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Factors Affecting the Semen Production and Semen Quality of Bulls Used for Artificial Insemination

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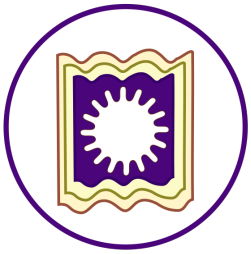
University of Rajshahi

FACTORS AFFECTING THE SEMEN PRODUCTION AND SEMEN QUALITY OF BULLS USED FOR ARTIFICIAL INSEMINATION



THESIS SUBMITTED FOR THE DEGREE
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SUBMITTED BY

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September, 2013

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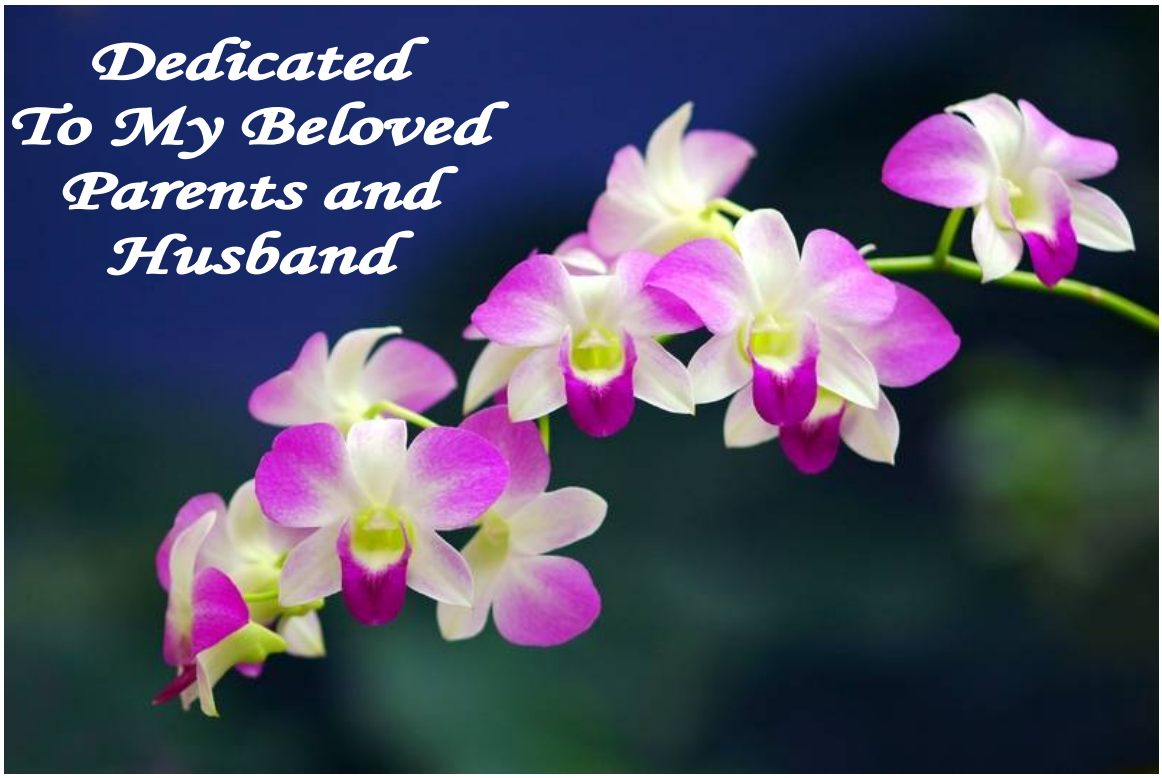
Dedicated
To My Beloved
Parents and
Husband

DECLARATION

I hereby declare that the thesis entitled "Factors Affecting the Semen Production and Semen Quality of Bulls used for Artificial Insemination" is the results of my own and original investigation in the Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh under the supervision of Professor Dr. Md. Jalal Uddin Sarder, & Co-supervisor Dr. Shah Md. Abdur Rauf, Associate Professor & Chairman, Department of Animal Husbandry and Veterinary Science, Rajshahi University, Rajshahi, Bangladesh in fulfillment of the requirement for the degree of Master of Philosophy (M. Phil).

I further declare that this research work has not been submitted in part or in full previously for any academic degree in this university or any university.

September, 2003 Rajshahi

(MAFRUZA SULTANA DINA)

Candidate

CERTIFICATE

We have the pleasure in certifying the thesis entitled "Factors Affecting the Semen Production and Semen Quality of Bulls used for Artificial Insemination" to the Institute of Biological Sciences, Rajshahi University for the Degree of Master of Philosophy.

We hereby certify that i) the candidate has fulfilled the residential requirement. ii) the research work embodied in the thesis were carried out by the candidate and iii) the data to the best of our knowledge is genuine and original. No part of the research work has been submitted in substance for any degree else.

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The Author
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ABSTRACT

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The present study was to evaluate the semen production and semen quality in relation to bull ID, breed, age, body weight, body condition, libido, testicular circumference, season and Artificial Insemination (AI) centres from 16 bulls used for AI programme in Bangladesh. The study was conducted at District AI Centres (DAIC), Rajshahi and Rajshahi Dairy & Cattle Improvement Farm (RDCIF) Rajabarihat, Rajshahi, AI sub centres / points under the Rajshahi district and Dept. of Animal Husbandry and Veterinary Science, University of Rajshahi duration from July, 2011 to June, 2013. The average values of semen production such as volume of ejaculate, colour, mass activity, density, sperm concentration, sperm motility, total number of sperm cells /ejaculate, number of semen doses per collection were 7.67±2.17ml, 3.06±0.98 (scale:1-4), 2.91±0.74 (scale:1-5), 3.56±0.77 (scale:1-4), 1330.46±313.32 million/ml, 62.94±5.32%, 10112.88±3521.12 million/ejaculate 337.10±117.37 respectively. Individual bull had significant effect ($P<0.05$) on only volume of ejaculate and colour of semen. Six breeds of the bull such as L×F×SL×F×F, SL×F, L×F×F, L×F, $(L\times F)_2$, L×F(L×F×F) had significant ($P<0.05$) effect on all the semen characteristics. The significant ($P<0.05$) highest volume (8.23±2.47 ml) was found in L×F×SL×F×F breed and lowest (5.97±2.36 ml) in L× F×(L×F×F) breed. Significantly ($P<0.05$) highest values of sperm motility (63.48±5.12%), total number of sperm cells/ejaculate (10523.03±3607.45million/ml) and the number of semen doses (350.70±120.25) were found in $(L\times F)_2$ breed of bull.

The semen quality was tested by using three type of extenders to evaluate sperm motility %, various duration of preservation of semen in relation to conception rate of cows. A total 189 cows were artificial inseminated in different AI sub centre/points of District AI centre and average conception rate was observed 50.77%. The three extenders of Egg Yolk Citrate (EYC), Cornell University Extender (CUE) and Coconut Milk Extender (CME) had significant effect ($P<0.05$) on sperm motility % and conception rate. The highest conception rate was found at EYC (57.82±7.00%) and lowest in CME (43.71±3.59%).

A total 64 semen sample was studied for the determination of sperm abnormalities in association to non return rate (NRR). The average head and tail abnormalities were obtained 4.29% and 13.90% respectively and total sperm abnormalities was observed 18.13 ±6.78%. A total of 1600 cows inseminated by that 16 bull semen were observed for NNR within 60 days. The overall NRR was obtained 78.50±11.95%. Bull ID, breed, testicular circumference, libido had significant effect ($P<0.05$) on sperm abnormalities and NR%.

It was concluded that the overall semen production performance was found better in $(L\times F)_2$ breed, young age, 500-<600 body weight, very good body condition, >38 cm testicular circumference, good libido, spring season and AI centres of RDCIF. Semen quality was suitable in the EYC extender for chilled semen preservation under field condition as well as L×F×F breed had shown satisfactory performance of sperm morphology and NR%.

LIST OF ABBREVIATIONS

%	=	Percentage
<	=	Less than
>	=	Greater than
°	=	Degree
µ	=	Microns (s)
AI	=	Artificial Insemination
ANOVA	=	Analysis of Variance
AV	=	Artificial Vagina
BCS	=	Body Condition Score
BW	=	Body Weight
C	=	Celsius
CCBSDF	=	Central Cattle Breeding Station and Dairy Farm
cm	=	Centimeter (s)
CME	=	Coconut Milk Extender
conc.	=	Concentration
CR	=	Conception Rate
CUE	=	Cornell University Extender
d	=	Day (s)
DAIC	=	District Artificial Insemination Center
DF	=	Degree of Freedom
DLS	=	Directorate of Livestock Services
DMRT	=	Duncan's Multiple Range Test
eja.	=	Ejaculate
et al	=	Et alia
EYC	=	Egg yolk Citrate
F	=	Friesian
FA(AI)	=	Field Assistant (Artificial Inseminator)
Fig.	=	Figure
gm	=	Gram (s)
i.u	=	International Unit
Id	=	Identification
Kg	=	Kilogram (s)
L	=	Local
l	=	Litre (s)
m	=	Month (s)
mg	=	Milligram (s)

mill.	=	Million
min	=	Minutes(s)
ml	=	Millilitre
mmol	=	Millimole
n or N	=	Number of Observation
no.	=	Number
NRR	=	Non-return Rate
P	=	Probability
RDCIF	=	Rajshahi Dairy and Cattle Improvement Farm
S/C	=	Service per Conception
S/N	=	Serial Number
SD	=	Standard Deviation (s)
SL	=	Sahiwal
SPSS	=	Statistical Package for the Social Sciences
TC	=	Testicular Circumference
v/v	=	Volume by Volume
yrs	=	Years

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CHAPTER 1

INTRODUCTION

Bangladesh is a densely populated and agro-based developing country. Poverty alleviation is one of the most important challenges of the twenty century in Bangladesh. Agricultural development is the main key to alleviate poverty from the country. Cultivated lands of the country are reducing @1% annually due to urbanization and other economic activities of the country (BBS, 2006). Due to lack of cultivated land a large number of people are interested to rearing livestock farm specially dairy and beef sector for animal protein, income generation, employment opportunity, poverty alleviation and foreign currency. Cattle is an important species of livestock and a member of genus Bos, under the family Bovidae.

Table 1.1. Livestock Population in Bangladesh.

Species	**Number in million**
Cattle	23.40
Buffalo	1.21
Goat	33.50
Sheep	2.57
Chicken	206.89
Ducks	39.08

The presently, livestock population contribution of livestock in national economy and production, requirement and deficits situation are given below:

Table 1.2. Contribution of Livestock in National Economy of Bangladesh

Items	1997-1998	1999-2000	2000-2001
GDP	6.5 %	3.86 %	3.12 %
Agricultural product	-	12.90 %	16.13 %
Land cultivation	95 %	95 %	75 %
Self employment (full time)	20 %	20 %	20 %
Foreign exchange earning	13 %	4.31 %	3.46 %*
Rural transport	50 %	50 %	50 %
Protein source	20 %	25 %	25 %
Fuel	20 %	25 %	25 %

Table 1.3. Production, Requirement and Deficits of Livestock Products in Bangladesh

Products	**Per capita need**	**Per capita[1] availability**	**Total need[2] (year)**	**Total production**	**% of total production**	**Total deficit[3]**	**Total deficit[3] (%)**
Milk	250 (ml /d)	44.06 (ml / d)	13.15 MMT	2.29 MMT	17.40	10.86 (MMT / year)	82.60
Meat	120 (g / d)	20.60 (g / d)	6.31 MMT	1.08 MMT	17.17	5.23 (MMT / year)	82.83
Eggs	104 (no. / Year)	33 (no. / Yr)	14997 M	4696 M	37.	8645 M	62.02

The consumption of livestock product is shown below:

Table 1.4. Scenario of consumption of Livestock products in Southern Asian countries.

Countries	Products		
	Milk (ml/h/d)	Meat(g/h/d)	Eggs (no./h/year)
Bangladesh	44	21	33
India	227	25	50
Nepal	140	38	30
Pakistan	520	45	55
Srilanka	142	27	75
Maldives	188	31.5	33

Asian countries per capita consumption of milk, meat and eggs are high in Pakistan followed by India. The position of Bangladesh are well below except the case of eggs for Maldives.

1.1 Protein Situation in Bangladesh:

Human population in Bangladesh is now approximately 160 million. Human growth rate is 1.6% and 19.65 million will be end of the 2025. Milk and meat will be deficit in 2013, 15.65 and 13.60 MMT respectively. Egg will be deficit 1099 million. Every year government spend a huge expenditure for export of milk, milk product, cattle and eggs which bed effect our economy. Sometimes, many animal and human diseases are outbreak in our country.

1.2 Breeds of Cattle in Bangladesh:

Inspire of huge population size, unfortunately there is no specific cattle and buffalo breed still now but Red Chittagong. All cattle are indigenous type and their productivity is very low average 0.75-1.5 litre per lactation.

Although, a few regions of BD particularly, Faridpur, Pabna, Munsigong, Manikgong, Chittagong having high producing cattle. Bangladesh consists a lot of cattle, buffalo, goat, sheep, chicken and ducks etc but their productivity is very low due to poor genetic potentials of native breed. Artificial insemination (AI) is a basic tool for rapid improvement of cattle in the world. So, AI may be alternative source of milk and meat to supply the increasing demand for milk/meat for this nation.

The majority of the rural households in Bangladesh have 2-3 dairy cows. Livestock are important to the food security of millions of people today. It will be important to the food security of millions more in the coming decades. Livestock source food is not essential to human nutrition but it is highly beneficial. In livestock systems that primarily consume roughage and agro-industrial waste products, livestock add to the food supply beyond what can be provided by crops. The role that livestock play in feeding the future will be shaped by three distinct human populations, each with its own particular needs, namely: urban dwellers, small-scale mixed farmers and livestock-dependent populations. The primary selection objective in dairy cattle breeding has been milk production. Milk, fat, and protein yields and fat and protein percentages are the main economic traits for selection in dairy cattle. Profitability of dairy cattle does not only depend on milk production but also on non-production characteristics such as fertility and health traits.

Cattle of Bangladesh is an inseparable and integrated part of agricultural operation and it ranks twelfth in cattle population in the world and in the Asian countries its position is third, but it yields only 21% of the world milk production and 34% of the beef production (Alam *et al.*, 1994; Rahman, 1992). Among the Asian countries, the condition of livestock in

Bangladesh is probably the worst. In the last decades, livestock sector has undergone a process of biotechnology incorporation with the main goal of enhancing productivity and improving the genetic makeup. In this sense, artificial insemination (AI) is considered as the most important biotechnology incorporated into livestock production systems because it implies the use and/or globalization of proven bulls, which represent a key tool in obtaining animals with higher genetic merit. The wide use of bovine AI was mainly attributed to the development of methods that ensured cell viability after storage for long periods by reducing sperm metabolism, due to important progresses in studies involving cryoprotectants. Nowadays, AI is considered as the most worldwide used reproductive biotechnology with an extremely interesting benefit-cost relationship. Despite the unquestionable role of this biotechnology in improving productivity, many causes have accounted for the range in results and/or some unsatisfactory indices of bovine AI programs, highlighting several factors inherent to female physiology and/or farm management. Nevertheless, another factor positively correlated with the AI outcomes that require appropriate attention, correspond to quality of semen samples used in the programs.

1.3 Cattle Development Programme in Bangladesh:

Most of the cattle originated from Bos taurus as hump-less cattle and Bos taurus as hump cattle, from the wild cattle of South East Asia. Hump less Longhorn cattle were first found in South Turkey. They were focous in Mesopotamia about 5000 BC and later in the Indus Valley (Pakistan). The small hump-less short horn cattle, originated some 2000 year later than the Longhorn, are the ancestors of the present dairy short-horn cattle found through out the Middle East and the North African-countries. The

Zebu probably originated in Western Asia (Udo, 1994). Mason and Buvanendran (1982) have suggested that the hump and pendulous dewlap, two of the distinguishing features of Zebu cattle, were originally selected by man to emphasize the appearance of size and strength in his bull and thus render them more suitable for religious and social rituals (Mason and Buvanendran, 1982). Zebu is believed to have been brought to India between 2200 and 1500 BC. From India they spread into South East Asia and Western Asia inter bred (Udo, 1994).

The cattle in Bangladesh are mostly non-descript indigenous types, believed to be originating from Indian cattle belonging to the genus Bos indicus. Sultana (1995) reported that only about 0.25 million cattle are pure breed and their crosses and rest are indigenous low producing Zebu type. In the hilly areas of Bangladesh the cattle species Gayal (Bos frontalis) and their crosses with local cattle are also available.

1.4 Characteristics of Cattle in Bangladesh:

The indigenous of cattle of Bangladesh come from primitive breeds. The characteristics are stunned growth, small size, late sexual maturity and poor production even under the good dietary and management condition. But they often exhibit remarkable heat tolerance, the ability to maintain body condition on poor quality feed stuff and to a good degree of resistance to local diseases. On the other hand, exotic breeds from Temperate zone are thought more productive in favorable environmental conditions but cannot adjust to our hot and humid condition (Taneja and Bhat, 1986). According to Taneja and Bhat (1986) the performance of temperate genotypes in tropical environment is 30-40% lower than that of the countries of their origin. Livestock performance depends mainly on genetic potential of the animal. Again optimum nutrition, disease control

and management practices permit full expression of this genetic potential. Climatic stress in the form of high temperature, high humidity and erratic or inadequate rainfall affect the productivity of dairy cattle in tropical countries (Matin, 1993).

1.5 Up-grading System of Cattle:

In order to increase the productive efficiency of indigenous cattle, specially at the small-holder level, for developing countries like Bangladesh is aiming to rapid improvement of production potentials. The breeders has two alternatives, either by introducing high performing Bos taurus cattle from the temperate zones or by grading up the indigenous low performing Bos indicus stock with high yielding breeds. Although, there are opinions that selection from within the indicus stock is preferable for sustainable improvement, but this method is too slow for the urgent and growing need for increased production. It has been estimated that in selection within indigenous stock, 2% increase achieved in milk yield per generation. This is of course compare unfavorably with productivity increase that can be achieved by upgrading with high yielding stock and selective breeding within the native cattle is necessary. Grading is the practice by which rapid changes in gene frequencies in the offspring and consequently rapid improvement of the whole stock is possible.

1.6 Cattle Breeding Policy in Bangladesh:

At first a scientific cattle breeding programme was undertaken by the Lord Linlithgow (British Viceroy for India) in 1935-36 to upgrade the small sized Zebu cattle of undivided Bengal by naturally mating the cows with Hariana bulls (Ali *et al.*, 1998). The cattle-upgrading project included Dhaka, Faridpur, Rajshahi, Pabna, Mursidabad and Midanapur-

Birbhum districts. With about one thousand pure Hariana stud bulls from Northern India the project was implemented and each district was given 100 bulls for natural service and for each bull a subsidy of Tk. 15.00 was allocated per month at the disposal of union board. Before Lord Linlithgow under took up-grading cattle development programme; two diary farm were established one in Syhlet in 1930 and other at Tejgoan with objectives to undertaken cattle breeding and milk supply programme. In the Syhlet farm initially Sahiwal breed was maintained. After partition of India in 1947 the programme for distribution of Hariana bull was not continued. But instead Sindhi and Sahiwal were brought to upgrade the local stock. Thus genetic improvement of the cattle with a definite blood line was not possible owing to the absence of real political will. With the introduction of Sindhi and Sahiwal breed in this Eastern Bengal the artificial insemination (AI) programme was launched during later part of the 50th decade with the objectives like i) better utilization of superior proven bulls ii) control of reproductive disease and iii) up-grading the local breeds with ease. The artificial insemination programme was later on extended to 21 district with Sub-centre at thana level with following objectives i) genetic improvement of local non-descript cattle (cows) through infusion of exotic blood in term of draught power, milk and beef ii) production of breeding bulls as per national breeding policy. In the meantime Savar dairy farm was established in 1960 to carry out fundamental research in cattle breeding problems to evolve on or to cattle breed suitable for the country, to supply milk to Dhaka city. Ahmed and Islam (1987) mentioned that cross-breeding performance with Sindhi and Shahiwal breed did not bring about satisfactory results either in Government farm or private sectors. Thence in 1973, 125 heads of Friesian and Jersey cattle were imported in Savar farm as gift from

Australian Society for these who have less'. Availability of these Bos taurus cattle is found ushered a new dimension in the cattle breeding programme. The performances of those animal F1 generations both by crossing with local and pure breeding gave satisfactory results. Mean while the Savar Dairy and Cattle Improvement Farm has been converted into Central Cattle Breeding Station (CCBS) to evolve a breed suitable for Bangladesh. The existing breeding policy envisages that the cattle of urban and milk potential area will be upgraded by using bull produced out of crossing between pure Friesian bulls and pure Sahiwal cows. The bulls having 50% Friesian blood and 50% Local blood will be put into breeding operation for up grading cattle of rural area. Deep frozen semen production has been planed at Central AI laboratory, at CCBS, Savar, Dhaka from 1992 and Deep frozen semen production laboratory, started from 1997 at Rajshahi dairy cattle improvement farm (RDCIF), Rajabarihat, Rajshahi. From that time there are commendable upgrading programme undertaken using various exotic breed with the same objectives to improve the productivity of local cattle (Ahmed and Islam, 1987; Ali *et al.*, 1998; Hoque *et al.*, 1997).

1.7 Artificial Insemination (AI) Programme in Bangladesh:

For grading up of indigenous stock, the technique of Artificial Insemination (AI) will surely play a vital role. AI is the best successful biotechnology for the improvement of cattle. To improve the cattle breed, AI method is used popularly around the world. The better characteristics are transmitted into the offspring if the cows are artificially inseminated with high yielding verities of bull semen. Now it is under practice continuing in our country. The artificial insemination technique was introduced in Bangladesh in 1958. For this purposes, first AI activities

started from 1958 at limited area with Sindhi and Sahiwal breed in Bangladesh. Actually, AI has expended during 1976-1977 all over the country. Currently a programme of artificial insemination has been operating through 24 A.I. centres and 3200 sub-centres points covering all the 64 districts of the country present. So, our dairy cows are increasing their productively (milk) day by day. During recent years thousands of organized private sector livestock farms with improved breeds have been established for commercial purposes. According to DLS, 1998 statistics, at present there are 29,649 cross-bred dairy farm in the country. As a result, the foreign expenditure has decreased up to 125 crore taka for importing the powder milk and milk products per year in our country. However, it was 500 crore taka a few years ago.

1.8 Government Activities of AI in Bangladesh:

AI breeding Station	02
AI Breeding centre	22
Breeding Bull	170
Frozen semen production cost	$1.6
Chilled semen production cost	$1.3
Liquid nitrogen cost per year	$221951
AI Sub center/Point	3200
Breeds of cattle used	Friesian, Shiwal, Jersey, Brahman, Red Chittagong and Local
Breed able cow	70,00,000
AI coverage	45%
Conception Rate	48-52%

1.9 AI Delivary System:

- Govt. provides AI service only for cattle
- BRAC provides AI service for cattle
- BRAC has recently started AI service for goat
- No breeding service for buffalo and sheep
- Farmers take their cows to the AI Point for insemination (Govt.)
- AI technicians extend door to door service (NGO and Cooperative)
- Farmers take their cow / does to private bull / buck keepers

Price of breeding service:

Govt. bull semen dose : Taka 30/= (Highly subsidized)
BRAC bull semen dose : Taka 70/=
BRAC buck semen dose : Taka 35/=
Private natural service : Taka 200/= (Bull)

Table 1.5. Semen production, achievement of Artificial Insemination (AI) and progenies production in lakh of Bangladesh.

Fiscal year	Total semen production (Lakh dose)	Artificial insemination (lakh)	Crossbred progeny production (Lakh)
2007-2008	23.11	18.11	6.10
2008-2009	25.10	19.99	6.29
2009-2010	26.10	22.71	6.83
2010-2011	29.66	28.44	7.53
2011-2012	34.36	26.89	7.96

1.10 On-going Programme by Government in Bangladesh:

Artificial Insemination (AI) and Embryo Transfer (ET) Project

Objectives :

1. Production of high productive animals
2. Increase the number of quality breeding bulls as well as quantity of semen
3. Familiarize and encourage AI activities at grass root level
4. Practice embryo transfer technology in the nucleus herd at CCBS
5. To establish a nucleus herd at CCBS

First phase : Period 2002 to 2007

- Training of more 1000 artificial inseminators (volunteers)
- DLS plans to cover the entire country with AI services
- MOET calves are being continually produced at the govt. CCBS

Second Phase: 2008-2013:

- Objective: Training of more 1000 artificial inseminators (volunteers).
- Selected 80 breeding bulls used for AI programme.
- Semen production target from 25 lakh dose to 40 lakh doses.
- To achieve this target, so a modern Artificial Insemination Laboratory has been established at Rajabarihat, Rajshahi.

1.11 Breed Upgradation through Progeny Testing Project

- The first phase project started from 2002 and end at 2007;
- The second phase project run among the 22 district artificial insemination centre.

- Main objective this project:
- Superior proven bull production
- Identification for the high yielding cows and heifers.
- Increase genetic potentially and production through plan breeding.
- Increase production of milk and meat.

1.12 Beef Breed Development Programme in Bangladesh

Research objectives:

- To develop a meat type cattle (Local x Brahman) for increasing meat production.
- To increase the meat production and fulfill the demand of protein.
- To study meat production and meat quality of Local x Brahman crossbred.
- For this purpose, a total 1400 doses semen of Brahman breed bull from America.

Study I: 200 local cows were inseminated at CCBDF to obtain 50%Local x 50% Brahman crossbred bulls calves.

- To evaluate the productive and reproductive performances, feed conversion efficiency, health status and meat production and quality of crossbred progeny of Brahman bulls upto 18-24 months.
- Semen quality of male progeny will be evaluated in Central AI Lab.

Study-II: a total 700 locals were inseminated among 10 districts covering 11 upazilla for production of crossbred offspring.

- To rear under the semi-intensive system at farmer level.
- Best bull calves will be brought to Central AI Lab. For testing semen quality.

1.13 The Bangladesh Rural Advancement Committee (BRAC):

- Providing cattle AI service since 1985
- Established own bull station
- Semen production from November, 2000
- Production capacity of 5, 00,000 doses of straw per annum
- Trained 1279 AI technicians
- Operating nationwide AI services through 89 points
- AI technicians extend door to door service
- The cost for single dose of home to home BRAC semen is TK. 70 = 1 $

1.14 AI Programme of Dairy Cooperative:

- Bangladesh Milk Producers' Cooperative Union Ltd. (Milk Vita)
- Have their own bull centre
- Different exotic cattle AnGRs (Sahiwal, Holstein Friesian, Jersey)
- Produce and distribute semen to their member farmers
- Free of cost for their member farmers
- AI technicians extend door to door service

Artificial insemination (AI) of cattle in Bangladesh is expanding day by day. Therefore, for improving the production potential and genetic merit of our nondescript indigenous cows, superior germ plasms have been introduced all over Bangladesh. Three AI centres /station (CCBSDF, Savar, Dhaka, RDCIF, Rajabarihat, Rajshahi and DAIC, Rajshahi) have been established in different region with the artificial insemination (AI) services. The success of Artificial Insemination Program mainly depends on the successful selection of properly fertile bulls. It is expected that the superior sire can only produce best quality semen. There are many factors that may affect the effectiveness of A.I in field condition. The semen characteristics are the major factors influence conception in cattle and

therefore, a determinant of efficiency of reproduction. Among the factors, quality of semen is an important consideration. The bulls used for AI at two centres or stations are mostly cross-bred (L×F, L×F×F, SL×F) and pure Sahiwal, Friesian and Local. The adaptability of such exotic and cross-bred bulls in hot and humid climate as prevailing in Dhaka and Rajshahi are not known. Some breeds like Holstein - Friesian and Sahiwal found to suitable for climatic condition of Bangladesh. In general, bulls having 50% exotic blood are used artificially on indigenous cows. This is because half bred bulls are found much suitable and treated as the best types for the most tropical region for improving indigenous animals (Nagarcenkar, 1982). Studies have reported that factors like genetic group, season and age of bull influence the semen quality (Hussain *et al.*, 1985; Stalhammar *et al.*, 1989 and Bhosrekar *et al.*, 1988). However, breed and season have been shown to have effects on semen production and its quality (Vincent, 1972; Meyerhoeffer *et al.*, 1976, Parkinson, 1987, Sekoni and Gustafsson, 1987). The general tendency was as the European inheritance increase, total sperm output, initial motility and freezability of semen increase (Abraham *et al.*, 1982). Raju and Rao (1982) also reported significant differences among Jersey, Brown Swiss and 50% Brown Swiss and 50% Ongole breed in terms of ejaculate volume, sperm motility and concentration of spermatozoa. Contracting the above finding Hussain *et al.* (1985) did not find any difference between 50% Local× 50% Friesian and 50%Sahiwal ×50%Friesian attributable to genetic constitution. Age of the bull affects semen volume and sperm concentration (Amann and Almquist, 1976; Fuente *et al.*, 1984; Siratskii, 1987; Siratskii, 1990). Tomar *et al.* (1966) reported that there was no seasonal trend in any semen characteristics of Hariana bulls. Seasonal variations in semen production have been

observed (Ibrahim *et al.*, 1983; Schwab *et al.*, 1987; Graffer *et al.*, 1988), but specific causes are not understood. As the seasons of our country varies markedly, special attention should be given to the fertility of crossbred bulls all the year round. Information are not available of the semen production characteristics such as volume, colour, density, mass activity, sperm concentration, motility, total number of sperm cells/ejaculates and total number of semen doses /ejaculate as influenced by breed, age, body weight, body condition, testicular circumference, libido season and District Artificial Insemination (AI) Centre, Rajshahi, and RDCIF, Rajabarihat, Rajshahi. A better knowledge of the influence of breed of the bull as well as age, body weight, body condition, testicular circumference (TC), as well as season of the year on semen characteristics of the two AI centres / stations will help the AI industry to adapt a standard management of bulls to improve semen output.

Retrieval of reliable fertility data in an artificial insemination programme serving smallholder cattle is extremely difficult. It is therefore important to ensure that high quality semen from high fertility bulls is used. In this way variation in pregnancy rates due to bull and semen factors (Soderquist, 1991) can be eliminated or at least be reduced as a contribution toward the success of the programme. No single test of semen has been found suitable for the prediction of fertilising capacity. Therefore, seeking for a test or combination to tests to predict accurately the fertilising capacity of semen has been the subject of continuous research in this field (Linford *et al.*, 1976; Budworth *et al.*, 1988; Sodrquist, 1991). The sperm characteristics evaluated for prediction of fertilising capacity should be relevant to the chance of oocyte penetration in the female genital tract (Den Daas, 1992). This can only be ensured by

deposition of optimum number of morphologically and functionally normal spermatozoa into the female genital trait in right time of the oestrous cycle (Saacke *et al.*, 1991). Irrespective of causes, morphologically abnormal spermatozoa are unable to fertilize the oocyte (Shamsuddin and Rodriguez-Martnez, 1994). The conception rate after insemination with frozen semen were slightly lower than those achieved with chilled semen (Foote, 1986; Shamsuddin *et al.*, 1987; Coulter, 1992). On the contrary, significantly higher conception rate have been reported after insemination with frozen semen in straws than with chilled semen (Howlader *et al.*, 1997; Shamsuddin *et al.*, 1997). Considering the facts the present study was conducted to compare the semen production characterstics, semen quality on the basis of conception rate and NRR in field condition of various AI Sub-centres or points under District Artificial Insemination Centre (DAIC), Rajshahi.

Fertility in dairy bulls is a complex trait that is made up of several physiological processes such as the development of the reproductive system from birth to puberty, spermatogenesis, ejaculation and mating behaviour (which involves libido and copulation). For optimum semen quality, all these physiological processes should be coordinated.

Sperm morphology, motility, sperm concentration and volume per ejaculate are common criteria for evaluating semen quality at most AI stations (Den Daas, 1992; Colenbrander *et al,* 1993; Bearden & Fuquay 1997). Sperm morphology, expressed as a percentage of normal sperm cells (Serrenson, 1979) plays an important role in fertilisation in humans (Kruger *et al.*, 1986; De Yi lui & Baker, 1982) and it also seems logical that the same will be true for domestic animals (Reinecke *et al,* 1995). Morrow (1980) defined the relationship between spermatozoa

morphology and the reproduction potential and indicated that when more than 30% of the ejaculated spermatozoa have structural defects, reduced fertility may occur in domestic animals.

Many structural abnormalities occur in the development of spermatozoa as a result of faulty spermatogenesis and also as due to semen handling both during and after collection (Salisbury *et al.*, 1978). Major factors that affect semen quality are also those related to the underdevelopment of the testis (King, 1993). All factors related to testicular degeneration, including hereditary and pathological conditions should be carefully considered as they may seriously affect semen quality via testicular development. Hoogenboezem & Swanepoel (2000) reported that testicular degeneration might be due to exposure to heat stress, nutritional deficiencies and management-related factors such as fat deposition in the scrotum and poor body condition.

The major contribution to the semen variation is environment (Curtis, 1983; Cupps, 1991; King, 1993; Bearden & Fuquay, 1997). To comprehend the impact of environment on semen quality, it is necessary to understand the biological significance of the term 'environment', which could be defined as all factors surrounding the animal (Bonsma, 1980), which include factors such as environmental temperature, nutrition, humidity, seasonal changes and management of the animal.

The frequency of abnormal sperm cells has been found to increase with factors such as extreme in temperature and heat stress (Rathore, 1970) and malnutrition (Brown, 1984), occurring mainly in the hot seasons (Anderson, 1941). This has been observed to result in a lower ejaculate volume and sperm motility, increase in the percentage of abnormal sperm

and a decrease in the total live sperm (Rathore, 1970). Malnutrition includes under-feeding and over-feeding, deficiencies of a specific nutrient and included amongst these may be the ingestion of toxic substances, which occurs largely as a consequence of poor management. The latter depresses appetite, and hence lowers feed intake. Lower feed intake is likely to result in impaired reproductive capability of bull in terms of libido and mating behaviour and semen production and quality (McDowell, 1972).

Mathevon *et al.* (1998) reported that season significantly affected semen morphological characteristics in young bulls but did not significantly affect ejaculate volume and sperm motility in mature bulls. Significant seasonal variation occurred in the incidence of sperm head abnormalities and total sperm abnormalities. Kumi-Diaka *et al.,* (1981) reported no significant seasonal variation in sperm cell concentration, percentage of live spermatozoa and sperm cell abnormalities in the indigenous breeds, while exotic breeds showed significant seasonal fluctuation, with regard to high sperm cell abnormalities, low percentage live sperm and lower sperm cell concentration during the hot season.

The food security is burning subject, according to FAO food security exists when all people, at all time, have access to sufficient, safe and nutritious food for healthy life. The issue of food security is now not only the concern of southern Asian countries but also the matter of whole world. Every man has fundamental right to get safety and nutritious food as per WFC at Rome, 1996. The food security position of Bangladesh is 81st out of 105 courtiers in the Global food security index-2012 published by the economist intelligence unit of Britain. Whatever, the Bangladesh is the lowest position in Food security among the southern Asian countries.

Food security mean not only produce rice and wheat because food security is safety and necessary nutritious food. The nutritious food contains in specially animal protein such as milk, meat and egg. FAO recommendation per capita at least 250 ml milk and 120 gm meat per day are required for human balanced diet. 87% people are suffering from malnutrition due to lack of demand of milk and meat in Bangladesh. So, this topic to evaluate the present situation and future prospect of milk and meat production through Artificial insemination Programme in food security of Bangladesh.

The success of Artificial Insemination programme mainly depends on the successful selection of properly fertile bulls. It is expected that the superior sire can only produce best quality semen. The success of AI programme depends on various factors such as selection of different breeds of bulls with age, body weight, body condition, testicular circumference and libido as well as semen characteristics in relation to bull, environmental and managemental factors, morphological characteristics of spermatozoa, semen quality, insemination technique and semen preservation, semen types, skillness of AI technicians, semen fertility in relation to conception rate and non-return rate.

In Bangladesh very little comprehensive work has been done regarding the “Factors Affecting the Semen Production and Semen Quality of Bulls used for Artificial Insemination”. Therefore, from all points of view the overall objectives of the present study were as follows.

1.15 Objectives:

1. To identify the suitable bulls used for Artificial Insemination (AI) programme on the basis of semen characteristics/production performances.
2. To evaluate the effect of breed, age, body weight, body condition, libido score and testicular circumference on semen production.
3. To find out the influence of season on semen production of AI bulls.
4. To compare the semen characteristics among AI centres.
5. To study the effect of extenders on semen quality on the basis of duration of preservation and conception rate.
6. To know the effect of breeds, age, body weight, libido score and season on semen quality.
7. To study the effect of breed, age, body weight, body condition, testicular circumference, libido score and season on sperm abnormalities in relation to non return rate (NRR).
8. To estimate the effect of season and AI centre on semen morphology in relation to NRR.

