 ± 3.82 & 295.11 ± 4.64 mg% and chloride 334.59 ± 15.56 & 269.13 ± 7.85 mg% respectively.

Nema *et al.* (1993) studied certain biochimical profile Static and Motile seminal ejaculates in Surti Buffalo bulls. They recorded mean value of inorganic phosphorus in static and motile semen sample as 19.58 ± 0.940 and 15.52 ± 0.640 mg percent respectively.

Dhami *et al.* (1994) studied relationships of seminal attributes and enzymatic profiles with biochemical and mineral constituents of seminal plasma in Friesian and Murrah buffalo

bulls. They found that among Friesian bulls, significant correlations ($P<0.05 = \pm 0.031$; $P<0.01 = \pm 0.41$) were observed for seminal fructose with ejaculate vol. density, sperm concentration, AKP and LDH (r = + 0.36, – 0.31, – 0.60, + 0.42 and ± 0.30 respectively), total protein with volume, mass activity and AKP (+ 0.43, + 0.38 and + 0.45), total cholesterol with pH and initial motility (+ 0.30 and – 0.39) seminal phosphates (inorganic, acid soluble and ester) with volume, sperm concentration, live sperm% and GOT (– 0.44, + 0.53, + 0.31 and + 0.35); zinc with motility (– 0.37); copper with volume and AKP (+ 0.30 and + 0.30) and iron and manganese with LDH (0.28 and – 0.26).

Patil *et al.* (1996) studied biochemical composition of Buck semen. The mean value of electrolytes viz. sodium, potassium, magnesium and inorganic phosphorus were observed as 42.56 ± 1.97 mEq per litre, 59.57 ± 2.25 mEq per litre, 4.23 ± 0.21 mg percent and 14.67 ± 0.74 mg percent respectively.

Gupta *et al.* (1997) studied distribution of electrolytes in the epididymal and ejaculated semen of buffalo bulls. They observed that sodium, potassium, calcium and chloride were 119.00 ± 1.40, 25.44 ± 0.56, 2.39 ± 0.02 and 35.78 ± 1.13 (mM/l) in ejaculated semen of buffalo.

Suresh Kumar *et al.* (1999) studied inorganic element and their seasonal variations in seminal plasma of Patanwadi rams. They recorded mean values of calcium, inorganic

phosphorus and magnesium concentration as 8.75 ± 0.28, 10.25 ± 0.21 and 4.94 ± 0.10 (mg%) respectively during different seasons.

Suther *et al.* (2000) studied the levels of certain ions and trace minerals in Patanwadi and crossbred ram semen. The average concentration of Zn was recorded higher in Patanwadi rams whereas, Fe and Cu levels remained elevated in crossbred rams. Iron and copper exihibited positive relationship with sperm concentration. A positive correlation of copper with pH was also recorded.

Vinaya B. Shelke and Dhami (2001) studied norms and correlations of seminal plasma biochemical, enzymatic and Macro-Micro mineral profiles with physical attributes and freezability of semen in Gir bulls. They observed average concentration of Ca, P, Mg, Zn, Cu, Co and Mn as 13.96 ± 0.23, 5.18 ± 0.25, 2.34 ± 0.20, 1.519 ± 0.090, 0.080 ± 0.004, 0.058 ± 0.002 and 0.022 ± 0.001 (mg%) respectively.

Dhami *et al.* (2001) studied trace minerals profile of blood and seminal plasma of breeding bulls. They reported that zinc levels were 10-15 fold higher in seminal plasma than that in the blood plasma. Iron and copper were identical in both blood & seminal plasma while copper and manganese level were 1.5 – 2.0 fold higher in blood than seminal plasma in all the breeds.

Singh *et al.* (2001) studied seminal plasma electrolytes and their correlation with seminal quality in the dromedary

camel. They observed mean value of calcium, inorganic phosphorus and magnesium 11.47 mg/dl, 3.21 mg/dl and 2.57 mg/dl respectively in seminal plasma of stud camels.

Taylor and Francis (2003) assessed the concentration of Copper, Iron, Zinc, Cadmium, lead and nickel in Boar semen and relation to the spermatozoa quality. They observed average concentration of copper and zinc in boar semen as 1.64 ± 0.28 mg. kg^{-1} and 171.74 ± 64.72 mg.$kg.^{-1}$ respectively.

Massanyi *et al.* (2003) studied seminal concentration of trace elements and their correlations in various animals. They observed was significantly higher seminal copper concentration in ram [(2.491i A' 0.18) mg/kg] and fox [(2.16iA' 0.53) mg/kg] than that in bull [(1.64iA'0.21) mg/kg], boar [(1.64iA'0.28) mg/kg.] and stallion (0.86 mg/kg). A significantly higher seminal zinc concentration [(171.74iA' 65.72) mg/kg] was found in boar in comparison with stallion [(86.20 iA' 45.88) mg/kg], bull [(83.15iA' 61.61) mg/kg], ram [(60.46iA' 35.37) mg/kg] and fox [(13.09iA' 5.22) mg/kg].

Massanyi *et al.* (2004) assessed the concentration of copper, zinc, iron, cadmium, lead and nickel in bull, ram, boar, stallion and fox semen. They observed that copper concentration was significantly higher in Rams (2.49 ± 0.18 mg/kg) and foxes (2.16 ± 0.53 mg/kg) in comparison with bulls (1.64 ± 0.21 mg/kg), boars (1.64 ± 0.28 mg/kg) and stallion (0.86 mg/kg). Boar semen had significantly higher zinc concentration (171.74 ± 65.72 mg/kg) in comparison with

stallion (86.20 ± 45.88 mg/kg), bull (83.15 ± 61.61 mg/kg), ram (60.46 ± 35.37 mg/kg.) and fox semen (13.09 mg/kg.).

Mohanty *et al.* (2004) studied trace minerals and freezability of crossbred bull semen. The mean concentration of copper, cobalt, zinc and iron in the neat seminal plasma of good freezable semen were 0.874 ± 0.092, 0.481 ± 0.038, 10.519 ± 0.648 and 48.98 ± 3.09 µg/ml whereas the corresponding value of these elements in the post thaw semen samples were 0.084 ± 0.011, 0.128 ± 0.039, 2.282 ± 0.241 and 15.73 ± 1.06 µg/ml respectively. Concentration of the above trace minerals in the poor freezable bull semen were 0.629 ± 0.071, 0.341 ± 0.037, 5.065 ± 0.446 and 45.45 ± 2.60 µg/ml and 0.066 ± 0.009, 0.103 ± 0.023, 1.993 ± 0.251 and 19.00 ± 1.01 µg/ml respectively in neat and post thaw semen samples.

Singh (2005) reported mean value of calcium and magnesium were reported as 6mg/100ml., 5-14 mg/100ml. respectively in boar semen.

Nayak *et al.* (2006) conducted cryopreservation and fertility trials of clarias batrachus spermatozoa. The concentration of sodium, potassium, calcium, magnesium and Zinc were found as (853.60 ± 0.75 ppm), (329.00 ± 0.51 ppm), (59.95 ± 0.23 ppm), (30.25 ± 0.07 ppm) and (9.74 ± 0.07 ppm) respectively.

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CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

3. 1. Experimental Animal :

The study was conducted on the semen samples obtained from four Large white Yorkshire and four Tamworth x Desi (T & D) boars maintained under the Adhoc Research Project at B.V.C. Patna. The selected boars were in the age group of 1.5-2.0 years and they were kept on approved ration schedule with identical managemental conditions. The selected Boars were trained for semen collection on oestrus sows.

3.2. Collection of Semen :

Semen samples were collected at 5 days interval with gloved hand technique following the procedure of Zavos and Liptrap (1987). Total 72 semen ejaculates, 9 from each boar were utilized for the study. The pre sperm fractions of ejaculate were collected in wide month sterilized large test tube of 30 ml. capacity. Whereas the sperm rich fractions were collected in thermos flask of 500 ml. capacity. The opening of the flask was covered with clean and sterilized muslin cloth. At the end of collection, the muslin cloth with gel mass was removed and thermos was capped immediately. The sperm rich fractions of boar ejaculates were brought to the laboratory and were evaluated.

3.3. Evaluation of neat semen :

The following seminal attributes were studied :

3.3.1. Macroscopic Evaluation :

3.3.1.a. Colour & Consistency :

The colour of ejaculates was observed by naked eye and recorded as creamy, milky and watery.

The consistency of ejaculates was observed by naked eye and recorded as thick, medium and thin.

3.3.1.b. Volume :

The volume of three fractions of the semen was recorded.

3.3.1.c. pH :

The pH of sperm rich fractions was determined by digital pH meter.

3.3.2. Microscopic Evaluation :

3.3.2.a. Mass activity :

Mass activity of neat semen was assessed following the procedure of Hancock (1959).

A clean and sterilized glass slide was taken. A drop of semen was poured over the glass slide and it was examined under low power objective of the microscope without using cover slips. The mass activity was graded from 0 to +5 based on the intensity of swirl under the following categories :-

(i) + 5 motility – There was very quick wave motion in the field. It was very difficult to trace when the eddies were formed or

disintegrated. There was only wave after wave in the field. Swirls were created with mass movement of spermatozoan.

(ii) + 4 motility – The swirls and eddies were not rapid as in +5 grade. The swirls were observed to move towards extremities.

(iii) +3 motility – The swirls were slow and scattered in the field.

(iv) + 2 motility – Swirls were absent. But individual movement of spermatozoa were more evident in the field.

(v) + 1 motility – No wave motion were observed in the field. Only 20% sperms had progressive motility. Rest of the spermatozoa showed throbbing movement. Semen samples showing mass activity of grade +4 and above were included for further processing.

3.3.2.b. Individual Sperm Motility :

The individual sperm motility percentage was calculated following standard method.

A drop of 2.94% sodium citrate dehydrate solution was taken on a clean glass slide. Then a drop of neat semen was placed on the slide and mixed with sodium citrate dehydrate solution. A thin smear was made using another slide and then covered with a cover slips. The smear of diluted semen was examined immediately under high power objective of microscope and non-motile spermatozoa were counted in three different fields randomly. Now, the slides was passed over a flame, so that all the spermatozoa were killed. Then, the total spermatozoa in

three different fields were counted randomly. The motile percentage of spermatozoa were calculated as

$$\% \text{ motile spermatozoa} = \frac{\text{Total spermatozoa} - \text{Non motile Spermatozoa}}{\text{Total spermatozoa}} \times 100$$

3.3.2.c. Live sperm percentage :

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

The compound stain of Eosin-nigrosin was prepared by dissolving 5 gm of Eosin, 30 gm of nigrosin (B.D.H.) in 300 ml. distill water. The solution was warmed in water bath for 30 minutes and was filtered after cooling.

Preparation of Slides :

On clean greese free glass slides, a drop of neat semen was taken, to it four drops of nigrosin-eosin were added. A thin smear was drawn on slide and was dried in the air. The smear was examined under oil immersion. Live spermatozoa were colourless, whereas dead spermatozoa took pink stain of Eosin. A total of 200 sperms were counted at random in different fields of microscope and the percentage of live spermatozoa was calculated as

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

3.3.2. d. Concentration of spermatozoa :

The concentration of spermatozoa was measured by photoelectric calourimeter method following the method of Willet and Buckner (1951).

0.1 ml. of the semen was transferred into an test tube and 9.9 ml. of 3% sodium citrate solution was added in it and was mixed by gently shaking. 3% sodium citrate solution was used as "Blank".

For measuring the light transmission through diluted semen samples, photo electric colorimeter with red filter was used at wave length of 600 milli micron. Blank was adjusted to 100% transmission and then optical density of the diluted semen was recorded after through mixing. Finally the determination of sperm concentration was made with the help of standard curve.

Preparation of standard curve :

Semen samples were pooled separately and sperm count were made from each sample following the haemocytometer method. After mixing semen samples thoroughly the following quantities of them were taken separately in test tube and 3% sodium citrate solution was added in each test tube to make finally the volume to 10 ml.

Test tube no.	Vol. of the semen (ml.)	Sodium citrate solution (ml.)	Total (ml.)
1	0.05	9.95	10.00
2	0.1	9.9	10.00
3	0.15	9.85	10.00
4	0.2	9.8	10.00
5	0.0	10.0	10.00

Readings of the diluted semen samples were taken at wavelength of 600 milli micron. The standard curve was prepared by plotting optical density against different sperm concentration, observed following the haemocytometer method. The optical density of semen samples of different boars obtained during the course of the study was compared with the standard curve and thus concentrations of spermatozoa in semen samples were calculated. Total number of spermatozoa per ejaculate was obtained by multiplying the concentration to the volume of the ejaculate.