CHAPTER 2

REVIEW OF LITERATURE

The Artificial Insemination (AI) may be defined, the semen collection through Artificial Vagina (AV) from high potential bulls then processing the semen preservation and AI into a estrus (heat) cows for the achievement of conception. In other word, the deposition of spermatozoa in the female genitalia by artificial rather than by natural means. There is a series of process involved in this technique. The success of AI programme mainly depends on the semen quality of bulls, timing of AI, uterine conditions of female and skillness of AI techniques. Semen is liquid cellular suspension containing spermatozoa and seminal plasma. Good quality semen ensure the higher conception rate. To obtain high milk yielding cow, beef cattle the upgraded bull semen is collected and processed for future use through AI. Many scientists have done research on artificial insemination and seminology. A few reviews of literature has been cited in relation to "Factors Affecting the Semen Production and Semen Quality of Bulls used for Artificial Insemination" in broad and abroad. To make it easy and understandable the reviews is divided as several sections as follows.

2.1 Factors Affecting the Variation in Semen Production and the Quality of Semen:

There are many factors associated with the variation in semen characteristics in relation to fertility of bulls used for artificial insemination (AI). The factors such as bulls as donor of semen, breed of AI bulls, age of bulls, body weight of bulls, testicular circumference of bull, season, bull selection and evaluation, sexual preparation of bulls and

semen processing for preservation (semen extender or diluents, cooling, equilibration and freezing, packing of semen and optimum number of spermatozoa per insemination dose, preservation, storage and transportation of semen thawing of semen and evaluation, AI technicians, chilled vs. frozen semen and hygienic management).

2.2 Growth and Sexual Development of Bull:

The production of semen by dairy bulls is dependent largely on the growth and sexual development of the bull from birth to puberty (Salisbury *et al.,* 1978). Bearden & Fuquay (1997) reported the endocrine related changes in body conformation, increase in aggressiveness and sexual desire, rapid growth of the testes and separation of the penis from the prepuce so that the extension of the penis is possible. These changes occur before the spermatozoa are present in the ejaculate, prior to puberty.

The sexual development of bulls before puberty can be divided into an infantile and juvenile stage, which are determined by luteinizing hormone (LH) and steroid hormone secretion. The infantile stage, from birth to 10 weeks of age, is characterised by the infrequent secretion of LH. The juvenile stage, from 10-12 weeks of age, is characterised by an increase in the frequency and amplitude of LH secretion (Cupps, 1991) which stimulates testicular growth and induces spermatogenesis (Ahmad & Noakes, 1996).

Al -Gedawy & Afiefy (1984) reported that as a bull grows from birth to puberty, the rate of growth of the testicles increases rapidly until 9 months of age, slows down at 11 months of age and then accelerates again at 13 months of age. Evan *et al.*, (1995) reported that in pre-

pubertal bull calves, there is a transient rise in the secretion of the gonadotrophin, LH and follicle stimulating hormone (FSH), which have been suggested to play a major role in the regulation of sexual development and semen production. Furthermore, Marnabolo (1999) reported that in the absence of these gonadotrophins, neither testicular development nor spermatogenesis may proceed. Evans *et al.* (1995) also reported that early increases in these gonadotrophins are dependent on the maturity-type of the bull. It was indicated that there is a significant increase in the rise of LH and FSH in early maturing bulls, compared to late maturing bulls at the age of 2 and 24 weeks.

Dairy calves enter the pre-puberty period at about 10-12 months of age (Bearden & Fuquay, 1997). At the onset of puberty, gonocytes migrate to the periphery of the seminiferous tubules and produce two types of cells or spermatogonia (King, 1993), namely:

(1) Stem cells or spermatogonia, which give rise to spermatogonia and succeeding spermatocytes, spermatids and spermatozoa and

(2) Reversed stem cells, which contribute to the increase in population of stem cells between puberty and adulthood.

Hafez (1974) reported that spermatogonia and a small number of spermatocytes are present in the seminiferous tubules at 63 days of age and Leydig cells are formed at approximately three and half months of age. McMillan & Hafs (1968) reported an increase in plasma LII and hypothalamic LH releasing factor until the age of puberty, while pituitary FSH secretion declines after six (6) months. The production and release of testosterone from the testis generally increases from birth to puberty. This hormone is responsible for the maintenance of male reproductive

system, initiation of spermatogenesis and male mating behaviour (Bearden & Fuquay, 1997). Foote *et al,* (1976) reported higher seasonal variation in the secretion of testosterone in Holstein bulls, where the average level for spring of 8.0 ng/ml. Higher than the value of 5.7 ng/ml recorded in the fall. It was also indicated that testosterone levels increase with age of the bull, up to 6 to 7 years of age. Further more, Penfold *et al.* (2000) indicated the testosterone concentration to be positively correlated with the percentage of normal sperm and total number of sperm produced.

2.3 Spermatogenesis of Bulls:

Spermatogenesis is defined as the process of formation and liberation of spermatozoa from the undifferentiated germ cells in the seminiferous tubules of the testis (King, 1993). The spermatozoa undergo maturation in the epididymis where they are stored until ejaculation takes place (Bearden & Fuquay, 1997).

2.4 Process of Spermatogenesis:

Spermatogenesis is defined as a lengthy chronological process whereby a few stem cell spermatogonia, lining the base of seminiferous tubules, divide by mitosis to maintain their own stem cell numbers and to produce primary spermatocytes that undergo ineiosis to produce spermatids which differentiate into a spermatozoa (Cupps, 1991; Fig. 2.1).

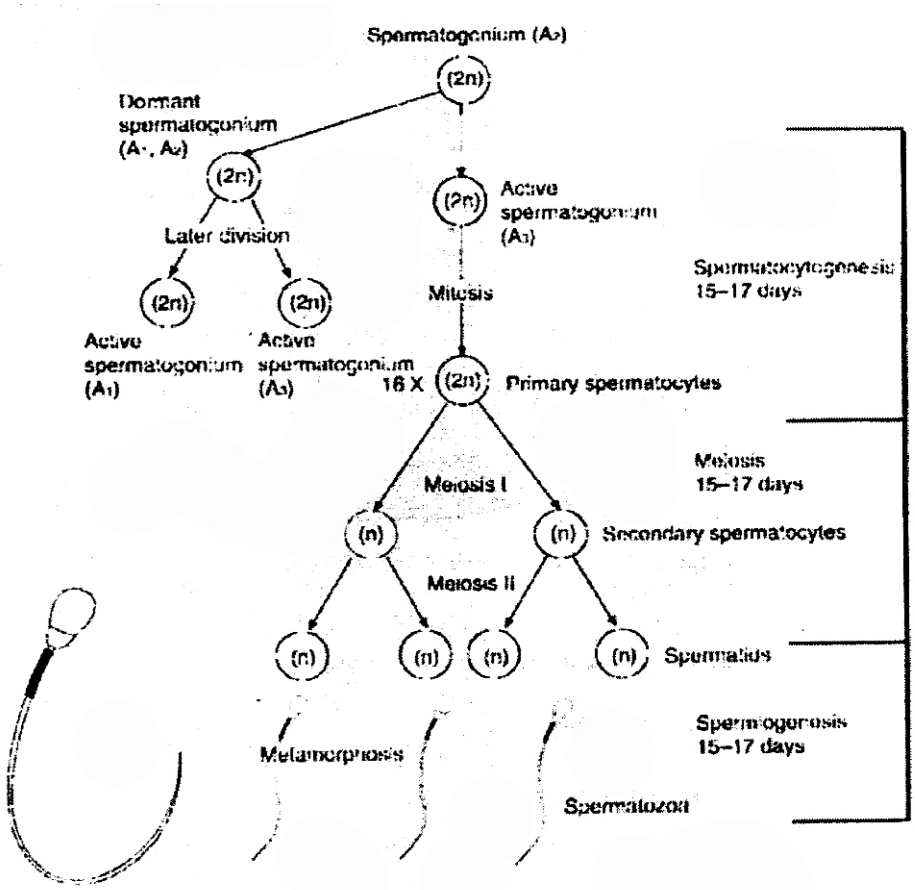

Fig. 2.1. Process of spermatogenesis in domestic animal (Bearden & Fuquay, 1997)

In the bull, the process of spermatogenesis takes approximately 56-63 days and is divided into three phases namely spennatocytogenesis, meosis and spermiogenesis (Foote, 1978; Bearden & Fuquay, 1997). Spermatogenesis starts before birth when primordial germ cells of the early developing embryo migrate to the undifferentiated fetal sex cord from the yolk sac and undergo several divisions to produce gonocytes. (Cupps, 1991; King, 1993). Gonocytes persist in the male sex cord until just before puberty, after which time they divide to provide spermatogonia (King, 1993).

The produced spermatogonia undergo mitotic divisions during the process of spermatocytogenesis (first phase of spermatogenesis) to form primary spermatocytes (Fig.. 2.1). Meiosis is the phase where the primary spermatocytes formed during spermatocytogenesis undergo meiotic division forming two secondary spermatocytes (Bearden & Fuquay, 1997). With this division, the chromosome complement in the nucleus is reduced to half so that the nuclei in the secondary spernatocytes contain unpaired haploid chromosomes (Cupps, 1991; King, 1993). Each secondary spermatocyte will undergo a second meiosis division, each forming two spermatids (Bearden & Fuquay, 1997).

During spermiogenesis, the last phase of spermatogenesis, the spermatids undergo a metamorphosis, which is defined as the process by which spermatids undergo changes in morphology to form spermatozoa. During this phase, spermatids are attached to the Sertoli cells. After metamorphosis, the newly formed spermatozoa will then be released from Sertoli ceLls through the process called “spermiation” and be forced out through seminiferous tubules into the rete testis. From the rete testis, spermatozoa are forced to epididymis via vas efferents (Bearden & Fuquay, 1997).

During the passage through the epididymis, spermatozoa undergo maturation whereby they gain the ability to be motile, lose the cytoplasmic droplets and also become fertile (Cupps, 1991; Bearden & Fuquay, 1997; Mamabolo, 1999; Dombo, 2002). At the same time spermatozoa undergo changes in fine structures and composition involving both lipids and protein metabolism and in surface properties (Robaire & Herino, 1988).

Dombo (2002) reported that the time required for spermatozoa to transverse the epididymis is from 4-12 days and their effective life span is 40 days. Hunter (1980) and Arthur *et al.* (1982) associated the frequent sexual activity and the small interval between semen collection with the acceleration of the movement of spermatozoa in the epididymis. This may lead to the appearance of the immature spermatozoa in the ejaculate.

2.5 Constituents of Bull Semen:

Immediately after ejacu1ation samples should be viewed with naked eyes and the volume, density and colour noted. Den Daas (1992); Coleubrander *et al* (1993) and Beardert & Fuquay (1997) reported that the percentage of normal sperm, motility, sperm concentration and volume per ejaculates are common criteria for evaluating semen quality at most Al stations.

Analysis of sperm morphology is an important part of routine spermiogramme, which gives an overall picture of the degree of semen quality (Serrenson, 1979). Phillis (1976) reported that semen evaluation should take place at the body temperature of approximately 37 °C.

2.6 Properties of Normal Semen in Dairy Bulls:

Table 2.1. Average chemical composition of fresh bull semen (Bearden & Fuquay, 1997)

Constituent or property	Proportion [mg per ml]
Sodium	230
Potassium	140
Calcium	144
Magnesium	9

Constituent or property	Proportion [mg per ml]
Chlorine	180
Fructose	530
Citric acid	720
Glycerylphoshorylcholine (GPC)	35×10^4
Protein [gram per ml]	6.8
Sorbitol	75

Table 2.2. Characteristics of fresh semen in dairy bulls (LSM±SE) (Serrenson, 1984)

	Friesland	Jersey
Concentration	71.2±2.7	75.6±1.3
Motility (%)	74.6±1.4	81.3±4.1
Morphological normal (%)	70-80	70-80
Abnormality (%)	13.7±1.3	10.3±1.5
Volume (ml)	5.7±0.38	4.3±0.21

2.7 Bulls as Donor of Semen:

Haque (1968) observed significant difference between bulls in concentration of spermatozoa, initial motility, volume of ejaculate, pH of semen and percentage of abnormal spermatozoa.

Kulkarni and Bhosrekar (1973) stated that reaction time was significantly less in Brown-Swiss bull than in Tharparkar bulls. No significant difference was noticed regarding ejaculate volume and sperm density. Initial motility was significantly different between bulls.

Amir *et al.* (1982) worked with 100 Friesian bulls of proven fertility in every winter (January to March) and summer (July to September) for a period of 3 years. They reported that the bulls and years significantly affected semen volume, sperm concentration and sperm motility before and after freezing. There were no significant differences between samples collected in summer and winter for semen characteristics.

Al-Hakim *et al.* (1984) studied over a 9 months period, 569 ejaculates collected from 9 Karadi (Kurdi) bulls. For first ejaculate semen volume averaged 2.62±0.06ml, mass motility 56.63±1.4%, sperm concentration 115.0±3.51×10^7 /ml, the percentage of abnormal spermatozoa 5.24±0.25, the percentage of dead spermatozoa 10. ±0.39 and pH 6.77±0.01. Corresponding Figs. for the 2nd ejaculate were 2.06±0.06 ml, 49.7±1.6%, 87.77±2.80×10^7 /ml, 4.61±0.22%, 9.63±0.39% and 6.85±0.01. All traits varied significantly between bulls and months. Season had a significant effect on ejaculate volume, pH and the percentage of abnormal spermatozoa and there were significant bull× seasons interactions.

Ziegler (1984) concluded that bull and month significantly affected semen volume and sperm motility.

Individual bulls differed with respect to semen volume ($P<0.01$), sperm concentration ($P<0.05$), and total spermatozoa per ejaculate ($P>0.01$). The differences between bulls may be attributed to the variation in their SC, breed, age, body size and body weight and secretory activities of their sex glands (Leon *et al.*, 1991; Sharma *et al.*, 1991). More over, collection frequency, pre collection, sexual stimulation, feeding regime and climatic conditions can also influence the semen volume and sperm concentration (Al-Hakim *et al.*, 1986).

2.8 Breed of AI Bulls:

Eckardt *et al.* (1973) studied the first 5 ejaculate of 419 German Black Pied (GBP), GBP×Jersey and GBP×Holstein-Friesian (HF) bulls at 10 months of age. Ejaculate volume was lowest in the GBP×Jersey and highest in the GBP×HF bulls, whereas sperm concentration and forward and mass motility were highest in the Jersey crosses.

Rao and Rao (1978) studied on 4 cross-bred (F1) two Brown Swiss × Ongle (BS×O) and two Holstein × Ongle (HF×O) bulls and that the ejaculate volume of Holstein × Ongle cross-bred and Brown Swiss×Ongle bulls were found 4.17±1.37 ml and 4.33±1.20 ml respectively. The initial motility of spermatozoa recorded for Brown Swiss × Ongle and Holstein Friesian × Ongle cross-bred bulls were 84.13±4.17 and 80.80±3.24 and the concentration of spermatozoa recorded ml of semen were 984.93±312.51 million and 611.84±164.26 million, respectively.

Bujarbaruah *et al.* (1982) noticed significant breed difference in sperm motility between Jersey and Danish Red bulls. Semen volume and motility varied significantly ($P<0.05$) between bulls within breeds. The sperm concentration and the proportion of spermatozoa resistant to cold shock varied significantly between Jerseys bulls, while the proportion of live spermatozoa and the mid-piece abnormalities varied significantly between Danish Red bulls. The semen volume, sperm motility, sperm concentration and proportion live spermatozoa of abnormal spermatozoa decreased significantly.

Djimde and Weniger (1984) examined semen and sexual behavior of Sahiwal, Red Sindhi (RS), Friesian, Jersey, Local ×Friesian, RS×Friesian, Sahiwal ×Jersey and RS×Jersey bulls. Differences in semen quality were

found between breeding groups and season. The Zebu breeds had significantly better semen quality and poor libido than other groups. Cross-breeds especially of local breeds with Friesian or Jersey had better semen quality and libido than bulls of imported breeds.

Gavthier and Varo (1985) observed that Creole bulls had a greater ($P<0.01$) ejaculate volume than Friesian bulls (6.20±1.90 vs. 4.66±1.5ml) but a lower ($P<0.01$) sperm concentration (0.68±0.30 × 10^9 vs. 0.83±0.30 × 10^9 spermatozoa/ml). They found no significant differences between breeds for total number of spermatozoa per ejaculate and motility score.

Schwab *et al.* (1987) observed data on 118000 ejaculates, collected in Switzerland over a period of 11 years from 1384 bulls of 6 breed types. Overall ejaculate volume averaged 5.87ml, sperm concentration 1.21×10^9/ml and the number of spermatozoa ejaculate 7.23 $\times10^9$. All 3 traits were affected ($P<0.001$) by breed type bulls (Simmental×Red Holstein performing better than Holstein

Friesian×European Friesian crossbreeds and Herens and Simmental purebreds), bull within breed age of bull (bulls aged 31-54 months having the best performance), season (best in January to June) and intervals between semen collections (poorest at intervals of <4 days). None of the traits was significantly affected by weather conditions.

Hazarika *et al.* (1988) observed that in bulls aged 3 to 4 years from 10 adult crossbred bulls, 2 from each of genotypes (group 1, 1/4 Sahiwal (S) ×1/4 HolsteinFriesian(HF) × 1/2 Red Dane(RD); group 2, 1/4 S×1/4 Jersey(J) ×1/2HF; group 3. 1/4 S×1/4J×1/2 RD; group 4, 1/4 S×1/4 RD×1/ 2J; and group 5, 1/2 S×1/2 J). The bulls of group 1, ejaculate semen of superior quality in respect of sperm concentration, live

spermatozoa, head abnormalities and total abnormalities in comparison to bull of other groups. The bulls of group 2, with 50%HF inheritance donated 5.21 ml semen per ejaculate. The introduction of Jersey blood (50%) in Sahiwal breed (group 5) resulted in lowest semen volume, sperm motility, sperm concentration, live spermatozoa and maximum of sperm abnormalities in comparison to the bulls of other genotypes groups. They also observed that correlation coefficients between semen characteristics (all bulls pooled) revealed that sperm motility and live spermatozoa were significantly positively related with sperm concentration (0.552 and 0.495 respectively). The total sperm abnormalities were positively significantly influenced by abnormalities in head (0.651) and mid-piece (0.723) region spermatozoa.

Piirimaee (1994) observed in 40125 ejaculates from 109 bulls for evaluate the semen traits depending on the breed of bulls, to estimate the dependency of the semen traits on the sire of the bulls, to evaluate the dependency of the insemination results on the genotype of the bulls, correlation's between inseminations results and semen traits. Sperm concentration and motility and the number of doses per ejaculate decrease by intensive using of the bulls, but the annual production of semen increases. Insemination results do not depend much on genotype of bull and semen traits.

Zawawi *et al.* (1996) evaluated semen quality in 11 bulls that had 25, 50, 62.5 or 75 percent of Friesian inheritance and found that there were significant differences between some breed types for ejaculate volume.

Hanada *et al.* (1997) studied 24 Japanese Black bull for artificial insemination of which 4 were bulls heterozyous for 7/21 translocation

and on was homozygous bull. There was no significant difference in semen characteristics between chromosomally normal bulls and bull with translocation. Overall ejaculate volume averaged 5.9±1.2 ml, concentration 13.6±2.9 x10^8/ml, no. of spermat./ejaculate 79.6±20.2 x10^6, abnormal sperm 8.4±1.1%, motility before freezing 83.3±5.1% and motility after freezing 43.9±3.6% in normal bulls.

Kibria *et al.* (1997) worked in 575 ejaculates semen sample from 7 genetic groups of bulls belonging to 5 age groups of various seasons viz. spring, summer, autumn and winter of Central Cattle Breeding Station (CCBS), Savar, Dhaka. Genetic groups had significant (P<0.01) effect on semen characteristics. Highest volume (8.46ml) of ejaculate was found in 50%Sahiwal-50%Holstein Friesian crossbred bull with the lowest (5.15ml) in 50% Sindhi-50%Sahiwal. The concentration of sperm was found highest in 100%Sahiwal (pure breed) and 50%Sindhi50%Sahiwal (1234.77 and 1125.44 mil/ml respectively). The concentration was highest (P<0.01) in spring season (1129.40mill/ml) and lowest in summer season (947.49mill/ml). The age of bull had significant (P<0.01) effect on total number of semen collection, volume (ml)/ejaculate and sperm concentration (mill/ml). Genetic group, season and age groups had no significant effect on initial motility.

Panayotova *et al.* (1998) observed semen production on 61 Holstein, 21 Bulgarian Brown (BB) and 8 Rodopi bulls at the Sliven AI station. For 14285, 3496 and 496 ejaculates that had been collected in 1987-1995 from the 3 breeds bulls, the effects of year, season, age, the number of ejaculates per collection day and the individual bull on ejaculate volume were significant in all 3 breeds except the effect of season in the Bulgarian Browns; the same effects were significant for sperm

concentration except for the effect of year and season in Rodopis.

Sarder *et al.* (2000) studied in 1320 ejaculates from 14 breeding bulls belonging to 8 genetic groups and 5 age groups of different seasons in District AI centre, Rajshahi. The highest volume (9.90 ml) of ejaculation was found in 50%Sindhi50%Sahiwal cross-bred bull with the lowest (3.77ml) in 75% Friesian-25%Local. The concentration of sperm was found highest in 50%Sindhi-50%Sahiwal (1447.03 and 1428.66 mill./ml, respectively). The concentration was highest in spring season (1279.98 mill./ml) and lowest in summer season (1140.52mill./ml). The age of bull had significant ($P<0.01$) effect on total number of semen collection, volume /ejaculation and sperm concentration (mill. /ml). The highest volume was found in age group of 10 and above (7.36ml) followed by 8-<10 yrs (6.70 ml). The highest sperm concentration was found in age group 8-<10 yrs (1367.28 mill./ml) and lowest sperm concentration was found in age group of 0-<4 yrs. Genetic group, season and age of bull had no significant effect on initial sperm motility.

2.9 Age of Bull:

The definition of the age at puberty differs between genders. In males, it can be defined as the age at which spermatozoa are present in the ejaculate, while in females it is defined as the age at first behavioural estrus (Bearden & Fuquay, 1997).

Dairy bulls reach puberty at the age of 10-12 months, at a body weight of 160-270kg (Bearden & Fuquay, 1997). Hafez (1974) reported to be much more difficult to determine the exact time of puberty in males because the first differentiation of spermatogenetic cells precedes the release of the first spermatozoa from the seminiferous tubules by a month or more.

Size and weight at puberty is more important than age in determining the onset of puberty. Mamabolo (1999) claimed testis weight, which is correlated with sperm production and quality, to be also positively correlated with body weight. It was also reported that approximately 80% of the variation in testicular weight to be due to the variation in body weight. Bongso *et al.* (1982) identified testicular measurements as an indicator of the reproductive potential and spermatogenic capacity of ruminants. This may explain why weight is more important than age at puberty.

Factors affecting age and weight at puberty are of major importance in the evaluation of semen quality as Day *et al.* (1984) reported puberty to be the major factor that dictates reproductive competence in bulls and heifers.

Differences in the weight at puberty from year to year have been observed. These differences have mainly been attributed to genotype or breed variation (Bearden & Fuquay, 1997) and a difference in environmental conditions which include factors such as temperature, nutrition, photoperiod and humidity (Paterson, 1981).

The effects of breed on weight and age at puberty may be as a result of factors such as adaptability, average daily gain (ADO) and maturity type of the breed which are all related to the growth rate and hence puberty. In beef calves, growth up to the age of puberty is a measure of adaptability and weight at puberty is regarded as an important post weaning trait that can be used in the final selection of bull calves as herd sires at phase C and P at performance testing stations (Bearden & Fuquay, 1997) and on the veld (Van Zyl *et al.*, 1992; Lubout, 1987; Nauhaus, 1992).

Under tropical and sub-tropical conditions, poor nutrition accompanied by high temperatures result in stress, which reduces the feed intake and affects growth rate in the bull (McDowell, 1972), and hence lowering of the weight and delaying of the age at puberty (Lammond, 1970). If overfeeding accelerates growth rate, body weight at the time of puberty will be higher than normal and the animal reaches puberty at a younger age. On the other hand, if underfeeding retards growth rate, puberty is delayed and body weight will not reach that of a normal fed animal. Hafez (1974) reported that if nutritional status of an animal is maintained at normal levels, puberty might occur when body weight reaches approximately 45% of adult weight in cattle. In males under-nutrition results in a delay in the onset of puberty due to poor testicular development and sperm production (Bearden & Fuquay, 1997). Low energy intake and protein deficiencies alter the rate of gain and might result in a lowering of the body weight and delay the age at which puberty is attained. Vitamins and minerals are important for cellular metabolism, growth and maintenance, follicular and testicular development, all of which influence the onset of puberty in both males and females (Bearderi & Fuquay, 1997). Unfavourably high ambient temperatures lead to a restriction of nutrients in the diet of the animal through the depression of appetite and hence reduced feed intake, The nutrient restriction may be enough to substantially limit somatic growth, and hence increase the age and lower the weight at puberty (McDowell, 1974).

The endocrine mechanisms that regulate puberty are centrally regulated within the hypothalamus (Bearden & Fuquay, 1997). Alterations in hypothalamic function that may direct the cascade of the endocrinological

events resulting in puberty include changes in the populations of estradiol-receptors containing neurones that may also delay puberty. Hafez (1974) associated the onset of puberty with a decrease in the sensitivity of these steroid receptors in the brain and an increase in gonadotrophin secretion and subsequent activation of spermatogenesis. Day *et al.* (1984) reported temperature and nutrition to be the primary environmental factors that influence the timing of this change and hence influencing puberty. Bearden & Fuquay (1997) also reported that the pineal gland inhibits the onset of puberty. Sensitivity of the brain steroid receptors can be modulated by pineal gland, which is limited to counteracting the unfavourable effect of photoperiod on sexual function (which is related to the increase of pineal sensitivity). Changes in day length or photoperiod interact with the genotype of an animal in affecting the onset of puberty through its effect on sexual development of both males and females. Seasonal changes in spermatogenesis and ovarian cyclic activity are caused largely by a change in the secretion of LH and FSH, which is reduced by the exposure to the inhibitory photoperiods and elevated by exposure to stimulatory photopenods (Straus *et al,* 1979). Hafez (1974) also found a correlation between the attainment of puberty and the social status of the animal. It was indicated that the presence of individuals of the opposite sex hasten puberty, while the presence of individuals of the same sex delays the onset of puberty.

Rodionovskii (1977) studied semen characters in 2540 ejaculate of 39 bulls, aged 2 years and in ejaculates of surviving bulls up to 10 years of age. Ejaculate volume increased from 3.48 at 2 years to 5.70 ml at 9 years. Sperm concentration decreased gradually with age.

Rao and Rao (1978) and Raju and Rao (1983) have also observed

significant increased in the volume and motility % of spermatozoa and a significant decreased in the abnormalities % of sperm cells in the semen of Brown Swiss×Ongle bulls with a increased in their age over 15 months. The present report, however does not confirm their observation on an increased in concentration up to an age of 24 months.

Ramamohana Rao *et al.* (1979) investigated on the semen characteristics of 3 cross-bred (F1) bulls comprising of two Holstein Friesian×Ongole and on Brown Swiss×Ongole bulls aged 15 months. The semen characteristics of 3 cross-bred (F×) bulls from the age of 15-23 months consisting of 75 ejaculates were studied. The semen quality was poor at the beginning and improved gradually with age and was nearly normal at the age of 23 months in the cross-bred (F×) bulls studied.

Ziegler (1984) observed that in 990 ejaculates from 35 Simmental bulls and 32298 first inseminations during 1980-82 from 10 older and 25 young bulls, the main difference in semen characters was for ejaculate volume (7.03 Vs 4.22ml). Bull and age significantly affected almost all semen characters. The 90 days non-return rate after AI was significantly correlated with ejaculate volume (0.11) and with sperm concentration in the older bulls (0.22).

Tomar *et al.* (1985) reported the semen production from 9 Holstein Friesian ×Hariana cross-bred bulls in different age groups (from less than 24 months to more than 36 months) in all seasons of the years (for the semen production parameters). Ejaculate volume, mass motility and live-percentage of spermatozoa increased with growing age of the bulls, while the live-abnormal percentage spermatozoa increased simultaneously. Mass motility was poor during winter season, while sperm concentration

was less was during summer season. The age groups had highly significant effect on all the semen characteristics except the concentration on spermatozoa in the semen.

Stalhammar *et al.* (1988) studied 215 Swedish Red and White and Swedish Friesian bulls in 1983-85 in respect of libido, ejaculate volume, number of spermatozoa per ejaculate, sperm motility before and after freezing and the number of semen doses per collection was generally significantly affected by season and age of bull.

Swedish Red and White (SRB) and Swedish Friesian breed(SLB). The effects of bull's sire, bull, season and bull's age at collection were estimated for ejaculate volume, motility, total no. of sperm cells per ejaculate and no. of semen doses per collection. Age of bull at collection affected ejaculate volume, total no. of sperm cells and no. of semen doses per collection. For the quantitative traits, 5-15% of the variation due to bull's age at collection. Total no. of sperm cells collected per day doubled from 12 months to 30 months of age and older. The effect of bull's age at collection on the two motility characteristics was significant except for post-freezing motility. There was significant seasonal effect on the semen characteristics. The summer months June, July and August were the best months for quantitative semen traits.

Barbosa *et al.* (1991) evaluated semen traits at 27 and 39 months of age in 7 Canchim and Nelore bulls. Volume was significantly greater in Canchim than in Nelore bulls at 27 months, but not at 39 months of age. There were significant breed differences or breed×age interactions for ejaculate volume or semen motility, concentration or abnormalities, but semen quality improved with increasing age bulls.

Zhang *et al.* (1993) evaluated 33 Holstein bulls at the Beijing Bulls Station were grouped by age (11 animals per group): 1 to 2 years (group A), 2 to 4 years (group B) and >4 years (group C). The semen quality of each ejaculate (volume, sperm concentration, total number of spermatozoa and sperm motility) was recorded for 1 month. The results showed that scrotal circumference and ejaculate volume in groups B and C were greater ($P<0.01$) than those in group A.

Rao and Sreemannarayana (1996) calculated the effect of age (3 to 14 years) on seminal volume, sperm concentration, total sperm and semen doses per ejaculate, initial motility and post-thaw motility was studied from 32 Murrah bulls at the Frozen Semen Bull Station, Visakhapatnam. The average volume, total sperm and semen doses per ejaculate increased progressively with advancement of age up to 14 years, while sperm concentration declined in older bulls. The quality of semen in terms of initial motility was better in young and aged bulls, while post-thaw motility increased progressively with age.

Diarra *et al.* (1997) studied 294 young Holstein bulls between 10 and 18 months old. The Sire effect was significant for the volume ($P<0.01$), concentration ($P<0.05$) and the number of motile spermatozoa per ejaculate ($P<0.01$). Intraclass regression studies indicated that each increase of 1 day of age in young bulls increased the volume of the ejaculate by 0.004%, the concentration and the motility of the ejaculate by 0.024×10^8 spermatozoa per ml and 0.03% and the concentration of the ejaculate by 0.14×10^8 spermatozoa per ml.

Sarder (200b) studied 680 semen samples were collected from 8 bulls belonging 4 age groups in 3 different seasons at District AI centre, Rajshahi. Age groups had a significant effect ($P<0.05$) on semen

characteristics except sperm concentration. The highest volume of ejaculate, sperm concentration, total number of motile sperm cells per ejaculate and total number of semen doses per ejaculate were found in age groups of 6.5 to ≤8.5 years (7.34±0.15 ml, 1372.05±33.03 mill. /ml, 6624.63±258.24 million and 336±15.02, respectively) and lowest in age groups of 2.5 to ≤4.5 years (5.30±0.20 ml, 1239.55±41.42 mill. /ml, 4317.79±273.17 million and 209±14.07, respectively). The total number of semen doses per collection was highest ($P<0.05$) in summer (295±18.61) and lowest in winter (248±12.52) season.

2.10 Body Weight of Bulls:

Pawlina *et al.* (1989b) observed on 66 bulls maintained in Animal Breed and Insemination Station during the period 1978-87. There was found the negative correlation between weight gain and spermatozoa content in ejaculate of crossbred but not pure breed bulls were stated.

Pawlina and Nowicki (1990) observed to influence of growth rate on semen production on 75 Polish Black-and-White Lowland and 50 Polish Red-and-White Lowland bulls. The negative effect of high growth rate during the rearing on semen production in bulls was stated.

Siratskii (1992) studied over a 20-year period from 642 bulls belonging to the pedigree breeding associations in Sumy, Chernigov and Kharkov Oblasts was studied. A significant dependence of ejaculate volume and the total number of spermatozoa per ejaculate in live weight was found.

Asad (2001) reported that body weight groups 400-600 kg, >600 to700 kg, >700 to 900 kg had significant ($P<0.001$) effect on volume of ejaculate, concentration, forward movement and non-significant effect ($P>0.05$) on mass movement of the semen. The highest value of volume

(12.16±022) in 400 to 600 kg body with the lowest (11.35±0.24ml) for >600 to 700kg body weight. The maximum concentration (1158.19±16.39 mill. /ml) was for >700 to 900 kg body weight with the minimum 1074.64±15.82 mill/ml) for >600 to 700 kg body weight. The greatest forward movement (60.25±0.45%) in 400 to 600 kg body weight with the lowest (56.63±0.66%) for .600 to 700 kg body weight.

Sarder *et al.* (2001a) studied that on seven AI bull at the Deep-frozen semen production laboratory, Rajabarihat, Rajshahi for a period from October 1998 to June 2000 to find out the phenotypic and genotypic variation in the semen traits. Body weight of bulls had significant ($P<0.05$) effect on volume of ejaculate, sperm concentration, pre-freezing motility and post-freezing motility except total number of sperm/ejaculate. The highest volume of ejaculate and total number of sperm /ejaculate was for 550 to <650 kg (8.38ml and 9294.77 mill. /ejcul.) and lowest for >650 kg (6.25 ml and 8188.89 mill. /ejacul, resp.). Scrotal circumference of the bulls significantly ($P<0.05$) affected all the semen traits. The highest volume of ejaculate was found in size of SC group <36 cm (9.08) and lowest for SC group <32 cm (6.01 ml).

In the Normade breed, production data are collected only from bulls selected after performance test on growth. Some authors have reported a negative genetic correlation between weight of the animal and sperm quality parameters in beef breeds as well as a positive correlation between weight and volume of ejaculate (Barber and Almquist, 1975; Kinights *et al.*, 1984). However, other authors found that growth traits and sperm characteristics one independent (Labesse, 1986; Guillin, 1989). In order to check if there is a possible relationship between these two groups of traits, a preliminary analysis including growth rate and

final weight as well as sperm parameters was performed.

2.11 Testicular Circumferences (TC) of Bull:

Determination of testicular circumference or scrotal circumference is an essential aspect of breeding soundness examination of bulls because of its significance as indicators of puberty, total sperm production, semen quality and pathological conditions of the testes (Ott, 1991). Testicular size of the sire lines is an important traits in the selection of males practically those used in AI progarmmes (Ying and Johnson, 1996). Scrotal circumference has also been found to be correlated with fertility of bulls (Mateose *et al.*, 1978). A relationship between the size of testes and the sperm motility and total spermatozoa per ejaculate of bulls (Coulter *et al.*, 1976; Neely *et al.*, 1982; Bruner *et al.*, 1995). In many studies testicular measurements have been evaluated and related to some seminal parameters, usually sperm number, sperm concentration and sperm motility (Aehnelt *et al.*, 1964; Gipson *et al.*, 1985; Hahn *et al.*, 1969; Rossouw 1975; Ruttle *et al.*, 1982). Many studies show that young bulls with small scrotal circumferences have significantly lower fertility than the bull with larger scrotal circumferences. Bull with small testicles was shown to have semen with a higher percentage of sperm defects (Aehnelt *et al.*, 1964). The average scrotal circumference was larger in the Friesian than Ayrsires but the difference was not significant (32.6 cm Vs 32.2 cm). According to Downey *et al.* (1984), the scrotal circumference is smaller in summer than in winter (worthern hemisspere) being negatively correlated with day length to a significant degree. The SC was positively correlated with fertility, which is in accordance with the results achieved by Aehnelt *et al.* (1964), Coulter and Foote (1979), Jakubiec (1983) and Ruttle *et al.* (1982). Scrotal circumference should be

measured to identify bulls with testicular hypoplasia. In this study no bulls with testicular hypoplasia.