3.3.2.e. Spermatozoan abnormalities :

Spermatozoan abnormalities were assessed following the procedure of Hancock (1959).

Abnormal sperms were detected with the help of methylene blue and Eosin stains. Methylene blue stain was prepared by

dissolving 1 gm. Of methylene blue in 100 ml. of glass distilled water. Eosin stain was prepared by dissolving 2 gm. Of Eosin (B.D.H.) in 100ml. of glass distilled water. Smear of semen was prepared and dried in air. Then it was flooded with 1% methylene blue and left for 20 minutes and washed gently in running tap water. Again the smear was flooded with 2% eosin solution and left for 5 minutes. Then it was washed gently under running tap water. The wet slide was dried up by means of a blotting paper and examined under oil immession lens to find out the abnormalities of sperms.

3.3.2. f. Acrosomal Integrity :

Smears prepared from neat semen and they were stained with Giemsa according to Watson (1975).

Preparation of Giemsa stain :

The following ingredients were used

Giemsa stain (stock solution)	-	3 ml.
Sorenson's phosphate buffer	-	2 ml.
Distilled water	-	35 ml.

Giemsa stain (Stock solution) :

Giemsa stain powder	-	3.8 g
Methanol	-	375 ml.
Glycerol	-	125 ml.

Sorenson's Phosphate buffer :

Solution A :

$Na_2HPO_4,2H_2O$	-	21.68g
H_2O	-	500ml.

Solution B :

KH_2PO_4	-	22.25g
H_2O	-	500 ml.

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

Buffered formal saline :

The solution was prepared with the following composition.

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

Stock sodium chloride solution

The solution was prepared as follows :

Sodium chloride	-	9.01 g
Distilled water	-	500 ml.

Technique of staining :

Procedure followed for staining with Giemsa stain was as follows :

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

(b) Smear was fixed in buffered formal saline for 15 minutes and then was washed in running tap water for 15-20 minutes.

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

(d) Finally, the slide was rinsed in distilled water and dried in air.

The slides were examined at a magnification of 1000 x. Two slides were examined from each sample and from each slide 100 acrosome were examined. Besides acrosomal morphology, the slides were utilized for assessing of head and tail abnormalities. Acrosome morphology was classified into four types according to Pursel *et al.* (1974) which was as follows :

(i) Normal Apical ridge (NAR) :

The acrosomal cap smoothly adhered to the nucleus and possessed a smooth, crescentic apical ridge.

(ii) Damaged apical ridge (DAR) :

The acrosome possessing irregular shaped apical ridge.

(iii) Missing apical ridge (MAR) :

Spermatozoa lacked the apical ridge but the acrosomal cap remained tightly adhered to the nucleus.

(iv) Loose acrosomal cap (LAC) :

Which was characterized by the vesiculation of the anterior acrosomal cap.

3.3.2. g. Progressive motility :

Progressive motility of spermatozoa were determined using haemocytometer following the procedure of Brady and Gildow (1939).

Two pipettes were used. In one, the semen was diluted with physiological saline, and in the other alcohol was used. Alcohol treated spermatozoa died. Counting chambers were charged, one with saline diluted semen and other with alcohol diluted. Non motile spermatozoa were counted in both the chambers. The difference between the counts of two chambers was the number of motile spermatozoa.

Progressively motile spermatozoa were differentiated from the weakly motile with haemocytometer technique. The one chamber weakly motile plus non-motile spermatozoa were counted, and in the other total spermatozoa were counted. The difference between the two counts was the number of progressively motile spermatozoa.

3.3.3. Chemical Evaluation :

3.3.3. a. Estimation of Calcium :

Method of Webster (1962) was employed to determine the concentration of calcium in the semen with spectrophotometer.

0.1 ml. of the neat semen was taken in a test tube and 3.5ml. of 12% trichloroacetic acid was added. They were mixed and allowed to stand for 10 minutes and then the solutions were

centrifuzed rapidly at 3000 rpm for 20 minutes. A "Blank" containing 2.0 ml. of triple glass distilled water and 3.0 ml. of 12% trichloroacetic acid was also centrifuged. After centrifugation, 3.0 ml. of clear supernatent fluid was transferred to a cuvette and 2ml. of ferrous sulphate ammonium molybdate reagent was added. After one minute the reading of semen samples of different boars was recorded against "Blank" at wavelength of 660 milli micron.

Preparation of standard curve :

Calcium carbonate (A.R.) weighing 0.4390 gms was dissolved in water in one litre volumetric flask. 10ml. of 10N sulphuric acid was added to it and was mixed. Triple glass distilled water was added to make up the volume to the mark and was then mixed. The solution contained 100 micro grams of calcium in 1ml.

Optical density of these standard solution was recorded with the help of spectrophotometer at a wave length of 660 milli micron and the density was plotted against concentration in order to obtain the standard curve. Optical density of semen samples was compared with the standard curve and thus the concentration of calcium in semen samples were obtained.

The different quantities from this standard solution were taken separately as follows :-

Tube No.	Standard solution (ml.)	Amount of Ca (mg/100ml.)	TCA (ml.)	Ferrous sulphate ammonium molybdate reagent (ml.)
1	0.5	50	2.5	2.0
2	1.0	100	2.0	2.0
3	1.5	150	1.5	2.0
4	2.0	200	1.0	2.0

3.3.3.b. Estimation of inorganic phosphorus :

Method of Taussky & Shorr (1953) was employed to determine the concentration of calcium in the semen with spectrophotometer.

0.1 ml. of the neat semen was taken in a test tube and 3.5ml. of 12% trichloroacetic acid was added. They were mixed and allowed to stand for 10 minutes and then the solutions were centrifuzed rapidly at 3000 rpm for 20 minutes. A "Blank" containing 2.0 ml. of triple glass distilled water and 3.0 ml. of 12% trichloroacetic acid was also centrifuged. After centrifugation, 3.0 ml. of clear supernatent fluid was transferred to a cuvette and 2ml. of ferrous sulphate ammonium molybdate reagent was added. After one minute the reading of semen

samples of different boars was recorded against "Blank" at wavelength of 660 milli micron.

Preparation of standard curve :

Monopotassium phosphate weighing 0.4390 gms was dissolved in water in one litre volumetric flask. 10ml. of 10N sulphuric acid was added to it and was mixed. Triple glass distilled water was added to make up the volume to the mark and was then mixed. The solution contained 100 micro grams of phosphorus in 1ml.

The different quantities from this standard solution were taken separately as follows :-

Tube No.	**Standard solution (ml.)**	**Amount of P (mg/100ml.)**	**TCA (ml.)**	**Ferrous sulphate ammonium molybdate reagent (ml.)**
1	0.5	50	2.5	2.0
2	1.0	100	2.0	2.0
3	1.5	150	1.5	2.0
4	2.0	200	1.0	2.0

Optical density of these standard solution was recorded with the help of spectrophotometer at a wave length of 660 milli micron and the density was plotted against concentration in

order to obtain the standard curve. Optical density of semen samples was compared with the standard curve and thus the concentration of calcium in semen samples were obtained.

3.3.3. c. Estimation of Magnesium :

Method of Oser (1965) was employed to determine the concentration of magnesium in the semen samples with spectrophotometer.

0.1 ml. of the neat semen was taken in test tube and 3.5 ml. of 12% trichloroacetic acid was added. They were mixed and allowed to stand for 10 minutes and then the solutions were centrifuged rapidly at 3000 rpm for 20 minutes. A "Blank" containing 2.0 ml. of triple glass distilled water and 3.0 ml. of 12% trichloroacetic acid was also centrifuged. After centrifugation, 3.0 ml. of clear supernatent fluid was transferred to a cuvette and 2ml. of ferrous sulphate ammonium molybalate reagent was added. After one minute the reading of semen samples of different boars was recorded against "Blank" at wavelength of 660 milli micron.

Preparation of standard curve :

Magnesium sulphate weighing 0.4390 gms. Was dissolved in water in one litre volumetric flask. 10 ml. of 10N sulphuric acid was added to it and was mixed. Triple glass distilled water was added to make up the volume to the mark and was then

mixed. The solution contained 100 micro grams of magnesium in 1ml.

The different quantities from this standard solution were taken separately as follows :-

Tube No.	Standard solution (ml.)	Amount of Mg (mg/100ml)	TCA (ml.)	Ferrous sulphate ammonium molybdate reagent (ml.)
1	0.5	50	2.5	2.0
2	1.0	100	2.0	2.0
3	1.5	150	1.5	2.0
4	2.0	200	1.0	2.0

Optical density of these standard solutions was recorded with the help of spectrophotometer at a wavelength of 660 milli micron and the density was plotted against concentration in order to obtain the standard curve. Optical density of semen samples was compared with the standard curve and thus the concentration of magnesium in semen samples were obtained.

3.3.3.d. Estimation of zinc :

Atomic absorption spectrophotometer was used to determine the level of zinc in the semen following the method of Oser (1979).

0.1 ml. of the neat semen was pipetted into a test tube containing 9.9ml. of 12% trichloroacetic acid. It was mixed and allowed to stand for atleast 10 minutes. After centrifuging rapidly at 3000 rpm for 10 minutes, supernatent was transferred to an another test tube. The samples were kept at refrigeration temperature and were analysed collectively after a month.

Preparation of standard solution :

0.0507 gms of zinc sulphate was dissolved in 100 ml. of triple glass distilled water in a volumetric flask. The concentration of the stock solution was 5mg/100ml. From this stock solution 14,18,22,26 and 30 ml. were taken separately in one litre volumetric flask and the volume was made to the mark adding the triple glass distilled water. The concentration of these standard solutions were 7, 9, 11, 13 and 15 mg/100ml. respectively at a dilution of 1:100.

Standardization of the instrument :

The wavelength of the instrument was adjusted at 213.9 nm. The sampling beaker containing triple glass distilled water was placed beneath the suction pipe. Ethylene gas was used as fuel and then the instrument was ignited. As such the instrument was left for 30 minutes for warming up.

Standard solution of different known concentrations were allowed to pass through suction pipe and readings were noted. A calibration curve was prepared with the readings of these

standard solutions. Semen samples, prepared from the whole semen of different boars, were allowed to pass only after fully shaking and readings were noted. These readings were compared with the calibration curve and thus the concentration of zinc in the semen samples were determined.

3.3.3.e Estimation of copper :

Atomic absorption spectrophotometer was used to determine the level of copper in the semen following the method of Oser (1979).

0.1 ml. of the neat semen was pipetted into a test tube containing 9.9ml. of 12% trichloroacetic acid. It was mixed and allowed to stand for atleast 10 minutes. After centrifuging rapidly at 3000 rpm for 10 minutes, supernatent was transferred to an another test tube. The samples were kept at refrigeration temperature and were analysed collectively after a month.

Preparation of standard solution :

0.0507 gms of copper sulphate was dissolved in 100 ml. of triple glass distilled water in a volumetric flask. The concentration of the stock solution was 5 mg/100ml. From this stock solution 14,18,22,26 and 30ml. were taken separately in one litre volumetric flask and the volume was made to the mark adding the triple glass distilled water. The concentration of these standard solutions were 7, 9, 11, 13 and 15 mg/100ml. respectively at a dilution of 1:100.

Standardization of the instrument :

The wavelength of the instrument was adjusted at 324.8nm. The sampling beaker containing triple glass distilled water was placed beneath the suction pipe. Ethylene gas was used as fuel and then the instrument was ignited. As such the instrument was left for 30 minutes for warming up.

Standard solutions of different known concentrations were allowed to pass through suction pipe and readings were noted. A calibration curve was prepared with the readings of these standard solutions. Semen samples, prepared from the whole semen of different boars, were allowed to pass only after fully shaking and readings were noted. These readings were compared with the calibration curve and thus the concentration of copper in semen samples were determined.

3.3.3.f. Estimation of cobalt :

Atomic absorption spectrophotometer was used to determine the level of cobalt in the semen following the method of Oser (1979).

0.1 ml. of the neat semen was pipetted into a test tube containing 9.9ml. of 12% trichloracetic acid. It was mixed and allowed to stand for atleast 10 minutes. After centrifuging rapidly at 3000 rpm for 10 minutes, supernatent was transferred to an another test tube. The samples were kept at refrigeration temp and were analysed collectively after a month.

Preparation of standard solution :

0.0507 gms. Of cobalt sulphate was dissolved in 100ml. of triple glass distilled water in a volumetric flask. The concentration of the stock solution was 5mg/100ml. From this stock solution 14,18,22,26 and 30 ml. were taken separately in one litre volumetric flask and the volume was made to the Mark adding the triple glass distilled water. The concentration of these standard solutions were 7,9,11,13 and 15 mg/100ml respectively at a dilution of 1:100.