Scrotal circumference and age at puberty of Guzerat and Nellore bulls averaged 25.6 +/- 2.2 cm at 18.0 +/- 2.0 mo and 23.6 +/- 0.2 cm at 18.5 +/- 2.7 mo, respectively. Nellore bulls were 42 kg lighter than Guzerat at puberty. The percentages of abnormal spermatozoa were higher ($P < 0.01$) at 13 to 15 mo of age in Guzerat (11.1%) and Nellore (14.4%) bulls than at 22 to 24 mo (6.7 and 8.0%, respectively).

Thompson *et al.* (1992) studied the accuracy of scrotal circumference as a predictor of specific pathologic changes within testicular parenchyma was assessed by scoring 121 Hereford and Simmental bulls for scrotal circumference and semen quality. Thirty-two centimeters was the minimal acceptable scrotal circumference to ensure both a low prevalence of tubules with irreversible loss of germinal epithelium and acceptable semen quality. Scrotal circumference had a positive predictive value of 0.07 and a specificity of 0.89 for 25% Grade4+ tubules. For acceptable semen morphology (greater than or equal to 75% morphologically normal spermatozoa), scrotal circumference had a positive predictive value of 0.08, a sensitivity of 0.06, and a specificity of 0.88. For acceptable sperm motility (greater than or equal to 30% progressively motile spermatozoa) scrotal circumference had a positive predictive value of 0.0, a sensitivity of 0.0, and a specificity of 0.87.

2.12 Libido and Mating Behaviour:

Libido has been identified as one of the main factors causing a variation in bull fertility. Therefore management factors that affect libido are of importance (Hoogenboezem & Swanepoel, 2000). It is important to

consider the fertility of the bull via libido and mating behaviour in the dairy herd as the quality of the semen samples collected may vary according to the collection method used (Dombo, 2002). The artificial vagina method is seen as the best method for semen collection as far as the quality of sample is concerned (Bearden & Fuquay, 1997). The use of this instrument is dependent on the ability of a bull to mate and it is therefore logical to say that libido and mating behaviour determine which method is to be used.

Van Denmark & Free (1970) reported that it is obvious that the effects of season on bull fertility are complex. Its effects may be influenced by temperature, humidity and in many cases by the nutritional status of the animal. Seasonal variation in the reproduction of cattle breeds is the direct consequences of photo- periodic control of the pituitary function. Male sex drive is often less intense under hot conditions and this is evidently where libido is affected by both photoperiod and environmental temperature (Bearden & Fuquay, 1997).

Over fed and fat males may become less willing and able to mate (Cupps, 1991). Energy and protein deficiency may lead to the suppression of the endocrine system and testicular function, coupled with a diminished libido (Mann & Lutwak-Mann, 1981). Reduction in libido is associated with the nutritional deficiency of vitamin A and E (Hurley & Doane, 1989), excessive molybdenum in the diet (Brown, 1984), iodine and cobalt (Cupps, 1991).

Male sexual activities are also affected by previous sexual experience (Bearden & Fuquany, 1997). Inexperienced males are often more awkward during their first interaction with a receptive female (Cupps,

1991). Bearden & Fuquay (1997) also reported that sexual exhaustion and sexual satiety may be among the common factors that tend to lower libido when bulls are maintained exclusively for the purpose of providing semen for AI.

2.13 Season:

Singh *et al.* (1968) reported that there was no significant effect by the season on volume, initial motility, sperm concentration and reaction time. They also stated that percentage of abnormal spermatozoa was significantly affected by ejaculation regime.

Igboeli and Rakha (1971) reported that there was no significant seasonal effect on the indigenous bulls semen where as exotic breed shown significant seasonal fluctuation. They also found that ejaculate volume was high in rainy season, sperm concentration in maximum in hot season and morphologically normal spermatozoa were highest in winter.

Samoilo (1974) indicated that the semen quality of Holstein Friesian bulls under temperate climatic conditions was better in summer followed by spring, autumn and winter.

Smirnov and Kruglyak (1980) stated that volume of ejaculation and number of sperm per ejaculation was significantly higher in summer than winter. They also reported that the motility of spermatozoa were higher on autumn and winter than summer.

Saxena and Tripathi (1981) tabulated data on sperm motility according to season and reported that season had a significant effect on the incidence of sperm abnormalities, which was higher in spring, summer and rainy season (4.46 to 8.00 percent) than in autumn and winter (2.67 to 2.92 percent).

Hardin *et al.* (1982) reported that the mean ejaculated volume were significantly higher during autumn (September to November) and winter (December to February) than spring (March to August) in Angus (7.8 and 7.6 Vs 6.6 and 6.8 ml, respectively) and Brahman (6.3 and 6.2 Vs 5.2 and 5.4ml, respectively) bulls. Mean sperm concentration ($\times 10^6$/ml) was significantly higher during summer and autumn than winter and spring in Angus (498 and 462 Vs 397 and 413/ml) bulls.

Sharma *et al.* (1982) studied that ejaculate volume average 3.67±0.04ml, initial sperm motility 80.61±0.48% and sperm concentration ($\times 10^6$/ml) 1274.18±14.76 were not significantly affected by season.

Ibrahim *et al.* (1983) in worked in 400 ejaculates per season were examined from 100 bulls aged 3-5 yrs. Season significantly affected ejaculate volume, mass sperm motility (P less than 0.05) and progressive motility. Ejaculate volume and motility were superior in spring, and sperm concentration and the number of spermatozoa per ejaculate in autumn, compared with the other seasons.

Djimde and Weniger (1984) studied 9000 ejaculates from the 296 bulls for finding effect of genetic groups and season on the semen quality at Central Cattle Breeding Station, Savar, Dhaka from 1974 to 1980. The climate at this location was divided into three groups, Season I (May to November), Season II (December to February) and Season III (March to April). The significantly largest amount of semen was collected in season III and motility was dependent on season and sperm concentration and mass motility were not dependent on season.

Tomar and Gupta (1984) studied 3 Hariana bulls aged 4-6 years in

different seasons. Season had no effect on reaction time (sex derive), semen volume, and percentage of abnormalities as well as live sperms. The initial motility and sperm concentration were both found to be significantly higher during summer season than the cold season. The initial motility and sperm concentration was higher in summer (4.3±0.2, 1193±52.2 million/ml, respectively) and lower in winter season (3.8±0.2 and 822.7±39.9 million/ml, respectively). The reaction time (sex drive) had no association with semen volume and sperm concentration.

Gavthier and Varo (1985) indicated that season had no significant effect on volume and initial motility of semen from cross-bred bulls, but sperm concentration was affected significantly by season. They also reported that the percentage of abnormal spermatozoa was greater ($P<0.01$) in the September than in April (17 vs. 6).

Hussain *et al.* (1985) studied on 5 crossed bulls belonging to 2 genetic groups (3 Local× Friesian and 2 Sahiwal×Friesian) in three different seasons of the year. They observed that no significant seasonal effect ($P>0.05$) on volume and initial motility of the summer.

However, season had a significant effect ($P<0.05$) on sperm concentration. The highest concentration was found in winter and the lowest in summer for both genetic groups.

Tomar *et al.* (1985) observed that season had a significant effect on the mass motility score (lowest during November to February) and on sperm concentration (lowest during March to June).

Saxena and Tripathi (1986) observed 59 ejaculates collected over a 1-year period from 204 Danish Red bulls. In the rainy, autumn, winter, spring

and summer seasons semen volume ranged from 2.8 to 4.31ml, progressive sperm motility (scale 0-5) from 3.63 to 4.25, sperm motility from 70.31 percent to 77.50 percent, sperm concentration ($\times 10^6$/ml) from 650 to 1310 number of spermatozoa per ejaculate ($\times 10^6$/ml) from 2585 to 3352. The effect of season were significant for progressive motility and for the percentage of sperm head abnormalities.

Muhuyi (1991) investigated that factor affecting the quality of ejaculates collected from 39 bulls in 1983-1986. Year had a significant effect on ejaculate volume and there were significant year ×season effects on sperm concentration and post dilution sperm motility ($P<0.05$). Post thawing sperm motility was affected by season (higher in rainy than dry season $P<0.05$). There was no significant age of bull, year× age or season × age effects on any of the traits investigated.

Sarder (2001a) evaluated in 277 semen samples was collected from 6 Holstein Friesian cross and one Sahiwal bull of different season viz. spring, summer, autumn and winter at Dairy and Cattle Improvement Farm, Rajabarihat, Rajshahi. Semen parameters affect significantly ($P<0.05$) with individual AI bulls. The mean volume of ejaculate, progressive movement, sperm concentration, total number of spermatozoa/ ejaculate and total number of motile spermatozoa /ejaculate were varied from 4.16 to9.38ml, 2.48 to 3.00 (scale=1-4), 1000.41 to 1677.78 million / ml, 56.14 to 65.34%, 6037.07 to 1065.89 million and 3664.75 to 6917.75 million respectively depending of AI bulls. Similarly, season significantly ($P<0.05$) influenced the volume and the sperm concentration but did not significantly affect progressive movement, total number of spermatozoa/ejaculate and total number of motile spermatozoa/ ejaculate. The volume of ejaculate was highest ($P<0.05$) in

winter season (8.34ml) and lowest in summer season (7.02ml).

2.14 Artificial Vagina:

The artificial vagina is designed to simulate the vaginal orifice of the female. Bearden & Fuquay (1997) reported that artificial vagina is the fastest and the most sanitary method and it provides a good imitation of the natural vagina. Maule (1962) reported that most AI Stations prefer the use of short pattern artificial vagina to ensure that the semen is ejaculated directly into the cone or collecting tube and not in the liner. King (1993) found that in the use of this technique, the length of vagina should be chosen so that the bull will ejaculate directly into the tube to avoid semen loss. It was further indicated that if more than one ejaculation is needed, more than one vagina should be used. Ran & Haranat (1984) reported the importance of the size of artificial vagina used. It was indicated that the use of smaller artificial vagina in bulls resulted in significantly higher mean seminal plasma volume and also higher sperm output.

2.15 Semen Processing for Preservation:

The two major functions of semen processing are to preserve the fertilizing capacity of spermatozoa and to increase the volume of ejaculate so that maximum utilization of the bulls genetic merit can be ensured by properly dosing the number of spermatozoa in a volume that can be conveniently packaged and used for insemination. During processing of semen for AI and/or evaluation of the quality, it is important not to expose semen to excessive light, direct sunshine, fumes from volatile chemicals or disinfectants (Hunter, 1985). Bull semen needs to be diluted with the preserving medium soon after collection to provided with necessary ingredients before reserved energy in the

spermatozoa is exhausted. Both semen and extender should be at the same temperature during mixing. For liquid semen, the usual mixing temperature is +30°C; however, glycerol should be added at +4°C during processing of semen for freezing (Polge, 1953; Foote, 1978a).

2.16 Semen Extender/Diluents:

The basic ingredients of an extender for processing bull semen are 1) water that works as solvent, 2) dissolved ionic and nonionic substances to maintain osmolality and to buffer the pH of the medium 3) organic materials with the capacity to prevent cold shock (usually egg yolk or milk), 4) cryoprotectant (for freezing semen) for instance, glycerol, 5) simple sugar as the source of energy and 6) antibiotics to prevent microbial growth (Pickett and Berndtson, 1978). It is important to mention that the basic buffer used to prepare the extender must be isotonic with respect to the seminal plasma (Martin, 1963; Steinbach and Foote, 1967; Yassen and Foote, 1967). In the case of any osmotic insult, the frequency of spermatozoa with bent or coiled tail increases in the preserved semen (Sullivan, 1978). In the fifties and sixties, to preserve bull semen at chilling temperature, extensive researches of good quality were carried out in search for a suitable extender (Foote and Bratton, 1960; Rottensten, 1961). Data clearly indicated the favorable effect of citrate as buffer compared with others on the fertilizing capacity of the preserved spermatozoa (Foote and Bratton, 1960; Rottensten, 1961); this can be due to chealating function of citrate on heavy metal contaminants (Foote, 1978a). Since the first use of artificial insemination, penicillin and streptomycin have been unequivocally added as antibacterial agents in semen extender simply because of their harmless effect on spermatozoa (Salisbury and Van Demark 1961). The widely used doses of penicillin

and streptomycin are 55-1000UI/ml and 0.5-1.0 mg/ml, respectively (Foote, 1978a).

2.17 Preservation, Storage and Transportation of Semen:

The extended semen can be preserved either as liquid or as frozen. The semen package should be kept out of direct sunlight during storage and transportation (Paufler and Foote, 1967). The liquid semen is almost during storage at +4 to 5°C. This temperature can be maintained fairly with ice in thermo flask or in refrigerator and has been satisfactory to maintain good quality semen for up to 3 days provided the ice in the thermaoflask or power supply to the refrigerator is consistent. When liquid semen is stored at +4°C to 7°C, the spermatozoa can continue metabolic activities at a reduced rate resulting in exhaustion of reserved energy and the fertilizing capacity of spermatozoa begins to decrease after 2 days of preservation (Salisbury and Flerchinger, 1967; Shamsuddin *et al.*, 1987). During shipment of liquid semen stored at +4°C to 5°C, ice-containing thermo flasks are good containers; provide the ice is consistently present. Even after collecting a good quality ejaculate, sperm morphology can be affected by improper processing of semen during dilution from preservation (Sullivan, 1978).

Singh and Mishra (1975) tabulated to observe in effect of bulls, season, and hours of preservation and mode of transport on livability of bull spermatozoa from 6 Tharparkar bulls. The average live sperm percentage till 96th hour of preservation in summer was 68.2 and in rainy 70.9. Bull, collections, season, mode of transport and many interactions were found to be having highly significant influence on the live sperm percentage.

Bhuiyan and Shamsuddin (1999) investigated that the effects on dilution, transportation and preservation period on the quality of chilled semen used for Artificial Insemination (AI) 5 crossed bulls of District AI Centre, Mymensigh, Bangladesh were included in two experiments during January to December, 1996. In experiment I, the phase contrast microscopy of formol saline fixed semen revealed that the proportion of spermatozoa with normal acrosome, mid-piece and tail was significantly ($P<0.01$) lower (73.4%) in diluted semen than that fresh one (82.2%). The data indicated the detrimental effects of dilution of semen for AI on sperm morphology.

2.18 Motility of Spermatozoa:

Visual estimation of the percentage of motile spermatozoa is the most commonly used technique of semen evaluation. There is a no single test available by which the fertility of bull of semen can be accurately predicted (Garrett and Baker, 1995). Computer aided methods have been introduced to overcome human bias and thereby to obtain more precise, objective measurement of sperm motility than visual observation (Amann, 1989; Coulter, 1992). The use of commercial luminometer for the determination of ATP content and fluorescence microscopy helped estimation of viable spermatozoa in frozen-thawed semen (Januskauskas *et al.* 1996). Normal motility of bull spermatozoa is characterized by forward progression caused by tail lashings. Optimum motility occurs at about body temperature motility ceases completely at about +7°C (Sullivan, 1978). Kjaestad *et al.* (1993) found significant correlation with post-thaw motility and NRs. The authors suggested that post-thaw motility was good predictor of freezability and fertilizing potential of bovine semen. However, sperm motility may not always correlate with

fertility (Lindford *et al.*, 1976; Elliott, 1978; Soderquist, 1991) simply because all motile spermatozoa may not be morphologically normal (Shamsuddin and Rodriguez-Martinez, 1994).

Spermatozoa obtain motility from the contractile elements located in the longitudinal fibres of a tail through the process of spermatozoa metabolism. Spermatozoa metabolism is defined as a process by which spermatozoa convert nutrients into usable forms of energy (Mamabolo, 1999). The enzymes for this conversion are situated in the mitochoridrial sheath. These principal energy substrates are fructose, sorbitol and GPC (Table 2.1) which are found in seminal plasma and a plasmologem (a lipid found within the spermatozoon as an energy reserve) that can be used when other substrates are limited (Bearden & Fuquay, 1997).

2.19 Morphology of Spermatozoa:

The morphology of spermatozoa is used as one of the important criteria in the evaluation of semen quality in domestic animals (Howard *et al.*, 1983). The fully formed spermatozoa are elongated cells consisting of a head containing DNA and a tail which provide the cell with motility (Mamabolo, 1999).

Sperm morphology is often used to assess the fertilizing capacity of spermatozoa in semen. The proportion of spermatozoa with abnormal morphology in semen of healthy bulls is usually low. Elliott (1978) recommended not tolerating more than 20% abnormalities in sperm head and / or mid-piece in bull semen to be used in routine AI. Abnormalities of bovine spermatozoa may be either compensable, those that are not able to participate in fertilization or uncompensable, those that are able to initiate but not complete fertilization or sustain embryo (Saacke, 1990;

Saacke *et al.*, 1991). The morphology of spermatozoa in individual bulls semen vary between ejaculates, season of collections and batches of frozen semen. Irrespective of causes, morphologically abnormal spermatozoa fail to fertilize the oocytes (Shamsuddin and Rodriguez-Martinez, 1994). However, at routine AI, the reduced fertility due to compensable sperm abnormalities can be manipulated by increasing the number of spermatozoa in an insemination dose and thereby to raise the total number of functionally normal spermatozoa (Saacke *et al.*, 1991). The frequency of abnormal spermatozoa in insemination dose was negatively correlated with the fertility of semen (Sullivan, 1978; Rao and Rao, 1979; Soderquist, 1991). Accordingly, Williams and Savage (1925) found impaired fertility of bulls with 18% or more abnormal sperm cells in the semen. Therefore, it seems rationale to evaluate the sperm morphology to determine the quality of semen to be inseminated.

2.20 Examination on Morphological Abnormalities of Spermatozoa:

For successful AI programme the importance of seminal characteristics needs to be considered. One of the most important of these has been well established, as having a high influence on fertility, is the percentage of spermatozoa with abnormal morphology. Many investigators (Saacke, 1970; Rao, 1971) observed the incidence of spermatozoa with abnormal morphology in fertile bulls to be 1018% where as Zemjanis (1970) reported the incidence of total spermatozoon abnormalities in the semen of fertile bulls to be 30-40%. Saacke (1970) reported that certain sperm morphological abnormalities had detrimental effects on the fertility of some bulls. Many types of morphological sperm cells can be observed readily (Barth and Okorj, 1989) and some forms, when present in a high proportion in semen, are associated with low fertility (Barth, 1989, Miller

et al., 1982; Pilip, 1996; Thundathil, 1998). A high incidence are abnormal spermatozoa produced by a bull appears to be associated with disease or stress (Coulter, 1976, 1978 and Thundathil, 1998).

Hancock (1959) reported there is a small but significant ($P<0.05$) direct relationship between the frequency of malformed heads and conception rate. He was also reported a significant ($P<0.05$) inverse correlation between the frequencies of cytoplasmic droplets and fertility. Swanson and Boyd (1962) indicated from studies of sperm morphology in bulls of varying fertility that high percentage of abnormal tails and mid-pieces cause reduced fertility and sterility. They also found that frequent service might reduce the percentage of coil tail and improve semen quality.

Cupps and Briggs (1965) found spermatozoa with looped or bent tails together with changes in the epididymal epithelium, after treatment with oestradiol. The coiling has been found to arise after mixing the spermatozoa with glycerol used as cryoperservatives.

Dott and Dingle (1968) shown that the cytoplamic droplets of bull spermatozoa containing many enzymes characteristics for lylosomes and that the free cytoplasmic droplet remain morphologically intact under physiological conditions.

Flies and Lutezyk (1973) indicated some common sperm defect i.e. faulty tails, head abnormalities, absences of tails, and the percentage of defective spermatozoa and highest percentage of abnormalities recorded here in Summer and lowest in December. They also found the effect of nutrition on the incidence of sperm abnormalities.

Mohana and Ramamohana (1975) observed 178 ejaculates from

Tharpakar and 212 ejaculates from Jersey bulls aged about 4-5 years were collected to study the semen characteristics over a period of one year. The mean values of different semen characteristics obtained in this study were; volume 3.4 and 4.02ml; motility 81.62 and 79.20 percent; concentration of spermatozoa 1332 and 1296 million per ml; head abnormalities 10.58 and 11.74 percent; loose head 2.11 and 3.75 per cent. mid-piece abnormalities 1.15 and 1.20 percent ; tail abnormalities 2.38 and 15.47 percent; proximal cytoplasmic droplet 1.50 and 2.20 percent for the Tharparkar and Jersey breeds, respectively. The variations in the characteristics of semen except volume, concentration and mid-piece abnormalities, between the Tharparkar and Jersey breeds were found to be statistically significant ($P<0.01$). The quality of the semen obtained from Jersey bulls during summer months was comparatively poorer than that of Tharparkar breed. However, the abnormalities were within the normal range prescribed for fertile bulls in both the breeds. The deterioration of semen quality during summer months was characteristics by the increased incidence of proximal protoplasmic droplet, loose head and reduction of live sperm count.

Bloom (1978) reported that head abnormalities, proximal cytoplasomic droplets, coiled tail, abaxial attachment of the mid-piece and corck screw sperm defects were accompanished by testicular degeneration caused impaired fertility. The author also stated that semen containing vary low sperm concentration exhibits an increased number of spermatozoa.

Wenkoff (1978) stated that the defect, which involves coiling, folding and splitting of sperm mid-piece, are found to have an epididymal origin.

Bujarbaruah *et al.* (1982) had under taken study on three Jersey and 2

Red Dane bulls of 60 weeks of age. The motility of spermatozoa, percentage live spermatozoa, head abnormalities and tail abnormalities were found significant differences between the Jersey and Red Dane breed bulls. The number of spermatozoa with head, mid-piece and tail abnormalities decreased significantly ($P<0.01$) with advancing age of bulls. The mean head, mid-piece and tail abnormalities were found in Jersey and Red Dane respectively (4.26±0.20 vs. 5.13±0.02, 5.06±0.12 vs. 6.26±0.17, and 7.29±0.20 vs. 6.66±0.14, respectively).

Raju and Rao (1983) studied in 405 ejaculate from 7 Brown-Swiss cross-bred bulls aged between 24 and 40 month for 1 year to evaluated the semen characteristics. The mean values of different semen abnormalities were sperm head abnormalities 3.43±0.300%, free loose head 4.91±0.439%, mid-piece abnormalities 0.65±0.45%, tail abnormalities 6.47±0.775%, proximal protoplasmic droplets 1.20±0.148%. Significant variation ($P<0.05$) was observed between bulls in respect of head abnormalities, free loose head, mid-piece abnormalities, tail abnormalities and proximal protoplasmic droplets. Seasonal effect, however, was evident only in the incidence of tail abnormality ($P<0.05$).

Orji *et al.* (1984) reported that in dry season (December to March) collection of semen showed rather low motility, poor sperm concentration and high percentage of morphologically abnormal spermatozoa.

Saxena and Tripathi (1984) studied 83 ejaculate collected from 4 bulls over a 12 months studied 83 ejaculates collected from 4 bulls over a 12 months period. There were no significant difference between seasons in number of live spermatozoa averaged 86.64%, 85.00%, 83.75%, 90.50% and 85.05% in spring, summer, the rainy season, autumn and winter

respectively and the proportion of abnormal spermatozoa was 13.27%, 13.13%, 13.00%, 8.56% and 7.15% (both P<0.01).

Tomar and Gupta (1984) have been reported that percentage of abnormal sperm range from 9.8±0.6 to 10.8±0.5 in different seasons.

Rao and Bane (1985) investigated to study the incidence of sperm abnormalities from 19 infertile bulls with varying degree of testicular degeneration. A direct relationship existed between the incidence of sperm abnormalities and testicular pathology. The incidence of abnormal sperm heads and proximal cytoplasmic droplets was considerably higher in bulls that suffered from testicular degeneration. No direct relationship between the incidence of abnormal sperm tails and the degree of pathological changes in the testis could be noticed.

Barth (1989) reported that the prevalence of abaxial tails, accessory tails and double tail from 600 Western Canadian bulls and 449 bulls in Canadian artificial insemination centre. Spermatozoa with abaxial tails were produced by 10.5% of bulls; however only 0.48% produced sperm abaxial tail.

Luthra and Marinoy (1995) tabulated to observe the incidence of sperm abnormalities during the different seasons from 10 Holstein Friesian bulls in both fresh and frozen semen. The percentage of sperm abnormalities in fresh and frozen semen was 12.50 vs. 17.22 for head, 0.69 vs 2.05 for mid-piece and 5.72 vs. 8.24 for tail. There was a 9% increased in the percent of total sperm abnormalities in frozen semen (27.4%) in comparison to the fresh semen (18.91%). The abnormalities of head and mid-piece in fresh semen were highest in autumn, and lowest in spring. The total abnormalities in thawed semen were highest 35.5% in summer

and lowest 25.93% in spring. The autumn and winter had 32.7 and 31.97 of total sperm abnormalities respectively.

Younis *et al.* (1998) investigated in semen quality during the low (May-July) and the peak (September-November) breeding seasons from 18 Nili-Ravi buffalo bulls varying in age from 3 to 15 years. Ejaculates collected from old bulls (14.29 plus-minus 1.03%) had higher ($P<0.05$) number of abnormal spermatozoa than those from young (13.02 plus-minus 0.85%) and adult (12.22 plus-minus 0.58%) bulls and in the low (13.74 plus-minus 0.61%) than the peak (1.94 plus-minus 1.61%) breeding season.

2.21 Methods Used for Semen Fertility:

Many methods of semen evaluation have been proposed for predicting the bull fertility and assess the techniques employed during processing and freezing /thawing of bull semen in AI practice (Saacke *et al.*, 1991).

A detailed assessment of sperm morphology in the domestic animals can be studied as the percentage of normal sperm cells and the occurrence of major and minor sperm defects (Van Rensburg, 1957; Van Denmark & Free, 1970 Serrenson, 1979). Spermatozoa defects are classified as major and minor defects (Table 23). Major defects are related to impaired fertility of abnormal conditions in the tests. Minor defects should be of concern only when they exceed 10% (Van Rensburg, 1957; Van Dark and Free, 1970; Bearden & Fuquay, 1997). Salisbury *et al.,* (1978) and Van Rensburg (1957) reported the following morphological abnormalities to be important in the reproduction of a bull (Table 2.3).

1. **Abnormal acrosomes:** e.g. kriobbed acrosomes, which are characterised by localised swellings on the apical ridge; ruffled acrosomes, which are characterised by a wrinkled appearance.
2. **Abnormal nucleus:** It appears as a dark necklace along anterior edge of the posterior nuclear cap and indicates abnormal spermiogenesis.
3. **Tailless sperm (Disintegrated or Decapitated sperm):** This entails when the tail separates within the caput epididymis. This defect is hereditary in some of the breed such as Guernsey. The appearances of tailless sperm in the ejaculate are the early indications of testicular degeneration as a result of exposure to heat stress and severe under-nutrition.

Table 2.3 Classification of sperm morphology according to major and minor defects in bulls (Salisbury *et al.*, 1978).

Major defects	Minor defects
1. Underdeveloped	14. Psuedodroplests
2. Double forms	15. Dag defects
3. Knobbed sperm defect	16. Narrow heads
4. Decapitated sperm	17. Small, normal heads
5. Diadem defects	18. Giants heads and short broad heads
6. Pear shaped head	19. Free heads (normal)
7. Narrow at the base	20. Detached acrosomal cap
8. Abnormal contour	21. Abaxial implantation
9. Small abnormal heads	22. Distal droplets
10. Free abnormal heads	23. Simply bent or coiled tail
11. Corkscrew defect	24. Terminally coiled tail
12. Other mid piece defects	
13. Proximal droplets	

4. **Abnormal mid piece:** This is where the mitochondrion helix is absent and the axial fibres are frayed at the distal portion of the mid-piece. Another type of mid piece defect may be termed "psuedodroplets", which is a major sperm defect in the semen of Friesland bulls. This defect is located near the centre of the mid-piece and appears as a rounded or elongated thickening that contains dense granules surrounded by mitochondria.

5. **Bent and coiled tails:** This may be the most common aberration in the ejaculated semen of the bull and could be associated with reduced fertility.

6. **Cytoplasmic droplets:** This is usually an indication of incomplete maturation in the epididymis (Hunter, 1980).

7. Van Rensburg (1957) also reported semen abnormalities such as loose heads, acrosome abnormalities, abnormal shape of the head, mid-piece abnormalities, cytplasmic droplets, coiled tails, deformity and medusa forms.

2.22 Evaluation of Semen Fertility (Non-return Rate and Conception Rate) of AI Bulls:

Almquist (1954) studied semen from 12 Guernsey and Holstein bull; six of relatively high fertility and sex of relatively low fertility was used to inseminate 6670 first service cows. The 60-to 90- days non-return rate average 64.1% for yolk citrate-penicillinstreptomycin dilute, 66.2% for heated homogenized milk diluter and 72.1% for heated milk diluter containing penicillin and streptomycin. Experiment-2 involved 240 semen samples from 30 Guernsey and Holstein bulls, which were used to inseminate 8400 first service cows. The average non-return rate was

63.5% for egg yolk citrate-penicillin streptomycin.

Almquist *et al.* (1954) studied 105 semen samples from 7 Holstein bulls of high fertility were used to inseminated 4689 first service cows. They reported that average NR rate were 66.1% for Holstein bull and 69.7% for Guernsey bull in the diluent of egg yolk citrate and NRR 73.2% for Day 1 semen and 76.4% for Day 2 in diluent of egg-yolk citrate.

Jenichen and Baum (1962) studied that the N.R rate to 13076 inseminations with 295 ejaculates obtained at different intervals from 5 groups each of 4 bulls was investigated; for a single ejaculate it was 60.6% at a 3 days interval, 67.5% at a 6 days intervals, 64.6% at an intervals of more than 6 days and 65.0% at irregular intervals: for 2 ejaculates per day at a 6 days intervals it was 67.1%. The difference between groups was not considered significant.

Van Snick *et al.* (1962) reported in 356011 cows inseminated from 207 bulls their age appox. 3 yrs. in Belgium, i.e. 26.7% of the total cows population; the 90-day N.R rate was 68.1%, East Flemish Red Pied having the poorest (66.0%) and Herve the best (69.9%).

Adler (1963) reported that the 60- to -90 day N.R rate to 1352 first inseminations with frozen semen was 53.1% vs. 65.7% to 426 first inseminations with the fresh half of the same ejaculates with fresh semen obtained from the same bulls during the period that the deep-frozen semen was being used.

Kelly and Hurst (1963) showed that equal effect of temperature on both the bulls and cows for reduced conception rate in summer. These correlations indicate that as temperature and humidity reduces fertility.

Ozkoca (1963b) studied with deep frozen semen in Turkey 102 out of 277 cows conceived. The conception rates attained by 6 technicians varied from 0 to 69.5%. This variation was attributed to lack of care on the part of some technicians and to the fact that as the study was carried out at the end of the breeding season some of the cows were repeat breeders.

Krishnan Nair (1975) was made in 87224 cows inseminated with Deep frozen semen from Brown Swiss bull during the period from 1970-1973. The mean conception rates varied from 38.88 to 41.48%. The mean number of AI with deep frozen semen required per conception varied from 2.41 to 2.57.

Tomar (1981) reported in 194 Hariana animals were inseminated with deep frozen semen (DJS) of 8 Holstein-Friesian bulls imported from USA, which were preserved in liquid nitrogen at -196°C. The conception rate averaged 61.34% with non-significant variation among bulls and about 3.4 inseminations required in one crossbred calf.

Papa (1982) analyzed on 7297 first inseminations with frozen semen thawed at 40°C and 8441 inseminations with semen thawed at 40° or 70°. Thawing at 70° resulted in 10% higher sperm motility and 30% more spermatozoa with undamaged acrosomes than thawing at 40°, both for cold-shocked and non-shocked semen. Prolonging the thawing time at 40°C from 10 to 30 s and that at 70°C from 10 to 15 s had no significant effect on semen characters. The 30 to 60 day NR rate to inseminations with semen thawed at 70°C was 77.09% vs. 76.32% for semen thawed at 40°C.

Raja and Rao (1983) observed that 7586 insemination data obtained from Cross breeding Research Station, Karala for calculating the fertility rate.

The inseminations were done in local cows with the extended frozen semen of Brown-Swiss cross-bred bull with 62.5% exotic inheritance. The overall fertility rate of Brown-Swiss cross-bred bull with 62.5% exotic inheritances was noted to be 56.06%. There was significant difference is the fertility between months/seasons of insemination. The highest conception rate was noticed during summer (58.59%) followed by winter (55.55%) and rainy (55.00%) in that order. The fertility rate was found to improve with advancing age.

Vivanco *et al.* (1983) investigated on 9447 inseminations of 3238 Holstein-Friesian cows, carried out over a 4-5 yr. There were highly significant differences in CR between herds, between cows and heifers and between imported and locally produced semen used on heifers but not on cows. When imported semen was used, the CR was 61.98% and 1.61 services were required per conception vs. 64.94% and 1.53 for local semen.

Bamualim *et al.* (1984) observed that significant differences were found between the bull in pregnancy rate of cows and no difference in bull age on the pregnancy rate.

Woods *et al.* (1986) studied to identify the relationship between semen characteristics and fertility in AI following 15320 inseminations. The mean 49-day non-return of the 242 in first ejaculate used in the field was 63.1±1.1% and no significant trends in semen quality on non-return rate.

Graham and Graham (1990) reported that A Sephadex G-15 filtration method was developed to remove abnormal and non-motile bull spermatozoa from entire ejaculates. Filtering increased motile spermatozoa from 51 to 57% and form 36 to 50% for the high-and low-

fertility bull respectively. The 60-90 day NR rate for filtered and unfiltered semen was 72 and 73% resp. for high-fertility bulls and 67 and 61% for low fertility bulls.

Humblot *et al.* (1991) observed the return rate (RR) in 856850 first inseminations by using 56 technicians from 103 bulls of 5 breeds for 1976-81. They were found (1) bull within breed and AI technicians has the highest influence on RR (2) year and hard size had less effect on RR than bull (3) results were similar for 24 day and 90 day RR and (4) the breed of bull and AI technicians had non-significant effect on 25 to 300 day RR.

Anderson *et al.* (1992) studied on comparisons between Ayrshire and Friesian bulls accepted for AI with regard to non-return rate. They obtained NR% of Ayrsire (n=286) was 64. ±3.7 and Friesian (n=80) was 68.2±3.2. They were found the SC classified into 4 groups got following results. Scrotal circumference were <29, 29-30, 31-33 and >33 cm having the NR 61.7±3.8, 63.9±4.3, 64.0±4.0 and 64.7±2.2, respectively. They stated that Friesian was shown to have significantly higher fertility (NR rate) than the Ayrshire.

Mahbub-E-Elahi *et al.* (1997) observed that a total 680 cows inseminated in different seasons such as winter, spring, summer and monsoon with the frozen semen of Sahiwal bull imported from New Zealand by cryocan. The non-return rate was found to be significantly high in winter (89.47%) and low in monsoon (86.47%).

CHAPTER 3

MATERIALS AND METHODS

The present study has been dealing with the semen production and semen quality of AI Bulls with various factors in relation to conception rate (CR) and non-return rate (NRR). The study was conducted in two Artificial Insemination (AI) centres /stations of greater Rajshahi district during the period from July, 2011 to June, 2013. A total 780 ejaculate collected from the District Artificial Insemination Centre (DAIC), Rajshahi and Rajshahi Dairy and Cattle Improvement Farm (RDCIF), Rajabarihat, Rajshahi.

The basic materials for the present study comprised sixteen (16) bulls belonging to six (6) genetic groups. Out of 16 bulls, 11 from RDCIF, Rajabarihat, Rajshahi and 5 bulls from DAIC, Rajshahi.

The methods used in study have been described under the following separate head:

3.1 Procedure of Selection of AI Bulls:

Selection of AI bulls was based on the calculation of heritability, dam's performance and individual performance. The following information was considered for final selection of a bull to be used for artificial insemination (AI).

3.1.1 Dam's performance:

Lactation of dam and grand mother, type and components of dam and sire, type and components of grand father, disease resistance of sire and

dam, age at first calving of dam, calving interval of dam, lactation period of dam, age at maturity of dam etc.

3.1.2 Individual performance of bull:

i) **Type and components**- Stature, head, front end, back, rump, hind leg, feet etc were examined thoroughly and selected the bulls which were satisfactory criteria.

ii) **Andrological condition of Bull**: The following points should considered-

1. **Prepuce**- Orifice, discharge of orifice, skin and subcutaneous tissue, swelling and prepuce length were examined.
2. **Penis**- Displaceability, swelling, male formation, neoplasia, haemorrhage, bending etc were examined.
3. **Scrotum**- freedom of movement, against each other, hair and skin of scrotum, colour, smell, temperature, swelling and infection etc. were examined.
4. **Testes**-Size, volume, shape, consistency, position and symmetrical.
5. **Epididymis**- Consistency, head, body and tail of epididymis.
6. **Spermatic cord**- Size, symmetry, consistency and displaceability.
7. **Accessory glands**- Size, shape, consistency of seminal vesicle by rectal palpation.

iii) **Health status**: Pulse rate- 60-70 /minute, respiration-15-35/ minute, body temperature-38.5°C, state of appetite-good, Rumination- 4/minute, growth rate, body weight, height etc, good temperament, posture-halting, walking and resting

iv) **Disease:** Resistance must be free from following diseases: T.B, Brucellosis, Trichomoniasis, Vibriosis, IBR/IPV and Leptopirosis.