Standardization of the instrument :

The wavelength of the instrument was adjusted at 240.7 nm. The sampling beaker containing triple glass distilled water was placed beneath the suction pipe. Ethylene gas was used as fuel and then the instrument was ignited. As such the instrument was left for 30 minutes for warming up.

Standard solutions of different known concentrations were allowed to pass through suction pipe and readings were noted. A calibration curve was prepared with the readings of these standard solutions. Semen samples, prepared from the whole semen of different boars, were allowed to pass only after fully shaking and readings were noted. These readings were compared with the calibration curve and thus the concentration of cobalt in semen samples were determined.

3.3.3.g. Estimation of manganese :

Atomic absorption spectrophotometer was used to determine the level of Manganese in the semen following the method of Oser (1979).

0.1 ml. of the neat semen was pipetted into a test tube containing 9.9 ml. of 12% trichloroacetic acid. It was mixed and allowed to stand for at least 10 minutes. After centrifuging rapidly at 3000 rpm for 10 minutes, supernatent was transferred to an another test tube. The samples were kept at refrigeration temperature and were analysed collectively after a month.

Preparation of standard solution :

0.0507 gm of manganese sulphate was dissolved in 100 ml. of triple glass distilled water in a volumetric flask. The concentration of the stock solution was 5mg/100ml. From this stock solution 14, 18, 22, 26 and 30ml. were taken separately in one litre volumetric flask and the volume was made to the mark adding the triple glass distilled water. The concentration of these standard solutions were 7, 9, 11, 13 and 15mg/100 ml. respectively at a dilution of 1:100.

Standardization of the instrument :

The wavelength of the instrument was adjusted at 235.4 nm. The sampling beaker containing triple glass distilled water was placed beneath the suction pipe. Ethylene gas was used as

fuel and then the instrument was ignited. As such the instrument was left for 30 minutes for warming up.

Standard solutions of different known concentrations were allowed to pass through suction pipe and readings were noted. A caliberation curve was prepared with the readings of these standard solutions. Semen samples, prepared from the whole semen of different boars, were allowed to pass only after fully shaking and readings were noted. These readings were compared with the calibration curve and thus the concentration of manganese in semen samples were determined.

4. Processing and preservation of semen :

The semen samples having 70 percent or more progressive motility were finally diluted with Kiev extender based on concentration of spermatozoa in the semen.

Composition of Kiev extender used for dilution of semen :

Glucose	-	60.00 gm.
Tri-sodium citrate	-	3.70 gm.
Sodium bicarbonate	-	1.20 gm.
EDTA	-	3.70 gm.
Penicillin-G sodium	-	1 lac I.U.
Streptomycin sulphate	-	1.00 gm.
Distilled water to	-	1.00 litre.

The extended semen were kept in 100ml. plastic jar and filled upto brim to maintain anaectobic condition. Then, these

samples were preserved in B.O.D. incubator at 18°C for the duration of 24, 48 and 72 hours of preservation. These samples were brought at room temperature and spermatozoan characteristics were evaluated at 24, 48 and 72 hours of preservation following the standard methods utilized for evaluation of neat semen.

Further, the levels of macro and micro minerals in seminal plasma were estimated at 24, 48 and 72 h of preservation using spectrophotometer and atomic absorption spectrophotometer respectively following standard methods as utilized for estimation of minerals in neat semen.

5. Statistical Analysis :

Results of the studies were analysed statistically according to Snedecor and Cochran (1967).

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CHAPTER - IV

RESULTS

RESULTS

During the course of study 72 semen samples from 4 large white Yorkshire and 4 Tamworth x Desi (T & D) boars were examined. The study was undertaken to assess seminal characteristics and seminal minerals of both breed in neat semen as well as preserved semen. The details of the results are given below.

1. Semen characteristics :

Mean values of semen characters for both breed are presented in Table-1.

1.1. Strained volume :

The mean strained volume were recorded as 160.34 ± 1.08 ml. in large white Yorkshire and 132.73 ± 3.89 ml. in T & D boars (Table-1).

Statistical analysis showed highly significant difference ($P<0.01$) in strained volume between large white Yorkshire and T & D breeds of boar (Table-5).

1.2 Gel volume :

The mean Gel volume were recorded as 41.47 ± 0.18ml. in large white Yorkshire and 34.70 ± 0.19 ml. in T & D boars (Table-1).

Statistical analysis showed highly significant difference ($P<0.01$) in gel volume between large white Yorkshire and T & D boars (Table-5).

Table - 1 : Semen characteristics (Mean ± S.E.) of Large white Yorkshire and Tamworth x Desi (T & D) Boars.

Semen Characters	Mean Value	
	Large white Yorkshire	T & D
Strained Volume (ml.)	$160.34^{b} \pm 1.08$	$132.73^{a} \pm 3.89$
Gel volume (ml.)	$41.47^{b} \pm 0.18$	$34.70^{a} \pm 0.19$
Total volume (ml.)	$220.38^{b} \pm 0.80$	$185.27^{a} \pm 0.79$
Individual sperm motility (Arc sin transformed value)	$66.59^{b} \pm 0.32$ (84.13 ± 0.41)	$65.16^{a} \pm 0.44$ (82.22 ± 0.58)
Live sperm (Arc sin transformed value)	$65.89^{b} \pm 0.37$ (83.22 ± 0.48)	$63.69^{a} \pm 0.49$ (80.19 ± 0.67)
Sperm concentration (Million/ml.)	$275.48^{b} \pm 0.35$	$268.70^{a} \pm 0.31$
Progressive motility (Arc sin transformed value)	$58.33^{a} \pm 0.59$ (72.27 ± 0.91)	$57.21^{a} \pm 0.52$ (70.55 ± 0.83)
pH	$7.60^{b} \pm 0.03$	$7.40^{a} \pm 0.04$

Each value is the average of 36 observations.

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

1.3 Total volume :

The mean total volume of semen were recorded as 220.38 ± 0.80 ml. in large white Yorkshire and 185.27 ± 0.79ml. in T & D boars (Table-1).

Statistical analysis showed highly significant difference ($P<0.01$) in total volume between large white Yorkshire and T & D boars (Table-5).

1.4 Colour and consistency :

The colour of the semen recorded during the present study varied between milky white to creamy white.

The consistency of semen recorded in the present study was thick.

1.5 Mass activity :

The mass activity of semen samples were recorded and graded from 0 to +5 based on intensity of swirl.

1.6 Individual sperm motility :

The average value of individual sperm motility in the present study were recorded as 84.13 ± 0.41 percent in large white Yorkshire and 82.22 ± 0.58 percent in T & D boars (Table-1).

Statistical analysis showed significant difference ($P<0.05$) in individual sperm motility between large white Yorkshire and T & D boars (Table-5).

1.7 Live sperm :

The average value of live sperm in the present study were recorded as 83.22 ± 0.48 percent in large white Yorkshire and 80.19 ± 0.67 percent in T & D boars (Table-1).

Statistical analysis showed highly significant difference ($P<0.01$) in live sperm between large white Yorkshire and T & D boars (Table – 5).

1.8 Sperm concentration :

The average sperm concentration were recorded as 275.48 ± 0.35 million per ml. in large white Yorkshire and T & D boars (Table-5) 268.70 ± 0.31 million per ml. in T & D boars (Table-1).

Statistical analysis showed highly significant difference ($P<0.01$) in sperm concentration between large white Yorkshire

1.9 Progressive motility :

The mean progressive motility of spermatozoa were recorded as 72.27 ± 0.91 percent is large white Yorkshire and 70.55 ± 0.83 percent in T & D boars (Table-1).

Statistical analysis showed non-significant difference in progressive motility of spermatozoa between large white Yorkshire and T and D breeds of boar (Table-5).

1.10 pH :

The pH of semen were recorded as 7.60 ± 0.03 in large white Yorkshire and 7.40 ± 0.04 in T & D boars (Table-1).

Statistical analysis showed highly significant difference ($P<0.01$) in pH of semen between large white Yorkshire and T & D boars (Table-5).

2. Sperm abnormality in neat semen :

The mean percentages of head, mid piece, tail, abnormalities, proximal cytoplasmic droplet and distal cytoplasmic droplet were recorded as 2.42 ± 0.03, 0.41 ± 0.03, 2.01 ± 0.02, 1.20 ± 0.002 and 1.14 ± 0.09 percent respectively in large white Yorkshire boars and 2.53 ± 0.02, 0.55 ± 0.02, 2.31 ± 0.01, 1.30 ± 0.02 and 1.19 ± 0.008 percent in T & D boars respectively (Table-2). The overall average sperm abnormalities were recorded as 7.17 ± 0.172 percent in large white Yorkshire and 7.88 ± 0.078 percent in T & D boars (Table-2).

Statistical analysis showed highly significant difference ($P<0.01$) in tail abnormality between large white Yorkshire and T & D boars (Table-5).

3. Acrosomal morphology in neat semen :

The average percentage of damaged apical ridge, missing apical ridge and loose acrosomal cap were recorded as 0.62 ± 0.03, 0.00 and 1.41 ± 0.06 percent respectively in large white Yorkshire and 0.63 ± 0.03, 0.00 and 1.42 ± 0.06 percent respectively in T & D boars (Table-3).

Statistical analysis showed no significant difference in acrosomal morphology of spermatozoa between large white Yorkshire and T & D boars (Table-5).

Table - 2 : Percentage (Mean ± S.E.) of sperm abnormalities in Large white Yorkshire and Tamworth x Desi (T & D) Boars. (Arc sin transformed value).

Sperm Abnormalities	Mean Percent	
	Large white Yorkshire	T & D
Head Abnormalities (HA)	$8.95^{a} \pm 0.07$ (2.42 ± 0.03)	$9.15^{a} \pm 0.05$ (2.53 ± 0.02)
Mid piece defect (MPD)	$3.41^{a} \pm 0.18$ (0.41 ± 0.03)	$4.13^{a} \pm 0.12$ (0.55 ± 0.02)
Tail Abnormalities (TA)	$8.15^{a} \pm 0.05$ (2.01 ± 0.02)	$8.74^{b} \pm 0.03$ (2.31 ± 0.01)
Proximal Cytoplasmic droplet (PCD)	$6.30^{a} \pm 0.02$ (1.20 ± 0.002)	$6.55^{a} \pm 0.06$ (1.30 ± 0.02)
Distal Cytoplasmic droplet (DCD)	$6.12^{a} \pm 0.06$ (1.14 ± 0.09)	$6.27^{a} \pm 0.02$ (1.19 ± 0.008)
Total	32.93 ± 0.38 (7.17 ± 0.172)	34.84 ± 0.28 (7.88 ± 0.078)

Each value is the average of 36 observations.

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Table - 3 : Percentage (Mean ± S.E.) of acrosomal morphology in Large white Yorkshire and Tamworth x Desi (T & D) Boars. (Arc sin transformed value).

Acrosomal Morphology	Mean Percentage	
	Large white Yorkshire	T & D
Damaged Apical ridge (DAR)	4.41[a] ± 0.17 (0.62 ± 0.03)	4.46[a] ± 0.16 (0.63 ± 0.03)
Missing Apical ridge (MAR)	0.00[a] (0.00)	0.00[a] (0.00)
Loose Acrosomal Cap (LAC)	6.73[a] ± 0.19 (1.41 ± 0.06)	6.76[a] ± 0.19 (1.42 ± 0.06)
Total	11.14 ± 0.36 (2.03 ± 0.09)	11.22 ± 0.35 (2.05 ± 0.09)

Each value is the average of 36 observations.

Value under parenthesis are percentage of original value.

Values bearing same small superscripts in a row did not differ significantly.

4. Seminal minerals :

4.1 The level of calcium :

The average value of Ca level in neat semen samples of large white Yorkshire and T & D boars were recorded 4.20 ± 0.25 mg. per 100 ml. and 4.09 ± 0.28 mg per 100ml. respectively (Table-4).

Statistical analysis showed non-significant difference in Ca level of semen between large white Yorkshire and T & D boars (Table-5).

4.2 The level of magnesium:

The average value of Mg level in neat semen samples of large white Yorkshire and T & D boars were recorded as 8.89 ± 0.418 mg. per 100 ml. and 8.85 ± 0.410 mg. per 100 ml. respectively (Table-4).

Statistical analysis showed non-significant difference in Mg level of semen between large white Yorkshire and T & D boars (Table-5).

4.3 The level of inorganic phosphorus :

The average value of P level in neat semen samples of large white Yorkshire and T & D boars were recorded as 1.79 ± 0.10mg. per 100ml. and 1.75 ± 0.04 mg. per 100ml. respectively (Table-4).

Statistical analysis showed non-significant difference in P level of semen between large white Yorkshire and T & D boars (Table-5).