3.1.3 Semen quality:

Libido or mating behavior- Sex desire, acceptance to A.V and thrusting of penis into A. V.

Volume of semen-	Average 4-10 ml
Color of semen-	Creamy
Density of semen-	Average 500 million /ml
Resistance of spermatozoa -	Average 48 hours and 50% motility
Motility of spermatozoa-	60-70%
Intensity of forward movement-	Good (+++)
Abnormal sperm cells -	<20%
Pathogenic sperm cell-	Nil
Preservating sperm cells -	Livability minimum 3 days for fresh semen.

The bulls were finally selected for AI programme meeting the above mentioned criteria. All the bulls were produced from CCBSDF, Savar and distributed to RDCIF, Rajabarihat, Rajshahi and DAIC, Rajshahi on the basis of indent

3.2 Factors Included in the Analysis:

The necessary data on semen production and semen characters in relation to conception and NRR of some AI bulls were analysed to see the effect of following factors. The bull factors were as individual bull, their breed, age, body weight, body condition, testicular circumference, libido, season and different AI stations where semen collection and processing.

3.2.1 Individual bull:

The present study was directly involved a total 16 bulls which were randomly used for AI programme in different AI centres/stations.

3.2.2 Breeds of bull:

Different types of breeds of bulls used for AI programme in Bangladesh (Fig. 3.1). The experimental AI bulls were divided into 6 (six) groups according to their genetic composition. Group-1 = L×F×SL×F×F, Group-2 =SL×F, Group-3 =L×F×F, Group-4 =L×F, Group-5 = $(L\times F)_2$, Group-6=L×F(L×F×F).

Fig. 3.1. Different types of Breeds of bull used for artificial insemination (AI) programme in Bangladesh.
A = L×F×F, B = L×F×SL×F×F, C = SL×F, D = $(L\times F)_2$,
E = L×F (L×F×F), F = L×F

3.2.3 Age of bulls:

These bulls were classified into five (5) groups on the basis of age categories. Group-A = <4 years, Group-B = 4 to<6 years, Group-C = 6 to<8 years, Group-D = 8 to <10years and Group-E = >10 years.

3.2.4 Body weight of bulls:

The body weight of bulls were divided into Three (3) groups such as Group I = <500 kg, Group II = 500 to<600 kg, Group III = >650 kg.

3.2.5 Body condition of bulls:

Among five scale (Nicholson and Butterworth, 1986) with slight modification the body condition of the bulls was scored into 1 to 3 scale; (average, good, very good).

3.2.6 Testicular circumference (TC) of bulls:

The scrotal circumference of the bulls were also separated into four (4) groups (Fig. 3.2) as follows 1st group = < 34 cm, 2nd group = 34 to <36 cm, 3rd group = 36 to <38 cm and 4th group = > 38 cm.

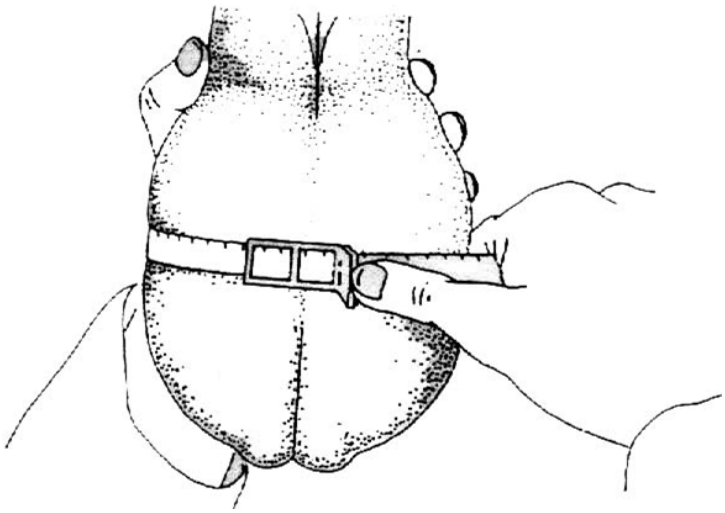

Fig. 3.2 Correct methods for measurement of Testicular Circumference (TC).

3.2.7 Libido of bulls:

The libido of each bull was recorded at the time of semen collection by assigning a numerical value as described by Singh and Pangawkar (1989), with slight modifications. A libido score was achieved by 1-3 scales (poor, good, very good).

3.2.8 Season:

Season of the whole year was divided into four seasons. Such as- 1) Spring season – from February to April. 2) Summer season - from May to July and 3) Autumn season- from August to October and 4) Winter season- from November to January.

3.2.9 AI Centres /Stations:

Two AI centres/stations such as District Artificial Insemination Centre (DAIC), Rajshahi and Deep frozen semen production laboratory and Rajshahi Dairy and Catttle Improvement Farm (RDCIF), Rajabarihat, Rajshahi were included in this study.

A total of 780 ejaculates (58 from L×F×SL×F×F, 76 from SL×F, 66 from L×F×F, 159 from L×F, 385 from $(L\times F)_2$, 36 from L×F(L×F×F) was obtained over a period of June, 2011 to July, 2013 from 16 AI bulls in two AI centres to study the various factors affecting semen production and semen quality of some AI bulls.

3.3 Preparation of Bulls before Semen Collection:

Semen collection was made early in the morning at 5 AM to 8.00AM due to avoid the thermal stress during hot seasons. The bulls were brought in to the semen collection hall for washing, cleaning and grooming at all the AI centres. Bulls were wiped with sanitary towel. Time was allowed to get the bulls dried from wet. Washing, cleaning and drying of the bulls

were done in the places where they were housed in all the AI centres. It was also hygienic to clean the hind quarters of the teasing animals by using antiseptic or detergent lotion.

3.4 Preparation of the Artificial Vagina (AV):

The artificial vagina should be the appropriate length for the bulls penis. It consist of a firm cylindrical tube with a thin-walled rubber inner Liner. Augmented portion of the inner liner at one end of AV holds a graduated semen collection glass vial. The graduated collecting tube was separated from the cone, covered with a plastic cap and labeled. After collection, semen samples were kept at 35°C in water - bath until the media and reagents were added to the samples. These procedures were all done at 35°C. Semen was collected from bulls generally twice in a week of all the AI centres. One every day one ejaculate was taken for schedule collection date of a bull (Fig. 3.3).

Fig. 3.3 Breeding bulls of RDCIF used for artificial insemination (AI) in Bangladesh.

3.5 Collection of Semen:

The bulls were trained to ejaculate in artificial vagina (AV) at homosexual mount using at two AI centres (Fig. 3.4 & 3.5). Briefly, the AV was assembled, warm water with a temperature of 45° to 50°C was poured through the filling aperture into the space between the rubber cylinder and liner. All the AI centres used a modified device of AV where the use of additional air pressure was avoided. The penis end of AV was lubricated with non spermicidal gel (# X B640, L'Aigle Cedex, France) and other end of the AV, a plastic cone with a calibrated collecting plastic tube was fixed. Immediately before collection the internal temperature of the AV was ensured to be +39°C to 45°C. Before collection the prepuce of the male was wiped clean to prevent semen contamination. The teaser bull was secured in a collection bail and his back-side was cleaned. The bulls were allowed usually at least 1 or 2 false mount. The semen collector took a crouching position at the right side of the teaser, and held the AV in the right hand along its flank, and with the open end facing towards the male and down wards at an angle of 45°. When the male mounted, the erected penis was directed into the open end of the AV to permit a vigorous upward and forward thrust which signifies that ejaculation has occurred, and male was allowed to withdraw his penis immediately after ejaculation in the AV. The semen collector touched the AV with glans penis only when the bull mounted with erect penis. The semen was obtained in a graduated tube previously assembled with the vaginal cone.

The graduated collecting tube was separated from the cone, covered with a plastic cap and labeled. After collection, semen samples were kept at 35°C in water - bath until the media and reagents were added to the samples.

These procedures were all done at 35°C. Semen was collected from bulls generally interval twice in a week of all the AI centres. One every day one or two ejaculates were taken for schedule collection date of a bull.

Fig. 3.4. Artificial vagina (AV) and bull preparation for semen collection

Fig. 3.5. Bull jump on dummy bull and AV used for semen collection

3.6 Semen Transportation and Distributions:

For chilled, individual AI doses in vials were placed in perforated sponge, the sponge was taken in a thermoflask / cooler box containing ice transportation to different AI Sub-centers and points. Public traffics namely bus and train, motor cycle, rickshaw and van etc was used for semen transportation. In AI Sub-centres and points, semen was preserved usually for 2-3 days either in refrigerator or within ice containing thermoflask.

Semen was collected early in the morning every 4 to 7 days intervals. Total one or two ejaculates were collected from each bull of three AI centres for the preparation of semen dose used for AI programmes. The semen was brought to the laboratory immediately after collection and was placed in water bath at 37°C for evaluation. The characteristics of semen were recorded were as follows: volume of ejaculate (ml), colour (scale:1-4), density (scale:1-5), mass activity (scale:1-4), sperm concentration (million/ml), sperm motility (%), total number of sperm cells/ejaculate (million) and total number of semen doses /ejaculate.

3.7 Semen evaluation:

A total 780 ejaculates (306 from District AI centre, 474 from Rajshahi Dairy and Cattle improvement farm, Rajabarihat, obtained over the period of June 2011 to July 2013 study the semen characters (Table 4.1). Immediately after collection semen was placed in a beaker containing lukewarm water and was examined for semen characteristics.

3.8 Semen Characteristics/Production Performance:

1) Volume: Volume of the ejaculate was measured directly from graduated collecting tube and recorded as ml.

2) Colour: The colour of semen was recorded as watery/opalescent, milky white, yellowish and creamy depending on the thickness and pigment the semen and was assigned a numerical weight from 1 to 4 for statistical analysis. A numerical weight of 1 was assigned to opalescent /watery, 2 to milky white, 3 to yellowish white and 4 to creamy white which were determined by eye estimation.

3) Density: The density of semen was scored into 1- 4 scales: 1= watery to cloudy 2= milky 3= thin creamy 4= creamy and 5= creamy grainy were determined also by eye estimation.

4) Mass activity/ Wave motion: The mass activity was evaluated in a drop of fresh undiluted semen placed on a pre-warmed slide without cover-slip at low magnification (100×). The mass activity was scored into 1- 4 scales:

1 = weak motion without forming any wave

2 = small, slow moving wave

3 = vigorous movement with moderate rapid waves and eddies and

4 = dense, very rapidly moving waves and eddies.

5) Sperm concentration: The concentration of spermatozoa (million/ml) in fresh semen was determined by using an improved Neubaur Haemocytometer (Salisbury *et al*., 1978) or Kara's scale at District AI centre, Rajshahi.

3.9 Method:

The heamocytometer using red blood count was used. Semen was drawn into the capillary tube of the dilution pipette with the mark of 1. The pipette tip was carefully wiped without drawing from the capillary. The semen was pulled into the bulb and pipette was filled with diluents,

usually 0.9% chloride solution (to mark of 101). The mixing pipette was then properly stippled and shaken gently by hand with a quick wrist motion while holding it between the thumb and forefinger. The first 3/4 drops of fluid withdrawn from the pipette was discarded after mixing. The cover glass was pressed firmly down on the slide and a drop of the diluted semen was allowed to flow under the cover glass by capillary action. The examination was delayed for a few minutes to let the cells settled. By convention, spermatozoa lying across the top and the left–hand–side grids of the squares were counted and those on the bottom and right-hand – side grids were ignored. The following formula was used for calculating total number of spermatozoa / ml of fresh semen.

$$N = C \times \frac{400}{S} \times d/ml$$

where,

N= number of spermatozoa counted per ml of semen.

C= number of spermatozoa counted a given number of small Squares.

S= number of small square counted

d= dilution ratio.

The sperm concentration/ml was directly recorded by using a Photometer at Deep frozen semen production laboratory, RDCIF, Rajabarihat, Rajshahi.

6) Sperm motility / Initial sperm motility: The motility of sperm was evaluated in small drop of diluted semen placed on a clean, prewarmed slide, covered with a cover slip and examined at a magnification of 400× under light microscope at District AI centre, Rajshahi and Video

microscope was used at Dairy and Cattle Improvement Farm, Rajabarihat, Rajshahi. Sperm motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement while those showing circling movements or oscillating at one place were regarded as immotile (Ahmad, 1994). Two examiners separately follow blind techniques for the evaluation. The average score of sperm motility given by the two examiners were recorded.

7) Total number of spermatozoa / ejaculate: Total number of sperm cell per ejaculation was calculated by multiplying the spermatozoon concentration per ml by volume (Bane, 1952).

8) Total number of semen doses per ejaculate: From the volume, motility and sperm concentration, total number of semen doses /ejaculate was also calculated automatically. The total number of insemination doses per ejaculate was calculated using the following formula for chilled semen at District AI centre, Rajshahi.

$$\text{Total No. of insemination dose/ejaculate} = \frac{\text{Volume} \times \text{Sperm conc.} \times \%\ \text{Motility}}{20 \times 100}$$

The total number of deep frozen semen insemination dose was calculated the following formula used at Frozen semen production AI Lab.

$$\text{Total dose for forzen semen} = \left\{\left(\frac{\text{Volume} \times \text{Sperm conc.}}{240} - \frac{\text{Volume}}{2}\right) \times 2 + \text{Volume}\right\} \times 4$$

The AI centres /stations had different set standards for the concentration, giving different numbers of sperm cells per semen dose (1 ml /0.25 ml). The standards were 20 million sperm cells/AI dose for chilled semen at District AI centre, Rajshahi and 30 million sperm cells/AI dose for frozen semen at DFSPL, RDCIF, Rajabarihat.

3.10 Semen Processing and Preservation Procedure in Different Extenders:

Two types of semen are produced in our country. One is chilled semen for short period preservation produced at District AI centre, Rajshahi and second is Deep frozen semen at Rajshahi Dairy and Central Cattle (RDCIF), Rajabarihat, Rajshahi.

Diluted to obtain 20×10^6 progressive motile spermatozoa per ml i.e. an insemination dose. Individual insemination doses were transferred into glass vial, covered by cork and preserved at +4°C to 8°C until used. Semen showing less than 50% motility after processing was not preserved for AI. For frozen semen of RDCIF, Rajabarihat were routinely used for Egg-yolk Tris- fructose acid glycerol extender.

The semen was collected from each bull twice a week, by the artificial vagina method. Three false mounts were allowed before each collection. Immediately after collection, the semen was assessed for volume, motility, concentration, % dead sperms and % abnormal sperms. From each, bull six ejaculates, each with motility greater than 60 % were used for preservation. Each ejaculate was divided into three parts and each part was diluted with either egg yolk citrate (EYC), cornell university extender (CUE) and Coconut milk extender (CME). The semen was extended with the above diluents to give a dilution rate of 1:20. Samples from extended semen were taken for examination of motility (%) count (Fig. 3.6 & 3.7). Each part of the diluted semen was then subdivided into six, put into 2 ml glass vials and sealed with cotton plugs. The vials were then kept in a dark place with temperature ranging from 4°C to 6°C. At 24 hour intervals one vial from the stored samples were examined for motility count. The length of time to which each diluent could maintain

sperm motility greater than 50% was noted. The composition of the diluents used in this study are given in below:

1. Egg-Yolk citrate diluent:

Distilled water	— 75 ml
Egg-yolk	— 25 ml
Sodium citrate	— 2.9 gm

To each diluent, 1000 1U of Pcnicillin and 1000 pg of Streptomycin were added per ml of diluent.

2. Cornell university extender (CUE):

(a)

Sodium citrate dhydrate	— 14.5g
Sodium bicarbonate	— 2.1 g
Potassium chloride	— 0.4 g
Glucose	— 3.0 g
Sulphanilamide	— 3.0 g
Glycine	— 9.37 g
Citric acid	— 0.87 g
Distilled water (final volume)	— 1000.00 ml

b) Add 20% (by volume) egg yolk and 80% of buffer solution (a) along with penicillin and dihydro streptomcin @ 100 I.U and 1000 microgram per ml of final dilution.

3. Coconut Milk Extender (CME):

Coconut Milk	— 100 ml
Distilled water	— 100 ml
Sodium citrate	— 4.32 g
Calcium carbonatc	— 100 mg

Fig. 3.6 Dilution of semen

Fig. 3.7 Examination of sperm motility and mass activity

3.11 Insemination of Cows and Pregnancy Diagnosis:

AI was performed in the study areas by 40 AI technicians. All AI technicians received a one-year training on AI and are involved in routine AI activities covering 90 to 450 AI monthly. The cow to be inseminated were detected in oestrus by the farmers based upon the clinical and behavioral sign and were delivered to AI centres or points. However, the cow in urban areas was often inseminated in the farm. The cows were inseminated transcervically by recto-vaginal method (Olds, 1978) with the help of inseminating gun in case of frozen semen or plastic AI tube/ pipette for chilled semen and cows were inseminated by inseminators at about 12-18 hour of oestrus. A total 189 cows were inseminated of different AI sub centre of Rajshahi district. Sixty to 120 days after insemination pregnancy was confirmed by measuring per rectal palpation of the genital tract (Ball, 1980). The data were calculated for the first service conception rate. The conception rate (CR) was calculated, in this study using the following formula:

$$C.R = \frac{\text{Number of animals conceived}}{\text{Number of animals inseminated and examined}} \times 100$$

3.12 Morphological Examination of Spermatozoa and Non Return Rate (NRR):

A total of 64 ejaculates of semen for head abnormities and tail abnormalities from 16 AI bulls among the six (6) breeds were obtained and following support chemical reagents viz. basic fucshin, alcohol, phenol, bluish eosin, chloramine for William's stain and disodium hydrophosphate, potassium di-hydrogen phosphate, sodium chloride and formaldehyde for formal saline used as fixative were used.

Morphological examination was done by staining the smear of semen with William's method (Williams, 1920; Lagerlof, 1934). A thin smear of fresh semen was prepared on a grease free slide for the study of morphological abnormalities of sperm head after staining with William's stain. William's stain was prepared by following way:

3.12.1 Composition of William stain:

Stock solution-I.

Basic fucshin-10 gm

95% alcohol.

Stock solution-II.

Saturated bluish eosin in 95% alcohol.

Stock solution-III.

Stock solution-I = 10 ml

5% phenol = 170 ml.

The final stain contain

Stock solution –II = 25 ml

Stock solution –III = 50 ml.

The stain was filtered before use.

3.12.2 Staining procedure:

The smear was air dried and fixed in flame and some information like bull ID and date of semen collection were marked on slide at any end with the help of permanent marker pen. Then the smear was treated with absolute alcohol for 3-4 minutes and washed with 0.5% chloramine for 1-2 min. until it appeared fairly clear and then washed in distilled water followed by rinsing in 95% alcohol and finally stained with Carbol-fucshin eosin (8-10 min.). After staining, the slides were washed in running tap water, dried off and examined under light microscope at 1000× (oil emersion). The proportion of sperm with normal head morphology included only those were free from any detectable abnormalities. The head abnormalities in spermatozoa were classified according to William's (1920) such as pear shape head, narrow at the base head, narrow head, broad, big and little short head, abaxial position of the mid piece, undeveloped head, abnormal contour and others abnormal head. At least 500 spermatozoa from individual smears were examined. The proportion of spermatozoa with abnormal head (Fig. 3.8) morphology included only those, which were any detectable abnormalities.

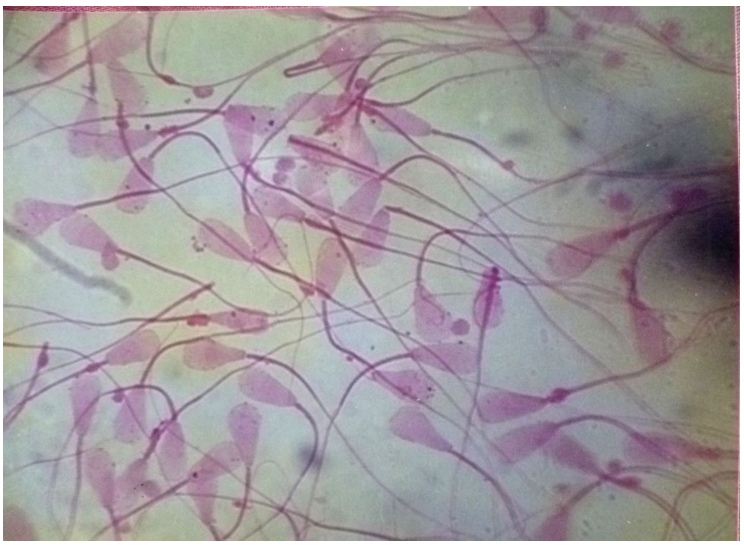

Fig. 3.8 Sperm head study by William's stain

The morphology of sperm mid-piece and tail was studied after fixed with buffered Formol–saline at the same temperature. Buffer- formol saline was prepared according to the method described by Hancock (1957).

3.12.3 Formol saline composition:

1. Di-sodium hydrogen phosphate ($Na_2 HPO_4. 2H_2O$)	34.7 mmol
2. Potassium di-hydrogen phosphate ($KH_2 PO_4$)	18.7 mmol.
3. Sodium chloride (NaCl)	92.6 mmol
4. Formaldehyde	1.54 mmol
5. Distilled water up-to	1000 ml

3.12.4 Procedures:

1 ml of formol-saline was taken in sample glass tube with plastic cork and a very small drop or 10 µml of fresh semen was mixed, shaked and marked the sample with the information like bull ID, breed and date of semen collection for later examination and sample was preserved for a long time in refrigerator (+4°C). The abnormalities of formol-saline fixed spermatozoa were observed under Phase contrast microscopy (Olympus

CH-2, Japan). The following abnormalities were found in the freshly collected and preserved semen viz. free loose head, abnormal mid-piece, abnormal tail (simple bent tail and coil tail), proximal and distal cytoplasomic droplets and double folded tail, broken neck, abnormal acrosome and others. At least 200 spermatozoa from individual replicates were examined at 1000× magnification. The proportion of abnormal spermatozoa at formol–saline preparation included only those, which had abnormalities in the mid-piece and tail. The spermatozoa were considered as normal having no mid-piece and tail defects.

3.13 Oestrus Detection and Insemination of Cows:

At first, the farmer of cattle detected the oestrus of cows on the basis of the following sign and symptoms; stand to be mounted, bellowing, restlessness, mounted to others and mucous discharge from vagina. Then the cows were brought into AI Sub-centres /points for AI. Finally, AI technicians confirmed the detection of oestrus cows after clinical examination. Several information like owner's name and address of cows, cows history, semen information were recorded in AI register and AI card issued for client after performing AI. The AI was performed as described Pickett and Beraudtson (1978). Briefly, after controlling the heated /estrous cows in the travice, perineum was washed with clean water, then with mild soap water to remove the dung from the vagina. The chilled semen was taken from semen vial with help of AI tube. The AI tube consist of poly valve. The left hand of the inseminator was inserted with the right hand per vaginum through the cervix to the uterus and semen was placed beyond the third ring into the body of the uterus by pushing the poly valve. Generally, AI technicians (Field Assistant AI) performed the AI between 12-18 hours after showing the first heat symptoms.

The AI technicians gave the instruction to the owner of cows after AI. The following instruction was for the farmer if the cows were not conceived within 60-days he/she must repeated AI by the same technician. The non-return rate (NRR) at 60-days after insemination was subsequently calculated from a total of 1600 first AI's. The non-return rate (NRR) was calculated in this study, by using the following formula:

$$\text{NR \%} = \frac{\text{Total no. of animals inseminated} - \text{Animal returning to service}}{\text{Total number of animals inseminate}} \times 100$$

3.14 Statistical Analysis:

Data obtained were subjected to analysis of variance test by SPSS computer software package (Anonymous, 1996). Data collected on percentage were transform into Z and all then analysis have made on transformed data. Re-transform were made and presented in tables. The results were presented as Mean±SD of 16 bulls as well as various factors. The means were compared by Duncan's multiple range test (Steel and Torrie, 1980). Analysis of Variance (ANOVA) was done by using one-way cross classification for breed, age, body weight, body condition, TC, libido, season and AI centres/stations groups with number of observations within the groups.

The following model was used for different semen parameters:

$$Y_{ijklmnopq} = \mu + A_i + B_j + Br_k + Bw_l + C_m + L_n + T_o + S_p + l_q + e_{ijklmnopqrs}$$

where,

$Y_{ijklmnopq}$ - observed semen characteristics

μ -overall mean

A_i – fixed effect of i^{th} age group of the bull at collection (i=1-5)

B_j – fixed effect of j^{th} bull ID of the AI bulls (j=1-16)

Br_k – fixed effect of k^{th} breed of the AI bulls (k=1-6)

Bw_l –fixed effect of l^{th} body weight of bulls at collection (l=1-3)

C_m – fixed effect of m^{th} body condition of bulls at collection (m=1-3)

L_n – fixed effect of n^{th} libido of bulls at collection (n=1-3)

T_o – fixed effect of o^{th} testicular circumference (o=1-4)

S_p – fixed effect of the p^{th} season of collection (p=1-4)

L_q – fixed effect of the q^{th} location at collection (q=1-2)

$e_{ijklmnop}$ = is a random residual effect

Mean effects were systematically included in the model. Random effects were assumed independently and identically distributed.

The extender of semen was judged on the motility % in various duration of preservation period in relation to various factors. Random effects were assumed independently and identically distributed. GLM test i.e. Univarite (Post Hoc, Duncan's test) for multiple comparisons for observed means was performed.

Mean and standard deviation in sperm abnormalities of tail and head among the factors such as individual bull, breed, age, body weight, body condition, testicular circumference, libido, season and AI centres/stations were also calculated.

The analysis of non-return (NR) at 60 days in relation with sperm abnormalities data was carried out by non orthogonal analysis of variance (ANOVA) with the MODEL program (Kobilinsky, 1980); each non-return rate 60-days post AI was weighted by the number of first AIs from which it was derived.

CHAPTER 4

RESULTS

4.1 Factors Affecting the Semen Production of Bulls:

A total 780 samples from 16 bulls belonging to six breeds from two AI centres or stations were used. The experiment was to evaluate the semen production of AI bulls regarding their breed, age, body weight, body condition, testicular circumference, libido, seasons and AI centre. The average values with standard deviation of volume of ejaculate, colour, mass activity, density, sperm concentration, sperm motility, total number of sperm cells /ejaculate, number of semen doses per collection were 7.67±2.17ml, 3.06±0.98, scale:1-4), 2.91±0.74, scale:1-5), 3.56±0.77, scale:1-4), 1330.46±313.32 million/ml, 62.94±5.32%, 10112.88±3521.12 million/ejaculate and 337.10±117.37 , respectively (Table 4.1). The volume of semen was found highest (8.81±2.41 ml) in bull ID 3209 and lowest (7.25±1.98 ml) was in bull ID 2872. Mass activity was found higher (3.05±0.83, scale:1-4) in bull ID D-028 and lower (2.81±0.72, scale:1-4) in bull ID D-04. Density was found greater (3.77±0.74, scale:1-5) in bull ID 3058 and less was found in bull ID 3209 (3.38±0.94, scale:1-5). Sperm concentration was found higher in bull ID 3165 (1382.87±287.63 million/ml) and lower was found in bull ID 3209(1244.53±397.97 million/ml). Sperm motility was found highest value was in bull ID 3381 (64.49±4.44%) and lowest value was found in bull ID 2872 (61.46±4.59%). Total sperm cells / ejaculate was found highest (10767.50±3965.68 million/ml) in bull ID 3209 and lowest was found in bull ID 2872 (9386.23±3680.64 million/ml). Number of semen doses per collection was found greater (356.87±126.70) in bull ID 3058 and less

was found in bull ID 2872 (312.87±122.69). The results of the semen characteristics analyzed in ANOVA is presented in Table 4.2. Bull ID had significant (P<0.05) effect on only volume and colour and there is no effect on other semen traits.

4.1.1 Breeds of bull:

The Mean±SD with comparison of the semen characteristics on the different breeds of bull is presented in the Tables 4.3 and Figs 4.1 & 4.2. Analysis of variance showed that breeds of bull had significant (P<0.05) effect (P<0.05) all the semen traits (Tables 4.4). The significant (P<0.05) highest volume (8.23±2.47ml) of semen was found for L×F×SL×F×F breed and lowest (5.97±2.36 ml) in L× F×(L×F×F) breed. The mean colour and density values were higher in L× F×(L×F×F) breed (3.19±0.98 and 3.67±0.63, respectively. Significantly (P<0.05) highest sperm concentration was found in SL×F (1367.11±313.91 million/ml). The mean sperm motility, total number of sperm cells/ejaculate and the number of semen doses were significantly highest in $(L\times F)_2$ breed (63.48±5.12, 10523.03±3607.45 and 350.70±120.25, respectively. The relation of the semen characteristics with breed analyzed and ANOVA are presented in Table 4.4.

Table 4.1. Semen characteristics of individual bull used for AI programme.

Bull ID	N	Semen Characteristics							
		Volume (ml)	Colour (1-4)*	Mass activity (1-4 scale)***	Density (1-5 scale)**	Sperm concentration ($\times 10^6$) /ml	Sperm motility (%)	Total sperm cells / ejaculate ($\times 10^6$)	Number of semen doses per collection
D-04	48	7.84± 2.35^{ab}	2.69± .99^{c}	2.81± .72	3.44± .77^{ab}	1278.65± 287.88	62.31± 5.80^{ab}	9978.65± 3704.89	332.62± 123.50
D-028	60	7.24± 2.16^{b}	2.98± 1.02^{abc}	3.05± .83	3.57± .77^{ab}	1338.25± 332.88	62.65± 4.26^{ab}	9595.15± 3459.94	319.84± 115.33
D229	69	7.44± 1.97^{b}	2.91± .98^{abc}	2.98± .75	3.64± .69^{ab}	1347.46± 284.98	62.61± 4.14^{ab}	10022.55± 3501.52	334.09± 116.72
1043	60	7.52± 1.85^{b}	3.05± 1.06^{abc}	2.94± .73	3.57± .85^{ab}	1355.00± 324.98	62.73± 4.04^{ab}	10191.77± 3514.96	339.73± 117.17
2872	69	7.25± 1.98^{b}	2.78± 1.00^{bc}	2.87± .80	3.42± .76^{ab}	1284.64± 302.14	61.46± 4.59^{b}	9386.23± 3680.64	312.87± 122.69
2227	38	7.78± 2.42^{b}	3.15± .99^{abc}	2.83± .74	3.54± .74^{ab}	1335.24± 313.75	63.29± 6.42^{ab}	10197.80± 3676.92	339.93± 122.56
2941	51	7.65± 1.85^{b}	3.25± .96^{ab}	2.84± .67	3.53± .83^{ab}	1321.96± 331.20	63.12± 5.39^{ab}	9997.94± 3173.88	333.26± 105.80
2979	51	8.00± 2.23^{ab}	3.20± .96^{ab}	2.83± .65	3.55± .67^{ab}	1308.82± 283.50	62.73± 4.82^{ab}	10448.92± 3540.76	348.30± 118.03
3037	48	7.84± 1.95^{ab}	3.15± .97^{abc}	2.86± .76	3.48± .87^{ab}	1317.71± 349.85	63.44± 5.86^{ab}	10257.81± 3570.73	341.93± 119.02
3058	39	7.80± 2.34^{b}	3.18± .94^{ab}	2.86± .69	3.77± .74^{a}	1376.15± 316.76	63.03± 7.17^{ab}	10706.15± 3801.13	356.87± 126.70
3079	36	7.48± 1.87^{b}	3.11± .98^{abc}	2.87± .61	3.56± .73^{ab}	1315.97± 289.53	63.94± 5.51^{ab}	9759.03± 3079.26	325.30± 102.64
3158	47	7.62± 2.18^{b}	3.30± .93^{a}	2.94± .70	3.57± .77^{ab}	1346.81± 297.94	63.45± 5.09^{ab}	10107.45± 3296.18	336.91± 109.87
3165	54	7.72± 2.19^{b}	3.11± .98^{abc}	3.01± .72	3.67± .67^{ab}	1382.87± 287.63	63.30± 5.23^{ab}	10612.04± 3611.21	353.73± 120.37
3209	32	8.81± 2.41^{a}	2.97± .90^{abc}	2.80± .79	3.38± .94^{b}	1244.53± 397.97	62.78± 7.21^{ab}	10767.50± 3965.68	358.92± 132.19
3211	36	7.98± 2.68^{ab}	3.17± .94^{ab}	3.04± .79	3.67± .76^{ab}	1381.00± 323.07	63.22± 6.65^{ab}	10590.50± 3251.06	353.02± 108.37
3381	39	7.52± 2.50^{b}	3.26± .97^{ab}	2.93± .81	3.62± .85^{ab}	1344.87± 330.63	64.49± 4.44^{a}	9912.69± 3648.60	330.42± 121.62
Overall	780	7.67± 2.17	3.06± .98	2.91± .74	3.56± .77	1330.46± 313.32	62.94± 5.32	10112.88± 3521.12	337.10± 117.37

N = Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b,c Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.2. Analysis of variance of semen characteristics individual AI bulls.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between bulls	85.54	15	5.703	1.214	
	Within Groups	3589.70	764	4.699		*
	Total	3675.25	779			
Colour (1-4)*	Between bulls	22.98	15	1.53	1.60	
	Within Groups	732.05	764	.95		*
	Total	755.04	779			
Mass activity (1-4 scale)***	Between bulls	6.83	15	.45	.76	
	Within Groups	457.57	764	.59		NS
	Total	464.40	779			
Density (1-5 scale)**	Between bulls	4.95	15	.33	.59	
	Within Groups	422.06	764	.55		NS
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between bulls	955982.03	15	63732.13	.64	
	Within Groups	75518771.88	764	98846.56		NS
	Total	76474753.92	779			
Sperm motility (%)	Between bulls	357.95	15	23.86	.84	
	Within Groups	21650.21	764	28.33		NS
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between bulls	117232899.64	15	7815526.64	.62	
	Within Groups	9541047263.61	764	12488281.75		NS
	Total	9658280163.25	779			
Number of semen doses per collection	Between bulls	130258.77	15	8683.91	.62	
	Within Groups	10601163.62	764	13875.86		NS
	Total	10731422.40	779			

* = P< 0.05 level of significant
NS = Non significant

Table 4.3. Effect of breeds on semen characteristics of Artificial Insemination (AI) bulls.