Table - 4 : Concentration (Mean ± S.E.) of Macro minerals and Micro minerals in Large white Yorkshire and Tamworth x Desi (T & D) Boars semen.

Seminal Minerals	Mean Value	
	Large white Yorkshire	T & D
MACRO MINERALS Calcium (mg/100ml)	$4.20^{a} \pm 0.25$	$4.09^{a} \pm 0.28$
Magnesium (mg/100ml.)	$8.89^{a} \pm 0.418$	$8.85^{a} \pm 0.410$
Inorganic phosphorus (mg/100ml.)	$1.79^{a} \pm 0.10$	$1.75^{a} \pm 0.04$
MICRO MINERALS Zinc (mg/100ml.)	$17.10^{a} \pm 0.11$	$16.98^{a} \pm 0.15$
Copper (mg/100ml.)	$0.156^{a} \pm 0.001$	$0.154^{a} \pm 0.001$
Cobalt (mg/100ml.)	$0.049^{a} \pm 0.005$	$0.044^{a} \pm 0.006$
Manganese (mg/100ml.)	$0.020^{a} \pm 0.003$	$0.018^{a} \pm 0.001$

Each value is the average of 36 observations.

Values bearing same small superscripts in a row did not differ significantly.

4.4 The level of zinc :

The average value of Zn level in neat semen samples of large white Yorkshire and T & D boars were recorded as 17.10 ± 0.11 mg. per 100ml. and 16.98 ± 0.15 mg. per 100 ml. respectively (Table-4).

Statistical analysis showed no significant difference in Zn level of semen between large white Yorkshire and T & D boars (Table-5).

4.5 The level of copper :

The average value of Cu level in neat semen samples of large white Yorkshire and T & D boars were recorded as 0.156 ± 0.001 mg. per 100ml. and 0.154 ± 0.001 mg. per 100ml. respectively (Table-4).

Statistical analysis showed no significant difference in Cu level of semen between large white Yorkshire and T & D boars (Table-5).

4.6 The level of cobalt :

The average value of Co level in neat semen samples of large white Yorkshire and T & D boars were recorded as 0.049 ± 0.005 mg. per 100ml. and 0.044 ± 0.006 mg. per 100 ml. respectively.

Statistical analysis showed no significant difference in co level of semen between large white Yorkshire and T & D boars (Table-5).

Table – 5 : Test of significance in different semen characteristics studied between large white Yorkshire and Tamworth x Desi (T & D) boars.

Semen Characters	't' value
Strained volume	6.83**
Gel volume	26.03**
Total volume	31.34**
Individual sperm motility	2.64*
Live sperm	3.54**
Sperm concentration	14.73**
Progressive motility	1.41
pH	4.00**
Head abnormalities	0.75
Mid piece defect	0.42
Tail abnormalities	9.83**
Proximal cytoplasmic droplet	0.53
Distal cytoplasmic droplet	0.57
Damaged apical ridge	0.21
Missing apical ridge	0.00
Loose acrosomal cap	0.11
Seminal calcium	0.28
Seminal magnesium	0.068
Seminal phosphorus	0.40
Seminal zinc	0.63
Seminal copper	1.05
Seminal cobalt	0.71
Seminal manganese	0.66

** : Highly significant (P<0.01)

* : Significant (P<0.05)

4.7 The level of manganese :

The average value of Mn level in neat semen samples of large white Yorkshire and T & D boars were recorded as 0.020 ± 0.003mg. 100ml. and 0.018 ± 0.001 mg. per 100ml. respectively (Table-4).

Statistical analysis showed no significant difference in Mn level of semen between large white Yorkshire and T & D boars (Table-5).

5. Effect of hours of preservation on seminal characters :

5.1 Motility of spermatozoa :

The average motility percentage in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 68.08 ± 0.67 and 68.18 ± 0.67, 62.08 ± 0.88 and 63.07 ± 0.48 and 56.07 ± 0.46 and 58.06 ± 0.70 percent in T & D and large white Yorkshire boars respectively (Table-6).

Analysis of variance (Table-10) indicated highly significant ($P<0.01$) effect of hours of preservation on motility percentage of spermatozoa. Critical difference test (Table-6) indicated significant difference among these stages with highest at 24 hour, which declined with increase in duration of preservation.

5.2 Live sperm percentage :

The average live sperm percentage in Kiev dilutor at 24, 48 and 72 hour of preservation was recorded as 76.66 ± 0.55 and 76.10 ± 0.59, 71.24 ± 1.02 and 70.13 ± 0.95 and 65.05 ±

Table - 6 : Motility percentage (Mean + S.E.) of boar semen at different hours of preservation in different breeds. (Arc sin transformed value).

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	$55.70^{Ac} \pm 0.41$ (68.18 ± 0.67)	$52.59^{Ab} \pm 0.29$ (63.07 ± 0.48)	$49.65^{Aa} \pm 0.41$ (58.06 ± 0.70)
T & D	$55.64^{Ac} \pm 0.41$ (68.08 ± 0.67)	$52.02^{Ab} \pm 0.45$ (62.08 ± 0.88)	$48.49^{Aa} \pm 0.27$ (56.07 ± 0.46)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing same capital superscripts in a column did not differ significantly.

Table - 7 : Live sperm percentage (Mean ± S.E.) of boar semen at different hours of preservation in different breeds. (Arc sin transformed value)

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	61.17^{Bc} ± 0.38 (76.66 ± 0.55)	57.69^{Bb} ± 0.66 (71.24 ± 1.02)	53.79^{Ba} ± 0.44 (65.05 ± 0.73)
T & D	60.79^{Ac} ± 0.39 (76.10 ± 0.59)	56.96^{Ab} ± 0.60 (70.13 ± 0.95)	53.22^{Aa} ± 0.52 (64.07 ± 0.88)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing different capital superscripts in a column differ significantly.

Table - 8 : Head abnormalities percentage (Mean ± S.E.) of boar semen at different hours of preservation in different breeds. (Arc sin transformed value)

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	$9.09^{Aa} \pm 0.06$ (2.50 ± 0.03)	$9.11^{Aa} \pm 0.18$ (2.57 ± 0.09)	$9.44^{Ab} \pm 0.12$ (2.70 ± 0.07)
T & D	$9.19^{Ba} \pm 0.06$ (2.55 ± 0.03)	$9.23^{Ba} \pm 0.15$ (2.60 ± 0.08)	$9.49^{Aa} \pm 0.12$ (2.73 ± 0.07)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing different capital superscripts in a column differ significantly.

0.73 and 64.07 ± 0.88 percent in large white Yorkshire and T & D boars respectively (Table-7).

Analysis of variance (Table-10) indicated highly significant ($P<0.01$) effect of hours of preservation on live sperm percentage. Critical difference test (Table-7) indicated significant difference among these stages with highest at 24 hour, which declined with duration of preservation.

5.3 Head abnormality :

The average head abnormality percentage in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 2.50 ± 0.03 and 2.55 ± 0.03, 2.57 ± 0.09 and 2.60 ± 0.08, and 2.70 ± 0.07 and 2.73 ± 0.07 percent in large white Yorkshire and T & D boars respectively (Table-8).

Analysis of variance (Table-10) showed significant ($P<0.05$) effect of hours of preservation on head abnormality percentage. Critical difference test (Table-8) indicated non significant difference among these stages, but significant difference between 48 and 72 hours of preservation in breed of large white Yorkshire boars.

5.4 Tail abnormality :

The average tail abnormality percentage in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 3.00 ± 0.03 and 3.27 ± 0.01, 5.61 ± 0.07 and 6.20 ± 0.18, and 10.09 ± 0.53 and 11.10 ± 0.77 percent in large white Yorkshire and T & D boars respectively (Table-9).

Table - 9 : Tail abnormalities percentage (Mean ± S.E.) of boar semen at different hours of preservation in different breeds. (Arc sin transformed value)

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	9.97^{Aa} ± 0.06 (3.00 ± 0.03)	13.70^{Ab} ± 0.08 (5.61 ± 0.07)	18.28^{Ac} ± 0.53 (10.09 ± 0.53)
T & D	10.41^{Ba} ± 0.03 (3.27 ± 0.01)	14.37^{Bb} ± 0.20 (6.20 ± 0.18)	19.01^{Bc} ± 0.75 (11.10 ± 0.77)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing different capital superscripts in a column differ significantly.

Table - 10 : Analysis of variance showing the effect of hours of preservation and breeds on the semen characters.

Source of variation	D.F.	Mean Squares			
		Sperm motility	Live sperm	Head Abnormality	Tail Abnormality
Between hours of preservation	2	21.78**	27.94**	0.0645*	35.84**
Between Breeds	1	0.54	0.47	0.0121	0.56**
Error	2	0.14	0.01	0.0007	0.005

** : Highly significant (P<0.01).

* : Significant (P<0.05).

Table - 11 : Percentage (Mean ± S.E.) of Damaged apical ridge (DAR) at different hours of preservation in different breeds. (Arc sin transformed value)

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	8.99^{Aa} ± 0.01 (2.45 ± 0.01)	18.85^{Ab} ± 0.20 (10.44 ± 0.22)	22.71^{Ac} ± 0.01 (14.90 ± 0.01)
T & D	9.09^{Aa} ± 0.01 (2.50 ± 0.01)	18.90^{Ab} ± 0.05 (10.50 ± 0.05)	22.76^{Ac} ± 0.06 (14.97 ± 0.08)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing same capital superscripts in a column did not differ significantly.

Analysis of variance (Table-10) indicated highly significant ($P<0.01$) effect of hours of preservation on tail abnormality percentage. Critical difference test (Table-9) showed significant difference among these stages of preservation with highest at 72 hours.

5.5 Acrosomal morphology ;

Damaged apical ridge (DAR) :

Average percentage of DAR with Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 2.45 ± 0.01 and 2.50 ± 0.01, 10.44 ± 0.22 and 10.50 ± 0.05, and 14.90 ± 0.01 and 14.97 ± 0.08 percent in large white Yorkshire and T & D boars respectively (Table-11).

Analysis of variance (Table-14) revealed highly significant ($P<0.01$) effect of hours of preservation on the occurance of DAR. Critical difference test (Table-11) indicated significant difference in the percentage of damaged apical ridge among these stages.

Missing apical ridge (MAR) :

Average percentage of MAR Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 0.00 and 0.00, 0.44 ± 0.03 and 0.49 ± 0.03, and 1.10 ± 0.06 and 1.20 ± 0.08 percent in large white Yorkshire and T & D breeds of boar respectively (Table-12).

Analysis of variance (Table-14) revealed highly significant ($P<0.01$) effect of hours of preservation on the occurrence of MAR. Critical difference test (Table-12) indicated significant

Table - 12 : Percentage (Mean ± S.E.) of Missing apical ridge (MAR) at different hours of preservation in different breeds. (Arc sin transformed value)

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	0.00^{Aa} (0.00)	$3.71^{Ab} \pm 0.13$ (0.44 ± 0.03)	$5.93^{Ac} \pm 0.17$ (1.10 ± 0.06)
T & D	0.00^{Aa} (0.00)	$3.88^{Ab} \pm 0.17$ (0.49 ± 0.03)	$6.09^{Ac} \pm 0.26$ (1.20 ± 0.08)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing same capital superscripts in a column did not differ significantly.

Table - 13 : Percentage (Mean ± S.E.) of Loose acrosomal cap(LAC) at different hours of preservation in different breeds. (Arc sin transformed value)

Breed	Hours of preservation		
	24 hour	48 hour	72 hour
Large white Yorkshire	7.50^{Aa} ± 0.02 (1.71 ± 0.01)	11.04^{Ab} ± 0.05 (3.67 ± 0.03)	11.96^{Ac} ± 0.03 (4.29 ± 0.02)
T & D	7.65^{Aa} ± 0.02 (1.78 ± 0.01)	11.63^{Bb} ± 0.23 (4.13 ± 0.16)	12.27^{Ac} ± 0.09 (4.49 ± 0.07)

Value under parenthesis are percentage of original value.

Values bearing different small superscripts in a row differ significantly.

Values bearing same capital superscripts in a column did not differ significantly.

Table – 14 : Analysis of variance showing the effect of hours of preservation and breeds on acrosomal morphology.

Source of variation	D.F.	Mean Squares		
		DAR	MAR	LAC
Between hours of preservation	2	99.72**	18.47**	11.78**
Between Breeds	1	0.006	0.018	0.18
Error	2	0.0008	0.004	0.02

** : Highly significant (P<0.01).

difference in the percentage of missing apical ridge among these stages.

Loose acrosomal cap :

Average percentage of LAC with Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 1.71 ± 0.01 and 1.78 ± 0.01, 3.67 ± 0.03 and 4.13 ± 0.16, and 4.29 ± 0.02 and 4.49 ± 0.07 percent in large white Yorkshire and T & D boars respectively (Table-13).