Semen Characteristics	Breeds						
	L×F×SL ×F×F	**SL×F**	**L×F×F**	**L×F**	**$(L\times F)_2$**	**L×F (L×F×F)**	**Overall**
Volume (ml)	8.23± 2.47^{a} n = 58	7.4 0± 1.95^{b} n = 76	7.41± 2.04^{b} n = 66	7.41± 2.14^{b} n = 159	7.94± 2.08^{ab} n = 385	5.97± 2.36^{c} n = 36	7.67± 2.17 n = 780
Colour (1-4)*	2.74± .93^{b} n = 58	3.16± 1.03^{a} n = 76	2.92± 1.01^{ab} n = 66	3.19± .93^{a} n = 159	3.05± .99^{ab} n = 385	3.19± .98^{a} n = 36	3.06± .98 n = 780
Mass activity (1-4 scale)***	2.68± .74^{b} n = 58	2.98± .74^{a} n = 76	2.99± .78^{a} n = 66	3.05± .77 ^{a} n = 159	2.85± .71^{ab} n = 385	2.95± .65^{a} n = 36	2.91± .74 n = 780
Density (1-5 scale)**	3.24± .88^{b} n = 58	3.62± .80^{a} n = 76	3.55± .75^{a} n = 66	3.62± .76^{a} n = 159	3.56± .76^{a} n = 385	3.67± .63^{a} n = 36	3.56± .77 n = 780
Sperm concentration ($\times10^{6}$) /ml	1209.05± 316.49^{b} n = 58	1367.11± 313.91^{a} n = 76	1323.41± 315.66^{a} n = 66	1356.89± 313.28^{a} n = 159	1328.82± 313.02^{a} n = 385	1362.50± 275.52^{a} n = 36	1330.46± 313.32 n = 780
Sperm motility (%)	61.67± 5.52^{ab} n = 58	62.75± 4.02^{ab} n = 76	63.09± 4.18^{ac} n = 66	62.53± 5.92^{ab} n = 159	63.48± 5.12^{a} n = 385	61.08± 7.47^{b} n = 36	62.94± 5.32 n = 780
Total sperm cells / ejaculate ($\times10^{6}$)	9780.60± 3561.34^{a} n = 58	10146.13± 3578.84^{a} n = 76	9702.78± 3322.23^{a} n = 66	9840.20± 3090.13^{a} n = 159	10523.03± 3607.45^{a} n = 385	8147.92± 3847.67^{b} n = 36	10112.88± 3521.12 n = 780
Number of semen doses per collection	326.02± 118.71^{a} n = 58	338.20± 119.29^{a} n = 76	323.43± 110.74^{a} n = 66	328.01± 103.00^{a} n = 159	350.77± 120.25^{a} n = 385	271.60± 128.26^{b} n = 36	337.10± 117.37 n = 780

N = Number of observation, L = Local, F = Friesian, SL = Sahiwal, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b,c Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.4. Analysis of variance of semen characteristics of different breeds of bulls used for Artificial Insemination (AI) in Bangladesh.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between breed	172.47	5	34.49	7.62	
	Within Groups	3502.77	774	4.52		***
	Total	3675.25	779			
Colour (1-4)*	Between breed	11.15	5	2.23	2.32	
	Within Groups	743.88	774	.96		*
	Total	755.04	779			
Mass activity (1-4 scale)***	Between breed	7.19	5	1.43	2.43	
	Within Groups	457.21	774	.59		*
	Total	464.40	779			
Density (1-5 scale)**	Between breed	8.56	5	1.71	3.16	
	Within Groups	418.45	774	.54		**
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between breed	1109290.36	5	221858.07	2.27	
	Within Groups	75365463.55	774	97371.40		*
	Total	76474753.92	779			
Sperm motility (%)	Between breed	361.23	5	72.24	2.58	*
	Within Groups	21646.93	774	27.96		
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between breed	233176097.85	5	46635219.57	3.83	
	Within Groups	9425104065.40	774	12177137.03		**
	Total	9658280163.25	779			
Number of semen doses per collection	Between breed	259084.55	5	51816.91	3.83	
	Within Groups	10472337.85	774	13530.15		**
	Total	10731422.40	779			

* = P<0.05 level of significant
** = P<0.01 level of significant
*** = P<0.001 level of significant

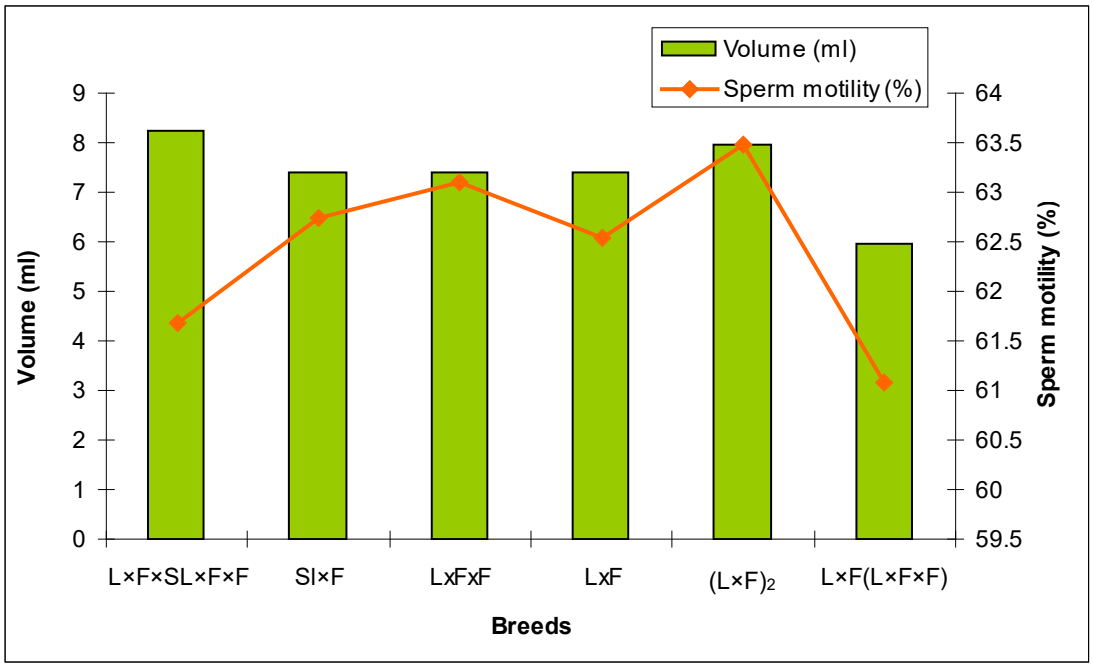

Fig. 4.1. Graph showing average volume and sperm motility influenced by breed of bulls.

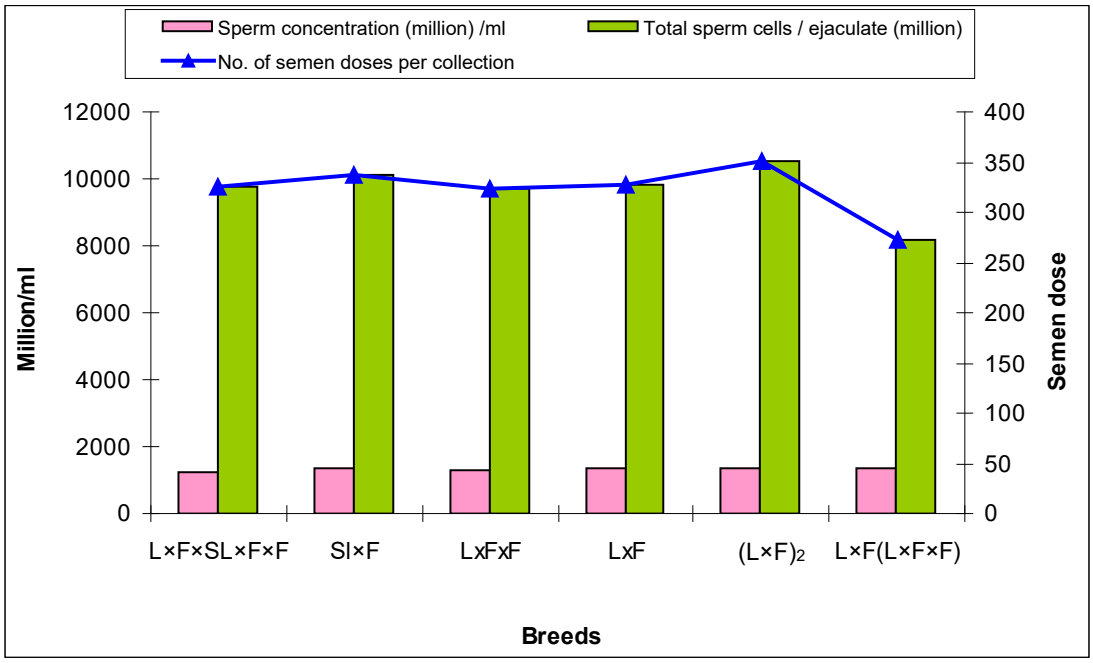

Fig. 4.2. Graph showing average Sperm concentration ($\times 10^6$) /ml, Total sperm cells / ejaculate ($\times 10^6$) and Number of semen doses per collection influenced by breed of bulls.

4.1.2 Age of the AI bulls:

Average volume of ejaculate, mass activity, density, sperm concentration sperm motility, total no. of sperm cells/eja., number of semen doses per collection as influenced by age of bulls is presented in Table 4.5 and Fig. 4.3 & 4.4. The volume of semen was greater at age groups <4 years age groups; (8.14±2.60 ml). Sperm motility, total sperm cells / ejaculate ($\times 10^6$) and number of semen doses per collection were greater at age groups <4 years age groups (63.7±5.95%, 10392.54±3652.68 million/ml, 346.42±121.76, respectively and lower at 6-<8 years age group (62.06±5.35%, 9728.56±3700.42 million/ml, 324.292±123.35, respectively. The mass activity, density and sperm concentration were highest values at age groups of >10 years for (2.98±0.75, scale:1-4), (3.64±0.69, scale:1-5), 1347.46±284.98 (million/ml), respectively and mass activity lower at age groups < 4 years (2.83±0.73, scale:1-4) and density and sperm concentration were lower at age group 6-<8 years (3.45±0.75, scale:1-5) and 1298.37±304.54 (million/ml), respectively. The relation of the semen characteristics with age group analyzed and ANOVA are presented in Table 4.6.

Table 4.5. Effect of age on semen characteristics of bulls used for AI programme in Bangladesh.

Semen Characteristics	Age Group					
	<4 yrs	**4-<6 yrs**	**6-<8 yrs**	**8-<10 years**	**>10 yrs**	**Overall**
Volume (ml)	8.14± 2.60[a] n = 65	7.73± 2.12[ab] n = 377	7.51± 2.22[ab] n = 101	7.51± 2.12[ab] n = 168	7.44± 1.97[b] n = 69	7.67± 2.17 n = 780
Colour (1-4)*	3.17± .93[ab] n = 65	3.18± .95[a] n = 377	2.88± 1.00[b] n = 101	2.92± 1.03[ab] n = 168	2.91± .98[ab] n = 69	3.06± .98 n = 780
Mass activity (1-4 scale)***	2.83± .73 n = 65	2.91± .71 n = 377	2.84± .77 n = 101	2.94± .77 n = 168	2.98± .75 n = 69	2.91± .74 n = 780
Density (1-5 scale)**	3.54± .79 n = 65	3.59± .78 n = 377	3.45± .75 n = 101	3.53± .80 n = 168	3.64± .69 n = 69	3.56± .77 n = 780
Sperm concentration ($\times 10^6$) /ml	1305.00± 309.40 n = 65	1341.79± 320.01 n = 377	1298.37± 304.54 n = 101	1327.20± 317.46 n = 168	1347.46± 284.98 n = 69	1330.46± 313.32 n = 780
Sperm motility (%)	63.71± 5.95[a] n = 65	63.26± 5.63[ab] n = 377	62.06± 5.35[b] n = 101	62.58± 4.66[ab] n = 168	62.61± 4.14[ab] n = 69	62.94± 5.32 n = 780
Total sperm cells / ejaculate ($\times 10^6$)	10392.54± 3652.68 n = 65	10271.10± 3449.35 n = 377	9728.56± 3700.42 n = 101	9917.80± 3538.75 n = 168	10022.55± 3501.52 n = 69	10112.88± 3521.12 n = 780
Number of semen doses per collection	346.42± 121.76 n = 65	342.37± 114.98 n = 377	324.29± 123.35 n = 101	330.59± 117.96 n = 168	334.09± 116.72 n = 69	337.10± 117.37 n = 780

N = Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.6. Analysis of variance for the semen parameters of various age groups of bulls used for AI programme in Bangladesh.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between age groups	26.57	4	6.64	1.41	
	Within Groups	3648.67	775	4.70		*
	Total	3675.25	779			
Colour (1-4)*	Between age groups	14.12	4	3.53	3.69	
	Within Groups	740.92	775	.95		**
	Total	755.04	779			
Mass activity (1-4 scale)***	Between age groups	2.23	4	.55	.93	
	Within Groups	462.17	775	.59		NS
	Total	464.40	779			
Density (1-5 scale)**	Between age groups	1.48	4	.37	.67	
	Within Groups	425.52	775	.54		NS
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between age groups	216318.33	4	54079.58	.55	NS
	Within Groups	76258435.58	775	98397.98		
	Total	76474753.92	779			
Sperm motility (%)	Between age groups	184.80	4	46.20	1.64	
	Within Groups	21823.36	775	28.15		*
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between age groups	36394793.47	4	9098698.37	.73	
	Within Groups	9621885369.77	775	12415335.96		NS
	Total	9658280163.25	779			
Number of semen doses per collection	Between age groups	40438.65	4	10109.66	.73	
	Within Groups	10690983.74	775	13794.81		NS
	Total	10731422.40	779			

** = P<0.01 level of significant
NS = Non significant

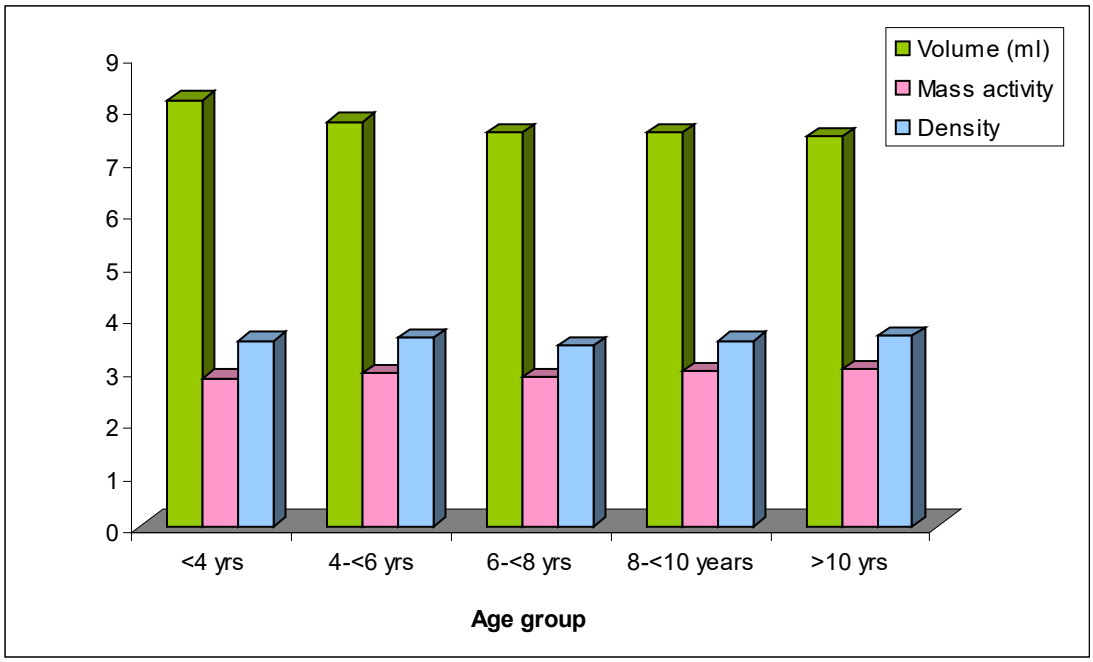

Fig. 4.3. Graph representing average volume (ml), mass activity (1-4 scale)*** and density (1-5 scale)** influenced by age group of bulls.

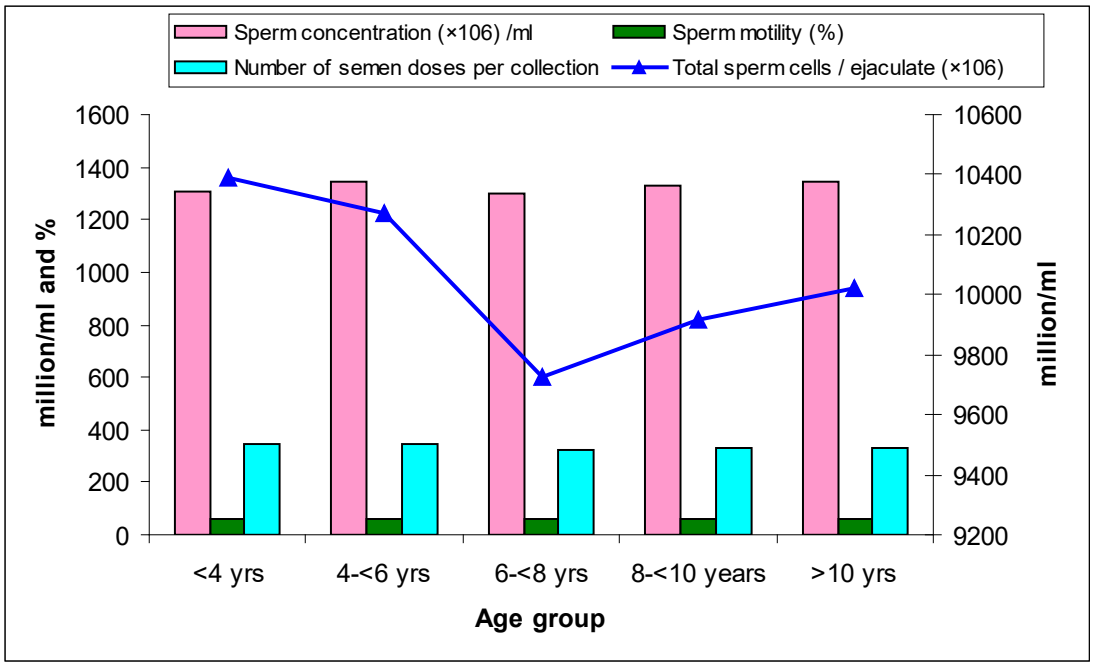

Fig. 4.4. Graph representing average sperm concentration ($\times 10^6$) /ml, sperm motility (%), number of semen doses per collection and total sperm cells / ejaculate ($\times 10^6$) influenced by age group of bulls.

4.1.3 Body weight of AI bulls:

Mean±SD with comparison of semen characteristics on body weight of bulls is presented in Table 4.7 and Fig. 4.5 & 4.6. Analysis of variance showed that body weight of bulls had significant (P<0.05) effect on semen characteristics except sperm concentration and sperm motility (Table 4.8). The highest value of volume, colour, mass activity, sperm concentration, total sperm cells/ejaculate, number of semen doses per collection were found at the body weight groups of 500-<600 kg 7.84±2.21 ml, (3.35±0.92, scale:1-4), (3.65±0.74, scale:1-4), 1361.11±312.47 million/ml, 10431.94±3178.4 million/ml and 347.73±105.93, respectively and density, motility higher at the body weight groups of <500 kg (3.04±0.69, scale:1-5), 63.55±4.52%, respectively. The lowest value of volume, mass activity, total sperm cells/ejaculate, number of semen doses per collection were found at the body weight groups of <500 kg 6.39±2.02ml, (3.53±0.74, scale:1-4), 8439.67±2759.56 million/ml, 281.32±91.99, respectively and colour, density, sperm concentration, sperm motility were found at the body weight groups of >600 kg (3.01±99, scale:1-4), (2.88±0.73, scale:1-5), 1325.22±316.14 million/ml, 62.84±5.36%, respectively. The relation of the semen characteristics with body weight group analyzed and ANOVA are presented in Table 4.8.

4.1.4 Body condition score of bull:

Body condition score effect on semen characteristics of artificial insemination (AI) bull used in Bangladesh is presented in the Table 4.9 and Fig. 4.7 & 4.8. From the Table 4.9 showed the body condition score had significant (P<0.05) effect on all the semen characteristics. The values of semen characteristics were found increased at body condition score at very good condition.

Table 4.7. Body weight effects on semen characteristics of AI bulls used in Bangladesh.

Semen Characteristics	Body Weight (kg)			
	<500 kg	**500-<600 kg**	**>600 kg**	**Overall**
Volume (ml)	6.39±2.02[b] n = 75	7.84±2.21[a] n = 81	7.80±2.13[a] n = 624	7.67±2.17a n = 780
Colour (1-4)*	3.19±.98[ab] n = 75	3.35±.92[a] n = 81	3.01±.99[b] n = 624	3.06±.98 n = 780
Mass activity (1-4 scale)***	3.53±.74 n = 75	3.65±.74 n = 81	3.55±.78 n = 624	3.56±.77 n = 780
Density (1-5 scale)**	3.04±.69 n = 75	3.02±.81 n = 81	2.88±.73 n = 624	2.91±.74 n = 780
Sperm concentration ($\times 10^6$) /ml	1341.00± 291.65 n = 75	1361.11± 312.47 n = 81	1325.22± 316.14 n = 624	1330.46± 313.32 n = 780
Sperm motility (%)	63.55±4.52 n = 75	63.11±5.65 n = 81	62.84±5.36 n = 624	62.94±5.32 n = 780
Total sperm cells / ejaculate ($\times 10^6$)	8439.67± 2759.56[b] n = 75	10431.94± 3178.04[a] n = 81	10272.57± 3595.22[a] n = 624	10112.88± 3521.12 n = 780
Number of semen doses per collection	281.32± 91.99[b] n = 75	347.73± 105.93[a] n = 81	342.42± 119.84[a] n = 624	337.10± 117.37 n = 780

N= Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.8. Analysis of variance for the semen characteristics of different body weight groups of bulls used for AI in Bangladesh.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between body weight	137.11	3	45.70	10.02	
	Within Groups	3538.13	776	4.55		***
	Total	3675.25	779			
Colour (1-4)*	Between body weight	12.16	3	4.05	4.23	
	Within Groups	742.87	776	.95		***
	Total	755.04	779			
Mass activity (1-4 scale)***	Between body weight	2.09	3	.69	1.17	
	Within Groups	462.30	776	.59		*
	Total	464.40	779			
Density (1-5 scale)**	Between body weight	4.03	3	1.34	2.46	
	Within Groups	422.97	776	.54		*
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between body weight	269422.51	3	89807.50	.91	
	Within Groups	76205331.41	776	98202.74		NS
	Total	76474753.92	779			
Sperm motility (%)	Between body weight	38.44	3	12.81	.45	
	Within Groups	21969.72	776	28.31		NS
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between body weight	262200207.76	3	87400069.25	7.21	
	Within Groups	9396079955.48	776	12108350.45		***
	Total	9658280163.25	779			
Number of semen doses per collection	Between body weight	291333.56	3	97111.18	7.21	
	Within Groups	10440088.83	776	13453.72		***
	Total	10731422.40	779			

* = P<0.05 level of significant
*** = P<0.001 level of significant
NS = Non significant

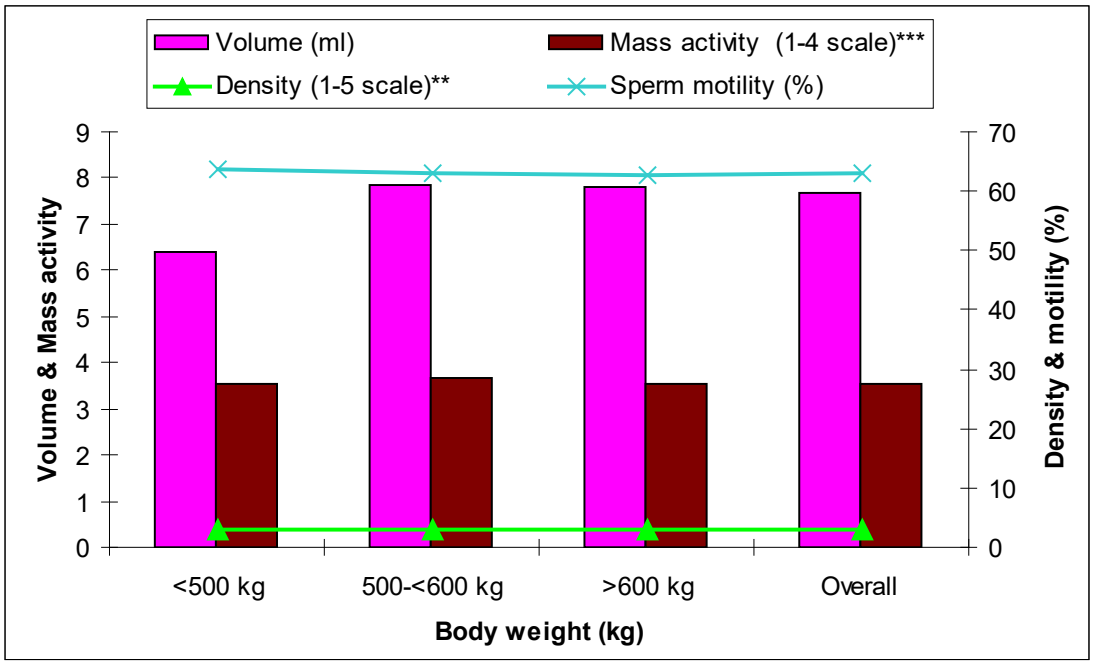

Fig. 4.5. Graph representing average volume (ml), mass activity (1-4 scale)***, density (1-5 scale)** and sperm motility influenced by body weight (kg) of bulls.

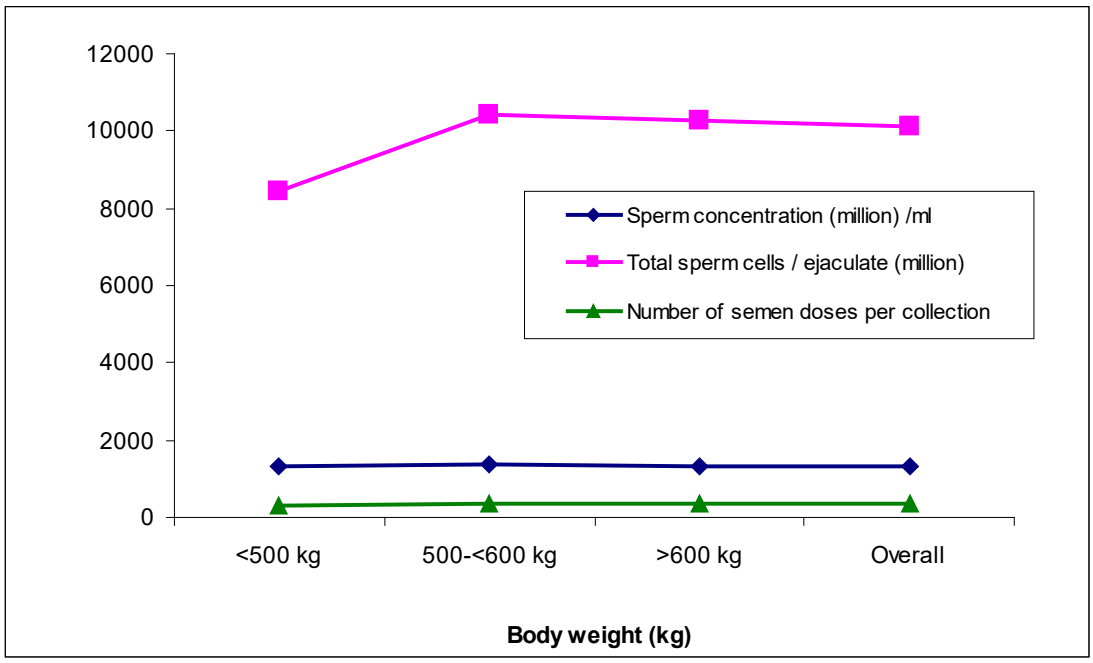

Fig. 4.6. Graph representing average sperm concentration ($\times 10^6$) /ml, total sperm cells / ejaculate ($\times 10^6$) and number of semen doses per collection influenced by body weight (kg) of bulls.

Table 4.9. Effect of body condition score on semen characteristics of bulls used for AI programme in Bangladesh.

Semen Characteristics	Body Condition Score			
	Average	**Good**	**Very**	**Overall**
Volume (ml)	5.32±1.49[b] n = 32	7.62±2.15[a] n = 453	7.99±2.10[a] n = 295	7.67±2.17 n = 780
Colour (1-4)*	3.37±.94[a] N = 32	2.94±.97[b] n = 453	3.21±.98[ab] n = 295	3.06±.98 n = 780
Mass activity (1-4 scale)***	3.31±.94[a] n = 32	2.86±.76[b] n = 453	2.94±.65[b] n = 295	2.91±.74 n = 780
Density (1-5 scale)**	3.75±.72[a] n = 32	3.40±.84[b] n = 453	3.77±.59[a] n = 295	3.56±.77 n = 780
Sperm concentration ($\times10^6$) /ml	1410.94± 353.26[a] n = 32	1274.26± 328.82[b] n = 453	1408.03± 262.91[a] n = 295	1330.46± 313.32 n = 780
Sperm motility (%)	61.44±2.88[b] n = 32	62.41±5.74[ab] n = 453	63.92±4.65[a] n = 295	62.94±5.32 n = 780
Total sperm cells / ejaculate ($\times10^6$)	7293.75± 2309.47[c] n = 32	9614.58± 3480.55[b] n = 453	11183.88± 3356.45[a] n = 295	10112.88± 3521.12 n = 780
Number of semen doses per collection	243.12± 76.98[c] n = 32	320.49± 116.02[b] n = 453	372.80± 111.88[a] n = 295	337.10± 117.37 n = 780

N = Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b,c Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.10. Analysis of variance of body condition score on semen characteristics of bulls used for AI programme in Bangladesh.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between body condition	209.42	2	104.71	23.47	
	Within Groups	3465.82	777	4.46		***
	Total	3675.25	779			
Colour (1-4)*	Between body condition	15.53	2	7.77	8.16	
	Within Groups	739.50	777	.95		**
	Total	755.04	779			
Mass activity (1-4 scale)***	Between body condition	25.54	2	12.77	22.61	
	Within Groups	438.85	777	.56		***
	Total	464.40	779			
Density (1-5 scale)**	Between body condition	6.65	2	3.32	6.14	
	Within Groups	420.36	777	.54		**
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between body condition	3413112.64	2	1706556.32	18.14	
	Within Groups	73061641.27	777	94030.42		***
	Total	76474753.92	779			
Sperm motility (%)	Between body condition	482.79	2	241.39	8.71	
	Within Groups	21525.37	777	27.70		**
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between body condition	705179455.91	2	352589727.95	30.60	
	Within Groups	8953100707.33	777	11522652.13		***
	Total	9658280163.25	779			
Number of semen doses per collection	Between body condition	783532.72	2	391766.36	30.60	
	Within Groups	9947889.67	777	12802.94		***
	Total	10731422.40	779			

** = P<0.01 level of significant
*** = P<0.001 level of significant

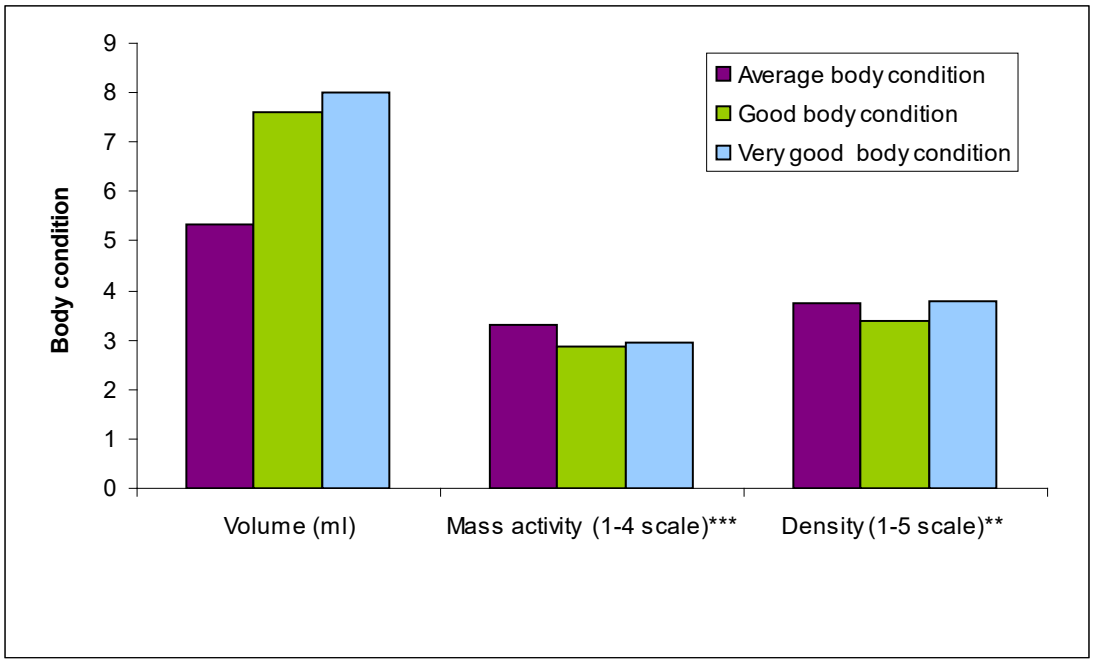

Fig. 4.7. Graph showing average, good and very good body condition influenced by bulls.

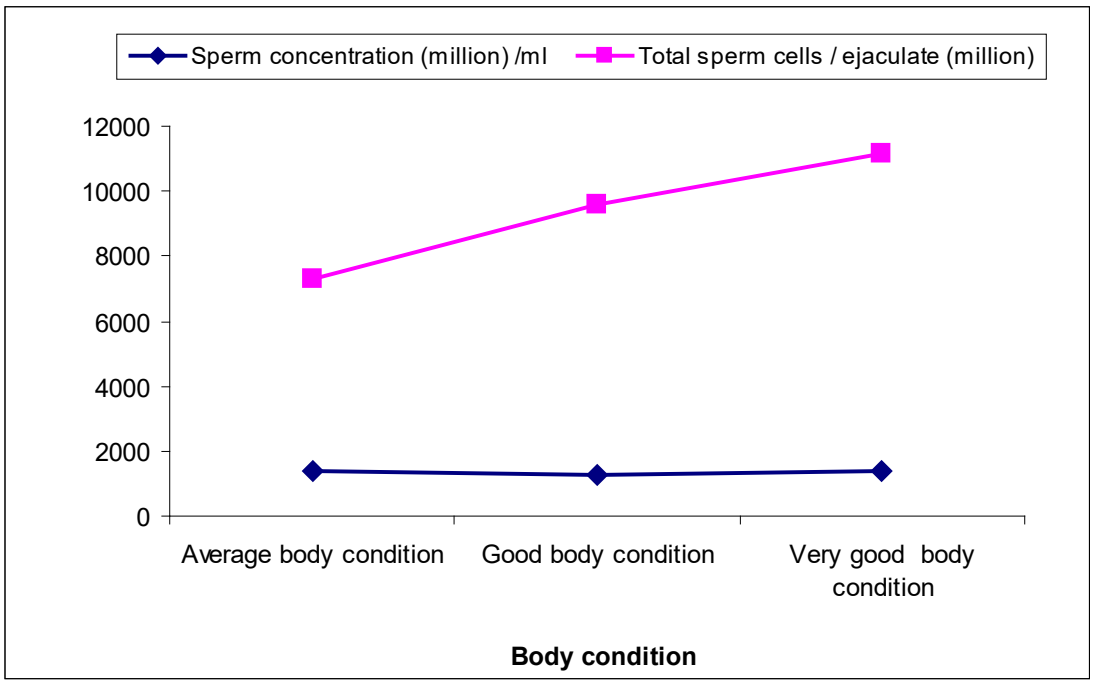

Fig. 4.8. Graph showing average sperm concentration ($\times 10^6$) /ml, total sperm cells / ejaculate ($\times 10^6$) influenced by body condition of bulls.

4.1.5 Testicular circumferences (TC) of AI bulls:

Mean±SD with comparisons of semen characteristics according to sizes of testicular circumferences (TC) is shown in Table 4.11 and Fig. 4.9 & 4.10. Testicular circumference of the AI bulls had significant ($P<0.05$) effect on all the semen traits (Table 4.12). The highest volume of ejaculate was found in TC groups of >38 cm (8.76±2.11 ml). On the other hand, the volume of ejaculate was lowest (5.35±1.53 ml) of the TC groups of <34 cm. The total sperm cells/ejaculate, and number of semen doses per collection were gradually increased with increasing sizes of TC groups of bulls. Colour, density and sperm concentration were at TC groups of <34 cm (3.33±0.95, scale:1-4), (3.81±0.52, scale:1-5) and 1409.71±259.17 million/ml, respectively.

4.1.6 Libido of AI bulls:

The effect of libido of bull on semen characteristic is furnished in the Table 4.13 and Fig. 4.11. Analysis of variance indicated that libido of bull had significant ($P<0.05$) effect on all the semen characteristics (Table 4.14). The mean±SD volume, colour, density were higher in libido score at very good 7.78±2.25 ml, (3.25±0.94, scale:1-4), (2.94±0.79, scale:1-5), respectively. Mass activity, sperm concentration, total sperm cells per ejaculate and number of semen doses per collection were higher values in libido score good (3.60±0.74, scale:1-4), 1352.42±304.65 million/ml, 10421.65±3442.08 million/ml and 347.39±116.07, respectively.

Table 4.11. Comparison of semen characteristics of testicular circumference (TC) of bulls used AI for programme.