Analysis of variance (Table-14) revealed highly significant ($P<0.01$) effect of hours of preservation on the occurance of LAC. Critical difference test (Table-13) indicated significant difference in the percentage of loose acrosomal cap among these stages.

5.6 Seminal minerals :

5.6.1 The level of calcium :

The mean value of Ca level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 4.10 ± 0.08 and 4.08 ± 0.04, 4.08 ± 0.01 and 4.06 ± 0.06, and 4.05 ± 0.07 and 4.04 ± 0.04 mg. per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed non-significant variation in the level of Ca in boar semen at different hours of preservation. Critical difference test (Table-15) revealed that the level of Ca decreased non-significantly with the increase in hours of preservation.

5.6.2 The level of magnesium :

The mean value of Mg level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 8.60 ± 0.20 and 8.58 ± 0.06, 8.58 ± 0.08 and 8.57 ± 0.03 and 8.555 ± 0.09 and 8.546 ± 0.20 mg per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Mg in boar semen at different hours of preservation. Critical difference test (Table-15) revealed that the level of Mg decreased non significantly with the increase in hours of preservation.

5.6.3 The level of inorganic phosphorus :

The mean value of P level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 1.74 ± 0.12 and 1.718 ± 0.12, 1.712 ± 0.05 and 1.692 ± 0.006, and 1.688 ± 0.05 and 1.666 ± 0.12 mg. per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed that there was no significant variation in the level of P in boar semen at different hours of preservation, critical difference test (Table-15) revealed that the level of P decreased non significantly with the increase in hours of preservation.

5.6.4 The level of zinc :

The mean value of Zn level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 16.860 ± 0.94 and 16.837 ± 0.05, 16.832 ± 0.06 and 16.814 ± 0.008, and 16.808

± 1.21 and 16.793 ± 0.16 mg per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Zn in boar semen at different hours of preservation. Critical difference test (Table-15) revealed that the level of Zn decreased non significantly with the increase in hours of preservation.

5.6.5 The level of copper :

The mean value of Cu level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 0.136 ± 0.02 and 0.135 ± 0.02, 0.134 ± 0.005 and 0.133 ± 0.003, 0.133 ± 0.013 and 0.132 ± 0.012 mg per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed that there was non significant variation in the level of Cu in boar semen at different hours of preservation. Critical difference test (Table-15) revealed that the level of Cu decreased non significantly with the increase in hours of preservation.

5.6.6 The level of cobalt :

The mean value of Co level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 0.040 ± 0.005 and 0.039 ± 0.003, 0.026 ± 0.01 and 0.025 ± 0.004 and 0.016 ± 0.001 and 0.015 ± 0.0003 mg. per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed that there was non significant variation in the level of Co in boar semen at

different hours of preservation. Critical difference test (Table-15) revealed that the level of Cu decreased non significantly with the increase in hours of preservation.

5.6.7 The level of manganese :

The mean value of Mn level in Kiev dilutor at 24, 48 and 72 hours of preservation was recorded as 0.018 ± 0.001 and 0.008 ± 0.0006, 0.012 ± 0.0008 and 0.007 ± 0.0005, and 0.008 ± 0.0005 and 0.005 ± 0.0003 mg. per 100ml. in large white Yorkshire and T & D boars respectively (Table-15).

Analysis of variance (Table-16) revealed that there was non significant variation in the level of Mn in boar semen at different hours of preservation. Critical difference test (Table-

Table – 15 : Concentration (Mean ± S.E.) of Boar seminal minerals at different hours of preservation in different breeds.

Seminal Minerals	Breeds	Hours of Preservation		
		24 hour	48 hour	72 hour
MACRO MINERALS Calcium (mg/100ml)	Large white Yorkshire	$4.10^{Aa} \pm 0.08$	$4.08^{Aa} \pm 0.01$	$4.05^{Aa} \pm 0.07$
	T & D	$4.08^{Aa} \pm 0.04$	$4.06^{Aa} \pm 0.06$	$4.04^{Aa} \pm 0.04$
Magnesium (mg/100ml.)	Large white Yorkshire	$8.60^{Aa} \pm 0.20$	$8.58^{Aa} \pm 0.08$	$8.555^{Aa} \pm 0.09$
	T & D	$8.58^{Aa} \pm 0.06$	$8.57^{Aa} \pm 0.03$	$8.546^{Aa} \pm 0.20$
Inorganic phosphorus (mg/100ml)	Large white Yorkshire	$1.74^{Aa} \pm 0.12$	$1.712^{Aa} \pm 0.05$	$1.688^{Aa} \pm 0.05$
	T & D	$1.718^{Aa} \pm 0.12$	$1.692^{Aa} \pm 0.006$	$1.666^{Aa} \pm 0.12$
MICRO MINERALS Zinc (mg/100ml.)	Large white Yorkshire	$16.860^{Aa} \pm 0.94$	$16.832^{Aa} \pm 0.06$	$16.808^{Aa} \pm 1.21$
	T & D	$16.837^{Aa} \pm 0.05$	$16.814^{Aa} \pm 0.008$	$16.793^{Aa} \pm 0.16$
Copper (mg/100ml)	Large white Yorkshire	$0.136^{Aa} \pm 0.02$	$0.134^{Aa} \pm 0.005$	$0.133^{Aa} \pm 0.013$
	T & D	$0.135^{Aa} \pm 0.02$	$0.133^{Aa} \pm 0.003$	$0.132^{Aa} \pm 0.012$
Cobalt (mg/100ml)	Large white Yorkshire	$0.040^{Aa} \pm 0.005$	$0.026^{Aa} \pm 0.01$	$0.016^{Aa} \pm 0.001$
	T & D	$0.039^{Aa} \pm 0.003$	$0.025^{Aa} \pm 0.004$	$0.015^{Aa} \pm 0.0003$
Manganese (mg/100ml)	Large white Yorkshire	$0.018^{Aa} \pm 0.001$	$0.012^{Aa} \pm 0.0008$	$0.008^{Aa} \pm 0.0005$
	T & D	$0.008^{Aa} \pm 0.0006$	$0.007^{Aa} \pm 0.0005$	$0.005^{Aa} \pm 0.0003$

Values bearing same small superscripts in a row did not differ significantly.

Values bearing same capital superscripts in a column did not differ significantly.

Table – 16 : Analysis of variance showing the effect of hours of preservation and breeds on the seminal minerals concentration.

Source of variation	D.F.	Mean Squares						
		Ca	Mg	P	Zn	Cu	Co	Mn
Between hours of preservation	2	0.0009	0.0008	0.0013	0.0010	0.000005	0.000241	0.000021
Between breeds	1	0.0004	0.0003	0.0002	0.0003	0.000001	0.000073	0.000054
Error	2	0.00005	0.00005	0.00015	0.0001	0.000014	0.000014	0.000006

15) revealed that the level of Mn decreased non significantly with the increase in hours of preservation.

6. Effect of breeds on seminal characters :

6.1 Motility of spermatozoa :

Analysis of variance (Table-10) indicated non significant effect of breeds on motility percentage of spermatozoa. Critical difference test (Table-6) indicated non significant difference between breeds. The percentage of motility was non significantly higher in large white Yorkshire than T & D breeds of boars (Table-6).

6.2 Live sperm percentage :

Analysis of variance (Table-10) indicated non significant effect of breeds on live sperm percentage. Critical difference test (Table-7) indicated significant difference between breeds. The percentage of live sperm was significantly higher in large white Yorkshire than in T & D boars (Table-7).

6.3 Head abnormality ;

Analysis of variance (Table-10) showed non significant effect of breeds on head abnormality percentage. Critical difference test (Table-8) indicated significant difference between breeds at 24 and 48 h preservation. The percentage of head abnormality was significantly higher at 24 and 48 hour of preservation in T & D than in large white Yorkshire boars, but non significantly higher at 72 h of preservation (Table-8).

6.4 Tail abnormality :

Analysis of variance (Table-10) showed highly significant effect of breeds on tail abnormality. Critical difference test (Table-9) indicated significant difference between breeds. The percentage of tail abnormality was significantly higher in T & D than in large white Yorkshire boars (Table-9).

6.5 Acrosomal morphology :

Damaged apical ridge (DAR) :

Analysis of variance (Table-14) revealed non significant effect of breeds on this parameter. Critical difference test (Table-11) indicated non significant difference between breeds at 48 and 72 hours of preservation. The percentage of DAR was non significantly higher at 48 and 72 hours of preservation in T & D than in large white Yorkshire boars, but significantly higher at 24 hours of preservation (Table-11).

Missing apical ridge (MAR) :

Analysis of variance (Table-14) indicated non significant effect of breeds on this parameter. Critical difference test (Table-12) indicated non-significant difference between breeds. The percentage of MAR was non significantly higher in T & D than in large white Yorkshire boars Table-12).

Loose acrosomal cap (LAC) :

Analysis of variance (Table-14) indicated non significant effect of breeds on this parameter. Critical difference test (Table-12) indicated significant difference between breeds at 48 hours of preservation only. The percentage of LAC was non

significantly higher at 24 and 72 hours of preservation in T & D than in large white Yorkshire boars, but significantly higher at 48 hours of preservation (Table-12).

6.6 Seminal minerals :

6.6.1 The level of calcium :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Ca between breeds. Critical difference test (Table-15) indicated non significant difference between breeds. The level of Ca was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

6.6.2 The level of Magnesium :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Mg between breeds. Critical difference test (Table-15) indicated non significant difference between breeds. The level of Mg was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

6.6.3 The level of inorganic phosphorus :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of P between breeds. Critical difference test (Table-15) indicated non significant difference between breeds. The level of P was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

6.6.4 The level of zinc :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Zn between breeds. Critical difference test (Table-15) indicated non significant difference

between breeds. The level of Zn was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

6.6.5 The level of copper :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Cu between breeds. Critical difference test (Table-15) indicated non significant difference between breeds. The level of Cu was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

6.6.6 The level of cobalt :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Co between breeds. Critical difference test (Table-15) indicated non significant difference between breeds. The level of Co was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

6.6.7 The level of Manganese :

Analysis of variance (Table-16) revealed that there was no significant variation in the level of Mn between breeds. Critical difference test (Table-15) indicated non significant difference between breeds. The level of Mn was non significantly higher in large white Yorkshire than in T & D boars (Table-15).

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CHAPTER - V

DISCUSSION

DISCUSSION

In this study attempts have been made to assess the Macro and Micro minerals concentration in Boar semen and its influence on preservability of large white Yorkshire and Tamworth x Desi (T & D) boars semen. The quality of neat as well as preserved semen for 24, 48 and 72 hours were assessed on the basis of motility, live sperm percentage, sperm abnormalities, acrosomal morphology, spermatozoan membrane integrity, concentration of spermatozoa and concentration of some macro and micro minerals. These minerals were also assessed at different stages of preservation to measure their profile.

1. Seminal characters :

1.1. Volume :

The mean of volume of different fractions of seminal ejaculates had been presented in Table-1. These findings are in agreement with the findings of Tamuli *et al.* (1982), Pandey and Singh (1998) and Kansi (2007). The observed value in the present study were higher than that of Bhuyan (1989), Rao *et al.* (1992) and Michalski and Polanska (1983) and lower than Tamuli (1982). Ejaculate volume has been found to be affected by many intrinsic and extrinsic factors viz. agroclimatic conditions, age, body weight, feeding, management and functional status of accessory sex glands (Prasad, 1969 and

Mittal, 1987). This might be the reason for variation in ejaculate volume as recorded during the study.

1.2. Individual sperm motility :

The mean percentage of individual sperm motility has been presented in Table-1. The observed mean percentage of individual sperm motility in the present study is in agreement with the findings of Rao *et al.* (1992) and Pandey and Singh (1998). The mean percent of individual sperm motility recorded in the present study is higher than those reported by Autonyuk *et al.* (1982), Michalski and Polanska (1983), Dubiel *et al.* (1985), Bhuyan (1989) and Kansi (2007). The mean percentage of individual sperm motility recorded for T & D boar semen in the present study is lower than that reported by Kansi (2007).

Influence of breed as well as individuals on sperm motility had been recorded and this might be reasons for variation in sperm motility recorded in the present finding.

1.3 Live sperm percentage :

The mean percentage of live sperm has been presented in Table-1. The observed mean live sperm percentage in the present study is in agreement with the findings of Rao *et al.* (1991) and Pandey and Singh (1998). However, the mean live sperm percentage recorded in the present study is lower than those reported by Tamuli (1982) and Bhuyan (1989). The mean live sperm percentage recorded for T & D boar semen in the present study is lower than that reported by Kansi (2007).

Variation in the mean live sperm percentage recorded in the present study from that of earlier reports may be due to factors like age (Luthra *et al.*, 1993), season (Kuni-Diaka *et al.*, 1980) and environment.

1.4 Sperm concentration :

The average sperm concentration recorded in the present study has been presented in Table-1. The observed average sperm concentration recorded in the present study is in agreement with the findings of Rao *et al.* (1992). However, the average sperm concentration recorded in the present study is lower than that reported by Pandey and Singh (1998) and Kansi (2007) and higher than that reported by Tamuli (1982), Antonyuk *et al.* (1982) and Bhuyan (1989). The average sperm concentration recorded in the present study for T & D boar semen is lower than that reported by Kansi (2007).

Variation in the average sperm concentration recorded in the present study from that of earlier reports might be due to age (Amann *et al.*, 1974), season of collection (Pandey and Singh, 1998) and nutrition (Castillo and Lopez, 1988).

1.5 Progressive motility :

The mean progressive motility recorded in the present study has been presented in Table-1. The present findings are in agreement with the findings of Louda and Pavlik (1984) and Pandey and Singh (1998). However, the mean progressive motility recorded in the present study is lower than that reported by Tamuli (1982) and Bhuyan (1989).

The variation in the mean percentage of progressive motility, recorded in the present study from findings of earlier worker might be due to effect of age of sire or season as reported by Kazantaeva (1971) or due to individual variations as reported by Murthy (1977).

1.6 pH :

The average pH of semen recorded in the present study has been presented in Table-1. The present findings are comparable to the findings of Pandey and Singh (1998). However, the average pH recorded in the present study is lower than that reported by Tamuli (1982) and Rao *et al.* (1991).

The variation in the average pH of semen recorded in the present study from that of earlier reports may be due to agroclimatic differences or genetic make up of sires.

2. Sperm abnormalities in neat semen :

The mean head abnormality percentage has been presented in Table-2. These findings are in accordance comparable to the findings of Pandey and Singh (1997) and Kansi (2007). However, Tamuli (1982) had reported lower head abnormality percentage in semen of landrace boar.

The mean mid piece defect, Tail abnormality and incidence of proximal cytoplasmic droplet and Distal cytoplasmic droplet percentage have been presented in Table-2. The recorded mean mid piece defect percentage is in agreement with the findings of Pandey and Singh (1997) and Kansi (2007). However, Tamuli (1982) and Bhuyan (1989) had

reported higher incidence of mid piece defect. The incidence of tail abnormality in the present study is in agreement with the findings of Cerovsky (1981), Pandey (1997) and Kansi (2007). However, Tamuli (1982) reported lower tail abnormality percentage, whereas Bhuyan (1989) reported higher tail abnormality percentage. The incidence of proximal cytoplasmic droplet in the present study is in agreement with the findings of Pandey (1997) and Kansi (2007). The incidence of Distal cytoplasmic droplet in the present study is higher than that reported by Pandey (1997) and Kansi (2007). The mean distal cytoplasmic droplet percentage of T & D boar semen is in agreement with the findings of Kansi (2007).

The variation in sperm abnormalities observed by different worker might be due to individual variations as reported by Murthy (1977) and Tamuli and Raj Konwar (1987).

3. Acrosomal defect in neat semen :

The average acrosomal defect recorded in the present study has been presented in Table-3. The total average acrosomal defect recorded in the present study was 2.03 ± 0.09 and 2.05 ± 0.09 percent in large white Yorkshire and T & D boars respectively (Table-3). The observed value is lower in comparison with findings of Tamuli and Raj Konwar (1987), but higher than that reported by Cerovsky (1981).

The influence of breed on the incidence of acrosomal defects as well as maintaining of spermatozoan membrane integrity had been reported (Rana and Dhami, 2003). The

variation recorded in the present study might be due to variation in breed of boars.

4. Seminal minerals :

4.1 The level of calcium :

The average calcium concentration recorded in the present study has been presented in Table-4. The observed average calcium concentration recorded in the present study is in close agreement with the findings of Mann (1964) and Singh (2005).

Studies on breed wise distribution of Ca level in boar semen were scanty and thus findings of the present study are uncomparable. However, significant variation in Ca level of semen samples of different breeds of goat and cattle had been reported by Pandey and Singh (1982) and Markhandeya *et al.* (1991) respectively.

4.2 The level of magnesium :

The level magnesium concentration recorded in the present study has been presented in Table-4. The observed concentration of magnesium, recorded in the present study is in close agreement to the findings of Mann (1964) and Singh (2005).

Studies on breedwise distribution of Mg level in boar semen had not been recorded and thus the findings of the present study can not be compared.

4.3 The level of inorganic phosphorus :

The average inorganic phosphorus concentration recorded in the present study has been presented in Table-4. These findings are in agreement with the findings of Gottardi and Brunel (1978). However, the average inorganic phosphorus concentration recorded in the present study is lower than that reported by Mann (1964).

The variation in the average inorganic phosphorus concentration recorded in the present study might either due to variation in nutritional status or breed variation of boars.

4.4 The level of zinc :

The average zinc concentration recorded in the present study has been presented in Table-4. The zinc concentration recorded in the present study is almost equal to the findings of Taylor & Francis (2003), Massanyi *et al.* (2003) and Massanyi *et al.* (2004).

The breedwise distribution of Zn, recorded in the present study is uncomparable in lack of available informations. However, significantly higher level of Zn concentration in boar semen in comparison of bull, stallion and ram semen had been reported by Massanyi *et al.* (2004).

4.5 The level of copper :

The average copper concentration recorded in the present study has been presented in Table-4. The copper concentration, recorded in the present study is in agreement

with the findings of Taylor & Francis (2003), Massanyi *et al.* (2003) and Massanyi *et al.* (2004).

The breedwise distribution of Cu, recorded in the present study can not be compared in lack of information. However, significantly higher level of Cu concentration in Rams (2.49 ± 0.18 mg/kg) and foxes (2.16 ± 0.53 mg/kg) in comparison with bulls (1.64 ± 0.21 mg/kg), boars (1.64 ± 0.28 mg/kg) and stallion (0.86 mg/kg) were reported by Massanyi *et al.* (2004).

4.6 The level of cobalt :

The average cobalt concentration recorded in the present study has been presented in Table-4. The cobalt concentration recorded in the present study is almost similar to the level of cobalt in bull semen (0.481 ± 0.038 µg/ml) as reported by Mohanty *et al.* (2004). However, the finding of average cobalt concentration, recorded in the present study is lower than findings of Vinaya B. Shelke and Dhami (2001) in bull semen.

The breedwise variation in level of Co level in semen samples of large white Yorkshire and T & D breed of boars are uncomparable.

4.7 The level of managanese :

The average manganese concentration recorded in the present study has been presented in Table-4. The observed manganese concentration recorded in the present study is in agreement with the findings of Vinaya B. Shelke *et al.* (2001) in bull semen.

The breed wise observations of the present study are uncomparable in lack of information. However, breed wise variation in Mn concentration in ram semen had been reported by Suther *et al.* (2000) and the non-significant variation recorded in the present finding might be due to variation in breed of boar.

5. Effect of hours and breeds on the preservability of Boar semen :

5.1 Motility of spermatozoa :

The mean sperm motility percentage has been presented in Table-6. The findings of present study are in close agreement with the findings of Bhuyan *et al.* (1992), Lalrintluanga *et al.* (2002), Das *et al.* (2006) and Kansi (2007). However, the present findings are lower than findings of Pandey (1993). Highly significant effect ($P<0.01$) of hours of preservation on motility percentage was observed which is in agreement with Tamuli *et al.* (1986) and Bhuyan *et al.* (1992).

It is evident from Table-6 that motility percentage of spermatozoa declined significantly with the advancement of preservation time with lowest motility at 72 hours of preservation.

5.2 Live sperm percentage :

The mean live sperm percentage has been presented in Table-7. The present findings are lower than that reported by Bhuyan *et al.* (1992), Pandey (1993) and Joshi and Kharche (1990). However, the present findings are higher than that

reported by Lalrintluanga *et al.* (1994) and Kansi (2007). The present findings of T & D boar semen is higher than that reported by Kansi (2007). Highly significant effect ($P<0.01$) of hours of preservation on live sperm percentage was observed which is in agreement with Bhuyan *et al.* (1992).

It is evident from Table-6 that live sperm percentage decline significantly with the advancement of preservation time with lowest live sperm percentage at 72 hours.

5.3 Head abnormality percentage :

The mean head abnormality percentage has been presented in Table-8. The finding of present study are in agreement with the findings of Pandey (1993) and Kansi (2007).

It is evident from Table-8 that head abnormality percentage of spermatozoa increased significantly with the advancement of preservation time with the highest incidence of head abnormality at 72 hours.

5.4 Tail abnormality percentage :

The mean tail abnormality percentage has been presented in Table-9. The findings of present study are in agreement with the findings of Pandey (1993) and Kansi (2007).

Highly significant effect of both hours of preservation and breeds on the incidence of such abnormality was noted during the study. Increase in the incidence of tail abnormalities after freezing and thawing have been reported by Baviskar (1985) and Sinha (1986) which supports the present findings.

5.5 Changes in acrosomal morphology :

The acrosome and its enzyme play essential role in the penetration and fertilization of Mammalian Ova (Bedforot, 1968; Stambaugh and Buckkley, 1969). Significant changes in the acrosome morphology of boar spermatozoa during *in vitro* storage with different dilutors was recorded by Pursel *et al.* (1974).

The mean value of Damaged apical ridge (DAR) recorded in the present study has been presented in Table-11. The present findings are in agreement with the findings of Pandey (1997). Highly significant effect ($P<0.01$) of hours of preservation on the occurrence of DAR has been recorded in the present study. Pursel *et al.* (1974) recorded gradual increase in the percentage of DAR as storage time increased, which supports the present findings.

The mean value of missing apical ridge (MAR) recorded in the present study has been presented in Table-12. The present findings are in agreement with the findings of Pandey (1997). Highly significant effect ($P<0.01$) of hours of preservation on the occurrence of MAR has been recorded in the present study. Pursel *et al.* (1974) recorded gradual increase in the percentage of MAR as storage time increased, which supports the present findings.

The mean value of loose acrosomal cap (LAC) recorded in the present study has been presented in Table-13. The present findings are in agreement with the findings of Pandey (1997).

Highly significant effect ($P<0.01$) of hours of preservation on the occurrence of LAC has been recorded in the present study. However, Pursel *et al.* (1974) did not find significant increase in the incidence of LAC type acrosome when we used BL-1 dilutor and studied upto 72 hours of preservation.

Observation on breed wise acrosomal morphology in boar semen were not recorded by previous workers and thus the results of the present study can not be compared due to lack of information on this aspect.

5.6 Seminal minerals :

Macrominerals :

The mean concentration of macrominerals in Boars semen recorded in the present study has been presented in Table-15. The non significant effect of hours of preservation and breeds on concentration of macrominerals has been observed in the present study.

The observation on seminal macrominerals concentration at different hours of preservation in boar semen were not recorded by previous workers and thus the results of the present study can not be compared in lack of informations.

Micro minerals (Trace minerals):

The mean concentration of micro minerals in boar semen recorded in the present study has been presented in Table-15. The non significant effect of hours of preservation and breeds, on concentration of microminerals (trace minerals) has been observed in the present study.

It is evident from Table-15 that mean concentration of micro minerals declined non-significantly with the advancement of preservation time with lowest concentration at 72 hours.

The distribution of seminal microminerals (Trace minerals) concentration at different hours of preservation in boar semen were scanty and so the present findings are uncomparable. However, Mohanty *et al.* (2004) reported non significant effect of duration of preservation on trace mineral concentration in crossbred Bull semen, which supports the present findings.

6. Effect of Breeds on the preservability of Boar semen :

Breed wise seminal characteristics of large white Yorkshire and T & D boars during the course of preservation have been presented in Table-6 to Table 14. It is evident from findings recorded in above mentioned table clearly showed the influence of breed on different characters of spermatozoa. Bhuyan (1989) and Kansi (2007) had also reported the influence of breed on seminal characteristics of boar semen Viz. progressive motility, live sperm %, head abnormality and tail abnormality during the preservation, which supports the present findings.

Breed wise seminal characteristics during 24, 48 and 72 hours of preservation in large white Yorkshire and T & D boar

semen showed superiority of large white Yorkshire boar semen in comparison of semen of T & D breed. However, Kansi (2007) had reported higher motility and live sperm percentage in semen of T & D boar. This variation might be due to change in agroclimatic and individual variation of boars.

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CHAPTER - VI

SUMMARY

SUMMARY

A study was conducted on 72 semen ejaculates of 4 large white Yorkshire and 4 Tamworth x Desi (T & D) boars maintained under the Adhoc Research Project at B.V.C., Patna. Semen collection was done by gloved hand technique over oestrus sows at 5 days interval.