Semen Characteristics	Testicular circumference				
	<34 cm	**34-<36 cm**	**36-<38 cm**	**>38 cm**	**Overall**
Volume (ml)	5.35±1.53^{c} n = 69	7.02±1.87^{b} n = 135	7.21±1.69^{b} n = 248	8.76±2.11^{a} n = 328	7.67±2.17 n = 780
Colour (1-4)*	3.33±.95^{a} n = 69	3.17±.99^{a} n = 135	2.85±1.01^{b} n = 248	3.12±.94^{a} n = 328	3.06±.98 n = 780
Mass activity (1-4 scale)***	3.17±.75^{a} n = 69	3.21±.81^{ab} n = 135	2.75±.70^{c} n = 248	2.85±.68^{b} n = 328	2.91±.74 n = 780
Density (1-5 scale)**	3.81±.52^{a} n = 69	3.69±.70^{a} n = 135	3.35±.84^{b} n = 248	3.60±.76^{b} n = 328	3.56±.77 n = 780
Sperm concentration ($\times10^{6}$) /ml	1409.71± 259.17^{a} n = 69	1404.04± 305.26^{a} n = 135	1256.59± 328.86^{b} n = 248	1339.36± 303.72^{a} n = 328	1330.46± 313.32 n = 780
Sperm motility (%)	62.48±5.25^{b} n = 69	65.27±3.11^{a} n = 135	62.57±5.25^{b} n = 248	62.36±5.84^{b} n = 328	62.94±5.32 n = 780
Total sperm cells / ejaculate ($\times10^{6}$)	7445.43± 2241.66^{d} n = 69	9788.72± 3099.29^{b} n = 135	8990.12± 2972.33^{c} n = 248	11656.37± 3604.31^{a} n = 328	10112.88± 3521.12 n = 780
Number of semen doses per collection	248.18± 74.72^{d} n = 69	326.29± 103.31^{b} n = 135	299.67± 99.08^{c} n = 248	388.55± 120.14^{a} n = 328	337.10± 117.37 n = 780

N= Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b,c,d Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.12. Analysis of variance of semen characteristics of testicular circumference of bulls used for AI programme.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between Groups	874.72	3	291.57	80.79	
	Within Groups	2800.52	776	3.60		***
	Total	3675.25	779			
Colour (1-4)*	Between Groups	19.33	3	6.44	6.79	
	Within Groups	735.71	776	.94		**
	Total	755.04	779			
Mass activity (1-4 scale)***	Between Groups	17.67	3	5.89	10.23	
	Within Groups	446.73	776	.57		**
	Total	464.40	779			
Density (1-5 scale)**	Between Groups	24.68	3	8.22	15.86	
	Within Groups	402.32	776	.51		***
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between Groups	2543371.21	3	847790.40	8.89	
	Within Groups	73931382.71	776	95272.40		**
	Total	76474753.92	779			
Sperm motility (%)	Between Groups	895.99	3	298.66	10.97	
	Within Groups	21112.17	776	27.20		***
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between Groups	1599177707.63	3	533059235.87	51.32	
	Within Groups	8059102455.61	776	10385441.30		***
	Total	9658280163.25	779			
Number of semen doses per collection	Between Groups	1776864.12	3	592288.04	51.32	
	Within Groups	8954558.28	776	11539.37		***
	Total	10731422.40	779			

** = P<0.01 level of significant
*** = P<0.001 level of significant

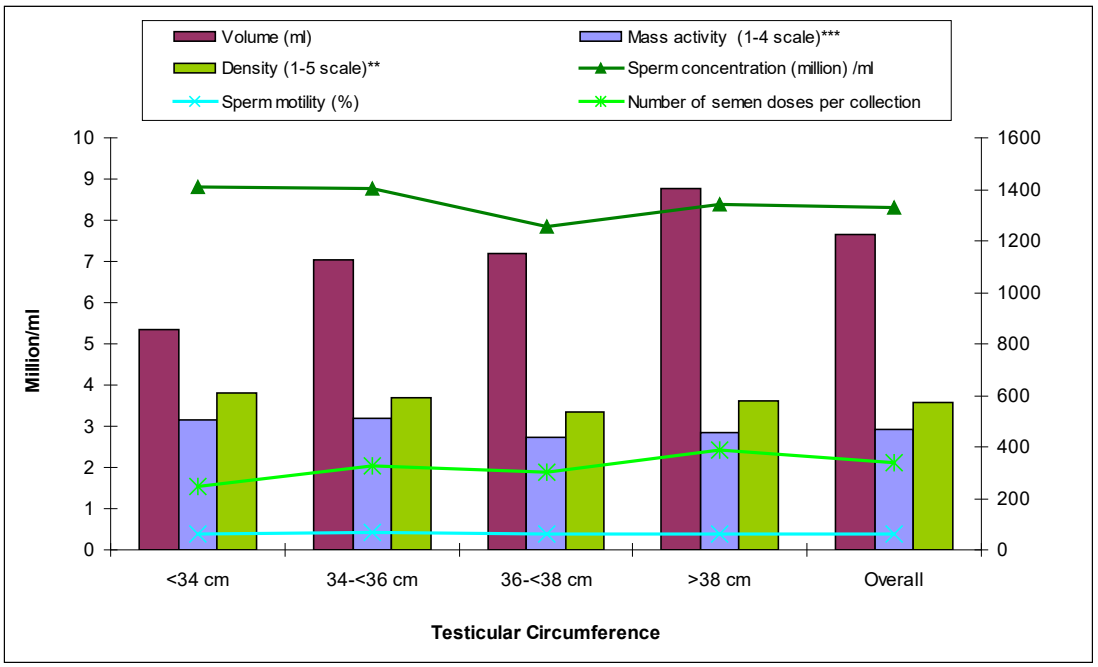

Fig. 4.9. Graph showing average volume (ml), mass activity (1-4 scale)***, density (1-5 scale)**, sperm concentration ($\times 10^6$) /ml, sperm motility (%), number of semen doses per collection influenced by testicular circumference of bulls.

Table 4.13. Comparison of semen characteristics of libido score of bulls used for AI programme.

Semen Characteristics	Libido Score			
	Poor	**Good libido**	**Very good libido**	**Overall**
Volume (ml)	7.04±2.38[b] n = 97	7.73±2.00[a] n = 364	7.78±2.25[a] n = 319	7.67±2.17 n = 780
Colour (1-4)*	2.91±.98[b] n = 97	2.93±1.00[b] n = 364	3.25±.94[a] n = 319	3.06±.98 n = 780
Mass activity (1-4 scale)***	3.46±.65 n = 97	3.60±.74 n = 364	3.54±.83 n = 319	3.56±.77 n = 780
Density (1-5 scale)**	2.79±.54 n = 97	2.92±.73 n = 364	2.94±.79 n = 319	2.91±.74 n = 780
Sperm concentration ($\times 10^6$) /ml	1265.21± 243.63[b] n = 97	1352.42± 304.65[a] n = 364	1325.25± 338.74[ab] n = 319	1330.46± 313.32 n = 780
Sperm motility (%)	63.96±5.28 n = 97	62.51±4.37 n = 364	63.12±6.20 n = 319	62.94±5.32 n = 780
Total sperm cells / ejaculate ($\times 10^6$)	8825.26± 3284.99[b] n = 97	10421.65± 3482.08[a] n = 364	10152.09± 3557.69[a] n = 319	10112.88± 3521.12 n = 780
Number of semen doses per collection	294.18± 109.50[b] n = 97	347.39± 116.07[a] n = 364	338.40± 118.59[ab] n = 319	337.10± 117.37 n = 780

N = Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.14. Analysis of variance of semen characteristics of libido score of bulls used for AI programme.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between Groups	44.40	2	22.20	4.75	
	Within Groups	3630.85	777	4.67		**
	Total	3675.25	779			
Colour (1-4)*	Between Groups	20.03	2	10.01	10.58	
	Within Groups	735.01	777	.94		***
	Total	755.04	779			
Mass activity (1-4 scale)***	Between Groups	1.70	2	.85	1.43	
	Within Groups	462.69	777	.59		*
	Total	464.40	779			
Density (1-5 scale)**	Between Groups	1.63	2	.81	1.48	
	Within Groups	425.38	777	.54		*
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between Groups	597175.08	2	298587.54	3.05	
	Within Groups	75877578.83	777	97654.54		*
	Total	76474753.92	779			
Sperm motility (%)	Between Groups	179.12	2	89.56	3.18	
	Within Groups	21829.04	777	28.09		*
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between Groups	196017502.34	2	98008751.17	8.04	
	Within Groups	9462262660.91	777	12177944.22		**
	Total	9658280163.25	779			
Number of semen doses per collection	Between Groups	217797.22	2	108898.61	8.04	
	Within Groups	10513625.17	777	13531.04		**
	Total	10731422.40	779			

* = P<0.05 level of significant
** = P<0.01 level of significant
*** = P<0.001 level of significant

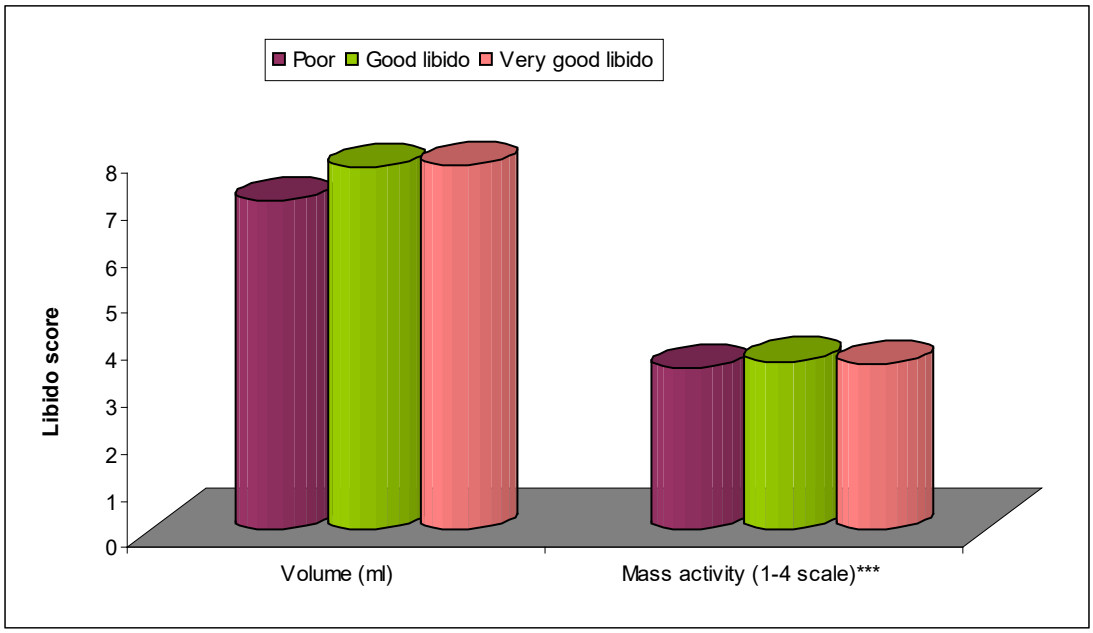

Fig. 4.10. Graph showing average poor, good and very good influenced by libido score of bulls.

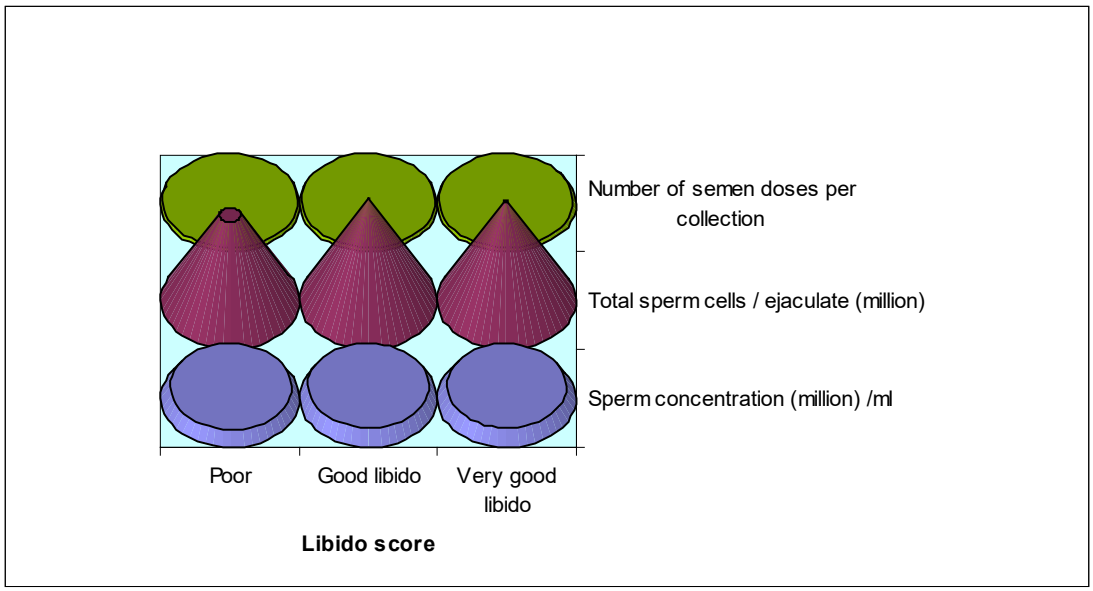

Fig. 4.11. Graph showing average sperm concentration ($\times 10^6$) /ml, total sperm cells / ejaculate ($\times 10^6$), number of semen doses per collection influenced by libido score of bulls.

4.1.7 Season:

Seasonal effect on the semen characteristics of artificial insemination (AI) bulls used in Bangladesh is presented in Table 4.15 and Fig. 4.12. It was found that season have significant ($P<0.05$) effect on most of semen traits except mass activity and density (Table 4.16). The highest (7.89±2.13 ml) volume was found at spring season. Mass activity and density highest at summer season (2.96±0.84, scale:1-4), (3.59±0.82, scale:1-5), respectively. Sperm motility, total sperm cells per ejaculate and number of semen doses per collection values were highest in spring season 63.63±3.96%, 10542.05±3640.71 million/ml, 351.40±121.36, respectively.

4.1.8 AI centres or stations:

Mean±SD with comparisons of the two AI centres or stations on semen traits is shown in Table 4.17 and Fig. 4.13 & 4.14. The highest significant ($P<0.05$) semen characteristics such as volume of ejaculate (7.81 ml) and colour (3.17, scale: 1-4) were found in RDCIF, Rajabarihat and lowest (7.43ml and 2.89, scale: 1-4, separately) at District AI centre, Rajshahi. The highest significant ($P<0.05$) mean density, sperm concentration, sperm motility, total sperm cells/ejaculate, number of semen doses per collection were (3.58±0.78, scale: 1-5), 1335.81±317.67million/ml, 63.33±5.73% 10300.96±3487.18 million/ml, 343.37±116.24 one by one in AI centre at RDCIDF, Rajshahi.

Table 4.15. Comparison of semen characteristics in different season of bulls used for AI programme.

Semen Characteristics	Season				
	Spring	**Summer**	**Autumn**	**Winter**	**Overall**
Volume (ml)	7.89±2.13^{a} n = 212	7.69±2.28^{ab} n = 218	7.36±2.10^{b} n = 180	7.68±2.13^{ab} n = 170	7.67±2.17 n = 780
Colour (1-4)*	2.92±.98^{b} n = 212	3.15±.98^{a} n = 218	3.17±.98^{a} n = 180	3.00±.97^{ab} n = 170	3.06±.98 n = 780
Mass activity (1-4 scale)***	2.88±.65 n = 212	2.96±.84 n = 218	2.94±.80 n = 180	2.85±.62 n = 170	2.91±.74 n = 780
Density (1-5 scale)**	3.55±.72 n = 212	3.59±.82 n = 218	3.58±.86 n = 180	3.51±.66 n = 170	3.56±.77 n = 780
Sperm concentration ($\times10^{6}$) /ml	1332.15± 298.08 n = 212	1344.70± 335.95 n = 218	1345.64± 340.77 n = 180	1294.03± 267.99 n = 170	1330.46± 313.32 n = 780
Sperm motility (%)	63.63± 3.96^{a} n = 212	63.47± 5.77^{a} n = 218	62.66± 6.46^{ab} n = 180	61.69± 4.59^{b} n = 170	62.94± 5.32 n = 780
Total sperm cells / ejaculate ($\times10^{6}$)	10542.05± 3640.71 n = 212	10078.92± 3371.65 n = 218	9829.63± 3629.09 n = 180	9921.15± 3420.42 n = 170	10112.88± 3521.12 n = 780
Number of semen doses per collection	351.40± 121.36 n = 212	335.96± 112.39 n = 218	327.65± 120.97 n = 180	330.71± 114.01 n = 170	337.10± 117.37 n = 780

N = Number of observation, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.16. Analysis of variance of semen characteristics in different season of bulls used for AI programme.

Semen Characteristics	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Volume (ml)	Between season	27.21	3	9.07	1.93	
	Within Groups	3648.03	776	4.70		*
	Total	3675.25	779			
Colour (1-4)*	Between season	8.58	3	2.86	2.97	
	Within Groups	746.45	776	.96		*
	Total	755.04	779			
Mass activity (1-4 scale)***	Between season	.80	3	.26	.44	
	Within Groups	463.59	776	.59		NS
	Total	464.40	779			
Density (1-5 scale)**	Between season	1.48	3	.49	.90	
	Within Groups	425.52	776	.54		NS
	Total	427.01	779			
Sperm concentration ($\times 10^6$) /ml	Between season	311943.73	3	103981.24	1.05	
	Within Groups	76162810.18	776	98147.95		*
	Total	76474753.92	779			
Sperm motility (%)	Between season	439.85	3	146.61	5.27	
	Within Groups	21568.31	776	27.79		**
	Total	22008.16	779			
Total sperm cells / ejaculate ($\times 10^6$)	Between season	59988275.28	3	19996091.76	1.61	
	Within Groups	9598291887.96	776	12368932.84		*
	Total	9658280163.25	779			
Number of semen doses per collection	Between season	66653.63	3	22217.88	1.61	
	Within Groups	10664768.76	776	13743.25		*
	Total	10731422.40	779			

* = P<0.05 level of significant
** = P<0.01 level of significant
NS = Non-significant

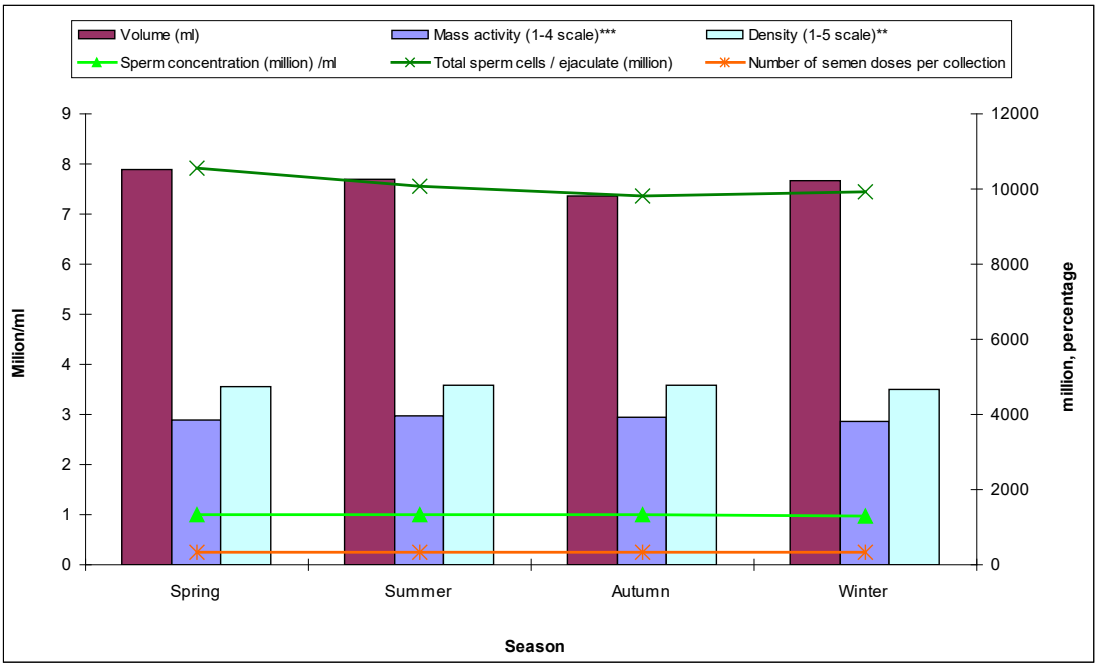

Fig. 4.12. Graph showing average volume (ml), mass activity (1-4 scale)***, density (1-5 scale)**, sperm concentration ($\times 10^6$) /ml, total sperm cells / ejaculate ($\times 10^6$) and number of semen doses per collection influenced by libido score of bulls.

Table 4.17. Comparison of semen characteristics in different AI centres of bulls used for AI programme.

Semen Characteristics	AI Centres/Sation		
	DAIC, Rajshahi	**RDCIDF, Rajshahi**	**Overall**
Volume (ml)	7.43±2.05[b] n = 306	7.81±2.23[a] n = 474	7.67±2.17 n = 780
Colour (1-4)*	2.89±1.01[b] n = 306	3.17±.95[a] n = 474	3.06±.98 n = 780
Mass activity (1-4 scale)***	2.94±.77[a] n = 306	2.89±.71[b] n = 474	2.91±.74 n = 780
Density (1-5 scale)**	3.53±.76 n = 306	3.58±.78 n = 474	3.56±.77 n = 780
Sperm concentration ($\times 10^6$) /ml	1322.17±306.79 n = 306	1335.81±317.67 n = 474	1330.46±313.32 n = 780
Sperm motility (%)	62.34±4.54[b] n = 306	63.33±5.73[a] n = 474	62.94±5.32 n = 780
Total sperm cells / ejaculate ($\times 10^6$)	9821.56±3559.17 n = 306	10300.96±3487.18 n = 474	10112.88±3521.12 n = 780
Number of semen doses per collection	327.39±118.64 n = 306	343.37±116.24 n = 474	337.10±117.37 n = 780

N = Number of observation, DAIC = District Artificial Insemination Centre, RDCIDF = Rajshahi Dairy Cattle Improvement and Dairy Farm, (1-4)*; 1=Opalescent, 2=Milky white, 3= yellowish white and 4=creamy white. (1-5)**; 1=watery, 2=milky, 3=mild creamy, 4=creamy and 5=creamy grainy. (1-4)***; 1= weak motion without forming any wave, 2=small, slow moving wave, 3=vigorous movement with moderate rapid waves and eddis and 5= dense, very rapidly moving wave and eddis. a,b Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

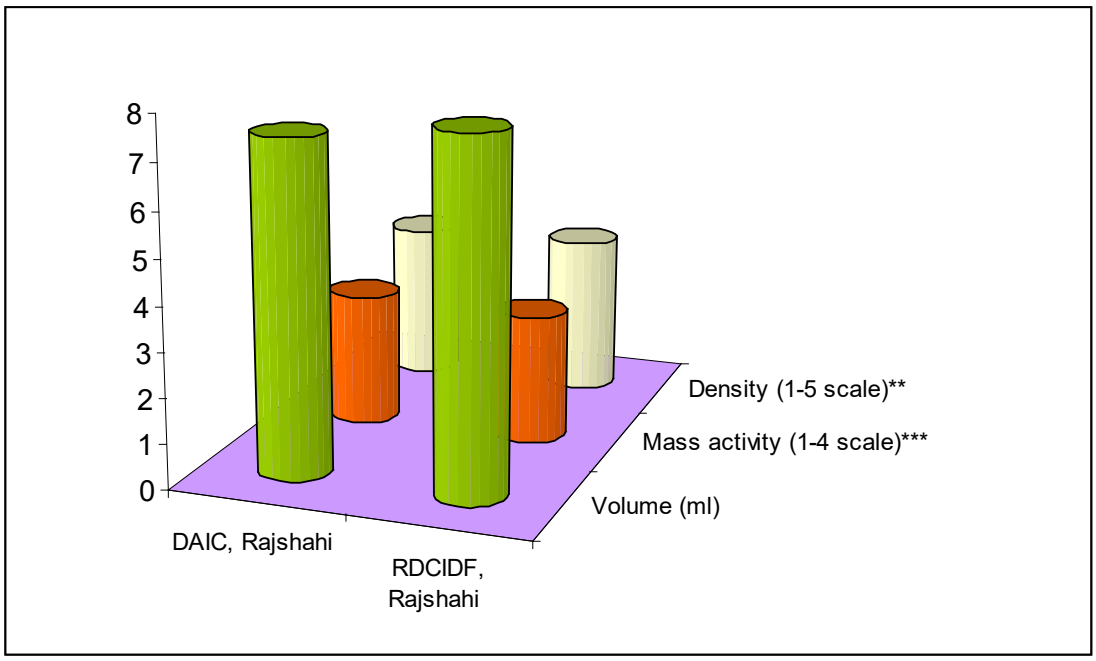

Fig. 4.13. Graph showing average volume (ml), mass activity (1-4 scale)*** and density (1-5 scale)** influenced by ai centre of bulls.

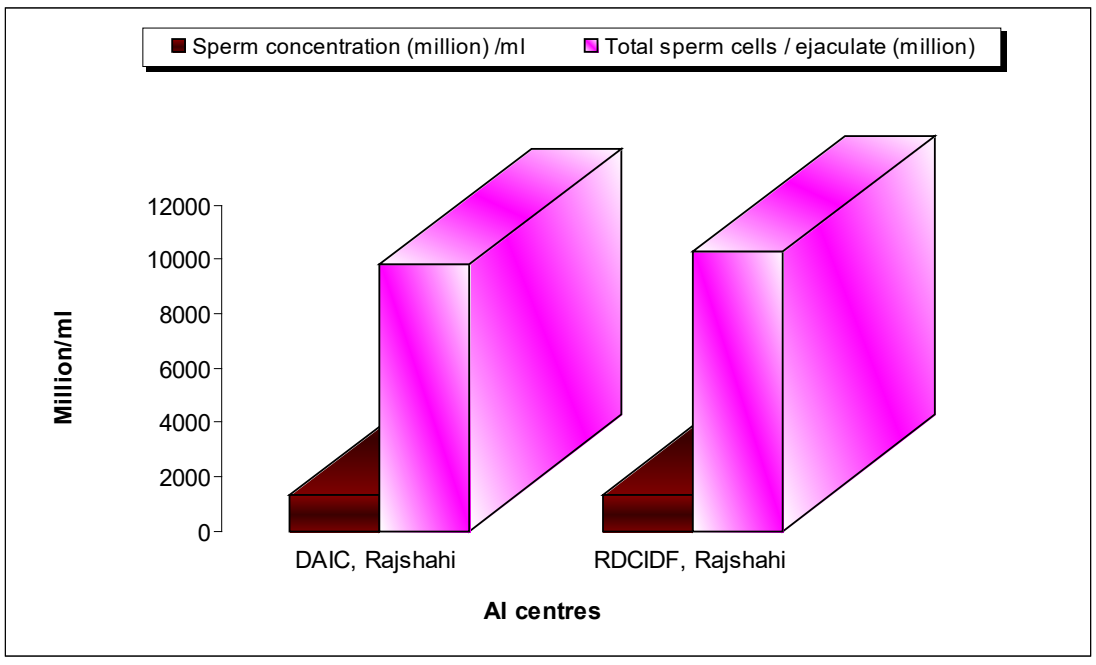

Fig. 4.14. Graph showing average sperm concentration ($\times 10^6$) /ml and total sperm cells / ejaculate ($\times 10^6$) influenced by ai centre of bulls.

4.2 Influence of Extender on Semen Quality on the Basis of Duration of Preservation:

The semen characteristics of freshly ejaculated semen of the 16 AI bulls used in this study are shown in Table 4.1. The percentage motility and the number of days that each diluent could maintain 50% sperm motility during storage in the three diluents are given in the Tables 4.18 and Fig. 4.15. The mean±SD value of conception rate was found higher by using extender egg yolk citrate solution (57.82±0.70). The relation of the extender with sperm motility and conception rate analyzed and ANOVA are presented in Table 4.19.

The highest conception rate (%) was found at D-04 (58.33±8.57%). The highest conception rate (%) for breeds, age of bull, body weight, libido score were found at L×F×F (55.67±9.24%), 8 - <10 years (56.88±8.93%), >600 kg (51.05±7.91%) and good libido (85.09±8.91%).

The bull ID, breed and body weight had significant effect ($P<0.05$) on conception rate.

Table 4.19. Effect of extender in relation with sperm motility and conception rate

Extender	N	Motility %				Conception rate (%)
		Day 1	Day 2	Day 3	Day 4	
Egg yolk citrate (EYC)	63	62.54± 6.26[a]	55.53± 5.19[a]	49.03± 4.83[a]	40.30± 2.64[a]	57.82± 7.00[a]
Cornel university extender (CUE)	63	59.33± 7.94[b]	50.73± 7.66[b]	44.79± 6.71[b]	37.79± 5.30[b]	50.87± 4.02[b]
Coconut milk extender (CME)	63	58.22± 3.01[b]	49.59± 3.18[b]	41.54± 3.34[c]	31.78± 2.78[c]	43.71± 3.59[c]
Total	189	60.03± 6.33	51.94± 6.17	45.10± 5.96	36.52± 5.20	50.77± 7.67

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.20. Analysis of variance of effect of extender in relation with sperm motility and conception rate.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Day 1	Between Groups	633.270	2	316.635	8.540	
	Within Groups	6896.540	186	37.078		**
	Total	7529.810	188			
Day 2	Between Groups	1240.186	2	620.093	19.527	
	Within Groups	5843.044	184	31.756		***
	Total	7083.230	186			
Day 3	Between Groups	1763.243	2	881.622	33.488	
	Within Groups	4817.816	183	26.327		***
	Total	6581.059	185			
Day 4	Between Groups	2365.949	2	1182.974	84.876	
	Within Groups	2466.963	177	13.938		***
	Total	4832.911	179			
Conception rate (%)	Between Groups	6220.812	2	3110.406	120.108	
	Within Groups	4790.890	185	25.897		***
	Total	11011.702	187			

** = P<0.01 level of significant
*** = P<0.001 level of significant

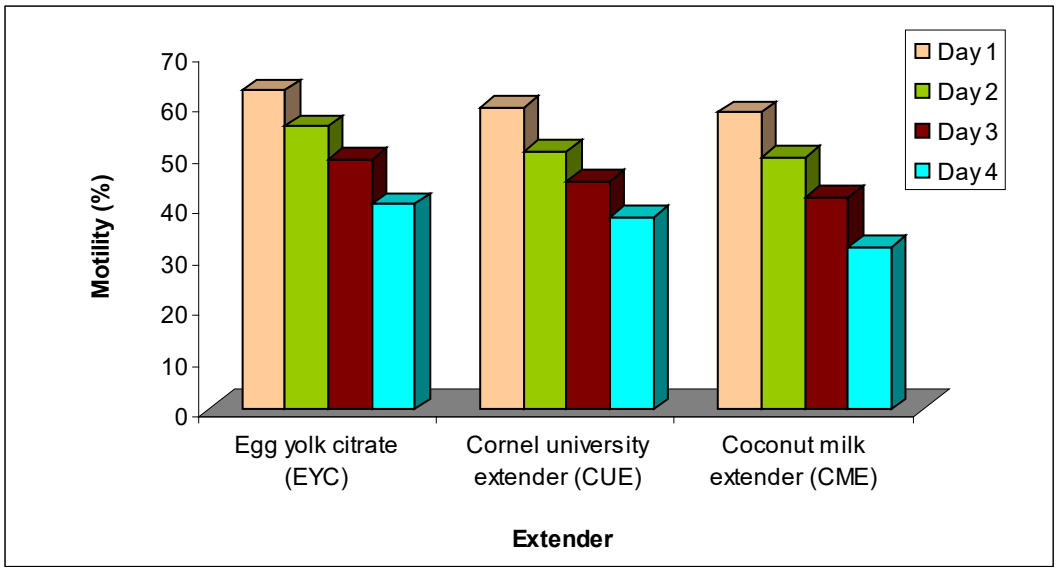

Fig. 4.15. Graph showing effect of various extender on sperm motility.

Table 4.21. Effect of duration of semen collection on motility and conception rate.

Bull ID	N	Motility %				Conception Rate (%)
		Day 1	Day-2	Day-3	Day-4	
D-04	12	61.50± 4.54	54.58± 3.37^{abc}	47.58± 2.68^{a}	38.33± 5.23	58.33± 8.57^{a}
D-028	12	61.75± 4.47	52.9167± 5.32^{abc}	45.5± 5.81^{ab}	37.0833± 6.33	55.66± 9.24^{a}
D229	12	62.33± 4.74	54.92± 5.25^{ab}	48.17± 6.51^{a}	38.75± 6.33	56.58± 7.32^{a}
1043	12	62.50± 4.95	53.33± 5.60^{abc}	46.75± 5.53^{ab}	37.45± 5.87	56.67± 9.54^{a}
2872	12	61.50± 4.89	52.92± 5.16^{abc}	47.25± 6.21^{ab}	38.42± 6.86	55.33± 7.84^{a}
2227	12	58.00± 6.57	49.54± 7.07^{abc}	42.85± 7.39^{ab}	35.58± 4.36	46.46± 5.47^{b}
2941	10	58.00± 7.16	49.00± 8.43^{bc}	41.70± 7.59^{b}	35.56± 4.95	49.00± 6.07^{b}
2979	12	59.83± 5.27	50.17± 5.20^{abc}	43.25± 5.85^{ab}	34.83± 5.83	48.33± 6.68^{b}
3037	12	57.83± 7.65	51.17± 7.60^{abc}	43.83± 6.90^{ab}	36.18± 4.85	48.50± 6.16^{b}
3058	11	58.00± 10.30	53.00± 5.48^{abc}	45.60± 4.84^{ab}	38.00± 3.16	48.82± 5.96^{b}
3079	12	62.92± 4.81	55.75± 5.21^{abc}	47.83± 5.41^{a}	36.50± 4.38	49.00± 6.24^{b}
3158	13	59.85± 3.67	50.85± 4.93^{abc}	44.46± 5.39^{ab}	35.62± 5.20	48.23± 5.60^{b}
3165	12	59.33± 7.74	49.92± 8.60^{abc}	42.50± 7.26^{ab}	36.20± 4.76	47.58± 5.05^{b}
3209	11	58.45± 6.89	48.73± 8.99^{c}	43.20± 6.18^{ab}	33.60± 6.04	46.91± 4.81^{b}
3211	11	56.91± 9.61	50.50± 1.58^{abc}	44.10± 2.56^{ab}	34.60± 2.68	48.00± 5.29^{b}
3381	12	61.08± 3.87	53.17± 4.11^{abc}	46.33± 4.33^{ab}	36.92± 4.10	48.25± 5.94
Total	189	60.03± 6.33	51.94± 6.17	45.10± 5.96	36.52± 5.20	50.76± 7.65^{b}

N = Number of observation, a,b,c Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.22. Analysis of variance preservation quality and conception rate at different bulls used for AI programme.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Day 1	Between bulls	676.731	15	45.115	1.139	
	Within Groups	6853.079	173	39.613		*
	Total	7529.810	188			
Day 2	Between bulls	845.125	15	56.342	1.544	
	Within Groups	6238.105	171	36.480		*
	Total	7083.230	186			
Day 3	Between bulls	761.803	15	50.787	1.484	
	Within Groups	5819.256	170	34.231		*
	Total	6581.059	185			
Day 4	Between bulls	367.598	15	24.507	.900	
	Within Groups	4465.313	164	27.228		NS
	Total	4832.911	179			
Conception rate (%)	Between bulls	3062.304	15	204.154	4.441	
	Within Groups	7952.501	173	45.968		**
	Total	11014.804	188			

* = P<0.05 level of significant
** = P<0.01 level of significant
NS = Non significant

Table 4.23. Effect of breed on sperm motility and conception rate of AI bull.