The mean value of different semen characteristics were strained volume 160.34 ± 1.08 and 132.73 ± 3.89 ml, Gel volume 41.47 ± 0.18 and 34.70 ± 0.19 ml, Total volume 220.38 ± 0.80 and 185.27 ± 0.79 ml, individual sperm motility 84.13 ± 0.41 and 82.22 ± 0.58 percent, Live sperm percentage 83.22 ± 0.48 and 80.19 ± 0.67, sperm concentration 275.48 ± 0.35 and 268.70 ± 0.31 million per ml, progressive motility 72.27 ± 0.91 and 70.55 ± 0.83 and pH 7.60 ± 0.03 and 7.40 ± 0.04 in large white Yorkshire and T & D boars respectively.

The stained volume, Gel volume, Total volume, individual sperm motility, live sperm, sperm concentration and pH differed significantly between breeds. There was no significant difference in progressive motility of spermatozoa between breeds.

The mean incidence of head abnormality, midpiece abnormality, tail abnormality, proximal cytoplasmic droplet and distal cytoplasmic droplet in large white Yorkshire and T & D boars were 2.42 ± 0.03 and 2.53 ± 0.02, 0.41 ± 0.03 and

0.55 ± 0.02, 2.01 ± 0.02 and 2.31 ± 0.01, 1.20 ± 0.002 and 1.30 ± 0.02 and 0.58 ± 0.09 and 1.19 ± 0.008 percent respectively.

Significant difference was observed in Tail abnormality of spermatozoa between breeds. But non-significant difference was observed for Head abnormality, midpice defect, proximal cytoplasmic droplet defect and distal cytoplasmic droplet defect.

The mean incidence of damaged apical ridge, missing apical ridge and loose acrosomal cap in large white Yorkshire and T & D boars were 0.62 ± 0.03 and 0.63 ± 0.03, 0.00 and 0.00 and 1.41 ± 0.06 and 1.42 ± 0.06 percent respectively.

No significant difference was observed in acrosomal morphology of spermatozoa between breeds.

The mean concentration of calcium, magnesium, inorganic phosphorus, zinc, copper, cobalt and manganese estimated in the present study were 4.20 ± 0.25 and 4.09 ± 0.28, 8.89 ± 0.418 and 8.85 ± 0.410, 1.79 ± 0.10 and 1.75 ± 0.04, 17.10 ± 0.11 and 16.98 ± 0.15, 0.156 ± 0.001 and 0.154 ± 0.001, 0.049 ± 0.005 and 0.044 ± 0.006 and 0.020 ± 0.003 and 0.018 ± 0.001 mg/ 100ml in large white Yorkshire and T & D boars respectively.

No significant difference was observed in seminal minerals concentration between breeds.

The sperm motility and live sperm percentage in large white Yorkshire boars were non-significantly higher than that in T & D boars semen. The tail abnormality in large white Yorkshire boar semen were significantly lower in comparison of T & D boars semen, whereas the incidence of head abnormality were non significantly lower in large white Yorkshire boar semen in comparison of T & D boar semen. Further, it was observed that a significant decrease in percentage of sperm motility and live sperm and significant increase in incidence of head and tail abnormality were recorded with increase in duration of preservation in both breeds.

The mean percentage of damaged apical ride, missing apical ridge and loose acrosomal cap in large white Yorkshire boars were non significantly lower than T & D boars semen.

A significant increase in percentage of damaged apical ridge, missing apical ridge and loose acrosomal cap were recorded with increase in preservation time in semen of both breeds.

The mean seminal minerals concentration in large white Yorkshire boars were non-significantly higher than T & D boars semen. Non-significant decrease in minerals concentration was recorded with increase in hours of preservation in semen samples of both breeds.

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CHAPTER - VII

CONCLUSION

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The following conclusion could be drawn from the present study :

1. A significant difference was observed in volume, individual sperm motility, live sperm, Tail abnormality sperm concentration and pH of semen in Large White Yorkshire and T & D boars.

   Non-significant difference was observed in progressive motility, head abnormality, mid piec defect, proximal cytoplasmic droplet defect and distal cytoplasmic droplet defect in large white Yorkshire and T & D boars.

2. Non-significant difference in seminal minerals concentration was observed between large white Yorkshire and T & D boars neat semen as well as preserved semen.

3. Significant decrease in percentage of sperm motility and live spermatozoa with increase is hours of preservation were observed in semen of both breeds.

4. There was significant increase in percentage of head, tail abnormality and acrosomal abnormalities with increase in hours of preservation in semen of both breeds.

5. Non-significant effect of breed was recorded on physical and mineral profile in preserved boar semen except tail abnormality.

6. Semen quality of large white Yorkshire boars were found better than semen of T & D boars under ecological condition of Patna. Spermatozoan abnormalities in semen of T & D boars showed non-significant difference in comparison of large white Yorkshire boar semen except tail abnormalities. Hence, successful utility of T & D boar semen could be practiced under environmental condition of Patna.

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CHAPTER - VIII

BIBLIOGRAPHY

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Anil Joshi, Sadhan Bag and Mittal, J.P. (1999). Computer assisted sperm analysis of freshly diluted and stored Garole ram liquid semen. *Indian J. Anim., Sci.*, **69** : 19-21.

Antonyuk, V.S., Inskaya, T.P. and Bezlyudnikov, L.G. (1982). The effects of season on semen quality of boars at large intensive farms. Nauchnye Osnovy azvitiya Zhivotnovodstva BSSR No. **11** : 28-30. (*Anim. Breed. Abstr.* **51** : 3755).

Bhattacharya, H.K., Subodh Kumar, Mandal, S.K., Saket Bhusan, Bajarbarnahi, K.M. and Chaudan Rajkhowa (2004). Estimation of some seminal attributes of Mithun. *Indian J. Anim. Sci.*, **74** : 514-515.

Bhavsar, B.K., Vadodaria, V.P., Dhami, A.J. and Kodagali, S.B. (1989). Seminal trace elements with reference to freezability and fertility of Mehsana buffalo bulls. *Indian Journal Anim. Sci.*, **59** (5) : 566-569.

Bhuyan, D. (1989). Studies on certain aspect of physico-Biochemical characteristics of Boar semen during preservation in three different axtenders. M.V.Sc. Thesis, Assam Agricultural University.

Bhuyan, D., Bargohain, B.N., Ahmed, K., Sarmal, B.C. and Chakravarty, P. (1991). Physico-morphological and

Biochemical characteristics of semen of Hampshire and crossbred Boars. *Indian J. Anim. Reprod.*, **12** (1) : 90-91.

Bhuyan, O., Borgohain, B.N., Ahmad, K. and Deka, B.C. (1992). Effect of Kiev, GPSE-1 and BL-1 extenders on the semen quality of crossbred boars. *Indian J. Anim. Reprod,* **13** (1) : 41-43.

Blackshaw, A.W. (1953). The effect of K and Ca salts on the motility of ram, rabbit and bull spermatozoa. *J. Physiol.*, **120** : 465-470.

Blom, E. and Wolstrup, C. (1976). Zinc as a possible causal factor in the sterilizing sperm tail defect, the 'Dag defect' in Jersey bulls. *Nordisk Veterinaermedicin*, **28** (10) : 515-518.

Brady, D.E. and Gildow, E.M. (1939). Characteristics of ram semen as influenced by the method of collection. *Am. Soc. Anim. Prod. Proc.*, **32** : 250-254.

Cerovsky, J. (1981). The development of sperm abnormalities in boar at insemination stations. Vedecke prace vykumneho ustavu pro chov prasat kostelci and orclici. 5 : 61-75 (*Anim. Breed. Abstr.* **31** : 3759).

Cerovsky, J. and Vinter, P. (1986). Sperm survival in diluents for short term storage of boar semen for insemination. *Zivocisna Vyroba Journal*, **31** (7) : 659-667.

Chandra, A.M., Sarkar, S., Bandopathyay, S.K., Chakrabarti, A., Ray, R. and Ghosh, B.B. (1992). Comparative study of certain Enzymatic and Biochemical constituents of Epididymis and Vas deferens between Buck and Boar. *Indian J. Anim. Reprod.*, **13** (2) : 144-146.

Chavan, A.T. and Kale, M.M. (2000). Computer assisted semen analysis of crossbred buck semen. Indian J. Anim. Sci., 70 : 936-937.

Das, B.C., Bujarbarauah, K.M., Sanyal, S., Das, P.K., Ghosh, P.R., Naskar, S., Khan, M.H., Das, A.B. and Bandopadhyay, S. (2006). Morphological characteristics of boar spermatozoa preserved with BTS diluent. Environment and Ecology 2006, 245 (Special 2) : 285-288.

Dhami, A.J. and Kodagali, S.B. (1986). Studies on seminal characteristics, Biochemical and Enzymatic constituents, freezability, Enzyme leakage and Fertility in Surti Bulls. *Indian J. Anim. Reprod.*, **17** : 94-97.

Dhami, A.J. and Sahni, K.L. (1993). Comparative assessment of certain Biochemical and Mineral constituents of seminal plasma and their inter relationships in and Buffalo bulls. *Indian J. Anim. Reprod.*, **14** (2) : 98-100.

Dhami, A.J., Gareesh Mohan and Sahni, K.L. (1994). Relationships of seminal attributes and Enzymatic profiles with Biochemical and Mineral constituents of

seminal plasma in Friesian and Murrah Buffalo Bulls. *Indian Vet. Med. J.*, **18** : 155-161.

Dhami, A.J., Shelke, V.B., Patel, K.P., Paradva, J.P. and Kavani, F.S. (2001). Trace minerals profile of blood and seminal plasma of breeding bulls. *Indian J. Anim. Sci.*, **71** : 761-763.

Dubiel, A., Barcikowski, B., Dziadek, K., Polanska, E., Ramanowicz, K. and Stanczyk, O.F. (1985). Sexual reflexes, semen quality and the concentration of testosterone in boars of several breeds. *Medicyna Weterynaryjna,* **41** (4) : 230-234. (*Anim. Breed. Abstr.* **55** : 5116).

Eaton, O.N. and Simmons, V.Z. (1952). A semen study of goats. *Am J. Vet. Res.*, **13** : 537-544.

Gamcik, P. (1981). A study of some characters of ejaculates of boars of different breeds in the course of a year. Veterinarstvi, **31** (9) : 396-398. (*Anim. Breed. Abstr.* **50** : 2097).

Gottardi, L. and Brunel, L. (1978). Mineral content and enzyme activity of Boar semen collected at different interval. *Archivio Veterinario Haliano Journal,* **29** (5/6) : 161-163.

Govt. of India (2003). Report on the 17th Livestock Census of Bihar. Animal Husbandary Department : 120-123.

Gupta, H.P., Sahni, K.L., Gareesh Mohan and Jodon, N.S. (1997). Distribution of electrolytes in the epididymal and

ejaculated semen of buffalo bulls. *Indian J. Anim. Sci.*, **67** : 511-512.

Hancock, J.L. (1951). A staining technique for the study of temperature shock in semen. Nature, **167** : 323-324.

Hancock, J.L. (1959). Semen and testis characteristics and sexual behaviour of boars. *J. Agril. Sci. Camb.*, **53** : 313.

Iritani, A. and Nishikawa, Y. (1964). Studies on the chemical properties of the ejaculated semen and the secretion of accessory sexual organs in the goat. *Jap. J. Anim. Reprod.*, **10** : 44-51.

Joshi, V.K. and Kharche, K.G. (1990). Effect of preservation of Diluted semen at varying intervals on semen quality in crossbred Bull. *Indian J. Dairy Sci.*, **43** (2) : 135-137.

Kansi, S. (2007). Study of seminal characters of different breed of Boars during preservation. M.V.Sc. Thesis, Birsa Agricultural University, Ranchi.

Karagiannidis, A. (1976). The distribution of Ca in bovine spermatozoa and seminal plasma in relation to cold shock. *J. Reprod. Fert.*, **46** : 83-90.

Kumar Suresh, Nakhashi, H.C., Vadodaria, V.P. and Kavani, F.S. (1999). Inorganic elements and their Seasonal variations in seminal plasma of Patanwadi Rams. *Indian J. Anim. Reprod.*, **20** (1) : 62-63.

Kuroman, B. and Stachowicz, R. (1984). The seasonality of semen quality of boars at a large farm. *Zootechnika* **26** : 73-83 (*Anim. Breed. Abstr.* **54** : 3925).

Kutty, C.I., Joseph, M. and Neelakanta Iyer, O.P. (1996). A simplified Nigrosine-Eosin-Giemsa staining technique to distinguish acrosome damaged live and dead sperms. *Indian J. Anim. Reprod.*, **17** (2) : 122-123.

Lalrintluanga, K. (1994). Preservation of Boar semen in liquid state. M. V.Sc. Thesis, Assam Agricultural University.

Lalrintluanga, K., Deka, B.C. Bargohain, B.N. and Sarmah, B.C. (2002). Study on preservation of Boars semen at 5^{0}C and 15^{0}C. *Indian Vet. J.*, **79** : 920-923.

Louda, F. and Pavlik, O. (1984). Semen quality of boars from a synthetic line. Sbornik. Vysoke, Skoly Zemedelskev praze Fakulta Agronomicka, **40** : 207-221. (*Anim. Breed. Abstr.* **52** : 5930).

Mann, T. (1964). The biochemistry of semen and the Male reproductive tract John Willey and sons, New York.

Markandeya, N.M., Pargaonkar, D.R., Bakshi, S.A. and Doijode, S.V. (1991). Studies on chemical composition of seminal plasma of Red Kandhari and crossbred Bulls. *Indian J. Anim. Reprod.*, **12** : 121-123.

Massanyi, P., Toman, R., Trandzik, J., Nad, P., Skalicka, M. and Korenekova, B. (2004). Concentration of copper, zinc, iron, cadmium, lead and nickel in bull, ram, boar, stallion and fox semen. *Journal Trace elements and electrolytes*, **21** (1) : 45-49.

Massayni, P., Trandzik, J., Nad, P., Toman, R., Skalick, M., and Korenekova, B. (2003). Seminal concentrations of

trace elements in various animals and their correlations. *Assian J. Androl.*, **5** : 101-104.

Mc. Grady, A.V., Nelson, L. and Ireland, M. (1974). Ionic effects on the motility of bull and Chimpanzee spermatozoa. *J. Reprod. Fertil.*, **40** : 71-76.

Michalski, Z. and Polanska, E. (1983). Semen characters of purebred and crossbred boars aged 6-8 months. Roczniki naukowe zootechniki, **10** (2) : 11-18. (*Anim. Breed. Abstr.* **55** : 5128).

Mohanty, D.N., Ansari, M.R. Patra, R.C. and Patthaik, A.K. (2004). Trace minerals and freezability of crossbred bull semen. *The Indian J. Anim. Reprod.*, **25** (2) : 140-142.

Murthy, P.R. (1977). Physical characteristics of boar semen, preservation and artificial insemination in swine. (*Anim. Breed. Abstr.* **48** (9) : 5437).

Murthy, P.R. and Rao, A.R. (1975). Preservation of Boar semen. *Indian Vet. J.*, **52** : 415-420.

Naidu, G.V. and Rao, K.B. (1999). Preservation of native boar semen. *Indian J. Anim. Reprod.*, **20** (1) : 64-65.

Naskar, S., Khan, M.K., Anubrata Das and Dilruba Hassin (2006). Physical and morphological characteristics of Boar semen of different genetic group. *Indian Vet. Journal,* **83** : 798-799.

Nayak, S.K., Gopal Krishma and Lakra, W.S. (2006). Cryopreservation and fertility trials of clarias batrachus spermatozoa. *Indian J. Anim. Sci.*, **76** (7) : 562-565.

Nema, S.P., Kodagali, S.B., Janakiraman, K. and Prabhu, G.A. (1993). Comparative studies on semen ejaculates (Static and motile) in Surti Buffalo Bulls. *Indian J. Anim. Reprod.*, **3** (1) : 5-8.

Orson, N.E. and Victor, L.S. (1952). A semen study of goats. *Am. J. Vet. Res.*, **13** : 537-544.

Oser, B.L. (1965). Howk's physiological chemistry 14th Edn., Blakistan Divi Mcbrow Hill Books can, Bombay.

Oser, B.L. (1979). Hawk's physiological chemistry, 14th Edn. Mc Grew Hill Publishing C. New Delhi, India.

Pandey, R.P. (1997). Abnormalities of large white Yorkshire Boar spermatozoa in different seasons. *Indian J. Dairy Sci.*, **50** (3) : 1-2.

Pandey, R.P. and Singh, B.K. (1997). Acrosomal changes during liquid preservation of boar semen with BTS, Kiev and BL-1 dilutors. *Indian J. Anim. Reprod.*, **18** (1) : 57-58.

Pandey, R.P. and Singh, B.K. (1998). Influence of season on coital Behaviour and seminal characters of large white Yorkshire Boars. *Indian Journal Anim. Reprod.*, **19** (2) : 146-148.

Pandey, R.P. and Singh, B.K. (1998). Preservability large white Yorkshire boar semen with BTS, Kiev and BLI dilutor from 18 to 20°C. *Indian J. Anim. Sci.*, **68** (4) : 365.

Pandey, R.P., Sinha, S.N., Mukherjee, S.K. and Balraj Singh (1982). The levels of certain ions in the semen of Saauen and Barbari Bucks. *Indian J. Dairy. Sci.*, **35** : 1-2.

Pangawkar, G.R., Sharma, R.D. and Rajvir Singh (1988). Protein, Sialic acid and Zinc concentration in the seminal plasma of Bulls in relation to freezability of semen. *Indian Vet. J.*, **65** : 58-60.

Pangawkar, G.R., Sharma, R.D., Biswas, R.K. and Dugwekar, Y.G. (1988). Electrolyte composition of seminal plasma in relation to freezability in HF Bulls. *Indian J. Dairy. Sci.*, **41**:4.

Patel, K.V., Dhami, A.J., Derashri, H.J. and Kodagali, S.B. (1989). Seminal biochemical profiles and their inter relationships in crossbred bulls. *Indian Journal Anim. Sci.*, **59** (5) : 524-529.

Patil, D.R., Chauhan, R.A.S. and Tiwari, S. (1996). Studies on Biochemical composition of Buck semen. *Indian J. Anim. Reprod.*, **17** (1) : 58-60.

Petzoldt, R. and Nehring, H. (1986). Ion levels in spermatozoa and seminal plasma of boar and ram. *Archi-fur-Experimentelle Veterinarmedizin Journal*, **40** (3) : 469-477.

Phillips, R.W. and Schoot, R.G. (1943). Seasonal variation in the semen of sheep and goat. *Cornell vet.*, **33** : 227-235.

Pursel, V.G., Johnson, L.A. and Schulman, L.L. (1974). Acrosome morphology of boar spermatozoa during in vitro aging. *J. Anim. Seoi.*, **38** (1) : 113-116.

Pushpendra Kumar and Balakrishnan, C.R. (1998). Effect of calcium ion on in vitro capacitation and acrosome reaction in cattle spermatozoa. *Indian J. Anim. Sci.*, **68** (1) : 60-62.

Rao, B.V., Venkatamunichetty, A., Ramachandraiah, S.V. and Sreeraman, P.K. (1991). Preservation of native boar semen. *Indian J. Anim. Reprod.*, **12** (2) : 148-150.

Rao, M.M., Ramachandraiah, S.V., Venkatamunichetty, A. and Sriraman, P.K. (1992). Studies on crossbred boar semen characteristics and preservation. *Indian J. Anim. Reprod.*, **13** (2) : 141-142.

Rattan, P.J.S., Rao, M.B. and Anantakrishnan, C.P. (1972). Biochemical studies on bovine semen. Annual Review, N.D.R.I., Karnal.

Robertson, J. and Watson, P.F. (1986). Calcium transport in diluted or cooled ram semen. *Journal of Reproduction and Fertility.*, **77** (1) : 177-185.

Saha, D.N. and Sidhu, N.S. (1982). A note on certain biochemical attributes of seminal plasma in different breeds of sheep. *Indian Veterinary Journal*, **59** (12) : 990-991.

Salisbury and Nakabayshi (1957). Dilution and Preservation of semen, 120-121. (Artificial insemination and reproduction of cattle and buffalo by Tomar, N.S. 1st edn).

Salisbury, G.W. and Cragle, R.G. (1956). Freezing print depression and mineral levels of fluids of the ruminant male reproductive tract. IIIrd Intern. *Congr. Anim. Reprod.*, **1** : 25-28.

Sane, C.R., Luktuke, S.N., Kaikini, A.S. and Despande, B.R., A Text Book of Reproduction in farm Animals (Theriogenology).

Schilling, E. and Vengust, M. (1987). Frequency of semen collection in boars and quality of ejaculates as evaluated by the osmotic resistance of acrosomal membrane. *Anim. Reprod. Sci.* **12** : 283.

Shelke, V.B. and Dhami, A.J. (2001). Norms and correlations of seminal plasma Biochemical, Enzymatic and Macro-Micro mineral profiles with physical attributes and freezability of semen in Gir Bulls. *Indian Vet. Med. Jour.*, **25** : 219-226.

Singh, B.K. (2005). Text Book of Andrology and Artificial insemination in Farm Animals. Jaypee Brothers Medical Publishers (P) Ltd., New Delhi.

Singh, J., Purohit, G.N., Pareek, P.K. (2001). Seminal plasma electrolytes in the dromedary camel their correlation with seminal quality. *Indian J. Anim. Sci.*, **71** : 782-783.

Snedecor, G.W. and Cochran (1967). Statistical method, 6th Edn, Oxford and I.B.H. Publishing Co. Calcutta, Bombay and New Delhi.

Souvenir. (2005), B.V.C., Patna. Rajendra Agricultural University, Pusa (Samastipur), 1 : 4-6.

Sreekumaran, T. and Raja, C.K.S.V. (1977). Biochemical characteristics of semen of Yorkshire boars. *Kerala J. Vet. Sci.*, **8** (2) : 211-213.

Strezezek, J. and Slaweta, R. (1984). Application of choosen biochemical indenes for biological quality of boar semen stored at 15-18°C. 10th int. cong. *Anim. Reprod.* A.I. paper, 67 (*Anim. Breed. Abstr.* **53** : 1544).

Suresh Kumar, Nakhashi, H.C., Vadodaria, V.P. and kavani, F.S. (1999). Inorganic elements and their seasonal variations in seminal plasma of Patanwadi Rams. *Indian J. Anim. Reprod.*, **20** (1) : 62-63.

Suthar, B.N., Sharma, V.K. and Kavani, F.S. (2000). Levels of certain ions and trace minerals in Patandwadi and crossbred Ram Semen. *Indian J. Anim. Reprod.*, **21** : 35-37.

Tamuli, M.K., Rajkonwar, C.K., Sarkar, A.B. and Nath, K.C. (1984). Semen characteristics in Landrace Boars. *Indian J. Anim. Sci.*, **54** (9) : 911.

Tamuli, N.K. (1982). Studies on semen characteristics and Artificial insemination in Pigs. M.V.Sc. Thesis, Assam Agricultural University.

Taussky, H.H. and Shorr, E. (1953). *J. Biochem* **202** : 675-685, Cited from clinical methods, Mannuals sepec-20 (1984), Bausch and Lomb, New York (1984).

Taylor & Francis (2003). Concentration of copper, Iron, Zinc, Cadmium, Lead and Nickel in Boar semen and relation to the spermatozoa quality. *Journal of Environmental science and Health*, Part A, **38** : 2643-2651.

Venumanohara Rao, B., Venkatmuaichetty, A., Ranachandraiah S.V. and Seeraman, P.K. (1991). Preservation of native Boar semen. *Indian J. Anim. Reprod.*, **23** : 125-126.

Vinaya B. Shelke and Dhami, A.J. (2001). Norms and correlations of seminal plasma biochemical, enzymatic and macro-micro minerals profiles with physical attributes and freezability of semen in Gir Bulls. *Indian Vet. Med. Jour.*, **25** : 219-226.

Waltere, J.R., Hooper, P.N., and Green, C.G. (1984). The utilization of A.I. in commercial pig improvement E.A.A.P. The Hague, Netherlands. (World Review of Animal Production Vol. XXII, No. 2, 1986).

Watson, P.F. (1975). Use of Giemsa stain to detect changes in acrosome in frozen ram spermatozoa. *Vet. Tec.*, **97** : 12-15.

Webster, Jr. W.W. (1962). Micro-spectrophotometric method for serum calcium Estimation. *Amer J. Clin. Path*, **37** : 330-333.

Willet, E.L. and Buckner, P.J. (1951). The determination of number of spermatozoa in bull semen by measurement of light transmission. *J. Anim. Sci.*, **10** : 219.

Winter halter, M. (1975). Artificial insemination has an established place in combating and preventing the occurrence of Brucellosis in swine. *Veterinarski Glosnik*, **29** (5) : 381-383.

Zavos, P.M. and Liptrap, D.O. (1987). Procedure for collection, evaluation, dilution and artificial insemination of boar spermatozoa. Agril. *Practice Swine Reproduction*, **8** (3) : 268.

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