Breed	N	Motility %				Conception Rate (%)
		Day 1	Day-2	Day-3	Day-4	
L×F×SL×F×F	21	60.43± 3.76	52.48± 4.06^{ab}	45.48± 4.31^{ab}	37.05± 5.27	53.48± 9.40^{ab}
SL×F	24	62.25± 4.66	53.63± 5.62^{a}	46.75± 5.12^{a}	37.43± 4.67	53.67± 8.56^{ab}
L×F×F	12	61.75± 4.47	52.92± 5.32^{ab}	45.50± 5.81^{ab}	37.08± 6.33	55.67± 9.24^{a}
L×F	62	59.73± 6.85	51.54± 6.82^{ab}	45.35± 6.05^{ab}	36.69± 5.08	50.81± 6.27^{abc}
$(L\times F)_2$	64	59.28± 6.95	51.63± 6.13^{ab}	44.43± 6.32^{ab}	35.82± 5.36	47.98± 6.87^{c}
L×F(L×F×F)	6	57.50± 8.80	48.50± 9.18^{b}	41.00± 8.88^{b}	35.20± 4.92	48.71± 4.79^{bc}
Total	189	60.03± 6.33	51.94± 6.17	45.10± 5.96	36.52± 5.20	50.76± 7.65

N = Number of observation, L = Local, F = Friesian, SL = Sahiwal, a,b,c Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.24. Analysis of variance effect of breed on sperm motility and conception rate of AI bull.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Day 1	Between Groups	237.140	5	47.428	1.190	
	Within Groups	7292.669	183	39.851		*
	Total	7529.810	188			
Day 2	Between Groups	172.199	5	34.440	.902	
	Within Groups	6911.030	181	38.182		NS
	Total	7083.230	186			
Day 3	Between Groups	203.242	5	40.648	1.147	
	Within Groups	6377.817	180	35.432		*
	Total	6581.059	185			
Day 4	Between Groups	69.098	5	13.820	.505	
	Within Groups	4763.813	174	27.378		NS
	Total	4832.911	179			
Conception rate (%)	Between Groups	1161.476	5	232.295	4.314	
	Within Groups	9853.328	183	53.843		**
	Total	11014.804	188			

* = P<0.1 level of significant
** = P<0.01 level of significant
NS = Non significant

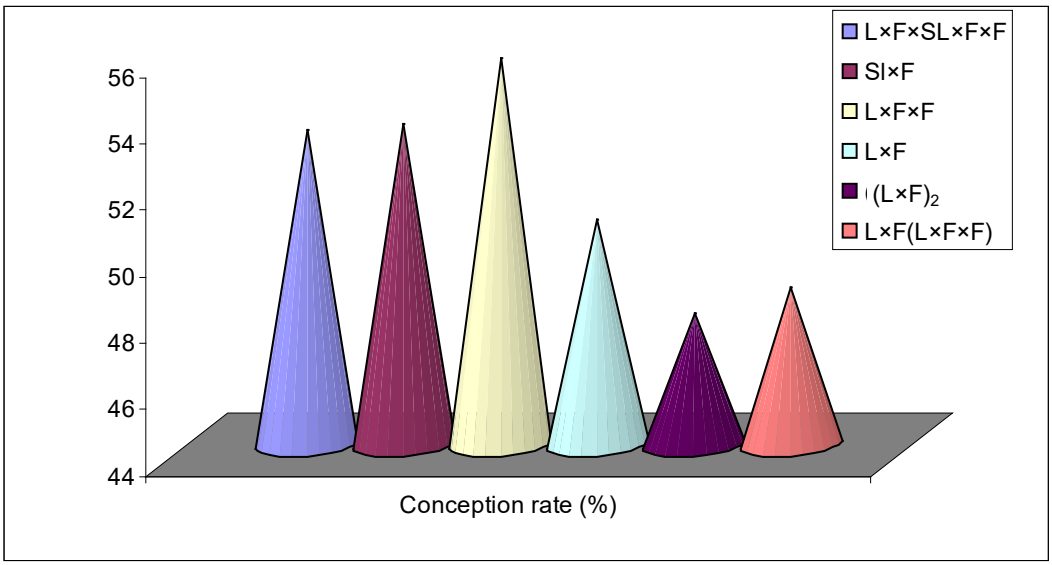

Fig. 4.16. Graph representing the influence of breed on conception rate of cows.

Table 4.25. Influence of age of bull on semen motility and conception rate.

Age of Bulls	N	Motility %				Conception Rate (%)
		Day 1	Day-2	Day-3	Day-4	
<4 yrs	21	59.42± 5.33	50.47± 7.07	44.60± 5.05	35.20± 4.87	47.19± 5.30
4-<6 yrs	98	59.13± 7.15	51.32± 6.35	44.12± 6.09	35.82± 4.65	48.29± 5.70
6-<8 yrs	22	60.27± 5.94	51.63± 6.38	45.68± 6.87	37.71± 5.75	51.90± 7.76
8-<10 years	36	61.91± 4.54	53.61± 4.77	46.61± 4.81	37.62± 5.67	56.88± 8.93
>10 yrs	12	62.33± 4.73	54.91± 5.24	48.16± 6.50	38.75± 6.32	56.58± 7.31
Total	189	60.03± 6.32	51.93± 6.17	45.10± 5.96	36.52± 5.19	50.75± 7.65

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

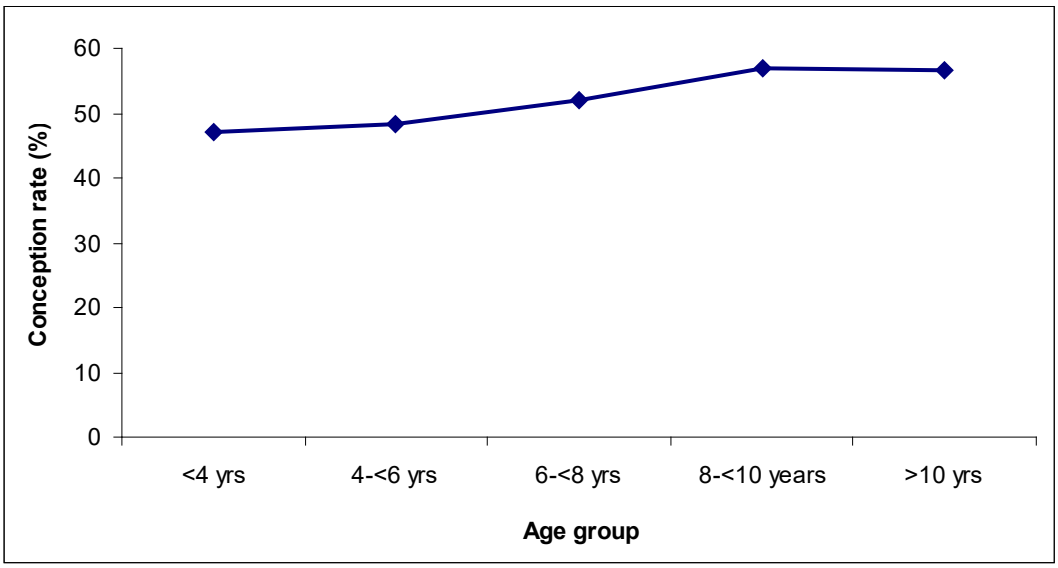

Fig. 4.17. Graph showing the influence of age group on conception rate of cows.

Table 4.26. Effect of body weight on motility and conception rate of AI Bull.

Body Weight	N	Motility %				Conception Rate (%)
		Day 1	Day-2	Day-3	Day-4	
<500 kg	9	59.44± 1.13^{ab}	50.22± 1.20^{ab}	42.22± 3.23^{ab}	32.67± 3.81	47.78± 4.76
500-<600 kg	10	55.30± 8.74^{b}	46.00± 8.38 ^{b}	39.50± 8.28 ^{b}	35.43± 3.95	48.40± 3.60
>600 kg	170	60.34± 6.24^{a}	52.38± 6.01 ^{a}	45.59± 5.73 ^{a}	36.78± 5.24	51.05± 7.91
Total	189	60.03± 6.33	51.94± 6.17	45.10± 5.96	36.52± 5.20	50.76± 7.65

N = Number of observation, a,b Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.27: Analysis of variance effect of body weight on motility and conception rate of AI Bull.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Day 1	Between Groups	243.276	2	121.638	3.105	
	Within Groups	7286.534	186	39.175		*
	Total	7529.810	188			
Day 2	Between Groups	412.055	2	206.028	5.683	
	Within Groups	6671.175	184	36.256		**
	Total	7083.230	186			
Day 3	Between Groups	428.692	2	214.346	6.376	
	Within Groups	6152.367	183	33.619		**
	Total	6581.059	185			
Day 4	Between Groups	153.099	2	76.550	2.895	
	Within Groups	4679.812	177	26.440		*
	Total	4832.911	179			
Conception rate (%)	Between Groups	150.325	2	75.163	1.287	
	Within Groups	10864.479	186	58.411		NS
	Total	11014.804	188			

* = P<0.05 level of significant
** = P<0.01 level of significant
NS = Non significant

Table 4.28. Bull libido relation with motility of spermatozoa and their conception rate

Libido score	N	Motility %				Conception Rate (%)
		Day 1	Day-2	Day-3	Day-4	
Poor	36	62.25± 4.25	54.47± 4.66	47.16± 4.77	37.36± 4.81	51.16± 3.82
Good libido	76	62.13± 4.72	54.03± 5.03	47.51± 5.33	38.88± 5.12	55.09± 8.91
Very good libido	77	56.92± 7.22	48.58± 6.38	41.62± 5.43	33.52± 3.86	46.28± 4.54
Total	189	60.03± 6.32	51.93± 6.17	45.10± 5.96	36.52± 5.19	50.75± 7.65

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.29. Seasonal effect on sperm motility and conception rate of AI bull.

Season	N	Motility %				Conception Rate (%)
		Day 1	Day 1	Day 1	Day 1	
Spring	63	62.16± 3.60	53.57± 4.84	46.52± 5.29	37.39± 4.94	53.37± 8.58
Summer	41	59.76± 7.51	51.73± 8.05	45.85± 6.59	37.42± 5.06	51.14± 7.79
Autumn	39	57.51± 9.08	50.38± 7.14	42.68± 6.91	34.40± 5.62	47.31± 5.51
Winter	46	59.50± 4.19	51.13± 4.51	44.46± 4.82	36.22± 4.96	49.73± 6.50
Total	189	60.03± 6.33	51.94± 6.17	45.10± 5.96	36.52± 5.20	50.76± 7.65

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

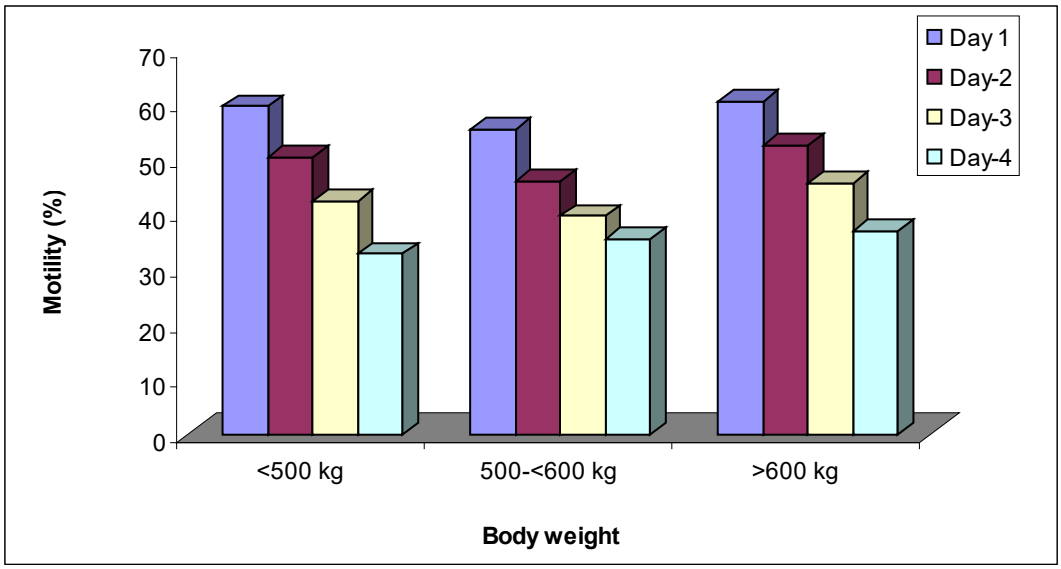

Fig. 4.18. Graph representing the influence of body weight on conception rate of cows.

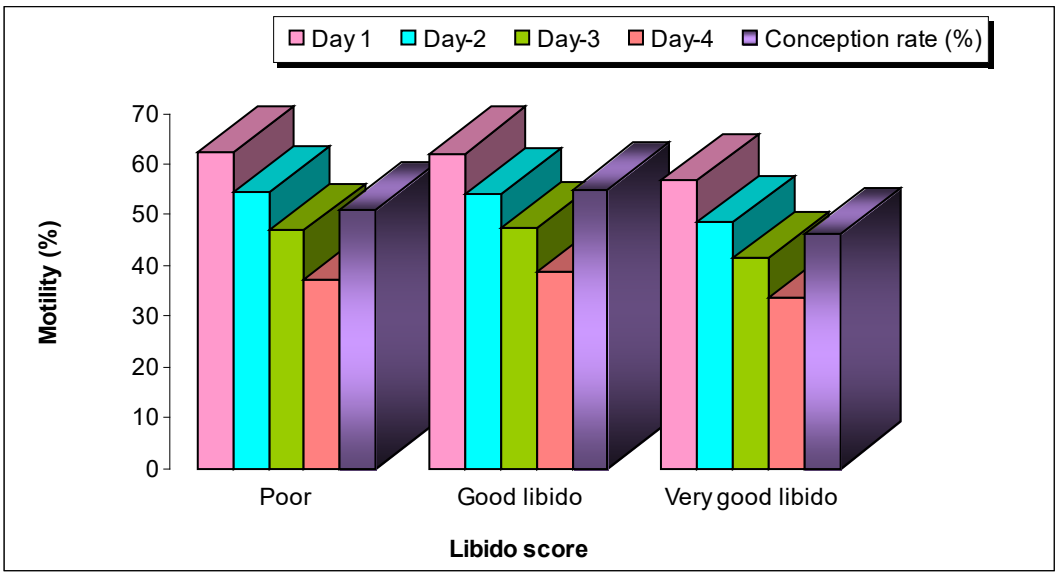

Fig. 4.19. Graph showing the influence of libido score on conception rate of cows.

4.3 Factors Affecting Sperm Abnormalities in Relations to NRR:

4.3.1 Individual bulls:

In total 164 ejaculates of semen from 16 bulls belongs six breeds collected during 1 year experiment, were studied for sperm abnormalities in head and tail regions of various bulls. Sperm abnormalities in relation to non return rate (NRR) of individual bulls used for AI program and ANOVA are shown in Tables 4.30 and 4.31. Significant ($P<0.05$) different was found at breed in relation with sperm abnormalities and non return rate. The average percentage of total sperm abnormalities was 18.13% in 16 AI bulls. The total sperm abnormalities was lower (12.95%) in bull Id 3058. The Fig. 4.27 represent the photographs of normal spermatozoa and Fig. from 4.28-4.34 represent some photographs of abnormal spermatozoa.

4.3.2 Breed effect:

The mean values of different sperm abnormalities obtained in this study were: total head abnormalities 4.29%, tail abnormalities 13.90%, and total sperm abnormalities 18.13% among the 6 breed of AI bulls (Tables 4.32). The average non return rate was found 78.50%. Breed of AI bulls had non significant effect ($P>0.05$) on all the morphological characteristic of spermatozoa except tail abnormalities (Table 4.33 and Fig. 4.30). The incidence of total sperm abnormalities was high (19.21%) in L×F breed and low in the breed of L×F×F (14.60%). The higher (83.50%) non return rate was found in L×F×F.

Table 4.30. Sperm abnormalities in relation to non-return rate of individual bulls used for AI programme.

Bull ID	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	N	Non Return Rate (%)
D-04	5	12.80± 7.19^{ab}	4.80± 4.13^{b}	17.60± 11.30^{abc}	100	79.00± 20.95^{ab}
D-028	4	11.25± 2.99^{ab}	3.35± 0.94^{b}	14.60± 3.83^{bc}	100	83.50± 5.97^{ab}
D229	4	11.75± 0.96^{ab}	3.75± 0.96^{b}	15.50± 1.41^{abc}	100	83.50± 2.38^{ab}
1043	4	12.50± 5.07^{ab}	4.25± 2.22^{b}	16.75± 7.14^{abc}	100	80.50± 13.00^{ab}
2872	4	17.75± 4.11^{ab}	5.00± 0.91^{b}	22.75± 5.01^{abc}	100	70.25± 9.03^{ab}
2227	3	14.00± 4.36^{ab}	3.17± 0.58^{b}	17.17± 4.73^{abc}	100	80.67± 7.57^{ab}
2941	4	13.75± 2.22^{ab}	3.40± 0.27^{b}	17.15± 2.46^{abc}	100	81.00± 3.16^{ab}
2979	4	12.50± 3.11^{ab}	3.75± 1.66^{b}	16.25± 1.55^{abc}	100	81.75± 2.36^{ab}
3037	4	13.25± 0.70^{ab}	4.50± 0.82^{b}	17.75± 1.25^{abc}	100	80.25± 2.06^{ab}
3058	4	10.00± 2.86^{b}	2.95± 0.67^{b}	12.95± 2.73^{c}	100	87.00± 4.76^{a}
3079	4	10.00± 4.24^{b}	3.25± 2.40^{b}	13.25± 6.59^{c}	100	87.50± 10.75^{a}
3158	4	15.50± 10.15^{ab}	4.58± 3.63^{b}	20.08± 13.69^{abc}	100	75.75± 21.33^{ab}
3165	4	12.38± 5.45^{ab}	2.98± 1.30^{b}	15.35± 6.60^{abc}	100	83.50± 11.09^{ab}
3209	4	19.00± 4.08^{a}	5.75± 2.60^{ab}	24.75± 6.66^{abc}	100	66.00± 13.95^{b}
3211	4	18.88± 0.63^{a}	4.67± 0.76^{b}	22.38± 2.56^{abc}	100	71.50± 3.42^{ab}
3381	4	17.38± 3.77^{ab}	8.25± 2.53^{a}	25.63± 6.26^{a}	100	64.75± 13.20^{b}
Total	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	1600	78.50± 11.95

N = Number of observation, a,b,c Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.31. Analysis of variance sperm abnormalities in relation to non-return rate of individual bulls used for AI programme.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Tail abnormality	Between Groups	521.977	15	34.798	1.641	
	Within Groups	1017.833	48	21.205		*
	Total	1539.810	63			
Head abnormality	Between Groups	106.749	15	7.117	1.623	
	Within Groups	206.028	47	4.384		*
	Total	312.777	62			
Total sperm abnormality	Between Groups	913.961	15	60.931	1.478	
	Within Groups	1979.419	48	41.238		*
	Total	2893.380	63			
Non return rate (%)	Between Groups	2903.583	15	193.572	1.524	
	Within Groups	6096.417	48	127.009		*
	Total	9000.000	63			

* = P<0.05 level of significant
NS = Non significant

Table 4.32. Influence of breed on sperm abnormalities and non return rate of various breeds of AI bulls

Breeds	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
L×F×SL×F×F	5	12.80± 7.19	4.80± 4.13	17.60± 11.30	79.00± 20.95
SL×F	10	11.60± 4.57	4.45± 2.44	16.05± 6.87	82.00± 11.96
L×F×F	4	11.25± 2.99	3.35± 0.94	14.60± 3.83	83.50± 5.97
L×F	26	15.13± 4.27	4.24± 1.78	19.21± 5.68	77.38± 8.99
$(L\times F)_2$	17	14.18± 5.59	4.51± 2.53	18.68± 7.65	76.76± 14.62
L×F(L×F×F)	2	15.00± 5.66	3.00± 0.71	18.00± 6.36	79.00± 9.90
Total	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50± 11.95

N = Number of observation, L = Local, F = Friesian, SL = Sahiwal, Mean±SD with different superscript letters in the same column differs significantly with each others ($P<0.001$).

Table 4.33. Analysis of variance influence of breed on Sperm abnormalities and non return rate of various breeds of AI bulls.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Tail abnormality	Between Groups	130.390	5	26.078	1.073	
	Within Groups	1409.419	58	24.300		*
	Total	1539.810	63			
Head abnormality	Between Groups	9.273	5	1.855	.348	
	Within Groups	303.504	57	5.325		NS
	Total	312.777	62			
Total sperm abnormality	Between Groups	130.144	5	26.029	.546	
	Within Groups	2763.236	58	47.642		NS
	Total	2893.380	63			
Non return rate (%)	Between Groups	307.787	5	61.557	.411	
	Within Groups	8692.213	58	149.866		NS
	Total	9000.000	63			

* = P<0.05 level of significant
NS = Non-significant

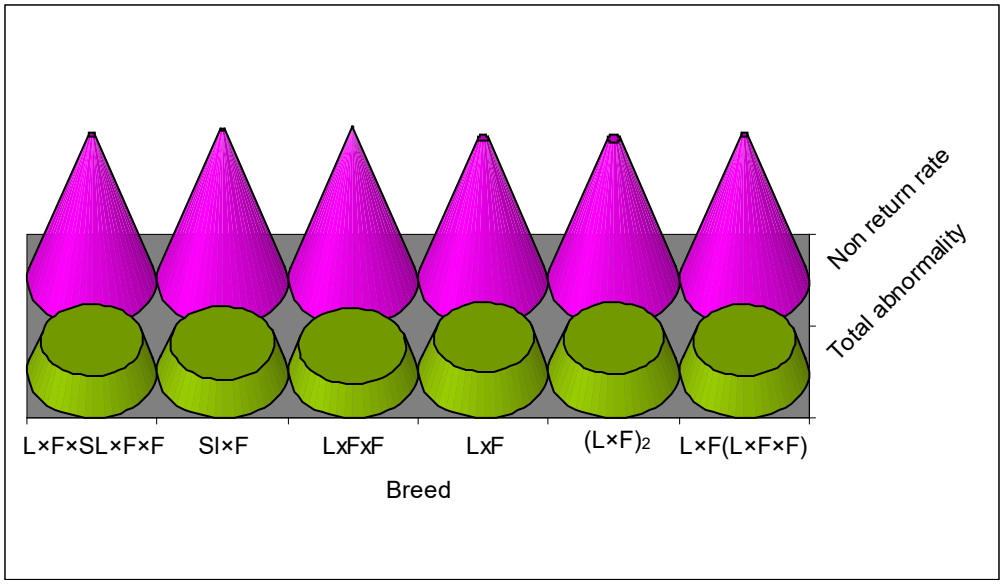

Fig. 4.20. Graph showing the influence of breed on total abnormalities in relation with NR(%).

4.3.3 Age effect:

The variations on the percentage of spermatozoon abnormalities in different age groups of bull studied are presented in the Table 4.34 and Fig. 4.31. The incidence of total sperm abnormalities was found higher (25.50%) at < 4 years and lower (15.50%) at >10 years. The higher (83.50%) non return rate was found in >10 years.

4.3.4 Body weight effect:

The data on the percentage of abnormalities of spermatozoa considering body weight groups are presented in Table 4.35 and Fig. 4.32. The incidence of total sperm abnormalities was found higher (26.50%) at 500-<600 kg and lower (12.75%) at <500 kg. The higher (88.00%) non return rate was found at <500 kg.

4.3.5 Body condition effect:

The mean value of different abnormalities of spermatozoa along with standard deviation among the body condition groups is furnished in Table 4.36. The incidence of total sperm abnormalities was found higher (19.84%) at good body condition. The higher (81.73%) non return rate was found in very good body condition.

4.3.6 Testicular circumference (TC) effect:

The data on percentage of abnormalities of spermatozoa for different TC groups of bull obtained in the present study are presented in Table 4.37 and Fig. 4.33. Analysis of variance showed that TC had non significant (P>0.05) effect on all the sperm abnormalities except head abnormalities (Table 4.38). The incidence of total sperm abnormalities was found higher (18.75%) at >38 cm of TC. The higher (80.85%) non return rate was found at 36-<38 cm.

Table 4.34. Age association with semen quality of bull.

Age	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
<4 yrs	7	18.57± 3.87	6.93± 2.94	25.50± 6.42	64.71± 13.45
4-<6 yrs	33	13.35± 4.89	3.85± 1.82	17.08± 6.18	80.70± 9.96
6-<8 yrs	7	16.14± 4.34	4.21± 1.22	20.36± 5.38	74.71± 9.53
8-<10 years	13	12.23± 5.13	4.18± 2.74	16.42± 7.79	80.85± 14.19
>10 yrs	4	11.75± 0.96	3.75± 0.96	15.50± 1.41	83.50± 2.38
Overall	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50± 11.95

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.35. Influence of body weight on semen quality of bull.

Body Weight	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
<500 kg	2	10.50± 6.36	2.25± 1.06	12.75± 7.42	88.00± 14.14
500-<600 kg	3	19.33± 3.79	7.17± 0.76	26.50± 4.09	62.00 11.14±
>600 kg	59	13.74± 4.84	4.22± 2.20	17.88± 6.61	79.02 11.40±
Total	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50 11.95±

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

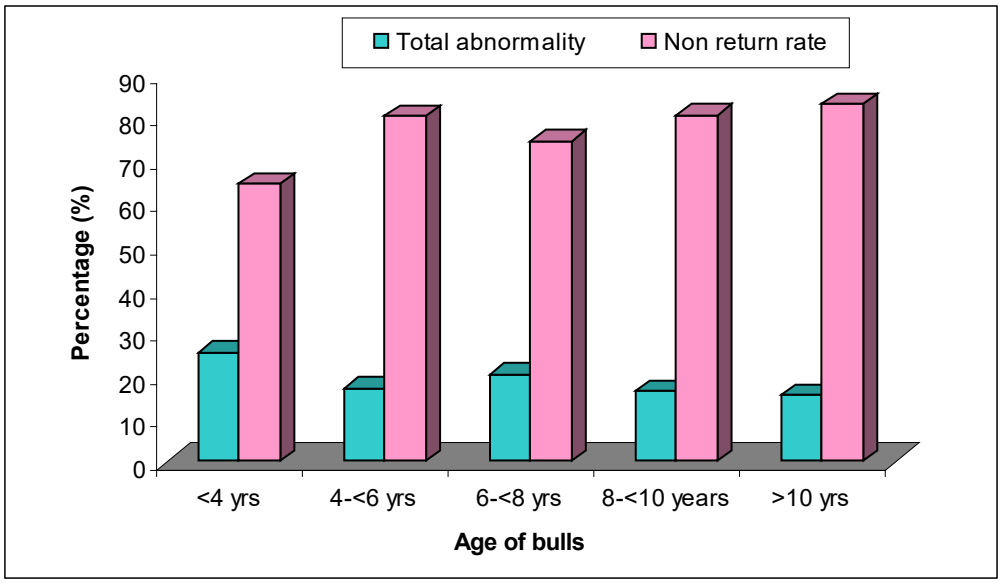

Fig. 4.21. Graph showing the effect of age on total abnormalities in relation with NR(%).

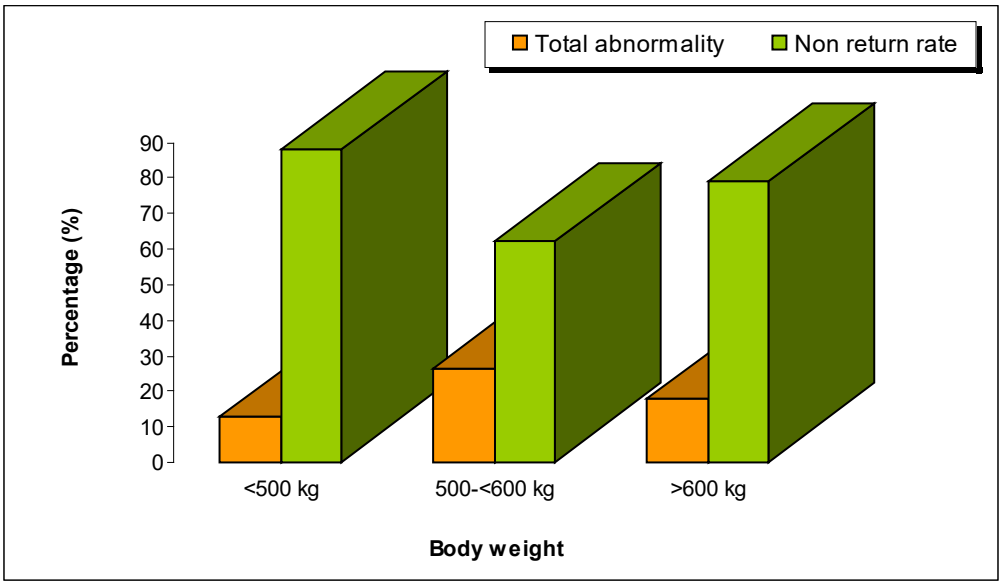

Fig. 4.22. Graph showing the effect of body weight on total abnormalities in relation with NR(%).

Table 4.36. Influence of body condition score on semen quality of bull.

Body Condition Score	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
Good body condition	31	14.94± 5.01	5.06± 2.56	19.84± 7.21	75.06 13.33
Very good body condition	33	12.92± 4.75	3.59± 1.67	16.52± 6.02	81.73 9.62
Total	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50 11.95

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.37. Effects of testicular circumference (TC) on semen quality of bull.

Testicular Circumference	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
36-<38 cm	20	12.78±6.17	3.98±2.74	16.76±8.49	80.85±14.44
>38 cm	44	14.41±4.26	4.44±1.99	18.75±5.84	77.43±10.65
Total	64	13.90±4.94	4.29±2.25	18.13±6.78	78.50±11.95

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.38. Analysis of variance effects of testicular circumference (TC) on semen quality of bull.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Tail abnormality	Between Groups	36.390	1	36.390	1.501	
	Within Groups	1503.420	62	24.249		*
	Total	1539.810	63			
Head abnormality	Between Groups	2.975	1	2.975	.586	
	Within Groups	309.802	61	5.079		NS
	Total	312.777	62			
Total sperm abnormality	Between Groups	54.601	1	54.601	1.193	
	Within Groups	2838.779	62	45.787		*
	Total	2893.380	63			
Non return rate (%)	Between Groups	160.655	1	160.655	1.127	
	Within Groups	8839.345	62	142.570		*
	Total	9000.000	63			

* = P<0.05 level of significant
NS = Non-significant

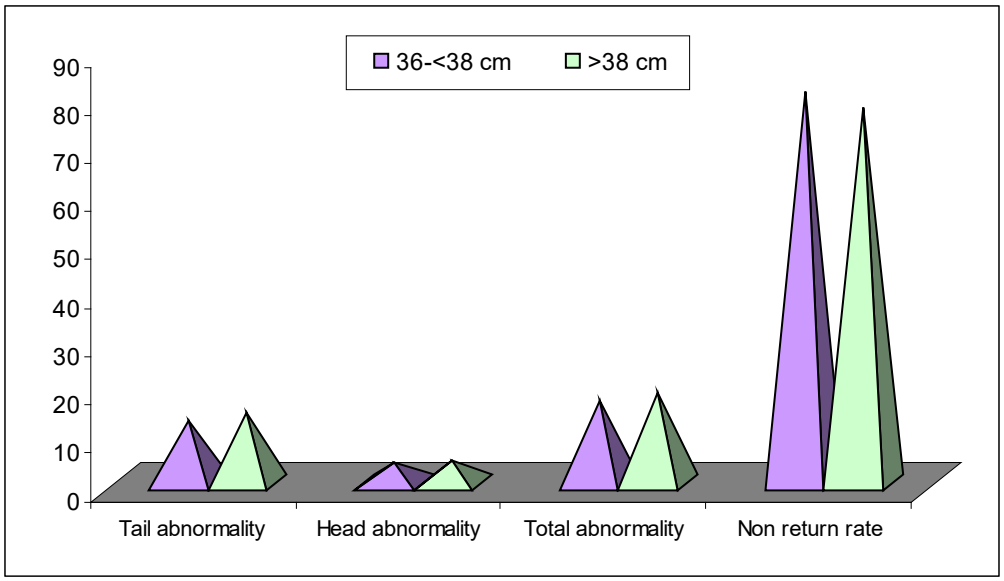

Fig. 4.23. Graph showing the effect of testicular circumference on total abnormalities in relation with NR(%).

4.3.7 Libido effect:

Mean and standard deviation (SD) of percentage of abnormalities of spermatozoa in different libido score are presented in Table 4.39 and Fig. 4.34. Analysis of variance showed that libido of bull significant ($P<0.05$) on sperm abnormalities (Table 4.40). The variation in the percentage of total head abnormalities in the different libido groups of bulls was found to be significant ($P<0.05$). The highest average % of head abnormalities was recorded (6.40%) in very good libido score and lowest in poor libido score (3.64%). The variation on the percentage of total tail abnormalities among libido was found to be significant ($P<0.05$). The highest average percentage of total tail abnormalities was recorded (17.30%) in very good libido score and lowest in poor libido score in (12.98%). The higher (81.85%) non return rate was found in poor libido.

4.3.8 Seasonal effect:

The mean±SD values of different percentage of abnormalities of spermatozoa pertaining to seasons are presented in Table 4.41 and Fig. 4.35 showed the effect on season on sperm abnormalities. Least squares analysis of variance showed that seasons had non significant ($P>0.05$) effect on total head abnormalities, tail abnormalities and total abnormalities and non return rate.

4.3.9 AI centres / stations effect:

The data on the effect of AI centre during semen collection on the percentage of abnormalities of spermatozoa are presented in the Table 4.43. Average total head abnormalities (4.31%) were a significantly highest in RDCIF, Rajabarihat followed by the District AI centre, Rajshahi (4.26%). The highest average % of abnormalities of spermatozoa was recorded (18.46%) in RDCIF, Rajabarihat, Rajshahi and lowest (17.45%) in DAIC, Rajshahi.

Table 4.39. Influence of libido with bull with semen quality.

Libido	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
Poor	20	12.98± 5.45	3.64± 1.96	16.43± 6.78	81.85± 10.70
Good libido	34	13.44± 4.31	4.04± 1.88	17.48± 5.90	79.53± 10.47
Very good libido	10	17.30± 4.98	6.40± 2.83	23.70± 7.39	68.30± 14.56
Overall	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50± 11.95

N = Number of observation, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.40. Analysis of variance influence of libido with bull with semen quality.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Tail abnormality	Between Groups	139.870	2	69.935	3.047	
	Within Groups	1399.940	61	22.950		*
	Total	1539.810	63			
Head abnormality	Between Groups	54.731	2	27.365	6.363	
	Within Groups	258.047	60	4.301		**
	Total	312.777	62			
Total sperm abnormality	Between Groups	382.309	2	191.154	4.644	
	Within Groups	2511.071	61	41.165		**
	Total	2893.380	63			
Non return rate (%)	Between Groups	1300.879	2	650.440	5.153	
	Within Groups	7699.121	61	126.215		***
	Total	9000.000	63			

* = P<0.05 level of significant
** = P<0.01 level of significant
*** = P<0.001 level of significant

Table 4.41. Seasonal variation of semen quality of bull.

Season	N	Tail Abnormality	Head Abnormality	Total sperm Abnormality	Non Return Rate (%)
Spring	24	13.40± 4.23	4.35± 2.10[ab]	17.75± 6.06	79.08± 10.75
Summer	15	12.97± 5.55	3.37± 2.09[b]	16.33± 7.45	81.33± 13.82
Autumn	10	13.46± 4.01	5.40± 2.84[a]	18.86± 6.52	77.20± 12.44
Winter	15	15.93± 5.78	4.40± 2.01[ab]	20.03± 7.45	75.60± 11.96
Overall	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50± 11.95

N = Number of observation, a,b Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

Table 4.42. Analysis of variance seasonal variation of semen quality of bull.

Parameters	Source of Variation	Sum of Squares	D.F.	Mean Square	F-value	P-value
Tail abnormality	Between Groups	82.714	3	27.571	1.135	
	Within Groups	1457.096	60	24.285		NS
	Total	1539.810	63			
Head abnormality	Between Groups	25.364	3	8.455	1.736	
	Within Groups	287.413	59	4.871		NS
	Total	312.777	62			
Total sperm abnormality	Between Groups	111.630	3	37.210	.803	
	Within Groups	2781.750	60	46.363		NS
	Total	2893.380	63			
Non return rate (%)	Between Groups	271.633	3	90.544	.622	
	Within Groups	8728.367	60	145.473		NS
	Total	9000.000	63			

NS = Non-significant

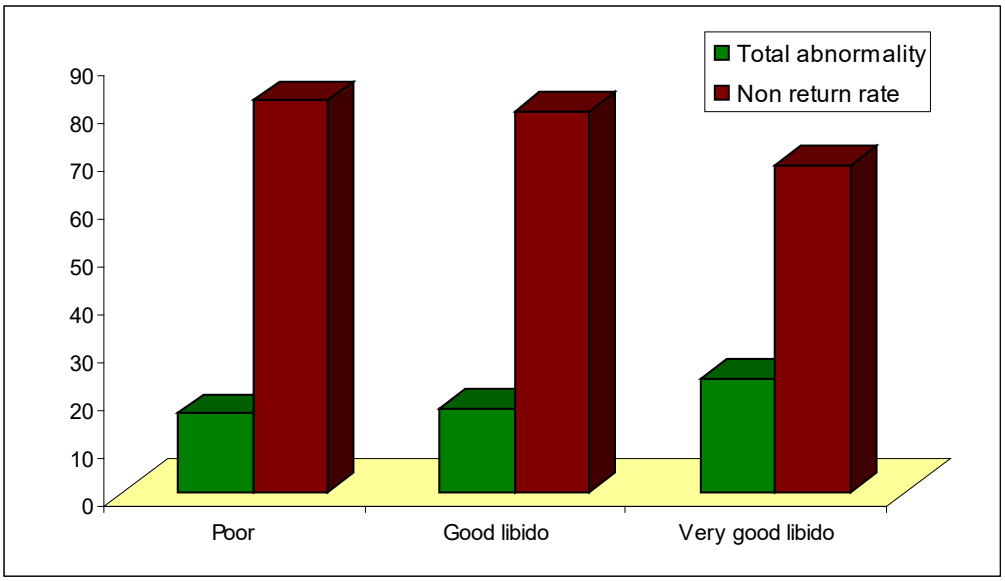

Fig. 4.24. Graph showing the influence of libido on total abnormalities in relation with NR(%).

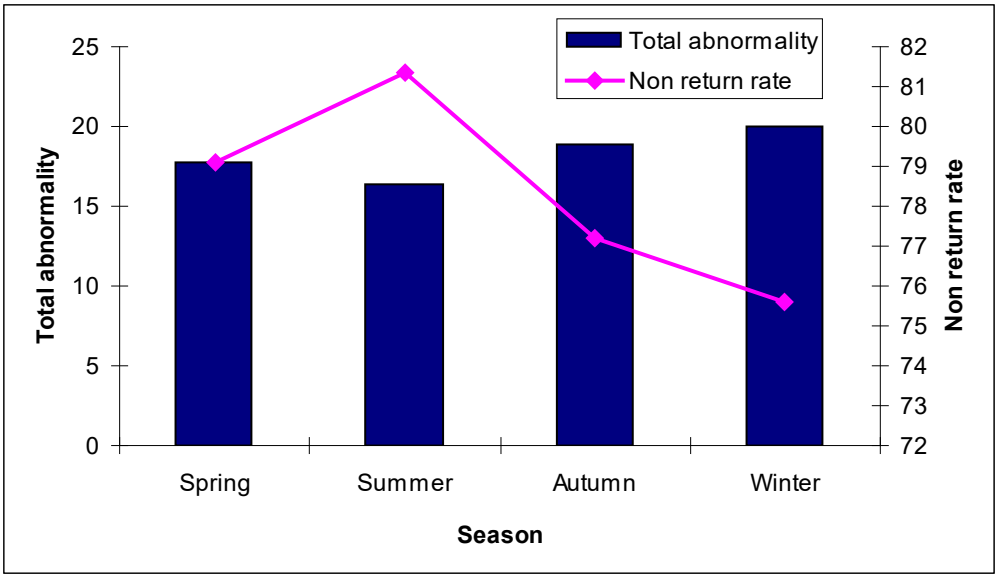

Fig. 4.25. Graph showing seasonal effect on total abnormalities in relation with NR(%).

Table 4.43. Analysis of variance influence of breed on Sperm abnormalities and non return rate of various breeds of AI bulls.

Location	N	Tail Abnormality	Head Abnormality	Total Sperm Abnormality	Non Return Rate (%)
DAIC, Rajshahi	21	13.19± 4.86	4.26± 2.22	17.45± 6.89	79.33± 12.46
RDCIDF, Rajshahi	43	14.24± 5.00	4.31± 2.28	18.46± 6.78	78.09± 11.82
Total	64	13.90± 4.94	4.29± 2.25	18.13± 6.78	78.50± 11.95

N = Number of observation, DAIC = District Artificial Insemination Centre, RDCIDF = Rajshahi Dairy Cattle Improvement and Dairy Farm, Mean±SD with different superscript letters in the same column differs significantly with each others (P<0.001).

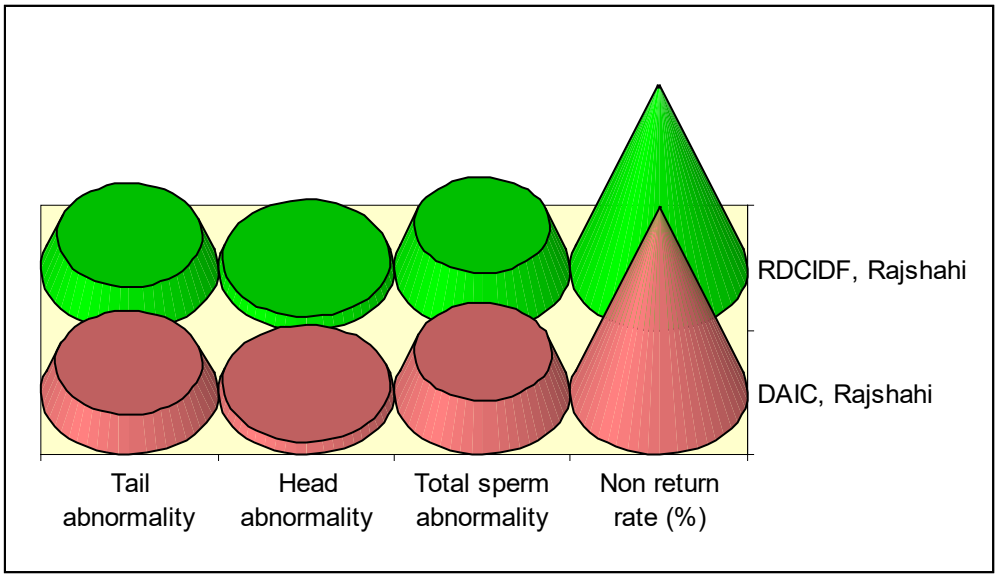

Fig. 4.26. Graph showing influence of AI centres on abnormalities in relation with NR(%).

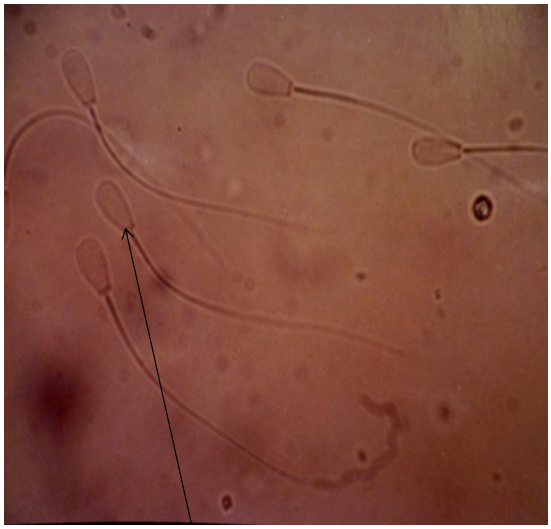

Fig. 4.27. Normal sperm head (William's stain 1000x)

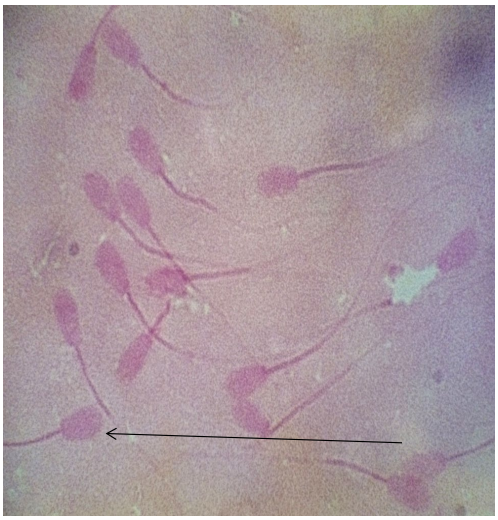

Fig. 4.28. Big sperm head (William's stain 1000x)

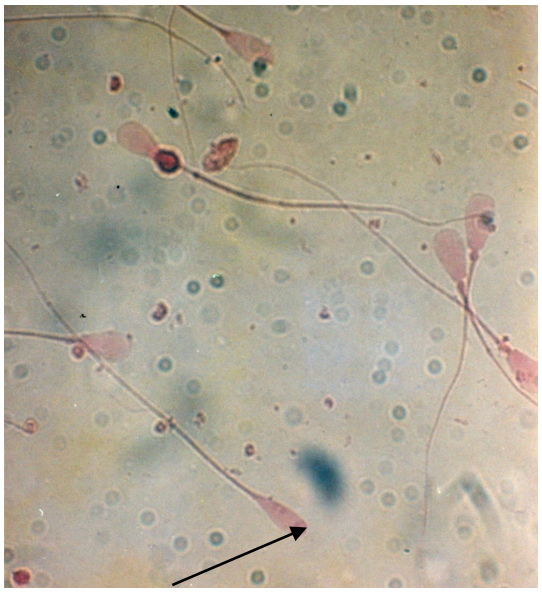

Fig. 4.29. Narrow sperm head (William's stain 1000x)

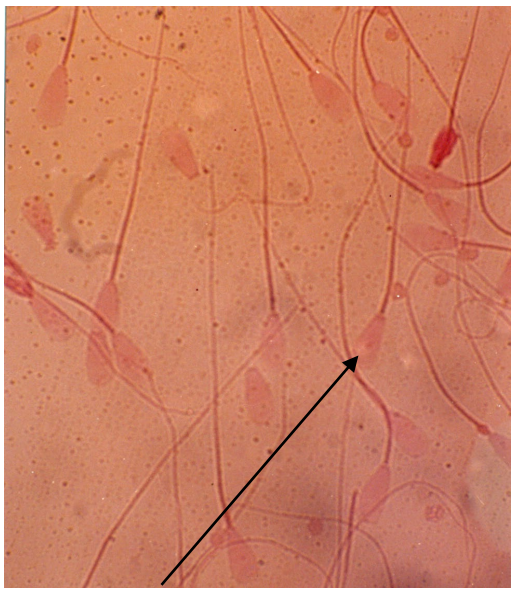

Fig. 4.30. Undeveloped sperm head (William's stain 1000x)

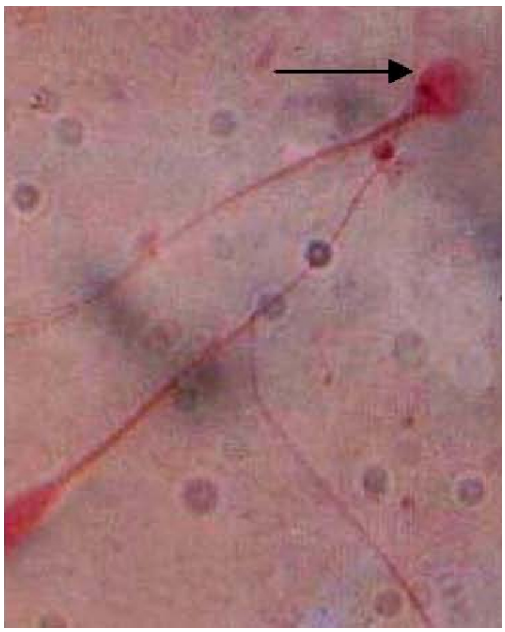

Fig. 4.31. Round sperm head (William's stain 1000x)

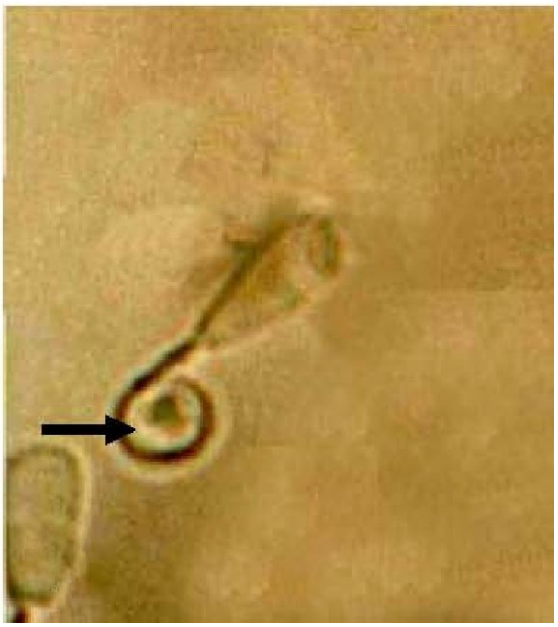

Fig. 4.32. Coil tail sperm (Phase contrast optics, 1000x)

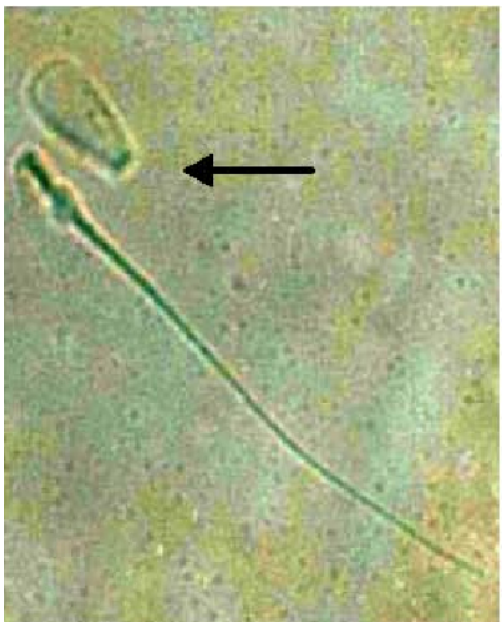

Fig. 4.33. Sperm head detached from mid-piece (Phase contrast optics, 1000x).

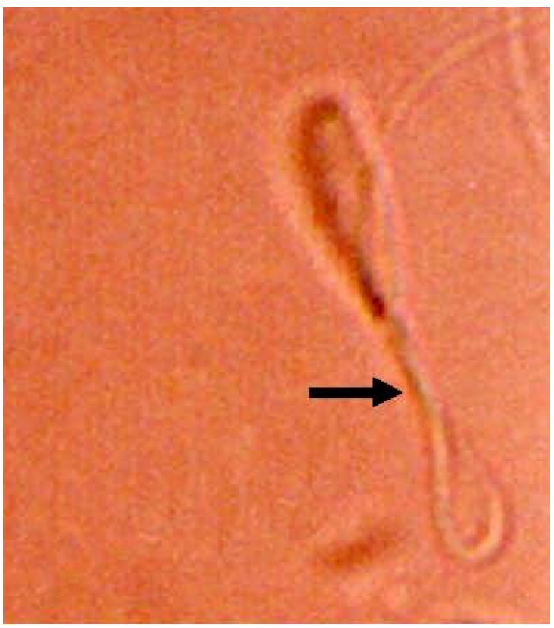

Fig. 4.34. Simple bent tail (Phase contrast optics, 1000x).

CHAPTER 5

DISCUSSION

5.1 Factors Affecting the Semen Production of Bulls:

The main findings of the present investigations are the significant effect on individual bull, breed, age, body weight, body condition, testicular circumference, libido, season and different AI centres on semen characteristics of AI bulls. Breed variation in semen characters refers to the observable or measurable differences among the individuals within a population for a population trait. Causes of breed variation in traits of farm animals are heredity, environment and joint effects of heredity and environment (Lasley, 1978). Through the application of artificial insemination practice, remarkable advances in the improvement of cattle population have been recorded. This however has placed more emphasis on genetic variation in semen characters of AI bulls used in AI programme. The breeding bulls for chilled semen in the present study can be regarded as normal based on the results of semen evaluation. However, individual group of bulls differ with regard to semen volume, sperm concentration and total number of spermatozoa /ejaculate. The differences between groups of bulls may be attributed to the variation in the secretory activities of the sex glands, age, body size and body weight (Graves, 1978; Leon *et al*., 1991; Sharma *et al*., 1991; Joinuddin and Hafez 1993). Moreover, collection frequency, pre-collection sexual stimulation, feeding regime and climatic condition can also influence the semen volume and sperm concentration (Graves, 1978; Al Hakim *et al*., 1986). In the present study, although the extrinsic factors were similar,

but the bulls were of different breed, age, body weight and testicular circumferences and also seasonal factors.

During processing, quality of preserved semen can be influenced by the extender used (Sullivan, 1978). Citrate-egg yolk diluent is proved to be preserved better quality and fertilizing capacity of spermatozoa than the others (Foote and Bratton, 1960; Rottensten, (1961). In the present study, citrate was used to produces chilled semen. For successful freezing of bull semen, a cooling period of at least two hours for lowering the temperature from +30° to +5°C recommended (Dhami and Sahni, 1993). When semen is subjected to cold shock, the spermatozoa with abnormal tail increases resulting in lower motility and reduced fertilizing capacity (Pickett and Berndston, 1978). Cold shock can also cause disruption of acrosome and fine structure of spermatozoa resulting lower fertilizing capacity (Shamsuddin and Rodrignez-Martnez, 1994; White, 1994).

The status of semen characteristics of the 16 bulls among six (6) breed groups maintained at two (2) different AI centres /station are presented in table 4.1. The individual bulls among the six breed had significant effect on only volume of ejaculate and colour of semen (Table 4.2). The volume of semen was found highest (8.81±2.41 ml) in bull ID 3209 and lowest (7.25±1.98 ml) was bull ID 2872. Semen volume depends on breed, age, body size, and body weight (Leon *et al*., 1991). All the semen traits were significantly affected by individual bull, which are related with finding of Graffer *et al*. (1988) and Sarder (2001a). In the present investigation, the semen characteristics range volume of semen was 7.24 to 8.81 ml, colour was 2.69 to 3.30 (scale:1-4), mass activity was 2.80 to 3.05 (scale:1-4), density was 3.38-3.77 (scale:1-5), sperm concentration was 1244.53 to 1382.87 million/ml, sperm motility was 61.46 to 64.49%, total sperm

cells/ejaculate was 9386.23 to 10767.50 million, number of semen doses/ejaculate was 312.87-358.92. Laing *et al*. (1988) stated that in case of bull semen, concentration of sperm varied from 500-2500 million/ml, where as Hafez, (1993) reported this concentration range of 1000-2000 and 800-1500 million/ml respectively for dairy and beef cattle which is almost similar as in the present study. Sarder (2001a) stated that in case of bull semen, volume, mass activity, sperm concentration, sperm motility and total sperm cells/ejaculate varied from 4.16 to 9.38 ml, 2.48 to 3.0 (scale:1-4), 1000.41 to 1677.78 million/ml, 56.14 to 65.34% and 6037.07 to10614.78 million/ml respectively. Again, Sarder (2003) who reported the total number of semen doses/ejaculates range of 217 to 314 which are almost similar as in the present study. However, Mathevon *et al*. (1998a) reported average total number of sperm cells/ejaculate of bovine semen 9310±4138 million. Individual bull differed with regard to the semen volume, colour, density, mass activity, sperm concentration, sperm motility, total sperm cells/ejaculate and number of semen doses /collection. The differences between bulls may be attributed to the variation in the secretory activity of the accessory glands, testicular circumference, age and body weight (Graves, 1978; Leon *et al*., 1991; Sharma *et al*., 1991). However, variation of semen characteristics among the bulls may be different location of AI centres / stations, nutritional status, semen collector, dummy, AV temperature and breed factors.

Six breeds of the bull such as L×F×SL×F×F, SL×F, L×F×F, L×F, (L×F)2, L×F and (L×F×F) had significant ($P< 0.05$) effect on all the semen characteristics. The significant highest volume (8.23±2.47 ml) was found in L×F×SL×F×F breed and lowest (5.97±2.36 ml) in L× F×(L×F×F) breed. Significantly ($P<0.05$) highest values of sperm

motility (63.48±5.12%), total number of sperm cells/ejaculate (10523.03±3607.45million/ml) and the number of semen doses (350.70±120.25) were found in (L×F)2 breed. Sarder (2003) also noticed significant (P<0.05) breed difference for volume of ejaculate in 100%SL, 75%F×25%L, 75%SL×25%HF, 50%SL×50%F and 50%F×50%L (6.84±2.2, 5.29±0.21, 6.99±0.16 and 5.87±0.21ml, respectively). Kibria *et al*. (1997) also noticed significant (P<0.05) breed difference for volume of ejaculate in 100%SL, 100%HF, 75%HF×25%L, 50%F×50%L, 75%SL×25%HF, 50%SL×50%HF and 50%S×50%SL. The colour, density and mass activity were significant affected by genetic groups. Sarder (2001a) also reported that the progressive movement of or mass activity of the sperm range from 2.48 to 3.0 which is the present result fall within the range. Again, Sarder (2003) noticed sperm concentration (1471±37 million/ml) in 100%SL and differ from 50%Fx50%L (1131±38) and did not differ between 75%F×25%L and 50%SL×50%F which are more close agreement to present study. Sarder *et al*. (2000) also noted that the sperm concentration (1447.03±132.32 mill./ml) was found in 50%SL×25%F×25%L and did not difference with 100%SL and 50%S×50%SL (1428.66±91.16 and 1384.09±172.23 mill./ml, respectively) which is more or less close to the present study. Laing *et al*. (1988) stated that in case of bull semen, concentration of sperm varied from 500-2500 million/ml, where as Hafez, (1993) reported this concentration range of 1000-2000 and 800-1500 million/ml respectively for dairy and beef cattle which is almost similar as in the present study. Variation among semen characteristics can also be due to skill of semen collector /attendant and temperature of AV.

Age groups of the bulls significantly affected volume, colour, sperm motility %. The volume of semen was highest at age groups of <4 years than that of >10 years ones (8.14 ml vs. 7.44 ml; P<0.05). This findings is varied from the study were made by Sarder *et al*. (2000). They reported that the volume of ejaculate for <4years, 4-6 years, 6-8 years, 8-10 years and >10 years were 4.31 ±0.87, 6.33±1.11, 6.33±1.82, 6.70±1.53 and 7.36±1.30 ml, respectively. Variation can be due to skill of semen collector, stress of bull and management factor. Mean colour, density, mass activity and sperm concentration were increased with medium age groups (4-6 years). Similar studies were made by Field *et al*.(1997), Hazarika *et al*. (1988), Eckardt *et al*. (1973), Hafez (1993), Rao *et al*.(1975), Stalhmmar *et al*. (1989) and Jamriska and Novy (1980). Total number of sperm /ejaculate increased at young age of the bulls. These results disagree with those of Siratskii (1992) who reported the semen volume and total sperm number per ejaculate increased with age, while sperm concentration remained relatively constant. Age of bull at collection affected ejaculate volume, sperm concentration, mass movement and forward movement (Stalhmmar *et al*., 1989). The sperm motility varied from 62.06±5.35 to 63.71±5.95% and lowest motility (62.06) was found in the age group at 6-<8 years. Sarder *et al*. (2000) reported that semen from young bull (>4 years old) had lower sperm motility than of older ones (>10 years) which is not similar result with present findings. However, Foote (1986) noted that semen from young bulls (3 to 6 years) had higher sperm motility than that of older ones (6 to 12 years) which is similar with present study. Variation in semen characteristics reported by different workers might be sample size, breed, age of bull, physiological condition of AI bull, body condition of bull and management.

Body weight of AI bull had significant affected all the semen traits except sperm concentration and sperm motlity. Volume of ejaculate, colour, mass activity, sperm concentration, total number sperm cells/ejaculate and total number of semen doses per collection were higher at the body weight groups at 500-<600 kg. Similar studies were made by Sarder *et al*. (2001a). They observed that the volume of ejaculate for <450 kg, 450 to 550kg, 550 to 650kg and >650 kg were 6.37±034ml, 7.72±0.5ml, 8.38±0.28ml and 6.25±0.50 ml, respectively. Asad (2001) reported that body weight groups 400-600 kg, >600 to 700 kg and >700 to 900 kg had significant (P<0.05) effect on volume, concentration, forward movement and non-significant effect on mass movement of semen. All the semen characteristics were found increased at body weight groups (500-<600 kg) may be due to proper feeding and management as well as large testes.

Body condition score of AI bulls had significantly affected all the semen characteristics. All the semen characteristics values were increasing with increasing body condition score in the study.

Testicular circumference groups of bulls significantly affected all the semen traits. The highest volume, density, total sperm cells/ejaculate and number of semen doses were gradually increased with increased of the sizes of the testicular circumferences and colour, mass activity and sperm motility decreased after 36-<38 cm groups of testicular circumference. Similar studies were made by Sarder *et al*. (2001a). They observed that the volume of ejaculate for <32 cm, 32-34 cm, 3436 cm and >36 cm groups were 6.01±0.36, 7.39±0.21, 6.31±0.54 and 9.08±0.30 ml respectively. The increase of the semen traits was probably due to the size of bull testes (Almquist, 1978). Bull tended to have small scrotum, less percentage of motile spermatozoa and higher proportion of abnormal

spermatozoa is classified as questionable breeders (Spitzer *et al*.,1988). Bull with small testes, have poor quality semen and after fail to produce no ejaculate in 4 separate Electro-ejaculate attempts, which are classified as unsatisfactory breeders (Spitzer *et al*.,1988).

Libido of AI bull had significant effect on all the semen characteristics. In the present investigation, most of the semen traits were increased at libido status good whereas volume, colour were higher values in libido score at very good.

Season of the year during semen collection significantly affected all the semen traits excluding mass activity and density. But Mukharjee and Bhattacharya (1952), Hussain *et al*. (1985), Hardin *et al*. (1982), Tomar and Gupta (1984), Samoilo (1974), Igboeli and Rakha (1971), Sinha and Prasad (1966) and Sarder *et al*. (2000) also reported that volume of semen does not differ with season. From the table 4.15 showed that the volume, sperm motility, total sperm cells/ejaculate, total number semen doses values were higher in spring season. Colour, mass activity, density values were higher in summer season. Sarder *et al*. (2000) observed that volume of ejaculate for spring, summer, autumn and winter were 6.69±1.78 ml, 6.91±1.41 ml, 6.27±1.97 ml and 6.74±1.62 ml, respectively. The highest significant sperm concentration was observed in autumn (1345.64±340.77 million /ml). On the other hand Tomar and Gupta (1984) reported that the initial motility and sperm concentration were both found to be significantly ($P<0.05$) higher during summer season than the cold season. The initial motility and sperm concentration was higher in summer (4.3±2.0, 1193±52.2 million/ml respectively) and lower win winter season (3.8±0.2 and 822.7±39.9 million/ml, respectively) supported the present study. Contrasting, Hussain *et al*. (1985) found

highest sperm concentration in winter and lowest in summer seasons and Kibria *et al*.(1997) also found highest sperm concentration in spring and lowest in summer. This variation might be related to geo-climatical condition of the area, feed of the bull and small number of observations. In this study, the season had significant effect on sperm motility which varied from 63.38±4.37 to 64.34±4.84%. Amir *et al*. (1982) also reported that the bulls and years significantly affected semen volume, sperm concentration and sperm motility before and after freezing. Tomar and Gupta (1984) also agree the present study. However, season had no significant effect on sperm motility reported by Hussain *et al*.(1985) and Mathevon *et al*.(1998b) Sarder *et al*. (2000), Saxena and Tripathi (1984).

Most of the semen parameters were better performances showed at RDCIDF, Rajshahi. Results of studies on the effects of AI stations on semen output are relatively diverse depending on location in terms of climate, altitude, and feeding and housing conditions. According to Godfrey *et al*. (1990), the influence of season on sperm concentration depends on breed and location. Semen characteristics seemed better in RDCIDF than the other remaining AI stations. The observed differences between AI centres / stations were greater in RDCIDF than the others. This could be either due to an improvement in bull management or climatic conditions.

5.2 Influence of Extender on Semen Quality on the Basis of Duration of Preservation:

Evaluation of the extender effect on sperm motility showed that there were differences ($P \leq 0.01$ or $P \leq 0.05$). Evaluation of semen quality is of great importance from the point of view of artificial insemination. It is

known that sperm originating from different breeds vary in many respects. During the time of storage a decrease in live morphologically normal spermatozoa and is a increase on dead spermatozoa and spermatozoa with bents neck were observed (Blesbois *et al.* 1999). The increasing importance of AI in cattle reproduction has caused investigators to become interested in developing the proper conditions for liquid (short term) and frozen (long term) semen storage. The possibility of dilution and storage of bull sperm would make the work of cattle breeder much easier, enabling them to transport semen even to distance farm to inseminate large groups of female and to improve the utilization of sperm from superior males. The most common procedure for short term bull semen storage (hour to day at refrigerator temperature) requires suspending sperm in an extender to retained their viability *in vitro* (Siudzinska and Lukarzeuiz, 2008).

The study was designate to evaluate an egg yolk citrate, cornell university extender and coconut milk extender of bull spermatozoa at 5º centre grate. The semen quality was tested by using three types of extenders on sperm motility % at duration of preservation in relation to conception rate of cows. The percentage motility and the number of days that each diluent could maintain 50% sperm motility during storage in the three diluents are given in the Tables 4.18 and Fig. 4.15. Average conception rate was observed 50.77%. The mean±SD value of conception rate was found higher by using extender egg yolk citrate solution. The three extender of egg yolk citrate (EYC), cornell university extender (CUE) and Coconut milk extender (CME) had significant effect ($P<0.05$) on sperm motility % and conception rate. Sperm motility and plasma membrane integrity are essential parameters used to assess the quality of

bovine semen. Ansari *et al.,* (2010) reported that the sperm motility was lower in powder egg yolk (68.3 ± 2.9) compared to the fresh egg yolk (71.7± 2.9). Gil *et al*., 2003a,b reported that sperm motility was found higher in Bioxcell® for five days of storage at 5 °C. A higher, although non-significantly different, sperm motility has been reported for Bioxcell® as compared to milk extender for ram semen. Using soya lecithin based extender Biociphos Plus® frozen-thawed semen is also reported to have higher percentage of sperm motility as compared to egg yolk based prepared extender Triladyl® (Gil *et al*., 2000; Moussa *et al*., 2002; Amirat *et al*., 2004; Amirat *et al*., 2005) The highest conception rate was found at EYC (57.82±7.00%) and lowest (43.71±3.59%) in CME. The highest conception rate (%) was found at D-04 (58.33±8.57%).

5.3 Factors Affecting Sperm Abnormalities in Relations to NRR:

For successful artificial insemination (AI) programme the importance of semen characteristics need to considered. One of the most important of these has been well established, as having a high influence on fertility is the percentage of spermatozoa with abnormal morphology. Many investigators (Saacke, 1970; Rao, 1971) observed the incidence of spermatozoa with abnormal morphology in fertile bulls to be 0-18% whereas Zemjanis (1970) reported the incidence of total spermatozoon abnormalities in the semen of fertile bulls to be 30-40%. Abnormalities of the spermatozoa occur due to following factors disorder of the semniferous or germina epithelium, during ejaculation or in manipulation of the ejaculate including excessive agitation, over-heating to rapid cooling, due to the presence of water, urine or antiseptic in the semen and etc. The primary abnormalities occur during spermatogenesis

either during spermatocytogenesis from spermatogonia to spermatids or during spermatogenesis from spematids to spermatogoa (Blom 1950 ; Lagerlof, 1934).

Individual bull of three AI centres had significant effect on morphological sperm characteristics. Individual bulls differed with respect to head abnormalities, tail abnormalities and total sperm abnormalities. The differences between bulls may be due to variation in their scrotal circumference, breed, age, body weight and body size and the secretory activities of their sex glands (Leon *et al.,* 1991; Sharma *et al*., 1991). Moreover, collection frequency, precollection sexual stimulation, feeding regume and climatic conditions can also influence the sperm abnormalities (Al-Hakim *et al*., 1986). The average percentage of total sperm abnormalities was 18.13% in 16 AI bulls.

Breeds of bull had significant effect on tail abnormalities of sperm in the tables 4.33. The average head and tail abnormalities were 4.29% and 13.90% respectively. The values recorded in present study for six breeds are lower than the average values reported in the literature for the normal fertile bulls (Mohana and Ramamohana, 1975). The lower value of head abnormalities (3.00±0.71%) and tail abnormalities (11.25±2.99%) were recorded in L×F×(L×F×F) and L×F×F respectively. The total sperm abnormalities was lowest values (14.60±3.83) at L×F×F.

The incidence of total head abnormalities, tail abnormalities and total sperm abnormalities decreased during the period of increasing the age of the bull. The quality of semen obtained during early period of maturity was poor and gradual improvement was noticed with the advancement of age which many related to the function of the testes.

The present findings are in general agreement wit the observations of Abdel-Raouf (loc.cit) who reported a similar improvements semen quality in growing Swedish bulls.

The data on the percentage of abnormalities of spermatozoa considering body weight groups are presented in Table 4.35 and Fig. 4.32. The lower value of tail abnormalities (10.50±6.36%), head abnormalities (2.25±1.06%) and total sperm abnormalities (12.75±7.42%) were recorded at <500 kg. The higher (88.00%) non return rate was found at <500 kg.

The testicular circumference (TC) had significant effect on tail abnormalities, total sperm abnormalities and non return rate. The TC groups of 36-<38 cm bull found to be significant at lower sperm abnormalities. The incidence of total abnormalities was found higher at >38 cm (18.75%). The higher NRR (80.85). The differences between TC groups of bulls may be attributed to the variation their scrotal circumference, bred, age body weight and body size and the scretory activity of the sex glands (Leon *et al*., 1991: Sharma *et al*., 1991).

Libido (score: 1-3) had a significant effect on the percentage of abnormalities of spermatozoa. The incidence of sperm abnormalities increased during the period of increased high libido score (1-3). The highest average % of head abnormalities was recorded (6.40%) in very good libido score and lowest in poor libido score (3.64%). The variation on the percentage of total tail abnormalities among libido was found to be significant. The highest average percentage of total tail abnormalities was recorded (17.30%) in very good libido score and

lowest in poor libido score in (12.98%). The higher (81.85%) non return rate was found in poor libido.

The incidence of tail abnormalities was low during summer seasons in comparison to their seasons. Krishna and Rao (1978) however, reported much higher incidence (6.39%) of tail abnormalities of spermatozoa in Murrah bulls. Perry (1965) however, reported high incidence of percentage of total abnormal spermatozoa during summer and low during autumn and rainy seasons which is not similar with that study. Flies and Lutezyk (1973) reported that highest percentage of abnormalities recorded in summer and lowest in December. Lunthra and Marinoy (1995) also reported the highest incidence of total abnormal spermatozoa (35.5%) in summer and lowest (25.93%) in spring. The differences in the investigation might possibly be due to different breeds of bulls used and climatic variation in the place of studies.

The data on the effect of AI centre during semen collection on the percentage of abnormalities of spermatozoa are presented in the Table 4.43. Average total head abnormalities (4.31%) were a significantly highest in RDCIF, Rajabarihat followed by the District AI centre, Rajshahi (4.26%). The highest average % of abnormalities of spermatozoa was recorded (18.46%) in RDCIF, Rajabarihat, Rajshahi and lowest (17.45%) in DAIC, Rajshahi.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions:

Results of the present study led to the following conclusions:

- Bull Id # 3209, 3058, 3165 and 3211 were the best performance of the semen production and semen quality.
- Breeds of $(L\times F)_2$ and SL×F had better semen traits. Other breed had improved performance of semen characteristics
- The age group of <4 and 4-<6 years of age had superior semen characteristics among the other groups.
- The heavier bull such as 500-<600 kg body weight had increased semen production performances.
- Very good body condition had more positive effect on semen production parameters.
- Testicular circumference of >38 had better semen quality.
- Good libido score was suitable for good semen production.
- Spring season had better performance of semen parameters.
- RDCIDF, Rajshahi had superior semen characteristics than other AI centres.
- Egg yolk citrated extender was suitable for chilled semen preservation.
- Use of egg yolk citrated (EYC) extender had better conception rate (%).

- Bull Id #: 3058 for RDCIF, Rajabarihat, Rajshahi, bull Id # D-028 for DAIC, Rajshahi shown the minimum % of total sperm abnormalities.
- The incidence of total sperm abnormalities was comparatively lower breed and non-return rate higher (83.50 × 597) in L×F×F.
- The middle and older age groups of 8 to 10 yrs and >10 yrs had shown the minimum sperm abnormalities and higher NRR than the young age groups.
- Body weight of <500 kg had the minimum sperm abnormalities and higher NRR than other groups.
- Very good body condition group of bull had the lowest sperm abnormalities and higher NRR.
- 36 to <38 cm testicular circumference (TC) had the minimum % of abnormal spermatozoa and maximum NRR than other TC groups.
- Libido score poor and good showed total sperm abnormalities lowest and higher NRR than the very good libido score.
- Spring and summer season was more favourable for the minimum % of sperm abnormalities (16.33%) and maximum NRR (81.33%) than other seasons.
- DAIC, Rajshahi showed the lowest % of abnormal spermatozoa in semen and highest NRR.

6.2 Constraints of AI Programme in Bangladesh:

- Selection of breed, semen quality, management, environment, feed, diseases, government policy etc.
- AI in animals is not having research and extension mandate under existing system of DLS.
- Poor maintenance of physical facility and lab equipments
- Manpower produced are less in number
- Financial constraints to meet the requirement for ensured quality of service
- AI centre is not well equipped and developed.
- Lack of awareness of farmers.
- Imperfect heat detection.
- Lack of skill persons for AI.
- Biodiversity issues.
- Available breeding bulls for National service.
- Necessity of proven bull.
- Traditional tale. Of farmer AI born calf are not survive.

6.3 Recommendation:

6.3.1 National animal breeding development priorities:

- Improvement of quality of animals through genetic means
- Characterization, conservation & improvement of dairy cattle
- Genetic improvement of smallholder livestock using ONBS with integration of MOET
- Evaluation of performance of exotic cattle & their crosses with native
- Development of simple method for genetic ranking of animals to be used in AI services
- Development of HYV livestock in the local environmental conditions

6.3.2 Farmers need:

- Farmers are the custodians of farm animal genetic resources
- Improved livestock breeds
- Quality breeding service (AI or breeding male)
- Training,
- Finance
- Feeds and fodder
- Animal health care
- Extension worker
- Marketing services

Finally the present study recommend the concern authority of artificially insemination programme should given the importance during selection of breeds, age, body weight, body condition, testicular circumference, libido of bull and season, management extender used for semen preservation, semen evaluation and conception rate and non-return rate of cows for the development of AI industry in Bangladesh.

CHAPTER 7

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